DEVELOPMENT AND PHARMACOLOGICAL EVALUATION OF HERBOMINERAL NANOEMULSION FOR THE TREATMENT OF SECOND DEGREE BURNS

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

AYURVEDIC PHARMACY

By

VIBHU KHANNA

Registration Number: 41900562

Supervised By

DR. Saurabh Singh

Ayurvedic Pharmacy (Professor)

Lovely Professional University

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2024

DECLARATION

I, hereby declared that the presented work in the thesis entitled "**Development and Pharmacological Evaluation of Herbo mineral Nano emulsion for the treatment of Second Degree Burns"** in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of **DR. Saurabh Singh**, working as Professor, in the **School of Pharmaceutical Sciences**of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

(Signature of Scholar)

Name of the Scholar : VIBHU KHANNA

Registration No. : 41900562

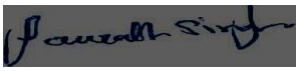
Department / School : Pharmaceutical Sciences

Lovely Professional University,

Punjab, India.

CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **"Development and Pharmacological Evaluation of Herbo Mineral Nanoemulsion for the Treatment of Second Degree Burns"** Submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the **Schol of Pharmaceutical Sciences** , is a research work carried out by **Vibhu Khanna**, Registration No. **41900562**, is a bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



Name of supervisor: Dr. Saurabh Singh

Designation: Professor

School: School of Pharmaceutical Sciences

University: Lovely Professional University.

ABSTRACT

Burn wounds are discussed in ancient literatures of Ayurveda as sushruta samhita as '*dagdha vrana'*, caused as accidental injuries during the parasurgical procedures of agnikarma. Classical literature of Ayurveda have quoted several references that elaborates the types, pathogenesis and management of vrana, and scar tissue under term *'vrana ropana'* along with healing herbs and various *'vrana upkrama'*. Treatment modalities and herbs derived from plant animal or mineral sources are reported to have vrana shodhana and ropana ability since ages. Ancient classics and current approach to burn wound treatment depends upon the depth of wound and wound infections.

Skin has always been the direct approach on surface area in classical and modern burn wound treatment strategies. Drug delivery from surface burn tissue using leaves bandaging, wound fumigations or application of ghrita, taila, malham, creams, gel, lotions dosage form or dressings has been challenging since skin favours penetration of smaller sized non-irritating amphiphilic drugs molecules. Credit to herbal drugs is ascribable to secondary metabolites and pharmacodynamic attributes. Recent innovations include NDDS (novel drug delivery system) in modern therapeutics for all types of burns. Research gap with translational approach for Classical polyherbomineral combinations (plant animal & mineral origin) using NDDS to resourcefully deliver the phytoconstituents with therapeutic efficacy is considered under this work. Compared to standard drugs, nanocarriers used for topical herbal remedies offer benefits with increased bioavailability, transdermal permeability, drug solubility, stability and absorption rates with enhanced pharmacotherapeutic activity. This makes improved drug delivery techniques more acceptable. The present study presents successful formulation of polyherbomineral nanoemulsion from herbal extracts, oils and mineral drug along with compatible excipients using high shear stress to formulate small droplets designed for burn wound treatments. initillay all drugs were subjected to authentication, physico-chemical phytochemical screening. Later screening the oil and surfactants were undertaken. Nanoemulsion was optimised using BBD. Further formulation was characterised using physical and chemical evaluation parameters. Stability studies were carried out as per ICH Q1A (R2) guidelines. In vitro and In vivo study was performed to check the burn wound healing effect of the formulation on second degree burn wounds. The results indicate that the polyherbomineral formulation is having anti-inflammatory, anti-oxidant and wound healing activity. Significant effect was observed when formulation was compared with standard drug.

Acknowledgement

I offer myself with deep sense of dedication **to Lord Sri Ram Ji** for giving me a golden opportunity to study in Lovely Professional University, Phagwara. My vocabulary falls short in expressing my profound sense of indebtedness to this great and wonderful institution, Lovely Professional University, Phagwara for creating such a platform for learning and execution for hundreds of students like me.

I consider it a privilege and honor to have a generous and conversant, respected, **Dr. Saurabh Singh**, Professor and H. O. D., Department of Ayurvedic pharmacy Faculty of Ayurveda, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, as my Guide / Supervisor. His perseverance, belief in my devotion for work, precious and constructive advice, insurmountable enthusiasm, painstaking efforts for me and expert supervision has smoothened my journey a lot and inspired me to strive towards perfection. I hold you as my idol who is always interested in growth of Ayurveda Science.

I acknowledge my sincere thanks to **Dr.Monica Gulati**, Professor and Senior, Dean, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, who allowed me to avail the departmental facilities and extended working hours that helped me to complete my research work

Undeniably, it is a pleasing privilege for me to express profound gratefulness and gratitude towards Head of the laboratory **Dr. Dileep Singh Bhagel**, professor, Department of Ayurvedic pharmacy, Faculty of Ayurveda, School of pharmaceutical Sciences, lovely professional University, Phagwara for his constant encouragement, expert and excellent laboratory supervision and purposive guidance helped me in every phase of the present thesis.

I am also thankful to Staff, School of pharmaceutical Sciences, lovely professional University, Phagwara specially **Dr. Bimlesh**, professor, Pharmacology Department for his critical support in animal study to complete my procedure of research work, **Dr. Sudhakar**, Associate professor, pharmaceutics department for the technical guidance.

I offer my honest obligation regards to incisive, Prof. **Dr. Ashwani Bhargav** Head of Department of Ras Shastra department, Dayanand Ayurvedic College Jallandhar for being my mentor and guiding me all the way through for professional and personal

doubts and responsibilities. A Journey becomes easy when someone joins you in the way. I am thankful to **Dr. Madhurima Bhargav,** for gleaned support. she stood by me in my journey. She guided me as an elder and was supportive as a true friend.

My thesis is virtually a result of love, care, advice and untiring support that I received from my husband, **Er. Ajay Arora** without his unwavering support in my family life and encouragement, this work could not have been completed. A special thanks for my Mother - in - law **Ms. Raj Arora** for her whole heartedly support and blessings. I Thank her for "keeping me sane" and "making the last five years fly by".

I could not forget to mention my sister and brother in law **Er. Deepti Arora** and **Er. Amit Arora** who always took the pressure of my family responsibilities. And the younger one **Aarush**, whose mischievous attitude made me smile after my exhausting day. They were supportive throughout my research work.

I feel overwhelmed with pleasure and gratitude in expressing my regards and thanks to My parents **Dr. Satish Khanna and Dr. Shashi Khanna** and my family members for their patience and blessing in every needful time.

It is my pleasure to thanks all my colleagues specially **Mr. Debojit, Ms. Rihana, Ms. Shashi, Ms. Sarvi, Ms. Neha and Ms. Priyanka** for their continue stimulation and zeal to carry out my work.

I express my sincere thanks to **Prof. Sanjeev Sood**, Principal D.A.V. Ayurvedic College Jallandhar for having permitted me to complete the PhD along with my job and helping me in every possible way.

I heartily thankful to my sweet loving little angel **Aarvi**, whose understanding patience and innocent smile always faded my problems and encouraged me to complete this herculean task well in time. Last but not the least, at this juncture I once again thank to all those who have given their pound of flesh directly and indirectly during this wonderful journey and helped a lot to glean up all that was needed in making of this project a great learning experience without any slipups.

Date : November, 2024 **Dr. Vibhu Khanna**

Place : Jalandhar

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF TECHNICAL TERMS

Abbreviations/ Symbols	Full form
MTT	3-[4,5- dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide
VAC	Vacuum assisted closure
NDDS	Novel drug delivery system
O/W	Oil in water emulsion
W/O	Water in oil emulsion
TBSA	Total body surface area
PG	Prostaglandins
TNF-α	Tumour necrosis factor- alpha
IL	Interleukins
EGF	Endothelial growth factor
FGF	Fibroblast growth factor
IGF	Insulin growth factor
GM-CSF	Granulocyte-monocyte colony stimulating factor
PDGF	Platelet derived growth factor
CO	Cyclooxygenase
ECM	Extra cellular matrix
MMP	Matrix metalloproteinases
TGF	Tissue growth factor
TIMB	Tissue inhibitors of metalloproteinases
KGF	Keratinocyte growth factor
VEGF	Vascular endothelial growth factor
GF	Growth factor
WHO	World health organisation
NO	Nitrous oxide
SOD	Superoxide dismutase
HPTLC	High performance thin layer chromatography
RL	Rubber latex
DL	Dialysable latex
NDL	Non-dialysable latex

LP	Latex proteins
HPLC	High performance thin layer Chromatography
ROS	Reactive oxygen species
APTT	Activated plasma thromboplastin time
TF	Tissue factor
DOE	Design of experiment
API	Active pharmceutical ingredient
CE	Curcuma longa extract
AE	Aloe vera extract
NSO	Neem seed oil
EYO	Egg yolk oil
T	Tuttha
PHMN	Polyherbomineral nanoemulsion
XRD	Xray diffraction studies
PG	Propylene glycol
FTIR	Fourier transform infrared spectroscopy
P-tpd	Pseudo-ternary phase diagram
ICH	International council of harmonisation
BBD	Box-behnken design
PDI	Polydispersity index
HUVEC	Human vascular endothelial cell line
ELISA	Enzyme-linked immunosorbent assay
PECAM	Platelet endothelial cell adhesion molecule
IEAC	Institute of ethics committee
RH	Relative density
CPSEA	Committee for the purpose of control and supevision of experiments on animals
MPO	Myeloperoxidases
CAT	Catalase
GSH	Glutathione peroxidase
LOD	Loss on drying
TA	Total ash
AIA	Acid insoluble ash

WSA	Water soluble ash
ASE	Alcohol soluble extractives
WSE	Water soluble extractives
JCPD	Joint committee on powedr diffraction standards
RF	Retardation factor
DOE	Design of experiment
CAACET	Calotropis procera, aloe vera, curcuma longa and tuttha
RI	Refractive index
MTT assay	3-(4,5-dimethyl thiazolyl-2)-2,5 diphenyltetrazolium bromide assay
ANOVA	Analysis of variance
UV	Ultraviolet
V/V	Volume / volume
w/w	Weight / weight
NMT	Not more than

LIST OF ANNEXURES

Chapter-1

Introduction

Ayurvedic science is a centuries-old health system with established deep roots thousands of years ago in India. This is one of the first ancient Vedic science that has been known as the traditional healing science. Though it has been commercially camouflaged and customized in various lucrative ways. However, people are fascinated only by the base roots of only ayurveda Etymological derivation of the term 'Ayurveda' states the meaning as the combination of two Sanskrit words 'Ayur' and 'Veda' means 'life' and 'science of life', the science focuses to bring harmony and balance in 'mind', 'body' and 'spirit'. Humans have been afflicted with wounds since the prehistoric era, and wound healing is an ancient art in biomedical science, Origin assumed in the pre-Vedic era (4000 B.C. – 1500 B.C). For ages, there has been a quest for solutions through herbal medications. Research nowadays promoting wound healing is escalating exponentially. The use of herbal remedies as therapeutics by Chinese, Greek & Egyptian, and Indian medicine has been already documented in Ayurvedic texts. (Parasuraman, Thing and Dhanaraj, 2014). wound is first mentioned in Charaka Samhita as 'vrana' and Sushruta Samhita later explained it in detail. Vrana is described in Shalya tantra of Ayurveda as the condition when the body tissues are destroyed leaving a scar mark after healing. The word 'Vrana' is obtained from the root prefix "vriya" having the meaning 'to recover' and suffix 'ach' meaning 'bhavya' '-ch' sound is omitted leaving behind 'vran'+'a'. shabdakalpadruma defines the word 'vrana' as 'Gatra vichoornena'.

'vrana gaatra vichurnane, vrana yati iti vrana ha' (su.chi.1/6)

The nirukti is stated as destruction of either the part of the body (tissue) or the body as a whole.

"vrunoti yasmaat roodhe api vrana vastu na nashyati I

Aadeha dhaaranaat tasmaat vrana ityuccyate budhaihi II" (su.su.21/40)

Conventional wound healing treatment entered a diversified approach since 5000 B.C. flaunting the popularity and importance of reparative treatment in the vedic era.

Ancient literature of Ayurveda (Sushruta Samhita 1000 B.C) has laid a strong underpinning for modern surgeons that funneled several references in various contexts in Ayurveda that explains aetiology, etiopathogenesis of wound, and scar tissue management in detail. Management of different types of wounds in classical texts are described with 'Vrana ropaka' drugs and 60 vrana kramas by Aacharya Sushruta (father of Indian surgery) (su.chi.1/8) and 36 types of procedures by Aacharya Charaka (father of Indian medicine) (Ch.chi.25/40-43). Ayurvedic treatment involves 70, 30, and nearly about 10 percent of drug usage from plant, mineral, and animal origin respectively.

Burn wounds are revealed under the term 'Dagdha Vrana' to occur either accidentally or as a result of para-surgical treatments like Agnikarma. Sushruta Sutra Sthana chapter 12, has detailed description with the clinical characteristics, kinds and recommended course of therapy for each form of burns. *Plushtadagdha* (first-degree burn), *Durdagdha* (second-degree partial thickness burn), *Samyakdagdha* (second-degree full-thickness burns), and *Atidagdha* (third-degree burn, involving muscle and joints).(Shindhe *et al.*, 2023). Use of Jatyadi ghrita in agantuja vrana management, neem seeds, mulethi, Haldi, plant latex or Aloe vera extracts, medicated ghrita and honey are few among the popular drugs used. They are known to be used individually or in polyherbal combinations for healing benefits in traditional system with promising results (Kotian *et al.*, 2018)

Current approach towards burns wound treatment Strategy for burn wound treatment involves drug penetration, wound recovery and to reconstruct the protective layers for appropriate healing which progresses in five phases. Starting with the use of immediate first aid for all categories of burns. Resuscitation therapy, burn wound coverage, supportive care and rehabilitation are used in later phases. Burn wound coverage uses the topical antimicrobials (Jeschke *et al.*, 2020). Anti-bacterial, non-toxic and non-reactive property of silver promoted the use of its salts and medicaments in market since long.(Atiyeh *et al.*, 2007) They includes silver sulphadiazine, silver impregnated dressings (acticoat, mepilex etc.). Other treatments include non-pharmacological procedures like NPWD(negative pressure wound device), VAC (vacuum assisted closure) (Argenta *et al.*, 2006) . non-healing wounds, hydrogel on moist wounds(Fumal *et al.*, 2002) or use of synthetic growth factors are popular

healing agents used in modern therapeutic procedures. Though abundant strategies have been considered to intervene the natural skin barrier and effectively deliver drugs through topical route. The Drug delivery to skin using the routine formulations like ointments, creams, gels, lotions and silicon impregnated dressings is challenging since Skin favors penetration of smaller sized, non-irritating amphiphilic drugs.

Ayurveda as an alternative approach to burn wound treatment plants have played a significant role in both conventional and alternative medicine for alomost 5,000 years. Sushruta Samhita has mentioned 43 medicinal herbs in nyagrodhadhi, sursadi gana, aragvadhadhi gana, arkadi gana, kakolyadi gana and priyanguambashthadi gana to have vrana ropana potential and 62 plants are found to have vrana shodhana and ropana ability(Kumar *et al.*, 2021). Dressing of the burn wound with medicated ghee preparations, bandaging with leaves or wound fumigation with medicinal drugs and polyherbal topical wound care formulations are important consideration under ancient treatments(Shindhe *et al.*, 2023). Herbal drugs to manufacture burn formulations are ascribable to secondary metabolites (flavonoids, terpenoids, tannins, saponins, alkaloids etc.) and the pharmacodynamic attributes (rasa, guna, veerya and vipaka) that provide promising treatment. (Kumar *et al.*, 2021). Arrival of in silico drug design technique, innovations in pharmaceutical chemistry, structure concentrated organic compounds and reduced industrial budget support between 1984-2003, decreased the popularity of plant derived natural products.

However, choice of drug used for dressing in modern and traditional treatment usually depends upon cost and availability of medicaments, clinician and their previous experiences with the drug.

Advantages of herbal drugs

1. *Herbal drugs* - The complex intricate makeup of herbal drugs allows them for synergistic action and better bioavailability that adds up to complete the therapeutic activity of the drug (Carmona and Pereira, 2013).
2. *Immunity booster*- They strengthen & boost the body's immune system for the physiology

of healing mechanism to preserve the body's 'milieu interieur' that is necessary for the recovery from disease

3. *No Adverse effects* – They continue to be popular because people believe, if consumed with proper prescription they have minimum adverse effects (Maver *et al.*, 2015).

4. *Natural remedies*- They present fascinating therapeutic rejuvenating options for anti-inflammatory, anti-microbial, immunomodulator and wound healing owing to the effect of primary and secondary metabolites present in herbs.

5. *Diet and nutrition*- The use of herbs has a a holistic body approach and potentiates 'agni' as a root cause that causes improved diet and nutrition.

6. *Reasonable price* – Herbal remedies formulated at home are reasonable in price so, they are largely preferred to synthetic drugs(Prajakta N. Dongare *et al.*, 2021)

7. *Widely accepted* - Due to their widespread availability as well as the vast empirical research data concerned with their traditional use.

Limitations of herbal drugs

1. Active pharmaceutical ingredients are not stated explicitly. An essential part of drug
   standardization exists to understand the active molecular interactions between active
   pharmaceutical ingredients.
2. Hidden substances with adulteration can cause harmful or unfavorable side effects due to
   drug incompatibilities with other drugs consumed along with them.
3. Popular Herbal dietary supplements freely available in the cosmetic market usually enter
   untraced without the approval of any regulatory authority.
4. Modern scientific methods to validate the claims about the therapeutic
   effects of plants in traditional systems of medicine.
5. Solubility, bioavailability, permeability, stability and targeted drug delivery create obstructions to efficient and fast delivery of herbal drugs to the target

site(Kesarwani and Gupta).

Need for novel drug delivery system in herbal drugs

In spite of these shortcomings, the herbal products are widely acceptable. Nanotechnology offers great advantage for recent innovations that include drugs formulated to nanometric scale to increase specific approach and patient acquiescence(Raina *et al.*, 2023). Ayurvedic herbal formulations are mushrooming under this canopy. In the last century, attention has been focused on the approaches through developing a novel drug delivery system (NDDS). Ayurveda herbals can resourcefully deliver plant metabolites only with the help of proper delivery vehicle and so rises the need of novel drug delivery system. The innovative formulations are made by combining the nanocarrier techniques in topical herbal remedies. They offer a number of noteworthy benefits to increase bioavailability and transdermal permeability with enhanced and consistent absorption rates, better drug solubility, targeted drug delivery and increased pharmacological activity through drug retention, sustained release profile with decreased drug toxicity (Raina *et al.*, 2023). The improved stability and helps easy uptake with improved delivery techniques more acceptable compared to previous standard techniques with enhanced pharmacokinetic and pharmacodynamics of herbal drugs. In comparison to traditional formulations. The active medieties are encapsulated in the polymers of excipients. The efficiency of topical formulations thus depends upon the drug delivery system used(Harwansh, Deshmukh and Rahman, 2019) to target epidermis and dermis for transdermal penetration. Recent Innovations in NDDS for herbal drugs include liposomes, transfersomes, ethosomes, niosomes, phytosomes, herbosomes, dendrimers, micro/nanoparticles, micro/nanoemulsions, micelles (Shaker *et al.*, 2019) etc. Research on effective burn wound healing formulation is a growing area in modern biomedical sciences.

A few advantages of NDDS with nanoemulsion prepared from non-toxic excipients include

1. Nanosized particles of nanoemulsion offer large interfacial area of droplets and improved solubility, absorption, biomembrane permeation and bioavailability of poorly soluble drugs

2. Bioactive principals of herbal drugs remain in suspended form in nanoemulsion that improves physical stability of novel herbal formulations.
3. Herbal oils used in formulations are biodegradable so safe for human health.
4. Better therapeutic activity is made available at a comparatively low dose. This can minimize the deleterious toxic effects of metals or minerals used in formulations
5. Improves Patient receptiveness

comparing the therapeutic potential of whole plant extract with isolated compounds has demonstrated that drug therapeutic potential decreases with compound isolation (Carmona and Pereira, 2013). A mixture of phytochemicals makeup of herbal compounds has a better effect than single isolated compounds (Gómez Castellanos, Prieto and Heinrich, 2009). The idea of pharmacological combinations is well-established in contemporary medicine with significant advancement over years. Though medications provided by contemporary medicine speed up the healing process, a dearth exists for proper tissue repair mechanisms using current medicaments. Polyherbal research formulation for burn wound healing in Ayurveda are a developing area in contemporary sciences with a great scope of research subjects for appropriate scarless burn wound healing process(Khanavi *et al.*, 2013).

Currently, a research gap exists for novel pharmacological therapeutic combinations with whole plant extracts. This facilitated to building up a theoretical hypothesis to amalgamate poly herbal drug extracts in novel drug delivery systems to introduce new boulevards for building novel herbal topical formulations that will be safe and cost-effective.

Vrana ropana drugs include plant animal and mineral resources from mines of Ayurveda. Empirical data exists for numerous among them, nevertheless potential of various remains unmapped. A thorough literature review listed Six such drugs, traced in ancient literature that were used individually or with polyherbal combinations for their beneficial benefits in wound healing. Neem seed oil, Egg yolk oil, Curcuma longa, Aloe vera, Calotropis procera latex and tuttha. Neem belongs to the family Meliaceae. plant native to India. Oil prepared from neem seeds is reported to have vranaropak properties. It is effective as an anti-inflammatory, anti-microbial and wound-healing activity(Sarkar, Singh and Bhattacharya, 2021). studies exist for scar-less burn wound healing with Egg

yolk oil in third-degree burns(Rastegar *et al.*, 2011) and combining Egg yolk oil with novel drug delivery techniques to increase epithelialization in dermal burn wounds(Yenilmez *et al.*, 2015). 'Aloe vera' is gel that belongs to the family Liliaceae. It is known for its moisturising effect(H.Hamman, 2008)(Dal'Belo, Rigo Gaspar and Maia Campos, 2006) and burn wound healing efficacy in various pharmacological models individually (Yam Mun F, Jamaludin Zainol, 2002) and compared to silver sulphadiazine(Akhoondinasab, Akhoondinasab and Saberi, 2014). Curcuma longa and tuttha is known as an important constituent of Jatyadi ghritam and Jatyadi tailam, potent ayurvedic formulations that have burn wound healing effect. Numerous pharmacological studies have been conducted with major bioactives of curcuma longa in burn wound healing It finds its appraisal as a wound healing herb in charaka and sushruta Samhita. Known to possess antibacterial, analgesic, anti-inflammatory and antioxidants effects and synthesizes collagen in burn wound healing. (Kulac *et al.*, 2013) (Kumar *et al.*, 2021)(Maver *et al.*, 2015). calotropis procera latex is commonly known as Ark ksheera belongs to family Asclepiadaceae. References exist for its use as an ingredient of samshodhana ghrita and oil prepared for healing chronic ulcers in siddha in sushruta Samhita and siddha yoga sangraha. The present study "Evaluation of herbomineral Formulation in the Management of Second degree Wound" was carried in an attempt to search out the more efficacious drug formulation in the management of second degree burn Wounds (Samyaka *DagdhaVrana*). These drugs are described in various places of *Ayurvedic* text with respect to management of *Vrana*. This formulation was prepared in the form of Nanoemulsion (O/W), one of the various therapeutic preparations. There are Wide descriptions of these drugs in many places of *Ayurvedic* texts but so for no clinical or experimental study was carried out to know the efficacy of these drugs in this combination of formulation for the management of second degree burn wounds (samyaka *dagdha Vrana*)

Chapter -2

Review of Literature

2.1 Skin

Skin is the first line of defence that blocks the direct microbial attack on the body. It is a competent drug delivery route to captivate researchers and is primarily thought for the wound healing due to its direct approach on the surface area. (Raina *et al.*, 2022). Anatomical barrier structure (epidermis, dermis, sweat glands and blood supply) along with Physiological functions determine penetration of topical applications. Most exposed and recognisable organ in human body is skin. It shields our body from alterations in environment and water loss (Wang *et al.*, 2019). Certain skin disorders affect epidermis that serves as a barrier to stop deterioration and microbial growth. Nutritional and electrolyte components try to perish in such circumstances. As a result, skin wounds can seriously harm a person's health. Skin can be damaged due to a number of conditions that include psoriasis, herpes, eczema rosacea etc. although burns are the prime contributor factor(Souto *et al.*, 2020).

2.1.1 Anatomical and physiological features of skin

The external layer of the body is called as skin. It is the heaviest organ in the body that forms 10% of body mass and $2m^2$ of body surface area. An interface in human body that act as a protection shield for organs tissues and gives covering to the body organs from external environmental factors. A barrier that makes skin selective as to what to let into and out through it. Immune system of the skin gets altered when it challenged by the (bacteria, fungus, etc.), otherwise it's undisturbed and in an inactive state. When there is some changes occurs in immune system, causes severe inflammation and hyper proliferation of skin(Dragicevic and Maibach, 2015). It is composed of stratified epithelium, with upper epidermis, dermis and underlying hypodermis. Additionally, appendageal system includes hair follicles and sweat ducts that navigate the several skin layers. Epidermis is further classified as stratum corneum, stratum granulosum, stratum Spinosum and stratum Germinativum. Stratum spinosum and stratum germinativum are collectively known as Malpighian layer(Venus, Waterman and McNab, 2010). Thickened body areas (palms and soles) contain an extra layer of stratum lucidium. Hair follicles and sweat glands constitute skin appendages.

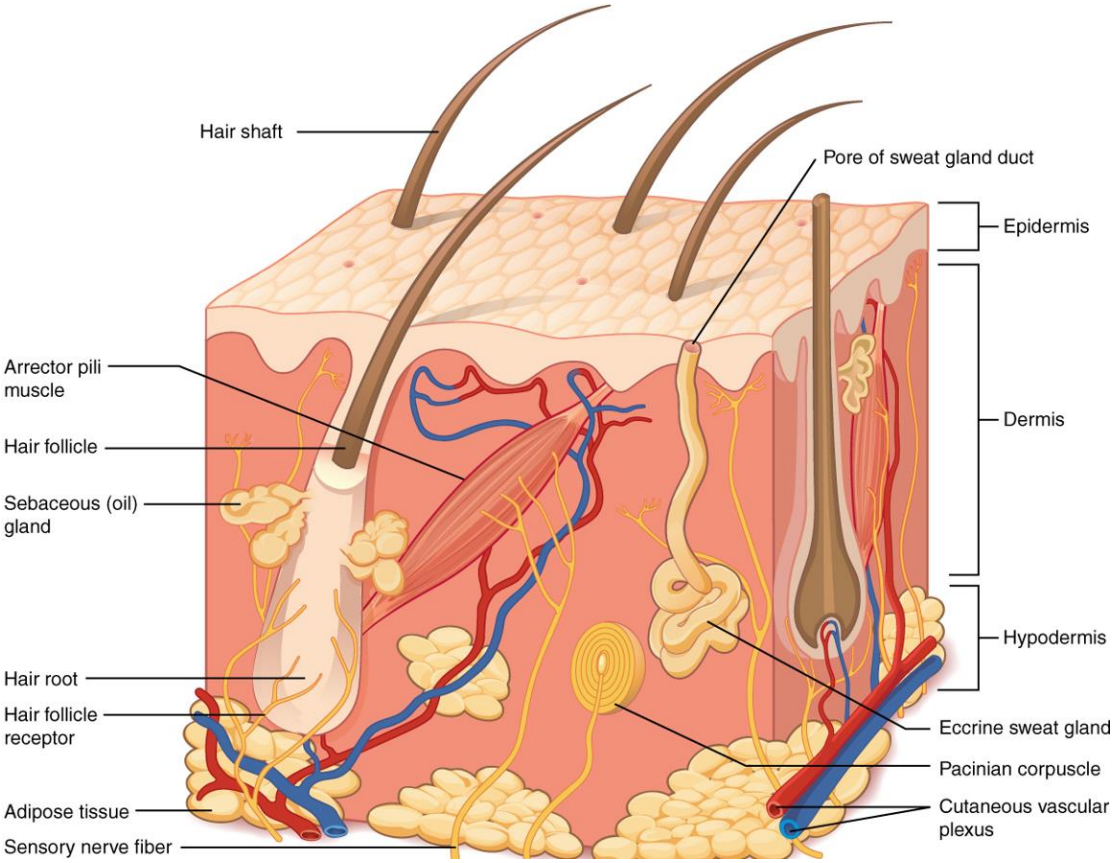


Figure 1 Different Layers and Structures of the Skin

2.1.2 **Stratum corneum** is the outermost skin layer 10-20 µm and is filled underneath with cells that originate from the deeper layers of epidermis, they are known as corneocytes arranged in about 10-15 layers. These corneocytes are flattened and Widened to 40-60 µm with 0.2 µm thickness, connected with corneodesmosomes (Kashibuchi *et al.*, 2002). They are under a continuous process of desquamation. The cornified envelope is occupied with keratin filaments, filled with ECM (extra cellular matrix) rich in lipids with proteins and peptides as constituent elements(Elias, 2012) and covered with membrane enriched in lipid moiety and lacks nuclei and organelles. This enriched stratum corneum is known for its continuity in stratum corneum, this is known as popular brick and mortar model(Michaels, Chandrasekaran and Shaw, 1975) (Elias, 1983). Diffusion across stratum corneum is the major pathway for substances to pass across skin. The lipids in intercellular layers are not only the limiting factor for substances and drugs penetration, but on the whole the microscopic structure of stratum corneum and its twisting aqueous and lipid channels between the cells and the polarities of various skin layers limit penetration of particles into the skin(Yu *et al.*, 2021).

stratum corneum is practically not permeable to everything. Small to moderate sized lipophilic particles can penetrate well compared to others that need a particular strategy to advance their skin penetration. The extra cellular matrix is composed of ceramides, cholesterol and free fatty acids as main constituents in approximately equal promotions. Ceramides make up 50% of stratum corneum lipids by weight, saturated free fatty acids and cholesterol makes this lipid bilayer liquid in nature

Various models have been proposed for stratum corneum molecular organization, that includes Swartzend ruber etal(Hill and Wertz, 2003), domain mosaic model (Forslind, 1994) sandwich model (Bouwstra *et al.*, 2000) (Bouwstra *et al.*, 2001), single gel phase model (Norlén, 2001), hill and wertz model (Hill and Wertz, 2003) model presented by Mcintosh (McIntosh, 2003), Schroter et al (Schröter *et al.*, 2009). Recent researches have revealed that ceramides extend in complete layer of stratum corneum, with hydrocarbon chains pointing in opposite directions and centre is occupied by spingoid moiety (Iwai *et al.*, 2012)

2.1.3 Epidermis 50-100 μm in thickness(Humbert *et al.*, 2004), lacks vascular and nervous supply. Mainly filled with a collection of 95% keratinocytes which has its origin from stratum basale and while moving towards stratum corneum undergoes the process of formation of intercellular meshwork of keratin fibres (keratinization) along with development of lamellar secreting lipids in stratum corneum lipids and slowly gets devoid of the nuclei and other organelles that ultimately forms corneocytes. Remaining 5% is composed of langerhan's cells, melanocytes and merkels discs (Dragicevic and Maibach, 2015)

2.1.4 Dermis About 2-3 mm thickness (Humbert *et al.*, 2004) but the thickness of dermis differs with 0.6mm on eyelids and upto 3mm on back palms and soles. Collagen protein makes about 70% of dermis. Synthesized from fibroblasts in dermis. Papillary dermis(upper layer) is composed of unorganized fibres of collagen while reticular dermis (lower layer) is filled with bundles of collagen as extensions from papillary layer upto hypodermis. Elastin fibres and proteoglycans are generated by fibroblasts as well that encase mast cells and macrophages. Elastin ensures elasticity whereas proteoglycan promotes viscosity and hydrates the dermis. Within fibrous dermis are located other elements that include blood and lymphatic vessels, sweat glands and and

hair roots (Nafisi and Maibach, 2018) Blood and lymph vessels allow flow of waste products and oxygen from the dermis to the epidermis(Ng and Lau, 2015)

2.1.5 Hypodermis Extends up to 3 cm into abdominal cavity. It is comprised of a mixture of loose connective tissue and fat. Two types of unique receptors possessed by hypodermis are Pacinian and Meissner's corpuscles. They enable dermis to receive vibration and touch sensations. Cutaneous nerves in dermis possess a unique cell body that sensory nerve culminates in sensory nerve endings enabling dermis to receive temperature and itching sensations. Adrenergic fibers in dermis contribute to muscles of hair roots, blood vessels and apocrine glands whereas cholinergic fibers stimulate eccrine sweat glands. Endocrine system manages the sebaceous glands at borders of upper and lower dermis. Papillary dermis is supplied by branches of this plexus that control skin temperature by alternat dilatation and constriction. Lymphatic vessels supply the nearby lymph nodes (Nafisi and Maibach, 2018) skin . Absence of dermis is noticed in lean skin. The layers and skin structures are represented in figure1.

2.2 Functions of Skin

2.2.1 Skin as a physical barrier separates internal physiological and external environment(Williams, 2003). physical contact to UV rays and outer materials is protected by skin. ultra violet rays are reflected by stratum corneum as it decreases absorption of UV radiations by increasing melanocyte activity. keratinocytes organized as a lattice resembling a scaffold and intercellular gaps are filled with a laminarly organized matrix that is rich in lipids and serve to act as a water tight barrier. But it is crucial to recognize that skin is not totally resistant to absorption (Casey, 2002). Skin is more permeable to aliphatic alcohols than to polar molecules like water, Na^+ and K^+ and other ions. Posterior part of hand, forehead and face areas with greatest permeability. At the same time palms of hands have lowest access to outer materials.(Venus, Waterman and McNab, 2010)

2.2.2 Skin as an immunological barrier- skin acts as a first line of defense against the invading organisms, skin performs this function effectively with the help of dendritic cells and macrophages present in skin and anti-microbial peptides secreted by epidermal workable cells that kill gram +ive, gram -ive, fungi and viruses. After injury they all leave epidermis towards draining lymph nodes to present antigen complex with MHC-II molecules to T cells. Along with these T-cells are found in small veins in dermis and

around hair follicles and sweat glands.(Venus, Waterman and McNab, 2010) (Lisa C. Zaba, James G Krueger, 2009)

2.2.3 Skin as a chemical barrier due to its acidic PH, it arrests the growth of pathogenic microorganisms that tend to survive best in alkaline environment. Sebum prepares the slippery, water proof and antimicrobial layer on the skin.(Ng and Lau, 2015)

2.2.4 Skin as a thermoregulator vital function of skin that maintains body temperature of about 37^0C via thermoregulatory mechanism of hypothalamus. Body hairs and adipose tissue serve to provide insulation from cold as they trap air and prevent loss of heat from the surface of the skin. Constriction and relaxation of blood vessels serve to loosen heat by adjusting the blood flow. sweat serves the loose heat from skin through evaporation mechanism conducted via pores on skin (Ng and Lau, 2015) (Venus, Waterman and McNab, 2010)

2.2.5 Skin as an excretory organ function is performed to excrete organic wastes released from skin in form of sweat. Sweating discards extra water and salts and small amounts of urea

2.2.6 Skin as a sense organ skin has a task to perceive diverse feelings of pressure, mechanical stress, pressure, warmth, icy touch, general touch, and discomfort. This helps to save body from any type of injury specially burn injuries except in third degree burns that are relatively painless because of destruction of nerve endings located in dermis.

2.2.7 Skin as a therapeutically important organ thousands of years back skin has been used as therapeutic target site for external cosmetic or therapeutic applications to either improve its appearance or heal specific ailments. (Benson and Watkinson, 2012)Recent innovations include transdermal delivery patches or devices for various disorders. External application applied to skin either enters deeper tissues or has systemic effects (Williams, 2003).

2.3 Chemistry of percutaneous absorption

2.3.1 Penetration term is used when the topically applied substance invades through the layers of the skin. The substance or particle may enter the bloodstream or gather in a specific layer of stratum corneum. *Permeation* refers to the passage of substances through hydrophilic channels from one layer into another.

2.3.2 Resorption is the process by which a material enters the blood vessel or

lymphatic system

2.3.3 Absorption is the summation of the above three processes. Dermal absorption is affected by applied dose, formulation, physical state, size and molecular weight of substances. The rate of the penetration through skin is referred to as flux (j), i.e., the amount of drug infiltrated per unit area, per unit time. (Singh, 2016). This depends upon the permeability of skin to the permeant and the concentration gradient of the permeant across the skin. As a general rule, molecules that infuse the skin voluntarily should have a molecular weight < 500 Da (Bos and Meinardi, 2000). Others factors affecting permeation are melting point, solubility and molecular volume(Ng and Lau, 2015).

2.3.4 Transcellular and intercellular routes of absorption Structure of skin permits the materials to be transferred through transcellular, intercellular or appendageal route. Mostly permeable material applied topically on skin incorporates itself by three possible routes to travel through skin. Sweat glands, hair follicles with attached sebaceous gland and epidermis. Relative importance of each pathway depends upon physical and chemical characteristics of permeable material (Nafisi and Maibach, 2018)

Trans appendageal route permits the materials through sweat, sebaceous and hair follicles. This provides the shortest pathway for diffusion. The route gains access to superficial blood vessels (Toll *et al.*, 2004) present in skin but occupies only 0.1% of available skin surface area So has least contribution towards transport of materials across skin. materials diffuse via this route in minor amounts (Roberts, M.S. and Walters, 2008)

Therapeutically used topical drugs can penetrate the skin through paracellular, transcellular or appendageal routes. Penetration through paracellular route takes place through lipid bilayer of the skin. It nearly covers around 1% of the stratum corneum(Sloan, 1989). Transcellular route favours the transfer of water-soluble drugs. Appendageal route contributes to 0.1 to 1% of the total substances absorbed via the skin.(Lai *et al.*, 2020)

The ability of "penetration enhancing agents" (ethanol, ethyl acetate, dimethyl suphoxide, sodium dodecyl sulphate etc.) to increase the penetration of various antimicrobial agents into the eschar of third-degree burns has been studied.(Manafi *et*

al., 2008)The selectivity of topical antimicrobials compared to their cytotoxicity towards host cells and tissue is a crucial factor to take into account. Trans epidermal route allows the materials to be passed through transcellular and intercellular routes. Transcellular is not considered a prominent pathway(D.I.J. Morrow, P.A. McCarron, 2007). The materials are permitted via the phospholipid layer and then the matrix of dead keratinocytes. This path creates an aqueous hydrophilic pathway that is suitable for transferring substances via a hydrophilic in nature are transferred through this pathway. Though this is the shortest available routes but materials must inevitably first travel through the lipid cell layer before it enters the hydrophilic keratin at inside cell. On the other hand, through intercellular route the materials penetrate through potential spaces available between the cells. This is an easy path for lipophilic materials. The rate of diffusion depends upon how readily they dissolve, their mass and their fluid nature. The intercellular pathway has been established to be suitable penetration route for majority of materials. It works most effectively for uncharged lipophilic molecules (Morganti *et al.*, 2001) (Scheuplein, 1978). Routes of Drug Delivery through Skin is presented in figure 2

2.4 Burns

The immunological barrier (Skin) when breaks destroy the Numerous nerve terminals and receptors in the skin allowing it to sense impulses linked to temperature, touch, ache, as well as pressure (feeling). Burns and other extreme injuries cause some or all of these functions to be lost or disrupted. Burns are a troublesome critical care problem affecting children to elderly people, a major problem of developed and developing countries attacking patients physically and psychologically. The distressing condition that leaves its visible and invisible sequelae for long periods. The heal of these injuries pose a great challenge to medical and paramedical staff as it requires 24 hrs. presence of an organized team of persons who completely understands that physical and mental consequences of burn injuries. Apart from these facts, an individual who suffers from burns is labeled physically and mentally as a patient of burn forever. 'The International Society of Burn Injuries' explains burn as a trauma to a living tissue due to heat (Barajas-Nava *et al.*, 2013). An excruciating painful, terrible injury causing tissue damage due to skin contact by hot liquid, steam, electricity, chemical radiations,

accidental fires and gases. 86% of burn injuries are caused by heat, 4% by electrical and chemicals cause around 3% of the burn injuries every year. India reports around 2-3 million burn injuries every year. Low and middle-income group people are more susceptible to these injuries (Yilmaz and Andsoy, 2020). Temperature and duration of revelation influence the impact on the skin with a collaborative effect (Evers, Bhavsar and Mailänder, 2010) (Żwierełło *et al.*, 2023)

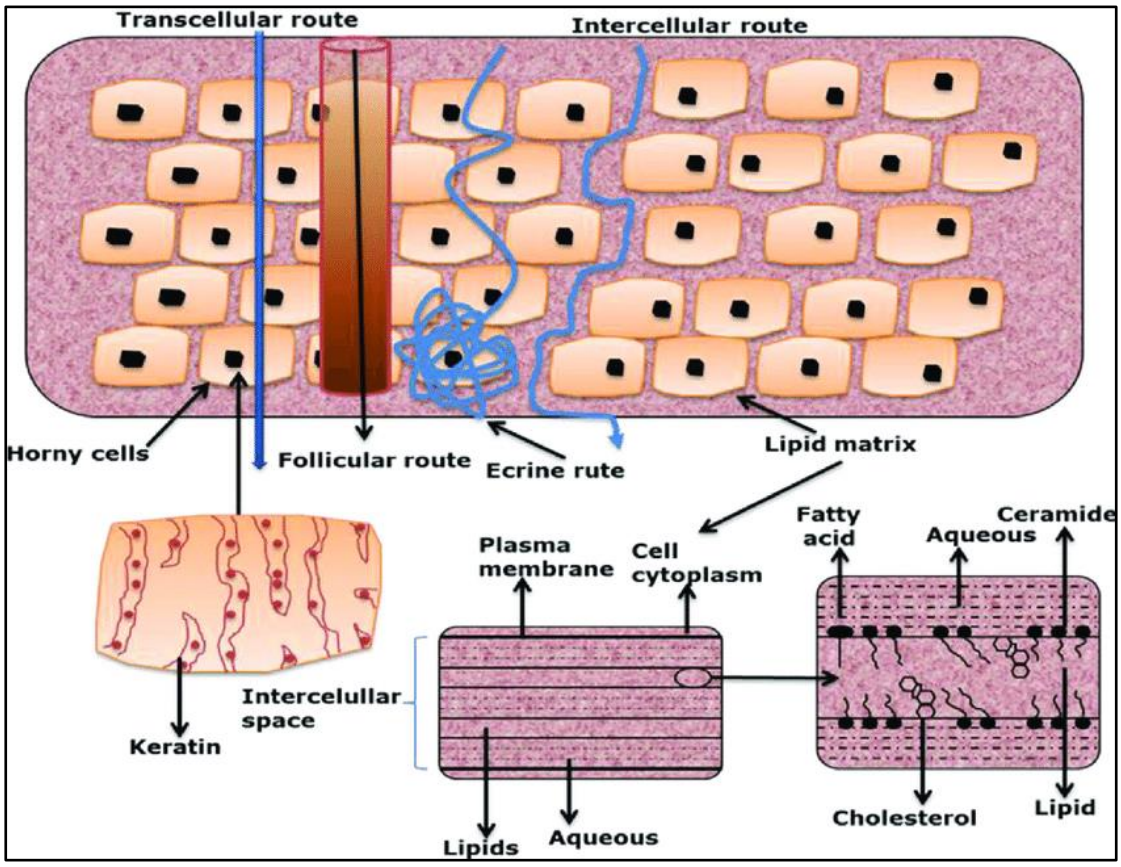


Figure 2 Transcellular and Intercellular Routes of Drug Delivery through Skin

2.4.1 Causes of burns

- Chemical injuries by strong acids or alkali
- Electrical injuries by voltage and amperes of current
- Thermal contact by fire
- Steam or Scalds from hot liquids
- Dry heat from Hot metal, glass or other objects
- Radiation from alpha beta or gamma rays or sunlight.

2.5 Classification of burns according to Ayurveda (Ts, Sweta and Surendranath, 2022)

Detailed description of burns is mentioned in Sushruta Samhita chapter 12, 16th verse onwards. Burns are classified into plushta dagdha, durdagdha, samyaka dagdha, and atidagdha.(Taifa Rozy, Barman Kumar Pankaj and Medhi Champak, 2020). the clinical features and the correlation according to the modern approach has been mentioned below.

2.5.1 Plushta Dagdha- Affected area has discolored skin with burning sensation. According to Dalhana commentary it can be correlated to first degree burns.

2.5.2 Durdagdha- affected area has redness and inflammation with burning pain and takes time to subside. It can be correlated to second degree burns.

2.5.3 Samyak Dagdha- affected area is compared with the color of Asian palm fruit (tala phala). These type of Burns are not deeply seated nor superficial.

2.5.4 Atidagdha- affected area involves skin, sub-cutaneous tissue, muscles, vessels, tendons, ligaments and deeper structures also. Injured person suffers from dehydration, burning pain and high fever. This can be co-related to the advanced stage of third degree burns.(Taifa Rozy, Barman Kumar Pankaj and Medhi Champak, 2020)

Apart from these types of dagdha vrana Harita Samhita has mentioned 'Isadagdha' and 'Madhyama dagdha' former to be treated with Kanji, Nimba patra, Tulsi patra and Dhatri patra application and the latter one can be treated with thin topical application of ghrita mixed with madhu, kustha and manjistha. Classification of burns according to the various samhitas of classical texts of Ayurveda is presented in table 1. Their clinical features and the line of treatment are mentioned in Tables 2 & 3.

2.6 Evaluation of burns depending upon TBSA

Assessment of the burned skin area is calculated by percentage of total body surface area (% TBSA) Wallace "Rule of Nine" and Lund and Browder approach are considered as methods to estimate total body surface area. Though the second method is considered as more accurate but the former it has been demonstrated as a fast, effortless, easily identifiable, sufficiently precise method to estimate the total body

surface area burnt (Thom, 2017). Various percentages are assigned percentages to various body locations forms the basis of the lund browder's scheme of calculation of burnt body surface area [figure 39(a) &(b)]. Anterior and posterior parts of the cranium account for estimated 9%, total trunk and upper extremities form a total of 32% each and 40% of the body is made up by lower extremities (rule of nine by moore,2022)

2.6.2 Classification of burns depending upon the severity of burns(Jeschke *et al.*, 2020) (Yastı *et al.*, 2015)

A burn injury is categorized as serious or minor depending upon the TBSA. Serious injuries require special attention and treated with topical anti-microbial or require repetitive surgical interventions of surgical reconstruction to release contractures (Oryan, Alemzadeh and Moshiri, 2017). Minor injuries are demarcated as those that typically heal without surgery. Guidelines for differentiating serious and minor burn injuries include >10% TBSA in older patients, > 20% TBSA in adults and > 30% TBSA in children

2.6.2.1 Superficial burns(Yastı *et al.*, 2015)

Excluded from TBSA percentage evaluation

2.6.2.2 Second-degree burns

a. *Minor burns*- with a surface area less than 10% in children and a surface area less than 15% in adults.
b. *Moderate burns*- with a surface area of 10-20% in children and a surface area of 15-25% in adults
c. *Major burns* – with a surface area of more than 20% in children and a surface area of more than 25% in adults

2.6.2.3 Third-degree

a. *Minor burns*- with a surface area less than 2% in children or adults
b. *Moderate burns*- with a surface area of 2-10%
c. *Major burns*- with a surface area of 10% or more

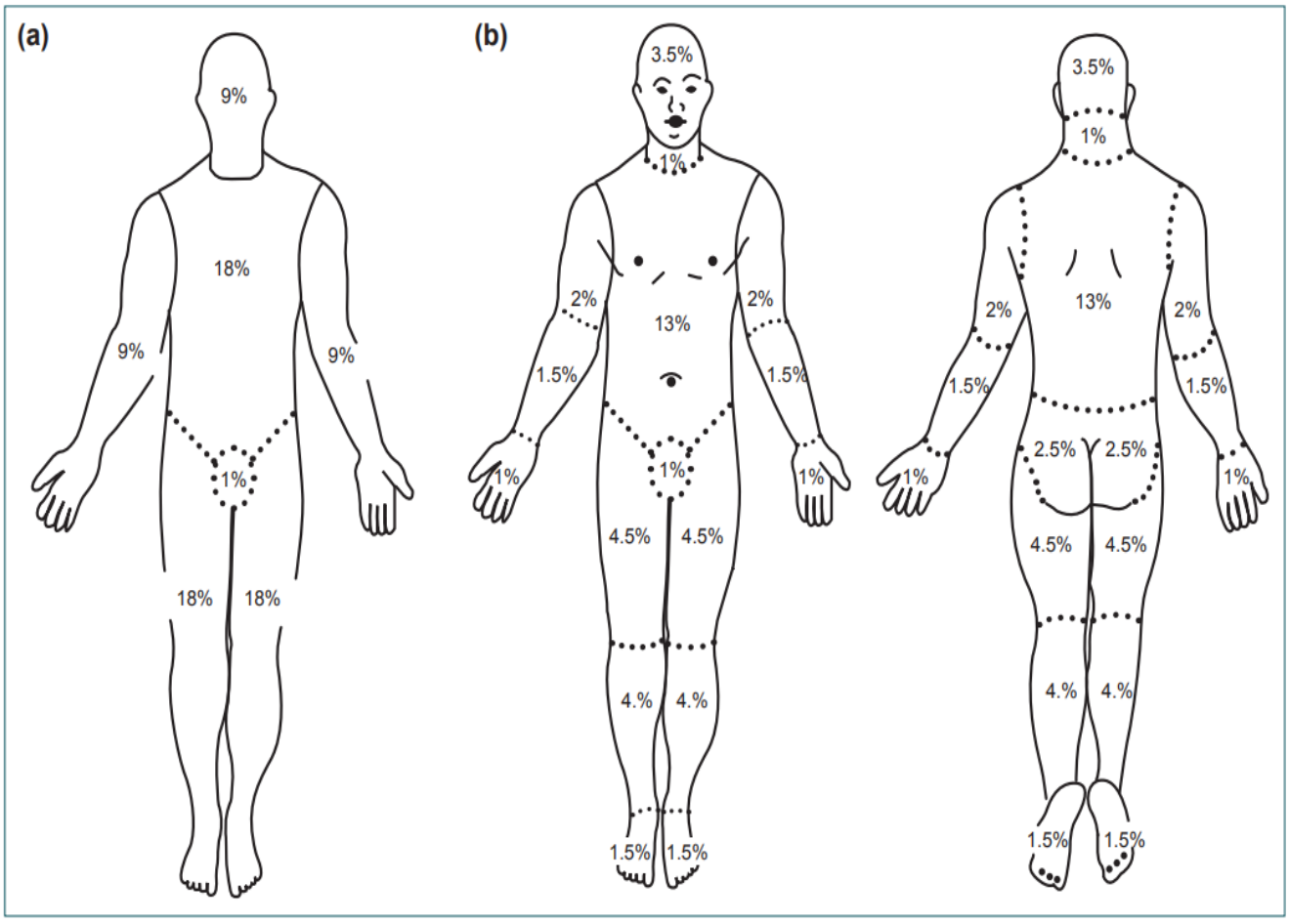


Figure 3(a). Rule of Nine (b). Lund Browder Scheme to Evaluate Total burn surface area in adults

Image source(Evers, Bhavsar and Mailänder, 2010): the biology of burns

Table 1 Classification of Dagdha vrana according to various classical texts of Ayurveda

Samhitas	Plusta	Tuttha	Tuccha	Isad Dagdha	Durd Dagdha	Madhya dagdha	Samyak dagdha	Ati dagdha
Sushruta Samhita	+	–	–	–	+	–	+	+
Charaka Samhita	–	–	–	–	–	–	–	–
Astanga Hridya	–	–	+	–	+	–	+	+
Astanga Sangraha	–	+	–	–	+	–	+	+
Madhav Nidana	–	–	–	–	–	–	–	–
Yog Ratnakar	+	–	–	–	+	–	+	+
Bhavprakash	+	–	–	–	+	–	+	+
Harita Samhita	–	–	–	+	–	+	+	+

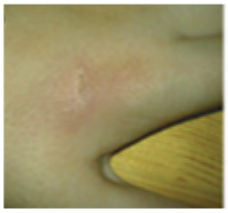
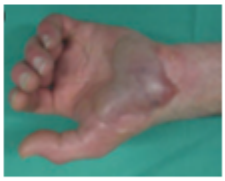
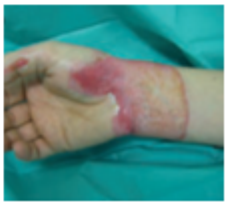
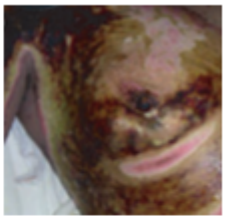
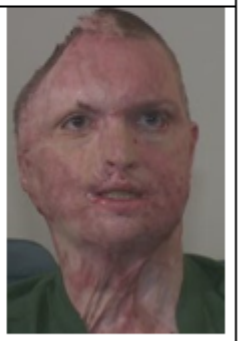
Table 2 Clinical features of different types of Dagdha vrana in various classical texts of Ayurveda

Clinical features	Sushruta Samhita	Astanga sangraha	Astanga hridya	Yog ratnakara	Harita Samhita
Plusta/ Tuttha/ Tuccha	Discoloration with severe burning sensations	Discoloration with severe burning sensations	Discoloration of skin, severe burning sensations, no eruptions	Discoloration	_
Durdagdha	Sores/ eruptions, severe pain with redness and suppurations, long lasting pains.	Sores, severe burning pain, needs longer time for healing	Sores, pain with a severe burning sensation.	Sores, red & inflamed skin, severe burning sensation & longlasting pain.	_
Samyaka dagdha	colour resembles like taal fruit colour, neither flattened nor raised lesions.	colour resembles like taal fruit colour, neither flattened nor raised lesions.	Colour resembles like taal fruit colour, neither flattened nor raised lesions, absence of severe pain, suitable healing	Neither elevated nor raised lesions, resembles like colour of ripe palm fruit	No dosha is involved
Ati dagdha	Removal of burned tissue, injured vessels, ligaments, bones or joints, fever with burning sensation, severe thirst and fainting	Deep wounds, burning sensation, severe pain,thirst, and fainting along with bleeding with various complications . difficult for wound to heal with scar formation, Smoke from throat,	Deep wounds with development of strictures, pain is felt with destruction of blood vessels, severe thirst and fainting and death	removal of burned tissue, destruction of vascular supply, ligament, joints & bones. Fever with severe thirst and fainting, Slow healing wounds with scar formation	Involvement of tridosha and dhatu- mamsa
Pittajadaha	–	–	–	–	Skin ruined, rakta & pitta involved

Table 3 Treatment principles of various types of dagdha vrana in different classical texts of Ayurveda

Clinical features	Sushruta Samhita	Astanga sangraha	Astanga hridya	Yog ratnakara	Bhavprakash	Harita Samhita
Plusta/ Tuttha/ Tuc0cha	Heat treatment and treatment with topical and internal medications hot in potency	Heat treatment and treatment with topical and internal medications hot in potency	Heat treatment and treatment with topical and internal medications hot in potency	Heat treatment and treatment with topical and internal medications hot in potency	Heat treatment and treatment with topical and internal medications hot in potency	_
Durdagdha	Hot & cold treatments and topical application of ghrita	Hot & cold treatments and topical application of ghrita	Hot & cold treatments	Hot & cold treatments and topical application of heat & ghrita	Hot & cold treatments and topical application of drugs hot in potency & ghrita	_
Samyaka dagdha	Thin and thick, topical applications of mamsa of gramya, Anoop and audak & pitta abscess treatment	Thin and thick topical applications of mamsa of gramya, Anoop and audak & pitta abscess treatment	Thin topical applications and pittaja abscess treatment	Only thin topical applications	Thin and thick topical applications of mamsa of gramya, Anoop and audak & pitta abscess treatment	_
Ati dagdha	Cold treatment, thick topical applications on line of treatment of pittaja erysipelas	Lepa application, Pittaja erysipelas treatment	Line of treatment of pittaja erysipelas	Cold treatment and thin topical applications	Cold treatments and thick topical applications with drugs hot in potency	_

Table 4 Classification of burn wounds, depending upon the depth the wound and clinical features (Noorbakhsh *et al.*, 2021)(Shupp *et al.*, 2010)

Types	A layer of skin involved	Clinical Features	Duration	Image
Superficial	Epidermis of Skin	Erythema and discomfort, followed by peeling of epidermis but no blisters.	3-5 days	
Superficial partial thickness	Epidermis and papillary dermis	Redness, blisters, painful, peeling, and edema. Turn pale with rapid capillary refill, no scarring or physical disability	3 weeks	
Deep partial thickness burns	Epidermis, papillary and Reticular dermis	Total analgesia and decreased pinprick sensation, Painful to pressure only, Wet and edematous wound with Slow capillary refill, scar formation	3-9 weeks	
Full thickness burns	Epidermis, papillary and reticular dermis & Subcutaneous adipose tissue	Waxy or black in colour, not painful, no capillary refill, no blisters with eschar formation	Need surgical interventio n	
Subdermal burns	Epidermis, dermis, sub-cutaneous adipose tissues, fascia, Bones, muscles, tendons and ligaments.	Waxy or black in colour, not painful, no capillary refill, no blisters with eschar formation	Surgical interventio n	

2.7 Pathophysiology of burns vis- a- vis other wounds

Pathophysiology of the burn wound can be critically better understood by three dimensional zones identified by Jackson (1959) in the injured burned tissue that spread to the deeper epidermis. (Żwierełło *et al.*, 2023)

- Coagulation zone- area in the middle of burn area acquired during injury with significant coagulative necrosis (shehan Hettiaratchy, 2004).
- Stasis zone- located at the boundary of coagulation zone. Circulation in this area is slow, elevated local inflammatory reactions with circulatory leakage impairs tissue permeability. It can improve with appropriate wound maintenance or progress towards necrosis.
- Hyperemia zone- located at the outermost location of zone of stasis. It is the consequence of severe vasodilation. Inflammatory phase results in improved blood flow. it reconciles completely until there is serious sepsis or protracted hypoperfusion

All burn wounds heal by three types of processes reactive, reparative and remodeling.
Surface area covered by burn wound, deep penetration of injury to wound tissue, delays in wound healing, hypertrophic sczars and dyspigmentation, contractures, infection and shock that influences the death rate and the disability in burn patients (Singh *et al.*, 2007).
Burn Wound healing is initiated by inflammation. Immediately after injury, Platelet degranulationreleases various glycoproteins. As platelet aggregation progresses coagulation cascade is activated. Fibrinogen is broken down to insoluble fibrin forming a clot. As healing progress, this clot serves as a provisional matrix. Damaged cells and platelets serve as chemotactic stimuli for fibroblasts, endothelial cells and keratinocytes. The provisional matrix is degraded by proteinases and is removed from the wounded site as a scab. Physiologically plasmin and MMP are key enzymes that dissolve fibrin clot (Ramos *et al.*, 2012). Growthfactors released from platelet degranulation activate fibroblasts to produce collagen in ECM and frames a supporting matrix. Thus, healing begins with hemostasis in a sequence of steps(Toriseva and Kähäri, 2009).

1. Inflammation.
2. Re-epithelialization
3. Tissue remodeling.

2.7.1 Inflammation- Beginning with vasoconstriction, platelet aggregation, adhesion and degranulation follow to stop the blood flow at the site of injury. after a few hours, the inflammatory cells begin to infiltrate in burn tissue. The inflammation and coagulation process

are found to be interrelated to each other. Inflammation is regulated by various mediators originating from blood plasma and from different kinds of leukocytes that reach at the wound site. Vasoactive amines and kinin system products are fast-acting mediators that regulate the acute response. vasoactive histamine in abundant amount released from mast cells are considered in mediating the early stages of post-burn edema by increasing vascular permeability (Noorbakhsh *et al.*, 2021) (Friedl *et al.*, 1989). Extensive areas involved in burns induce excessive that cause lesions in surrounding area (lucteot). This could be due to overactivation of the Hageman factor (F XII)(Arturson, 1996), which affects the coagulation-fibrinolytic, complement, and arachidonic acid responses in addition to increasing activity of the kallikrenin-bradykinin system. Bradykinin, a byproduct of the kinin system, increases venular dilatation and permeability, it significantly assists to the production of local edema. Likewise, prostaglandins (PGD2, PGE2, PG12), prostacyclin and thromboxane A2 are the products of arachidonic acid metabolism by cyclooxygenase enzyme. Prostaglandins lead towards increasing vascular permeability, development of local burn edema and local hypoxia in burn tissue respectively.(Teot *et al.*, 2012) (Khosroshahi *et al.*, 2019). Hemostasis intermediated by extrinsic and intrinsic pathways of blood clotting activate coagulation cascade. Clotting factors from the coagulation cascade act on damaged cells and platelets to progress towards the formation of insoluble fibrin fibers that forms a clot further. Meshwork of the temporary matrix is sealed off by fibronectin and vitronectin to halt bleeding. prominent blood loss by damaged blood vessels of the burn area is prevented by vasoconstriction from zone of necrosis that delays burn wound healing. (3,4 burn chapter). Complement system components activates alternative complement path with C3a and C5a in plasma. They co-ordinate the Polymorphonuclear cells in burn patients. (10, lucteot). In thermal burns, coagulation activity is stimulated within 2-3 hours due to heat (11- lucteot). Leucocyte arrival releases cytokine and growth factors that regulate the length and amplitude of the inflammatory response through proliferation,migration, Chemotaxis of fibroblasts and inflammatory cells, stimulation of endothelial cells, release of ROS, phagocytosis & angiogenesis. Cytokines include TNF-α, IL-1 and IL-6 and growth factors, PDGF, FGF, EGF, TGF-α, VEGF AND IGF-1, TGF-β,GM-CSF are presented to have a central role in cellular processes of acute inflammatory process (13-lucteot) (Khosroshahi *et al.*, 2019) (Noorbakhsh *et al.*, 2021). Macrophages activate TNF-α to release IL-1β for inflammatory response. IL-1β has a chemotactic property for neutrophils and stimulates gene expressionfor iNOS & COX-2 (Ren and Torres, 2009). IL-10, an anti-inflammatory cytokine (Müller *et al.*, 2002) released during uncontrolled bacterial infections, down regulates inflammatory cell response (Peñaloza *et al.*,

2016). Cellular infiltrations cause neutrophilsto release ROS, that leads to local response in burn tissue, has a protective effect against infection (16,17- roshangar) conversely higher ROS release is related to infection, sepsis, tissue damage, systemic organ failure and immunosuppression. Consequently, the level of balance between secretion and disposal of free radicals determines how effectively the burn wound heals. Released TNF-α, IL-1, IL-6 (pro-inflammatory cytokines) release chemokines and complement system activation promotes coagulation (Khosroshahi *et al.*, 2019) (Ramos *et al.*, 2012). Coagulation factors, complement system and chemotactic stimuli released by growth factors act on keratinocytes, endothelial and fibroblasts cells to accelerate wound healing that form a provisional matrix which is degraded by plasmin and the interweaving fibers are removed from site as a scab (Toriseva and Kähäri, 2009). Within a week of burn injury infections can counteract the burn wound area (Lachiewicz *et al.*, 2017) that protracts the inflammatory stage. Elevated levels of MMPs, collagen and ECM breakdown turns the burn wound to chronic stage and thus a non-healing wound (Demling, 2005)

2.7.2 Re-epithelialization- Platelet degranulation and macrophages activate cytokines (PDGF & TGF-β) (Schultz *et al.,* 2011). ECM deposition isthe task considered by Macrophages and platelets. In early stages, macrophages and platelets function to form fibronectin and in later stages, they function to form collagen and proteoglycans. (figure 4) The function performed by PDGF & TGF-β from macrophages and platelets activate fibroblasts for this process. (Schultz *et al.*, 2011). As fibroblasts travel into the wound site to produce granulation tissue and the fresh dermal layer. PDGF influences proliferation, chemotaxis and collagenase expression by fibroblasts. Synthesis of matrix proteins and proteases are increased by TGF-β that down-regulate the process of matrix degradation and stimulates the secretion of TIMP, for inhibition of matrix degradation (Schultz *et al.*, 2011).

2.7.3 Primarily PDGF, TGF-β along with IGF-I & FGF function to recruit fibroblasts to migrate to the matrix. Appointed fibroblasts change their morphology and begin tosynthesize reparative granulation tissue (collagen, elastin and proteoglycans) that helps invascular growth (Ravanti and Kähäri, 2000) revealing the fibrogenesis process. This allows a balance to be maintained between ECM production and degradation. Any disturbance in the balance between production and degradation phase leads to derange (Schultz *et al.,* 2011)the wound healing process and thus forming scars. The process of epithelialization is stimulated by EGFs, TGFα & KGFs (Keratinocyte's growth factors). correspondingly angiogenesis is encouraged by growth factors of inflammatory phase. Skin appendages have remnants of new epidermal cells located within undamaged dermis. They are readily available for progress of healing in

superficial burns (Linares). But in deep dermal burns heal at a comparatively slower rate due to loss of skin appendages. Re-epithelialization that can happen from the wound margins can happen only after necrosis is stopped (Cuttle *et al.*, 2006). Initially formed endothelial cells form new sprouts that interact with ECM. During healing process collagen levels eventually rise. The resulting tissue regains its blood supply identical to original tissue (Tonnesen, Feng and Clark, 2000) .

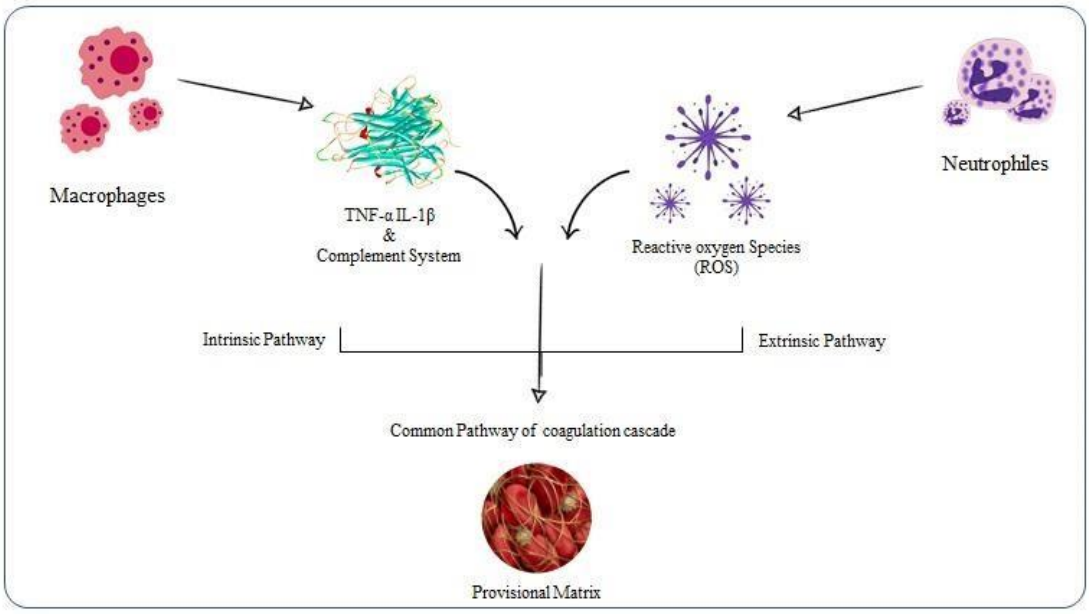


Figure 4- Graphical representation for steps between inflammation and wound healing

2.7.3 Remodeling process – During the last remodeling phase of healing. The myofibroblasts are generated from fibroblasts. They contract and eventually disappear from the newly formed extracellular matrix (ECM). The process persists even after the wound appears healed and the re-epithelialization process is finished(Amadeu *et al.*, 2003). It has two phases early and late inflammatory phase.

Early remodeling occurs during the formation of fibronectin. Capillary endothelium of damaged tissues decreases in oxygen levels (Toriseva and Kähäri, 2009). Macrophages, keratinocytes and endothelial cells release signals from TGF- β, VEGF and bFGFs stimulate angiogenesis. Angiogenesis depends upon plasmin, MMPs and oxygen availability(Schultz *et al.*, 2011). MMP-2 & MMP- 9 is seen to play an essential role in physiological angiogenesis. They activate TGF- β, an important growth factor that regulates matrix deposition (Toriseva and Kähäri, 2009). Neovascularization provides nutrition, signals and cells for coordinated

tissue repair (Schultz *et al.*, 2011). After wound closure MMP-2 & MMP-9 continues to playa significant role in remodeling process by their ability to digest ECM (Gauglitz *et al.*, 2011). Expression of MMPs during different phases of wound healing (Ravanti and Kähäri, 2000). In early phases, matrix metalloproteinases degrade collagen, fibronectin and elastin. It has an ability to cleave ECM(Parks, 2009). It plays an inhibitory role in wound healing. TIMP, bind all MMPs and inhibits them by adhering to ECM proteoglycans. *Late remodeling* requires MMP-2 to degrade collagen (Toriseva and Kähäri, 2009). It involves areduction in the number of fibroblasts by apoptosis. Fibroblasts change their phenotypeto my fibroblasts that express α-smooth muscle actin causing wound contraction that reduces the scar area (Schultz *et al.*, 2011). They are eliminated in later stages thus reducing myofibroblasts (Toriseva and Kähäri, 2009). Scar formation is prevented when collagen type III fibres are replaced by collagen type I fibres, bundles of which are arranged in the dermis. Plasmin and Proteinases digests ECM (laminin & fibronectin) making the release of GF and cytokines from it. TGF-β (Toriseva and Kähäri, 2009) regulate MMPs to down regulate ECM deposition. MMP-9 has a regulatory (inhibitory) rolein wound healing and is known to digest fibrin (Toriseva and Kähäri, 2009b). Physiologically plasmin and MMP help in wound healing (Lijnen H.R, 2002). The finished appearance of the healed tissue is determined by the balance between ECM formation and destruction. The remodelling process occurs in co-ordination with immune cells, endothelial cells, keratinocytes, fibroblasts and elimination of myofibroblasts In burn tissues the remodeling phase is considerably prolonged. The co-ordination fails to occur. The normal myofibroblasts are found elevated in dermis that leads to stiffness and abnormal contractures (Gabbiani, 2003). Diminished remodelling and significant matrix accumulation are characteristic features of healing with fibrosis in burn wounds, that is being typically observed in hypertrophic scarring(Lazarus *et al.*, 1994) How a wound progresses toward HTS & keloid.

Healing in deeper burns is difficult to treat and compared to other wounds it is comparatively slow(Tiwari, 2012). The slow wound healing accompanied with infection and pain with the hypertrophic scarring continue to remain a major challenge for studies on research and management of wounds. Burn tissue being initially sterile are more prone to infective complications and scarring. when the skin heals after burns it undergoes a remodeling phase which is equivalent to traumatic wound healing(Tiwari, 2012). Clinically, there are less chances of scars, if the wound healing occurs before 2 weeks after burn injury. Chances of scar are more when healing takes 2-3 weeks and scars are sure to develop when healing occurs after 3 weeks[3]. When compared to the normal wound healing process, significant differences exists

in inflammatory response. (Tiwari, 2012).

2.8 Treatment in classical Texts dagdha vrana explanations found in charaka Samhita has mentioned trauma to be a cause of dagdha vrana. Other classical texts have classified dagdha vrana according to the depth of burns. Plusta vrana is known by the name of 'Tuttha vrana' in astanga hridya and 'Tuccha vrana' in astanga sangraha. The clinical features of these vranas are as described by sushruta Samhita. Harita Samhita has not clearly mentioned dagdha vrana with its clinical features.(Taifa Rozy, Barman Kumar Pankaj and Medhi Champak, 2020). the details of description found in various classical texts of Ayurveda have been mentioned in table 1.Quest for the scar less appropriate healing has given scope for further research work that continues to grow in this concern. Many new single-drug formulations are being tested for the healing process to occur smoothly on burn wounds with early epithelialization and wound contraction. Table 5 presents various research formulations prepared with the single herbal drug in different dosage forms for topical applications in burn wound formulations along with the efficacious results achieved in the same framework. The various stages of wound healing along with the various physiological events mentioned are represented in figure 5

2.8.1 Polyherbalism: the concept of Ayurveda

The concept of polyherbal formulations was accomplished in 1300 B.C. The term is particular to Ayurveda mentioned in Sharangdhar Samhita. Mostly ayurvedic formulations are prepared with a combination of herbs. Synergism refers to beneficial effect in therapeutic action with two herb interaction. Research suggests that combination of various plants with diverse potency yield better results. Therapeutic effects are not discernible when used alone but are significant when combined with other plants(Parasuraman, Thing and Dhanaraj, 2014). Since. Literature specifies phytochemical constituents present in individual herbs but their contribution is inadequate for therapeutic activity. Since, the sum up of phytoconstituents of many plants is higher than that of single plant that provides the desired effect (Sharma, Verma and Misri, 2016). This advantage of the potentiating effect of the combination of active phytoconstituents in a single formulation is advantageous over the single drug medicaments, the major reason for the rising demand for ayurvedic products. The combinations from various plants can work in a synergistic, agonistic or antagonistic way depending upon the combinations of active principles to result with the best treatment efficiency with lesser side effects. Prolific combinations of polyherbal have higher benefits at low doses and are convenient to patients to reduce multiple drug intake. Thanks to the pharmaceutical combination therapies to acquaint hopes in patients for various disorders. From literature review of classical texts in Ayurveda polyhebal drug

combinations for topical burn wound therapy are made with certain herbs in a proportionate amount to yield good results with relevant pharmacological studies. Tables 6 and 7 present the various pharmacological research studies conducted by use of single and polyherbal formulations for burn wound therapy in burns. Various combinations of polyherbals are in various dosage forms that continue to ensure promising results for superficial and second-degree burns. A collection of such polyherbal marketed formulations is mentioned in Table 6.

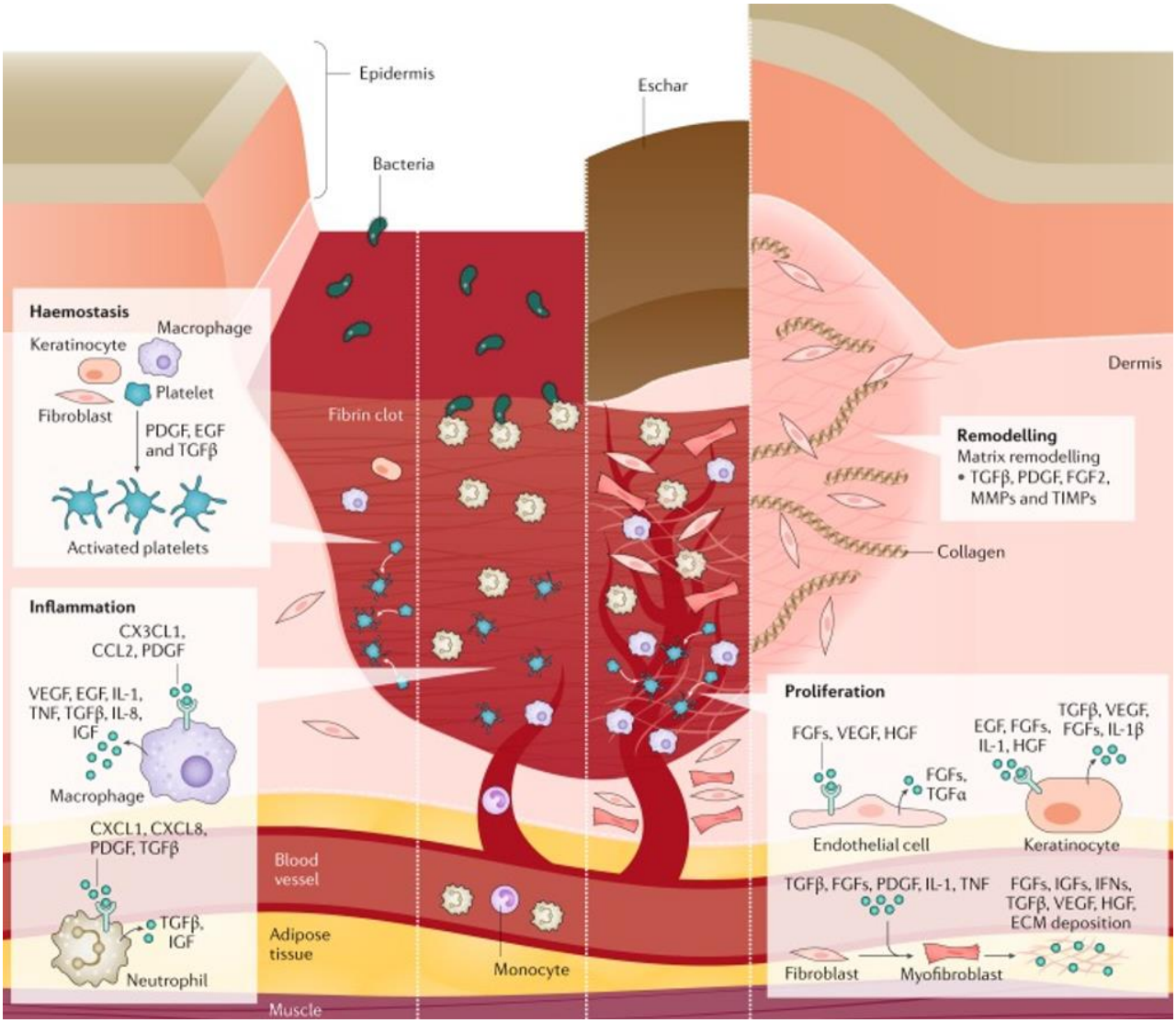


Figure 5 physiological events during various stages of wound healing.

Table 5: Pharmacological research studies data of single drug formulations in burns

Scientific name	Plant part/ extract	Burn model	Study design/animal used	Results	References
Aloe vera	Gel preparation	Second & third degree burn wounds	In vivo / Male SD rats	↓Healing time, ↑wound contraction	(Akhoondinasab, Akhoondinasab and Saberi, no date)
Aloe vera	Fresh mucilage gel	First and second degree burns	RCT	↑increased healing rate	(Maenthaisong *et al.*, 2007)
Aloe vera	Cream preparation	Thermal burn wound	In vivo / Rats	↑wound contraction ↑re-epithelialisation	(Seyed Jalal Hosseinimehr 1, Ghasemali Khorasani, 2010)
Allium sativum	Bulb/ethanolic extract	Thermal burn wound model	In-vivo/ Male white rabbit	↑Wound contraction ↓Microbial count	(Jalali *et al.*, 2009)
Brassica oleracea	Leaf/ aqueous extract	Deep second degree burn wounds	In-vivo / Female SD rats	↑Wound contraction ↑Efficient re-epithelialisation at higher dose	(Hassanzadeh *et al.*, 2013)
Calendula officinalis	Flower/ ethanolic extract	Thermal burn wound	In-vivo / Female wistar rats	↑Hydroxyproline ↑Hexosamine ↑GSH, ↓Lipid peroxidation ↓Haptoglobulin	(Chandran and Kuttan, 2008)
Camellia sinensis	Leaf / ethanolic extract	Type – II burn wound model	In-vivo / Male wistar rats	No significant difference in epithelialisation and angiogenesis ↑Wound contraction	(Karimi *et al.*, 2013)
Carica Papaya	Fruit / Latex	Thermal burn wound	In-vivo / Mouse	No difference in wound contraction, ↑ Re-epithelialisation	(Gomes *et al.*, 2010)
Centella asiatica	Aerial parts / ethanolic extract	Full thickness burn wound model	In-vitro Human monocyte cell line & human	↑IL-1β production in THP-1, ↑ MCP in HaCa cells, Angiogenesis- no effect	(Liu *et al.*, 2008)

			keratinocyte cell line		
Centella asiatica	Aerial parts / different extracts	Partial thickness burn wounds	In- vivo / Male SD Rats	↓wound healing time, ↑re-epithelialisation, keratin formation	(Somboonwong *et al.*, 2012)
Cocos nucifera	Fruit inner fresh oil	Partial thickness burn wounds	In-vivo / Both sex- Wistar rats	↑wound contraction ↓ Epithelialisation period	(Pallavi Srivastava 1, 2008)
Crocus sativus	Pollen Hydroethanolic extract	Second degree burn wounds	In vivo / Male wistar rats	↑wound contraction ↓ Epithelialisation period	(Khorasani *et al.*, 2008)
Datura Alba	Leaf / different ethanolic extract	Thermal burn	In vivo / Male wistar rats	↑wound contraction, Antimicrobial with chloroform fraction & crude extracts, ↑inflammatory cells, ↑blood vessels ↑MMP2 & MMP9, ↑Hexosamine, ↑collagen formation	(Priya *et al.*, 2002)
Euphorbia hirta	Whole plant / ethanolic extract	Thermal burn	In-vivo / Male albino wistar rats	↑ wound contraction	(Jaiprakash *et al.*, 2006)
Ginko bilob	Leaf	Second degree burn wounds	In-vivo / both sex albino wistar rats	↓ALT, AST, BUN, Creatinine, TNF-α & LDH	(Sakarcan *et al.*, 2005)
Galium odoratum	Aqueous and methanolic extracts	Second degree burn wounds	In vivo / male wistar rats	↓ inflammatory cell infiltration, ↑ re-epithelialisation ↑anti-oxidant activity ↑wound contraction- aqueous extracts	(Khanavi *et al.*, 2013)
Hippophae rhamnoids	Seed oil	Third degree burn wounds	In- vivo/ SD rats	↓ Edema, ↑tissue regeneration, ↑DNA, ↑ total protein, ↑ hydroxyproline, ↑hexosamine , ↑ ROS, ↑MMP2, ↑MMP9, ↑VEGF, ↑type III collagen	(Upadhyay *et al.*, 2009)

Hippohae rahmnoides	Leaf / aqueous extract	Third degree burn	In- vitro-chorioallantoic membrane , In- vivo/Male SD rats	↑Edema, ↑re-epithelialisation, ↑lipid peroxidation, ↑MMP2 & MMP9, ↑VEGF, ↑Hydroxyproline, ↑Hexosamine, ↑Type III collagen, ↑anti-oxidants assays	(Upadhyay *et al.*, 2011)
Malva sylvestris	Flower / Ethanolic extract	Thermal burn wound	In-vivo/ Male wistar rats	↑wound contraction, ↑re-epthelialisation, ↑collagen formation, ↑ fibroblasts migration, ↑ angiogenesis, ↑granulation tissue formation	(Ghasemi Pirbalouti, Azizi and Koohpayeh, 2012)
Nigella sativa	Seed oil	Third degree burns	In- vivo / Male albino wistar rat	↑thickness of granulation tissue, ↓ time of re-epithelialisation,	(Yaman *et al.*, 2010)
Phyllanthus nirui	Fruit / ethanolic extracts	Thermal burn wound	In- vivo / Male	Re-epithelialisation- no change, period of wound contraction – no change	(Shanbhag *et al.*, 2010)
Plantago major	Aqueous extract / seed extract	Third degree burn wound	Male SD rats	↑ burn wound model, ↑re-epithelialisation, ↑granulation tissue formation	(Amini *et al.*, 2010)
Punica granatum	Flower / ethanolic extract	Thermal burn wound	In-vivo / Male wistar rats	Wound contraction & re-epithelialisation - no change, ↑ fibroblasts migration, ↑angiogenesis ↑granulation tissue formation	(Ghasemi Pirbalouti, Azizi and Koohpayeh, 2012)
Pistacia lentiscus	Fruit/virgin fatty oil	Deep third degree burn	In vivo/ male rabbit	↑ wound contraction, ↓ epithelialisation time	(Djerrou *et al.*, 2010)
Pterocarpus santalinus	Wood powder	Thermal burn wound	In vivo / Diabetic rats	↑ wound contraction, ↑ collagen formation	(Biswas, Maity and Mukherjee, 2004)
Panax ginseng	Root / crude saponins	Thermal burn wound	In vivo/ Male Balb/c mice	↑wound contraction, ↑neovascularisation , ↑VEGF, ↑IL1b, ↑HIF-1a in keratinocytes	(Kawahira *et al.*, 2008)

Sesamum indicum	seed oil	Partial thickness burn wound	In vivo/ male albino wistar rats	↑wound contraction, ↓ epithelialisation time	(Kiran and Asad, 2008)
Silybum marianum	Fruit	Partial thickness burn wound	In vivo/ Both sex albino rats	↑TNF-α, ↓Lactase dehydrogenase, ↓Malonyl dialdehyde	(Toklu *et al.*, 2007)
Tribulus teresteris	Aqueous extract	Thermal burn wounds	In vivo /Rats	↑ wound contraction, ↓ epithelialisation time	(Wesley J, Christina A, 2009)
Tridax procumbens	Lead	Partial thickness burn wounds	In vivo / Rats	↑ wound contraction, ↓ epithelialisation time	(Ganesh Babu, Sanjeeva, 2003)
Tephrosia purpurea	Flavonoid rich aerial part	Partial thickness and full thickness burn wound		Partial thickness- ↑ wound contraction, ↑tensile strength Full thickness- ↑hydroxyproline, ↑collagen fibre, ↑fibroblasts, ↑angiogenesis of wound tissue.	(Lodhi *et al.*, 2010)
Thymus species	Essential oil mixed in olive oil	Thermal burn wound	In vivo / SD rats	↓ repair time, ↑granulation tissue formation, ↓NO levels	(Dursun *et al.*, 2003)
Matricaria chamomilla	Hydroalcoholic extract of aerial parts	Second degree burn wounds	In vivo / Male albino rats	↓wound repair time	(Jarrahi, 2008)
Actinidia deliciosa	Fresh fruit	Full thickness burns	In vivo /Male SD rats	↑ wound contraction ↑eschar sloughing	(Hafezi *et al.*, 2010)
Spathodea campanulate	Methanolic bark extract	Full thickness burn wounds	In vivo /Male rats	Early Complete wound healing	(G Y Sy 1, 2005)

Table 6 Pharmacological research studies on Polyherbal formulations for burn wound treatment

Scientific name	Plant part/ extract	Burn model	Study design/animal used / case study	Results	References
Malva sylvestris, Solanum nigrum Rosa damascene	Aqueous and oily extract of leaves	Second degree burns	In vivo/ male wistar rats	Antimicrobial, anti-inflammatory and anti-oxidant effect ↑epithelialisation ↑Angiogenesis	(Fahimi *et al.*, 2015)
Ambucus nigra L. Sanguisorba minor Bertol, Teucrium chamaedrys, Polypodium vulgarae	Aqueous extract	Second degree burns	In vivo/ female wistar rats	↑wound contration ↑epithelialisation ↓wound size, better healing, acceptable scars and keloids	(Becić *et al.*, 2005)
Aloe Barbadensis miler Glycyrrhiza glabra Jasmimum officinale Brassica juncea Apis mellifera and Azaderacta indica	Aqueous extracts	Burn wound model	In vivo / Swiss albino rats	↑wound contration ↑epithelialisation ↑hydroxyproline & ↑hexosamine content	(Jogpal *et al.*, 2022)
Punica granatum Coleus aromaticus	Methanolic & hydroalcoholic extracts	Partial thickness burn wounds	Swiss albino rats	↑wound contration	(Mishra *et al.*, 2022)
Thymus serpyllum, Macrophyllum Platonychium	Ointment	Second and third degree burns	In vivo / male wistar- albino rats	Improved wound area, ↓inflammation – second degree burns	(Akhoondinasab *et al.*, 2015)

				↑ epithelialisation – third degree wounds	
Rosa damacena Calendula officinalis	Ointment	Second and third degree burns	In vivo / male wistar- albino rats	↑ healing rate, improved wound area - second degree burns Less scar formation ↑ epithelialisation & improved wound area- third degree wounds	(Akhoondinasab *et al.*, 2015)
Azadirachta indica, Berberis aristata, Curcuma longa, Glycyrrhiza glabra, Jasminum officinale, Pongamia pinnata, Rubia cordifolia, Terminalia chebula, Trichosanthes dioica, Symplocos racemosa, Ichnocarpus frutescens, Capsicum abbreviata, Nymphaea lotus	Oil based formulation	Second degree burn wound	In vivo / male wistar rats	↑ wound contraction ↑vitamin c, ↑ protein levels ↑GSH ↓MDA, ↓NO, ↓Xanthine oxidase	(Dwivedi *et al.*, 2010)
Azadirachta indica, Berberis aristata, Curcuma longa, Glycyrrhiza glabra, Jasminum officinale, Pongamia pinnata, Rubia cordifolia, Terminalia chebula, Trichosanthes dioica, Symplocos racemosa, Ichnocarpus frutescens, Capsicum abbreviata, Nymphaea lotus	Oil based formulation	Partial thicknes burn wounds	In vivo/ SD rats	↑ wound contraction	(SSehgal Rajesh *, Chaudhary Manu, 2010)
Malva sylvestris, Punica granatum, Amygdalus communis	Ethanolic extracts	Thermal burn wound in diabetic rats	In vivo /male wistar rats	↑ wound contraction, ↑ collagen activity, ↑fibroblasts migration ↑blood vessels sprouting	(Ghasemi Pirbalouti, Azizi and Koohpayeh, 2012)

Achille millefolium Honey	Aqueous extracts	Thermal burn wounds	In vivo / male rabbits	↑wound contraction ↓microbial invasion	(Jalali *et al.*, 2009)
Jatyadi tailam	Oil	Partial thickness burns	In vivo/rats	↑wound contraction ↑epithelialization	(Dhande, 2017)
Jatyadi ghritam	Ghrita	Partial thickness burns	In vivo/rats	↑wound contraction ↑epithelialization	(Dhande, 2017)
Madhuchista,Madhuka, Lodhra , Sarjarasa , Manjistha, Chandana , Murva -Ghrita	Medicated ghrita	First and Second degree burns	In vivo- case study	Twachya, anti-microbial, vrana shodhana ↑ wound healing ↑complexion	(Shindhe *et al.*, 2023)

Table 7 Marketed dosage forms for burn treatment

Drug / Brand name	Chief Composition	Manufacturer
Murivenna ointment S	Moringa leaves, Indian shallots, Indian beech, Aloe vera	Sitaram ayurveda
AVP Murivenna oil	Turmeric, ginger, neem	The Arya Vaidya pharmacy, Ltd.
Nidagdha cream	Shorea robusta, shatdhaut ghrita	Inducare pharma pvt.ltd.
Murivenna oil	Aloe vera, tambula, sigrupatra, palandu, shatavari in coconut oil	Kerala Ayurveda Ltd.
Burnion ointment	Resina flava, mayur tutha	Spurtha pharma
Aloe gel	Aloe vera	Fission pharma
Burn cutter	Majitha, yashtimadhu, aloe vera	Vita health care pvt.ltd.
Ridan veda burn skin protection cream		Harsh ayurvedic pharma
Alocol cream	Kumari, gulab, Aloe, Turmeric, Mandukparni, and Sariva	Ayushadi wellness pvt. Ltd.

2.9 Topical treatment- modern therapeutic approach for Burns

A review study conducted among eleven clinical practice guidelines presented array in recommendations based on three determining factors: burn severity, depth of burn wound, and clinical scenario. Fluid renewal, safeguarding the air passage, use of wide spectrum antibiotics, dietary guidance, escharotomy, fasciotomy, replacing with skin grafts, and prophylactic treatment for tetanus are standard prescribed protocols for burn treatments (Jeschke *et al.*, 2020). Topical agents form an essential component for burn wound treatments.

Bacteria are supposed to colonize on burn wounds with an extreme potential to infect. This needs topical antimicrobial therapy and debridement of the wound or excision is needed immediately to combat infection. The considerable agreement among various guidelines, advise use of topical antimicrobials to avert infection. Silver containing dressing was one among the most commonly used treatment options. nevertheless, no topical antibiotic is found that can be prescribed as a perfect therapeutic in every situation. More standardized recommendations need to be created in order to standardize clinical practice in burns (guidelines). Readily accessible to be applied on burn injuries. They comprise short acting topical agents that have therapeutic efficiency for 24hr duration. and long acting topicals. Broadly classified as antiseptics, antimicrobials agents and enzymatic exfoliating agents.

2.9.1 Antiseptics substantially toxic to all living things compared to antimicrobial agents that have targeted action against bacteria, fungi and virus. Topicals with enzymatic actions are helpful to debride wounds (current state of topical anti-microbials). Early outcomes of burn wound are infections, dermatitis and kidney failures while late complications include hypertrophic scarring and keloids(Shenoy and Shenoy (Nileshwar), 2014). A french surgeon, Ambroise Pare, used onion paste for burn wound treatment in the 16^{th} century (Majno and Pas-, 2000). Antiseptics heal microbial growth and colony formation. Researches demonstrate that inappropriate use of antimicrobial agents leads to poor health or death (Kollef *et al.*, 1999). Microbial culture count at 10^2/g tissue needs topical agents. When the microbial count greater than 10^2/g tissue requires antimicrobial administration. Whether a burn wound needs excision or has to undergo grafting is significantly determined by use of antiseptic and antibiotic treatment. Antiseptics can be classified into four primary categories. They are mentioned in table 8.

2.9.1.1 Emulsifiers they protect extensively uncovered burn wounds. Hydrophobic end of emulsifiers penetrates lipid bilayer and the hydrophilic end creates micelles to solubilize lipid membranes. Members of this class include soaps and detergents. They act silent pathogen reserves that create biofilms. Existing guidelines recommend use of antiseptic on burn wound sites with pathogen biofilms but absence of clinical signs of infections. Though they are not selective to kill pathogens but, cleansing burn wounds with soaps disturbs microbial biofilms from burn wound surface(Bjarnsholt, 2013). Chlorhexidine, a popular dental antiseptic, prescribed in essential medicines mentioned by WHO (World Health Organization, 2021) is used to initially treat gram positive, gram negative and fungal microbial growth on burn wounds.

2.9.1.2 Oxidisers Penetrates biofilms on burn wounds through free radical mediators. Though they are compounds with wide spectrum activity but limited therapeutic utility. Hydrogen peroxide and sodium hypochlorite is a popular member of this class. Due to its short half-life of H_2O_2, its potential is not being appropriately used in burn wounds however it is used to remove blood and clotted material from wound site to remove organic matter as a feed for bacterial growth(Cambiaso-Daniel *et al.*, 2018). An aqueous solution of 3% H_2O_2 and 0.5% sodium hypochlorite is widely used to remove bacterial colonies due to their wide spectrum anti-microbial potential(Omidbakhsh and Sattar, 2006). Povidone- iodine is an oxidiser with a wide range of antimicrobial efficacy. Commonly known as the agent ‘Betadine’ is available in

liquid and cream forms. PVP-I decreases bacterial load and helps in wound healing(Fumal *et al.*, 2002).

2.9.1.3 Acids Weak acids are popular for their antimicrobial activity since ancient times. The antimicrobial action of 5 % aceitic acid against gram positive and gram negative microbes was earlier proposed by Hippocrates. Gauze soaked in aceitic acid works actively against biofilms. Limited antibacterial activity of boric acid is significant to reduce host DNA activity and plays an important role in wound healing. Since ancient times honey is popular wide spectrum antimicrobial agent for topical use (Bitter and Erickson, 2016). It became a topic of scientific interest in late 19^{th} century. It is popular for its wound healing activity in developing countries. Low pH, significant sugar levels and osmotic gradient works against microbes (Saikaly and Khachemoune, 2017)

2.9.1.4 Heavy metals use of noble metals as copper, silver, mercury and bismuth form a major class of heavy metal antiseptics (Cambiaso-Daniel *et al.*, 2018). Since 1000 B.C, silver is known to act as bactericidal. However, silver's appeal declined after 20^{th} century after the introduction of antibiotics. Copper and bismuth have limited use. Damage due to systemic toxicity issues has created a pause to its therapeutic use in burns. Metallic form of silver (Ag^0) is found in crystalline or nanocrystalline states (Khundkar, Malic and Burge, 2010) while in solution form silver exists in sub-crystalline states. Crystalline state of silver comparatively inert with low absorption by body self and host cells Silver ionises rapidly on binding with proteins or cell membranes. As a metal, silver is relatively inert and is poorly absorbed by mammalian or bacterial cells. However, in the presence of wound fluids or other secretions, it readily ionizes and becomes highly reactive in binding to proteins and cell membranes. (Atiyeh *et al.*, 2007) . Ionic silver binds to proteins, enzymes and DNA and undergoes fragmentation process through oxidative reactions of catalase production. Despite the fact stated in previous studies that, silver absorbed from the wound surface of extensive wound enters the circulatory system and gets deposited in the internal organs like liver and kidney(Wang *et al.*, 1985). Some studies suggest absorption of silver from wounded area do not enters the deeper layers but remains on superficial eschar of partial or full thickness burn wounds.

2.9.2 Topical antimicrobials Until treatment is directed with prescribed culture report antimicrobial regimen, antimicrobials administration is initiated with wide spectrum

antibiotics. Substantially decreased mortality rates have been observed by topical antimicrobial(Cambiaso-Daniel *et al.*, 2018)

2.9.2.1. Silver preparations Silver can be delivered to burn wounds in various forms of silver salts like chloride or silver calcium phosphate or metallic compounds like silver in nano sized form. To exhibit its antibacterial properties silver must be in its ionized form. Negatively charged proteins in the wound fluid easily bind to positively charged silver ions, preventing the transfer of silver ions to the wound bd. In fact, a significant concern with topical silver agents is less number of available species their limited capacity to enter the target area and release silver at low concentrations due to their poor penetration of the tissue and proinflammatory reactions of nitrates or cream base limit the use of topical silver preparations. To address these difficulties, a number of silver products with various innovations have been released to the market in the recent few years(Warriner and Burrell, 2005) (Atiyeh *et al.*, 2007).

2.9.2.2 Silver nitrate Most commonly used silver salt is silver nitrate, which has a strong toxic effect at concentrations above 1% and an antibacterial action (both in vitro and in vivo) below 0.5%. The most popular method of using silver nitrate topically is soaking gauzes and then applying them to serious burns. Silver nitrate gels, which are supposed to be simpler to give, have patents issued for them (Poon and Burd, 2004).

2.9.2.3 Silver sulphadiazine the most well-known topical medication for treating burns is silver sulfadiazine. Used since 1970's. widely used global treatment for burns treatment (17-Greenhalgh) This cream dissolves well in water and has 1% of insoluble silver sulfadiazine in micronized form. A combination of sodium sulfadiazine and silver nitrate is the active ingredient. Propylene glycol, stearyl alcohol, and isopropyl myrisolate are complexed with silver. In the sulfadiazine molecule, a hydrogen atom is swapped out for a silver atom. It rarely discolors tissues or clothing, however it may turn gray. The primary drawback of the agent is that it may hinder re-epithelialization, which raises doubts about its suitability for treating superficial partial-thickness burns. Additionally, the cream is rather hazardous to fibroblasts in vitro. It is less clear if these in vitro activities truly hinder the healing of wounds.

2.9.2.4 Long acting silver dressings

2.9.2.4.1 Silverlon this nylon dressing with a polymeric substrate surface plated with silver, was one of the first to be launched. Releases 10% of existing ionic silver (Ag^{+}) in dressing during first 24 hours and remaining until dressing is taken off. Efficiency of silver to penetrate most gram positive, some gram-negative bacteria and several fungal and yeast species. Ease of

use, stability in harsh environment, convenient to store and significantly less per unit weight compared to other short acting topicals make it convenient for usage. Dressing change is required every 3-7 days (Barillo, Pozza and Margaret-Brandt, 2014).

2.9.2.4.2 Acticoat it was made accessible in the nineties. It is a multilayered barrier dressing with antimicrobial qualities that uses silver. It is often used for donor and recipient graft sites in burn therapy. Acticoat is composed of three layers: an absorbent rayon and polyester core is encircled by two outer layers of non-adherent polyethylene net coated in silver. For three days, the dressing can be left in place(Khundkar, Malic and Burge, 2010). When the patient is being repositioned, it can be fastened to the tissues by sutures or staples to stop shear forces from upsetting the grafts and dressings underneath (Hamnett *et al.*, 2016).

2.9.2.4.3 Aquacel- a hydro fibre dressing of sodium carboxymethylcellulose with embedded silver. It is made up of hydro fibre with silver contained in it that forms a gel in the wound bed to encourage a moist environment favourable to healing and to combat infection with broad antibacterial action. The dressing's contours remove any empty space where possible infections could grow Aquacel Ag did show a statistically significant difference in ease of use. When applied, it resembles soft felt, but as it dries, it becomes firmer. With this material, the only problem is that this dressing sticks to the surface and needs some lubrication to remove it (Verbelen *et al.*, 2014).

2.9.2.4.4 Mepilex-Ag It is a flexible, soft foam silver containing dressing that contains silver. It functions to absorb exudate from wounds that exude from low to medium levels. It creates a moist environment that promotes wound healing while shielding the surrounding skin from wound drainage This reduces germs and need for dressing changes. Within 30 minutes, it starts to inactivate wound pathogens and its effects might extend for up to seven days. These silver foam dressings can be sliced into numerous shapes to cover a wide range of wounds(Hashmi and Haith, 2019).

2.9.2.4.5 Mepitel- Ag This is the newest long-acting dressing with a silver base. This soft silicone dressing with silver bonding offers broad spectrum antibacterial protection for up to eight days. The dressing is a good substance for joints and mobile areas and is highly compliant (Hallerstig *et al.*, 2017)

The "end of the antibiotic era" has been attributed to the current state of the global issue of rising antibiotic resistance(Poole, 1993). Over use of antibiotics and improper prescription

practices, poor patient compliance had led to rising antibiotic resistance. if nothing is done, many minor diseases that could have been cured with a straight forward course of antibiotics may become virtually incurable in future, just as they were in the days before to the discovery of antibiotics (Davies, 2006). Resistance is also becoming more prevalent in burn pathogens. Compared to other traumatic, surgical, and medicinal purposes that may be prone to infection, external antimicrobial medications have been used specifically to prevent and treat burn infections. While some of the medications are intended to be administered in a prophylactic manner to stop infections from occurring, while others are meant to be used in an infection's aftermath to eradicate the microbial cells that are actively multiplying within the burn.

Table 8 Modern approach for available treatment options in burns

Short acting topical agents			
Class	**Category**	**Examples**	**Action**
I Antiseptics			
	Emulsifiers	Surfactants, Soaps, Chlorhexidine	Reduce colonies of microbes(Barret and Herndon, 2003)
	Oxidizers	Hydrogen peroxide,sodium hypochlorite, povidone-iodine, Aceitic acid etc.	generates free radicals to damage cell walls & membranes (4- current state by Danielle Hashmi)
	Heavy metals	Bismuth	Inhibits synthesis of capsular structure of bacterial polysaccharide. (16- current state by danille Hashmi)
	Enzymatic exfoliaters	Collagenase	Destroys necrotic tissue and spares the viable tissue (Payne *et al.*, 2008)
II Longer acting topical agents			
Anti-microbials(Dai *et al.*, 2010)	Polybiguanides	Silver nitrate, Chlorhexidine	Penetrate cell walls and organelles of cytoplasm (Hashmi and Haith, 2019)
	Sulphonamide	0.5% Mafenide	Reduces bacterial thickness in wounds
	Sulpha antibiotics	1% Silver sulphadiazine	Binds with extracellular fluids, serum, cell wall to destroy bacteria cell

			membrane (Greenhalgh, 2009)
	Aminoglycoside antibiotics	Gentamycin sulphate, Neosporin	Reduces sepsis by preventing bacterial colonies formation(Charles F.T. snelling, Allian R . Ronald, 1969)
	Monocarboxylic acids	Bactobran, 2% Mupirocin	Interacts with bacterial isoleucyl-tRNA synthetase to prevent protein synthesis (Hashmi and Haith, 2019).
	Cyclic polypeptides	5-8% Bacitracin	Prevents formation of peptidoglycan cell walls of bacteria current state of (Hashmi and Haith, 2019)
	Polypeptides	Polymyxin B	Prevents bacterial cell wall formation and Hinders Tetrahydrofolic acid (THF) synthesis (McQuillan *et al.*, 2012).
	Nitrofuran	Nitrofurazone	Inhibits bacterial enzyme systems and damages their DNA (R.S.Vardanyan and V.J.Hruby, 2006)
III Antifungals	Polyenes	Nystatin	Attaches to the steroid components of fungal cell membrane(Dai *et al.*, 2010)

2.10 Proposed drugs in polyherbaomineral nanoemulsion (PHMN)- *Drug review*

2.10.1 Neem seed oil

Scientific name- Azadirachta indica

Kingdom- Plantae- plants

Super division - Spermatophyta- seed plants

Division- Magnoliophyte- flowering plants

Class- Magnoliopsida- Dicotyledons

Subclass- Rosidae

Order- Sapindales

Family- Meliaceae

Genus- Azadirachta

Vernacular names- Arishta, Sarbaroganivarini.

Popularly growing medicinal plant of India and neighbouring countries in the last 4500. This medicinal tree ranges from medium to large size. 1992 excavations discovered many medicinal items from the ruins of Mohenjo Dara and Harappa. The findings suggest its therapeutic use from Harapan period of civilization in India(Sarkar, Singh and Bhattacharya, 2021). In the start of 20th century, its cultivation extended to other countries. Well known in India by a common name 'Neem' has numerous medicinal uses. Neem oil contains Nimbidin, Nimbidal, Azadirachtin, Nimbin, Azadirine, Gedunin and Salanin contribute to its active principles among with Margosic acids, Butyric acid and fatty acids with traces of valeric acid. Neem oil has very strong antibacterial, anti-fungal, anti-viral, anti-inflammatory properties. It is an immune system booster and works an efficient insect repellent. Medicinally it is used to treat skin conditions, ringworms, scabies, eczema(Kaur, Alam and Athar, 2004). Oil is extracted from seed grains with yield of 50% of its dry weight. Currently many sophisticated methods exist to extract and formulate the neem extracts. It has a strong odour of sulphur and garlic and is composed of fatty acids oleic acid, stearic acid, palmitic acid, linoleic acid and other lower fatty acids. The percentage of fatty acids are different from seed oil recovered from various samples. Neem oil treatment is also used for certain plant diseases like mild dew and rust(1Ukaoma, A.A, 2019). Active components of neem seed oil include nimbidin, Nimbin, Nimbidinin, Nimbolide and

Nimbidic acid. with a significant role in inflammation, arthritis and ulcers comparable to phenylbutazone in treating carrageenin induced rat paw oedema(Pillai and Santhakumari, 1981).

2.10.1.1 Anti-inflammatory action- Nimbidin successfully combats inflammation by reducing macrophages and neutrophil activity on rats fed with oral Nimbidin at a dose between 5 to 25mg/kg for 3 days. It reduced number of macrophages migrated to peritoneal cavities. In-vitro studies also showed that phagocytosis and respiratory burst was prevented by Nimbidin on PMA induced cells. Nimbidin inhibits neutrophil functioning and so, decreases production of glucuronidase, myeloperoxidase and lysozyme Biochemical parameters presented reduction in IL-1, PGE_2 & NO synthesis. They suppresses inflammatory response(Kaur, Alam and Athar, 2004).

2.10.1.2 Antimicrobial activity- it has antibacterial, antifungal and antiviral properties(Alzohairy, 2016). It is effective against microsporum, epidermophyton, gestrichum and candida and various pathogenic bacterias. Activity of Neem seed oil against multidrug resistant organinsm were performed by agar well diffusion method. The study was conducted to determine MIC & MBC by ethanolic neem seed oil extracts showed multidrug resistant organisms vulnerable to it. The oil effectively works on Salmonella typhi and Staphylococcus, Escherichia coli and pseudomonas aeruginosa at maximum concentration (100%) compared to ampicillin control. Zone of inhibition observed were 15mm, 18mm, 19mm and 23 mm respectively(1Ukaoma, A.A, 2019).

2.10.1.3 Wound healing effect – Neem oil is effective to be applied on persistent, non-healing wounds. A study results presented 44% of patients regained 50% of wound healing with application of neem seed oil(Naik *et al.*, 2014). In one study, the benefits of neem oil on chronic, nonhealing wounds were investigated. The findings revealed that, after 8 weeks of treatment, nearly 44% of patients, the wounds recovered 50% (Singh *et al.*, 2014)

2.10.1.4 GC Analysis- methyl esters were prepared from neem oil to undergo gas chromatographic analysis using detection through mass spectrometry. Oven temperature was raised to 240^0C at a steady rate of 3^0C per minute after it was initially

set at 60^0C. The results were compared with NIST (national institute of standards and technology) revealed mostly the presence of Linoleic acid, Oleic acid, Stearic acid and palmitic acid as 34.69%, 20.46%, 20.42%, 18.66% respectively. other studies reveal that the percentage of oleic acid varies between 25-61.9%. (Ofoegbu and Uzo Anya, 2012), (Singh and Singh, 2010).

2.10.2 Curcuma Longa

Scientific name: Curcuma Longa
Kingdom- plantae-plants
Subdivision- Tracheobionta
Superdivision- spermatophyta
Division- Magnoliophyta
Class- liliopsida- Monocotyledons
Subclass- zingiberaceae
Order- zingiberales
Family- zingiberaceae
Genus- curcuma L
Species- curcuma longa L
Vernacular names- Haldi, Turmeric

Curcuma longa is popular by a common name turmeric. South east Asia and India are prime cultivators. A kitchen spice being widely used in India, Pakistani and Thai cuisine. Apart from this, it finds its popular use in tea in Japan, cosmetic in china, dye in Malaysia and as an antiseptic in India and Pakistan(Hay *et al.*, 2019). Since traditional times, it is being used for its medicinal properties. active principle found in rhizomes of curcuma longa is known by the common name 'curcumin'. Curcumin is a constituent of curcumoid family that makes approximately 2-8% of turmeric(Braga *et al.*, 2003). Chemically it is a phenol. Naturally phenols are found in a wide range of food sources and nutraceuticals. Curcumin is formed from phenylalanine. Current studies include the utility of phenolic compounds to inhibit cancer growth and work against oxidative damage. Traditional medicines used in Asian countries have highlighted therapeutic efficiency of turmeric to cure a variety of diseases though the

mode of action and active ingredients have been recently discovered. The plant has been used for its anti-inflammatory, anti-bacterial, anti-cancerous, and anti-oxidant properties. applications of the plant include of of turmeric are popular. It is avaialible in various formulations as capsules, tablets, ointments, drins, soaps and cosmetics(Gupta, Kismali and Aggarwal, 2013).

2.10.2.1 Components of Curcuma Longa

The composition of turmeric includes proteins, fats, minerals, carbohydrates and moisture and essential oils 6.3%. 5.1%,3.5%,69.4%,13.1% and 5.8% respectively. curcumin imparts the turmeric a yellow hue. Many biotechnology firms are now commercialised in isolating curcumin from turmeric(Prasad *et al.*, 2014). Curcumoids are the chemical component found in turmeric. It is acceptable and proved to be safe by US Food and Drug Administration (Hewlings and Kalman, 2017). It contains 77% curcumin, 17% di-methoxy curcumin and 3% bis-di-methoxy curcumin. Safe at a daily dose up to 8mg. its primary ingredient is curcumin which is denoted by the name deferuloylmethane. A yellow- orange coloured crystalline solid. It occurs in isomeric form and displays keto-enol tautomerisation. Predominated by enol form in dissolved state(Zhu *et al.*, 2017). Turmeric contains alkaloids, saponins, tannins, sterols, phytic acid, flavonoids and phenols to 0.76%,0.45%,1.08%,0.03%,0.82%,0.40% and 0.08% respectively. alkaloids provide turmeric the efficiency to manage inflammation, cholesterol, cold and cough, persistent headaches and migraine. Saponins, Flavonoids and Tannins provide the antioxidant property and used in treatment of GI tract disorders. flavonoids provide the property to scavenge free radicals. they also have a role as an anti-inflammatory, anti-allergic, analgesic and anti-oxidant property. 1.08% tannins provide turmeric the astringent and antimicrobial, anti-cancerous activity used to cure GI disorders. saponins act on inflammatory cells to manage inflammation in contrast to this advantageous property, they are cytotoxic in nature with requirement of researches in this context and Natural Steroids available in turmeric extract work in relation with various sex hormones. Minerals and vitamin composition of turmeric contains thiamine, riboflavin, niacin, calcium, phosphorous, potassium and iron in 0.89%, 0.16%, 2.30%,0.20%, 0.63%, 0.46% and 0.05% respectively. thiamine, riboflavin, potassium and iron provide the property for strong bones, muscle

movements, haemostatic and anti-hypertensive, haemopoietic property. Calcium strengthens the bones and plays an active role in muscle movements. Potassium and magnesium provide turmeric the anti-hypertensive property, controls nerve impulse transmission. Minerals function as a co-enzyme in certain biochemical reactions(Ikpeama, Onwuka and Nwankwo, 2014)

2.10.2.2 Antimicrobial action- Aqueous extracts of curcuma longa had antibacterial activity against various bacteria Escherichia coli, Staphylococcus aureus and Staphylococcus epidermidis and Krebsilla pneumonia at low concentrations(N.Niamsa and Sittiwet, 2009). Activity against gram-positive and gram-negative bacteria and some fungal isolates were identified using ampicillin and fungobacter standards. Agar well diffusion method was used for the study. Statistically significant Zone of inhibition against aspergillu niger with turmeric extract (20mm). other studies have presented it to be effective against pseudomonas aeruginosa, aeromonas hydrophila(Harikrishnan and Balasundaram, 2008), Heliobacter pylori(Zaidi *et al.*, 2009) listeria monocytogens, staphylococcus and Salmonella typhimurium (Kim *et al.*, 2005), Escherichia coli(Gupta, S. and Ravishankar, 2005), This supports the use of turmeric to stop bacterial spread or illness.

2.10.2.3 Anti-inflammatory agent- inflammation is the second stage in wound healing, a wound not recovered at this stage leads to undesirable consequences. Inflammatory disorders cause tissue damage as is evident in rheumatoid arthritis(Joe, Vijaykumar and Lokesh, 2004). Curcumin was found to decrease chronic inflammation by decreasing functions of macrophages and ROS production. was demonstrated in rat model for arthritis. As Turmeric finds its extensive use in traditional medicinal treatments of China and India as a potential anti-inflammatory agent. In vitro studies reveal the inhibition of TNF-α and IL-6 in macrophages of mouse when they were pre-treated with Lipopolysaccharide. TNF- α helps in induction of various proinflammatory cytokines like IL-6, IL-8 and IL-1β. It has been explored that curcumin present in turmeric can inhibit these cytokines and COX-2 and iNOS as other inflammatory mediators(Lantz *et al.*, 2005). The limitation to this property of curcumin exists due to its poor pharmacokinetics. (guang Liang, 2015). Inflammatory response was inhibited

in a mouse model by reducting the acivity of NF-(κ) B gene(Mohanty, Das and Sahoo, 2012) (Jagetia and Rajanikant, 2012). Different opinion exists in another research study where inflammatory response is activated due to increased NO production. (Kulac *et al.*, 2013). Most of the research papers have provided proofs that curcumin decreases inflammation so that the process of healing moves forward towards proliferative phase and then the remodelling phase commences. Prolonging the inflammatory phase delaying the healing process(Akbik *et al.*, 2014)

2.10.2.4 Antioxidant agent **–** superoxide and hydrogen peroxide form biomarkers for reactive oxygen species. They are essential to defend human cells against microorganisms. Prolonged presence in higher concentrations can cause injury to body's own cells and hampers the remodelling process(Thangapazham, Sharad and Maheshwari, 2013). Oxidative stress leads to lipid peroxidation process that inhibits the wound healing process. reactive oxygen species cause inflammation that ultimately damages tissues. SOD, glutathione peroxidase and catalase help to protect the healthy cells against their deleterious effects. Topical application of Curcumin in curcuma longa has the potential to remove these harmful radicals without the involvement of enzymatic path & shortens the wound healing phase(Panchatcharam *et al.*, 2006) (Ghoneim *et al.*, 2002)

2.10.2.5 Wound healing effect- The effect is contributed due to the anti-inflammatory(Lantz *et al.*, 2005), anti-oxidant, controls infection and proliferative and re-epithelialization effect(Kulac *et al.*, 2013) of active component curcumin present in curcuma longa extract. (Mohanty and Sahoo, 2017). Experimental studies have proved curcumin effective in burn wounds, diabetic wounds & cutaneous excision wounds (Panchatcharam *et al.*, 2006)(Sidhu *et al.*, 1998) (Kulac *et al.*, 2013)(Akbik *et al.*, 2014). It helps to deposit collagen for granulation tissue construction, angiogenesis and remodelling process to occur smoothly (Cheppudira *et al.*, 2013)·(Thangapazham, Sharad and Maheshwari, 2013).

2.10.2.5.1 Effect on Proliferative phase - fibroblasts start to prepare the bed for the new tissue formation, researches state that the delayed infiltration of fibroblasts at wounded site are the prime cause for delayed wound healing in chronic wounds

(Blakytny and Jude, 2006). It has been highlighted by Mohanty et al that in Curcumin treated wounds fibroblasts are launched within four days, Wound contraction happens when they change themselves into myofibroblasts(Mohanty, Das and Sahoo, 2012). The Invitro model depicted no such changes in the scratch test since there is a challenge to precisely replicate the healing process in the invitro settings.

2.10.2.5.2 Effect on granulation tissue formation- around fourth day the new blood vessels begin to sprout along with fibroblast being collected at the wounded site. They prepare the bed for the newly formed granulation tissue which further promotes the epithelial cells to cover the wounded area. Curcumin treated wounds present better Hydroxyproline content and efficient orientation and consistency of granulation tissue. Hydroxyproline content is a biomarker that signifies efficient collagen formation(Gopinath *et al.*, 2004). The wounds with topical curcumin infiltration present better consistency and organisation and firmness of collagen fibres(Mohanty, Das and Sahoo, 2012)(Panchatcharam *et al.*, 2006) (Sidhu *et al.*, 1998).

2.10.2.5.3 Collagen deposit activity- Experimental studies on rat model have shown that the collagen amount increase, mature soon, increase in strength of these fibres and cross linkage fibres are confirmed by estimating the aldehyde content of fibres(Mohanty, Das and Sahoo, 2012) (Panchatcharam *et al.*, 2006). Curcumin as topical drug synthesizes well compressed & symmetrically aligned fibres following 8 days of therapeutic treatment. Researches report local treatment of curcumin in rat models drastically shortens the time taken for complete epithelial layer formation in excision wounds, diabetic wounds & burn wounds

2.10.2.6 HPTLC analysis- This method is appropriate for identification of curcuma longa to differentiate it from other curcuma species. It is effectively used to identify and screen curcuma longa extracts for commercial uses in pharmaceutics. The extract recoverd with Soxhlet extraction was dissolved in methanol. Stationary phase of precoated aluminium plates $60F_{254}$ was taken and Chloroform: methanol was taken in a 48:2 ratio as mobile phase. Densitometric analytical technique was *used in* chromatography study. plates were scanned at 425nm. 3 different spots for curcumin,

demethoxycurcumin and bisdemethoxycurcumin at Rf values of 0.66, 0.48 and 0.30 were observed. (Paramasivam *et al.*, 2009)

2.10.3 Aloe vera

Kingdom- plantae

Sub kingdom- Tracheobionta

Super division- Spermatophyta

Division- Magnoliophyta

Class- Liliosida

Subclass- Lilidae

Order- Lilales

Family- Aloceae

Genus – Aloe L

Species- Aloe vera (L) Burm.f

Vernacular names- Aloe, ghrit kumari

2.10.3.1 Morphological features and traditional uses

Aloe vera is a xerophytic plant of family liliaceae of warm areas of tropical and sub-tropical regions of world with a huge collection for water storage.(Patel, Patel and Dhanabal, 2012) Among 400 different Aloe vera species(Radha and Laxmipriya, 2015). “Aloe Barbedensis Miller” is biologically most active kind in North America, Europe and Asia used for therapeutic and commercial use (Hu, Xu and Hu, 2003).

Water content ranges from 99.95% and solids amounts to only 0.5% with about 75 potentially active compounds including inorganic compounds and organic acids, anthraquinones, polysaccharides, phenols, essential amino acids, vitamins and minerals(Hamman, 2008). Cosmetic industry uses the mucilage tissue of inner part of leaf of Aloe vera(Vogler and Ernst, 1999). The plant can be separated into two parts: latex and Aloe gel. Technically speaking, "gel" or "mucous” denotes the viscous transparent liquid found inside the parenchyma cells, while "pulpy" or "parenchymatous tissue" refers to the complete fleshy inner section of the leaf.(H.Hamman, 2008) Cell walls, thick liquid of the cell and the degenerated cell

components contribute to the structural elements of the pulp. It has been established that these three may be distinguished from one another both in terms of shape and sugar composition (Hamman, 2008). Latex is an exudate that comes from the pericyclic tubules in the outer skin of the leaves.

2.10.3.2 Composition

Type of species cultivated, conditions of environment, leaf position on stem and the collection technique determine the chemical profile of the plant. Before the collection of leaves, a three-year growth period (Hęś and Gujska, 2019)is needed for the plant to gain highest content of polysaccharides and flavonoids approx.6.55 & 4.70g/kg respectively. Pulp of Aloe vera contributes to 70-80% of whole plant weight. Dry weight of pulp contains 57.6% polysaccharides, 4.2% lipids and 7.3% proteins.(Radha and Laxmipriya, 2015). Acemannan is the chief polysaccharide with molecular weight more than 30,000- 40,000 dalton. It is composed of glucose and mannose units in ratio of 1:3. Berberine, Rutin and Gallic acid are generally used as the standard marker compounds for quantitative screening (HPTLC) of Aloe vera extract at different concentrations in methanol(Patel, Patel and Dhanabal, 2012) . It has been predicted that the solid material that includes 75 important nutritional and therapeutic components like polysaccharides, vitamins, enzymes minerals, phenolic compounds, saponins, organic acids and fats soluble vitamins(Vogler and Ernst, 1999). Polysaccharides include pectin, cellulose, hemicelluloses, glucomannan, acemannan, and mannose derivatives. The parenchymatous tissue of the leaves has a moisture level of 0.95 to 0.99 g water/g d.m. Aloe vera pulp with moisture level of diverse compositions may be a factor in the variety of pharmacological and therapeutic effects of aloe gel products. It is frequently required to use certain processing procedures, to prevent permanent changes to its cosmetic, functional, medicinal and nutraceutical properties.(Singh, 2017). Review of literature indicates active components of Aloe vera (alkaloids, phenols, tannins, flavonoids and others aromatic compounds) are responsible for anti-bacterial, anti-fungal, anti-oxidant cytoprotective, cardiac stimulator, anti-diabetic and immunomodulator properties. It is used as a remedy in skin disorders and promotes wound healing, reduces oedema and pain (Heggers *et al.*,

1996). The identity of the active ingredients in aloe vera gel is a matter of debate. All the Functions of Aloe vera is attributed to the combined effect of polysaccharides and other components (Hamman, 2008). Commercial Aloe vera products need frequently processing before being used. Dried powdered samples can be combined with additional ingredients to create a wide range of nutritional, cosmetic, and medicinal products. Freeze drying was investigated as a very gentle process to deliver powdered samples with the least damage of phytochemicals. It preserves the polysaccharides, minerals and reduces the inorganic components(Krokida, Pappa and Agalioti, 2011).

2.10.3.3 Traditional uses of plant

Traditionally plant is used as a topical application to soothe small burn wounds and skin irritation. While orally the plant is used in constipation, cough, ulcer, diabetic treatment, used in arthritis and immune compromised diseases.(Vogler and Ernst, 1999)·(R H Davis, K Y Rosenthal, L R Cesario, 1989) (Maenthaisong *et al.*, 2007) According to Vega et al. (2007). The plant compounds have high antioxidant potential phenolic components (anthraquinones) and sugars in their chemical makeup and so the traditionally used Aloe vera, sometimes referred to as "nature's sun screen," is becoming more and more popular in the nutraceutical and cosmetic industries as a skin tonic and its products have been utilized for its therapeutic and curative purpose.

2.10.3.4 Aloe vera as a Permeation enhancer- They are the substances or chemicals that provide easy permeability of medicaments to reach the deeper tissues and blood stream thereafter. Recently transdermal drug transportation is a popular route nw-a-day because crossing the obstacle of stratum corneum will provide target delivery for drugs at therapeutic levels(Moser *et al.*, 2001). Aloe vera is a popular known herbal drug that functions as penetration enhancers because it is moisturising in nature and is safe to be applied on skin. In vitro studies state that within Aloe vera gel a complex is created with the penetration enhancer and compound used along with it. Aloe vera gel penetrates the skin and the compound accompanies it by pull effect depending upon the molecular weight of the compound applied along with the gel(Cole and Heard, 2007). 'Lignins & 'saponins' helps the compound used along with gel to reach cell levels to provide proper nourishment. Further research is needed to sustain these studies(Sharma, Mittal and Chauhan, 2015).

2.10.3.5 Anti-inflammatory- Aloe vera is rapidly used in skin care products. Various clinical studies exist to favour anti-inflammatory activity of Aloe vera gel (Maenthaisong *et al.*, 2007)(Akhoondinasab, Akhoondinasab and Saberi, no date)(Akhoondinasab *et al.*, 2015)(Hekmatpou *et al.*, 2019). The anti-inflammatory activity was evaluated by UV erythema test potential was studied through in-vivo studies in 40 volunteers with 1% hydrocortisone cream and gel as a control for 2 days. After 48 hours. Findings were suggestive of the fact that Aloe vera gel reduced erythema better and surpassed 1% hydrocortisone gel on the other hand hydrocortisone cream was better than Aloe vera gel to recover from inflammation in erythema(Reuter *et al.*, 2008).

2.10.3.6 Anti-oxidant potential-Aloe vera leaf gel present higher anti-oxidant potential. Hexane extracts have lowest activity. Researches explore that Aloe vera show its highest potential when used in form of emulsions (Hęś and Gujska, 2019). In- vitro studies have explored ethanolic extracts to have flavonoids and phenols that impart highest DPPH radical scavenging activity and ferric reducing power(Moniruzzaman *et al.*, 2012). Ethanolic extracts prepared by reflux technique exhibited enhanced anti-oxidant potential than those prepared by shaker approach (Hęś and Gujska, 2019).

2.10.3.7 Wound healing- Experimental studies conducted with lyophilised aloe vera used in dressing for second degree burns show successful treatment in 80% rats with no signs of infection. Increase tensile strength of (7.42 ± 0.687) on 7-10th day compared to controls (4.17 ± 1.332). The tensile strength decreases after this with decrease in fibroblasts number. Treated groups were completed with the regeneration process when the controls presented initial signs of regeneration process on 15-20th day. In vitro studies exist that used biomechanical assays to identify the mode of action of aloe vera, state that fibroblasts migration is not affected by its use(Topman, Lin and Gefen, 2013). Since, Treatment groups had early regeneration of hair follicles, sebaceous, sweat glands and blood vessels(Yam Mun F, Jamaludin Zainol, 2002). Another study with aloe vera gel topically applied on cutaneous excision wound model presented 80% healing on 14th day(Khan *et al.*, 2013). Further research with in-vivo models is needed to actually prove the mode of action of this natural medication(Topman, Lin and Gefen, 2013)(Oryan *et al.*, 2010).

2.10.3.8 Immunomodulator effect- Improved immunosuppression caused by UV radiations on mice skin. Structure and shape of skin cells remained unchanged. Research study demonstrated positive results with a halt in tumour progress and survival period was increased by use of Aloe vera and melatonin (Lissoni *et al.*, 1998).

2.10.4 Calotropis procera

Scientific name- Calotropis Procera
Kingdom- plantae
Subclass- tracheobionta
Super division- spermatophyta
Division- Magnoliophyta
Class- Magnoliopsida
Subclass- Asteridae
Order- Genitianales
Family- Asclepiadaceae Borkh.
Genus – Calotropis.R.Br.
Verancular names- Ark, suryarka

Ayurvedic medicinal plant. *Calotropis procera* (Asclepiadaceae), a golden gift for human beings, is an Indian shrub well grown in tropical and subtropical regions. Ayurveda accepts two varieties: *Shvetaarka* and *Raktaarka.* Shvetaarka, identified by synonyms Aak, Arka, Madar, Akavana, Akand, Akanda, Akan, Ravi, Tapana, Bhanu, Rui, and Erikku, has been included in Hindu texts since Vedic periods. (D., 2017). The leaves of the plant were used to worship the sun by Hindus and as a drug of choice for a variety of ailments. (Chundattu, Agrawal and Ganesh, 2016). It is a well-recognized plant in the pharmacopeial compendium of Ayurveda. (Shefali and Sumeet, 2010). The plant has its description in the early mediaeval period in Dhanwantari Nighantu, one of the oldest Indian materia medica. (10^{th}-13^{th} century A.D).

Traditional medical systems utilized plant latex from the Asclepiadaceae family as therapeutic anti-microbial and medicinal agents for stopping bleeding from minor wounds and promoting wound healing (Venkatesha, 2016). This plant has been

extensively used in the folklore and medicinal history of India. Topically, the latex of *Calotropis procera* was applied for gum bleeding and as a hemostatic and healer for fresh wounds by tribal and rural people. (Ramos et al., 2012). Leaves, latex, and flowers are traditionally used as a poultice on sores and toothache preparations. (Parihar and Balekar, 2016), cracked feet and as a vermifuge (Rasik *et al.*, 1999) (Catalogus *et al.*, no date). Root bark has been used by traditional therapist for elephantiasis. The secretions from rootbark were used for skin diseases, asthma, leprosy, hemostasis, intestinal worm infestations, ascites and anasarca (Mueen Ahmed, 1999). Flowers increase appetite and improves digestion. Flower tops were being used for asthma, while boiled and oil-treated leaves have been proven effective in the treatment of paralysis. Leaf powder is used as an alternative to ipecacuanha and is being used for wound healing, while juice is applied as an infanticide to induce abortion. Topically, the latex was applied to fresh wounds for hemostatic use and for healing wounds by tribal and rural people. (Ramos et al., 2012). It has been used as a topical application for skin infections in Brazil for ages. Information is available about drinking diluted latex with water as a traditional treatment for hyperglycemia. (Ramos *et al.*, 2007). Traditionally, milky latex was assumed to have purgative and caustic actions, thus being used as an anti-inflammatory, antimicrobial, analgesic, anti-diabetic, infanticide, anti-helminthic, anti-arthritic, and also to treat baldness and toothache. In comparison with chloroform extracts. The aqueous and ethanol extracts possess better bioactive components.(Merzaia *et al.*, 2017).

2.10.4.1 *Composition of latex of Calotropis procera*

The composition of latex is responsible for its various pharmacological activities. Latex has a specific gravity of 1.021, solids 14.8%, water-soluble parts 88.4–93%, and 0.8–2.5% coagulate. (Chaudhary preeti, 2017). Phytochemical analysis confirms latex extracts for the existence of biologically active cardiac glycosides, phenols, saponins, alkaloids, triterpenes, tannins, anthocyanins, and steroids(Cavalcante *et al.*, 2020).

Main cardenolide in crude latex includes uscharin, usharidin, calotropin, calotropagenin, 19-dihydrocalotropagenin, calotoxin, 12β-hydroxycoroglaucigenin,

calactin, 15β- hydroxycalactin, voruscharin, uscharin, uscharidin, uzarigenin, syriogenin, dihydrouscharin, 15β-hydroxyuscharin, afrogenin, afroside (Chan *et al.*, 2017). Flavonoids inthe aerial parts of the plant latex. Crude flavonoid fraction of dialyzed methanolic extract from aerial parts of plant identified as kaempferol-3-O-rutinoside, isorhamnetin-3-O- rutinoside, quercetin-3-O-rutinoside and the flavonoid 5-hydroxy-3,7-dimethoxyflavone- 4-O--glucopyranoside(Al-Snafi, 2015). Di and Tri terpenes, 3β, 27-dihydroxy-urs-18-en,13,28-olide, Urs - 19(29)-en-3yl acetate (calotropenyl acetate), multiflorenol, α,β- Calotropeol, 3-epimoretenol (Chundattu et al., 2016; Nenaah, 2013), β- amyrin, α-amyrin & germanicyl as terpenes (Merzaia *et al.*, 2017). Taraxast-20(30)-en-3(4-methyl 3-pentenoate). Enzymes detected as cysteine proteases included procerain and Trypsin an enzyme with invertase like activity (Madan Ranjit and Rao, 2012; Chundattu et al., 2016 .Procerain exhibited tryptophan and tyrosine residues along with cysteines. Hydrocarbons detected were 4-Hydroxy-4-methylpentan-2-one, 2,3,4-timethylhexane, Decane, n-Pentadecane, 2,6,dimethyltetra-1,5 Decane, n-Eicosane, 3,7,11-trimethyl-2,6,10,12-Petadecatrien-1-ol,2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22- tetra co sahexaene, 1,2,5-triisopropylbenzene, Z-2-propenyl-2-hydroxyethylcarbonate (Parihar and Balekar, 2016). Other compounds detected are Phenols that can be used as anti-oxidant, nutraceuticals and anti-inflammatory. phenols present in latex are gallic acid (Cavalcante *et al.*, 2020), heperidin, rutin and naringin (Merzaia *et al.*, 2017). They can be used as a good anti-inflammatory agent. Saponins detected are 3- epimoretenol (Parihar and Balekar, 2016).

Latex Protein section of *Calotropis procera* contains basic proteins of molecular masses between5 to 95 kDa (Freitas *et al.*, 2007). Two cysteine peptidases named Procerain (Kumar Dubey and Jagannadham, 2003) and Procerain B (Singh *et al.*, 2010). Enzymatic activities of the Soluble Latex proteins were obtained by biochemical and spectroscopic studies through protocols decided by Ramos et al (Ramos *et al.*, 2013). Major proteins recognized in *Calotropis procera's latex* are a group of three cysteine proteinases known by the name peroxidases, chitinases, osmotin and germin. LP_{PI} is mainly composed of chitinases, LP_{PII},is composed of

cysteine proteinases and less amount of osmotin (Bezerra *et al.*, 2017). LP_{PIII} with residual protein activity. Dialyzable fraction of crude latex is composed of amino acids glutamic acid, aspartic acid, glycine, serine, histidine, arginine, alanine, threonine, tyrosine, proline, valine, ethionine, leucine, isoleucine, lysine and phenylalanine (Al-Snafi, 2015).

2.10.4.2 Functions of RL, DL & NDLStudies suggest DL & RL to be involved in inflammation. NDL, a rubber free section is formed of high molecular weight soluble proteins that do not have any adverse effects and are supposed to be a cause for the anti-inflammatory activity.

DL when subjected to electrophoresis on a PD-10 desalting column was found to induce migration of neutrophils while no such activity was seen in RL and NDL. Carrageenan induced peritonitis was reversed with a pre-treatment with NDL and RL but the same activity was absent with DL. DL induced inflammation was prevented by prior administration of NDL(Alencar *et al.*, 2006). The fraction with soluble protein part was not able to induce toxicity in rats inducing an oral dose for about 35 days (Ramos *et al.*, 2006). Latex non-protein section was screened for the presence of Phytochemicals and secondary metabolites. LNP section when subjected to HPLC, yielded the presence of gallic acid and Quercetin as phenolic compounds (Cavalcante *et al.*, 2020).

2.10.4.3 Latex as a hemostatic for wound healing Promoted by inflammatory response of the body hemostasis process involves endothelialcell damage, platelet aggregation, coagulation cascade, neutrophil infiltrations, productionof ROS, release of proteases and coagulation (Singh *et al.*, 2010). Defensive role of plant latex is attributed to hydrolytic enzymes among which proteases play a major role. Aqueous extracts of dry latex and proteins recovered from fresh latex both contain this activity. (Alencar *et al.*, 2004). Initial in-vitro studies explored Latex of Asclepiadaceae family to possess proteolytic activity due to cysteine peptidases present in them. According to research by Shivaprasad and co-workers (Shivaprasad *et al.*, 2009). They were discovered to cleave Arg-16-gly and Arg-14-gly bonds in the A, B, and sub-parts of fibrinogen chains and release fibrinopeptide A & B, which causes fibrin to form. Invitro studies of latex of calotropis procera using azocasein as a substrate revealed the

proteolyticactivity of latex proteins contributed by 4 cysteine peptidases (Freitas *et al.*, 2007). Further,in-vitro studies by marico Ramos in 2012(Ramos *et al.*, 2012) were conducted to explore cysteine peptidases by fibrinogen agarose plate and spectrophotometric assays in sub-fractions PII & PIII of thelatex of *calotropis procera*. Latex proteins (LPP_{II} & LPP_{III}) exhibited thrombin and plasmin like property thus promoting hemostasis process and clot hydrolysis and reduce the clotting time in a dose dependent manner (Ramos et al., 2012). However prolonged exposure of cysteine peptidases to fibrinogen exhibit hydrolysis of fibrinogen chains in order of Aα > Bβ > ϒ. The cysteine peptidases can induce fibrin clot when exposed to fibrinogen (Ramos *et al.*, 2012). The same study's fibrinogen experiments demonstrated that the latex proteins LPP_{II} & LPP_{III} had a procoagulant impact similar to APTT assays presented reduced plasma coagulation time while the PT assays provide no change so, they were believed to trigger coagulation factors IX, XI & XII by latex protein sections (LPP_{II} & LPP_{III}) that reduce clotting time via intrinsic pathway (Ramos *et al.*, 2012). In the absence of inflammation latex presented procoagulant effect without any change in platelet count(Alencar *et al.*, 2006). The activity of LPP_{II} and LPP_{III} sections on plasma coagulation time was concurrently examined in a recent in-vivo study conducted to explore the effect of latex proteins to downregulate inflammation in acute infections (Sousa *et al.*, 2020). LPP_{III} section of latex works in acute inflammatory process and is believed to trigger a factor VII through TF signaling, which aids in the development of procoagulant activity in latex. Similar results were seen on treatment with LPP_{III} & LPP_{III} -IAA, ruling out role of LPP_{III} in proteolysis.

2.10.4.4 Anti-inflammatory, anti-oxidant and bactericidal action-

Considering the Studies conducted two decades back. Insoluble and soluble proteins present in latex of *calotropis procera* were suggested to be a cause for the pro and Anti-inflammatory activity seen. The anti-inflammatory activity was considered in dried latex (Kumar and Basu, 1994). Methanolic extracts of dried latex were tested on various inflammatory mediators (Arya and Kumar, 2005) and inflammatory models (Alencar *et al.*, 2004). The Pro inflammatory activity was seen in dried latex later acknowledged as dialysed latex (DL)(Alencar et al., 2006; Shivkar and Kumar, 2003) and anti-inflammatory activity was to be due to non-dialysable latex NDL (Alencar et al., 2006; (Freitas *et al.*, 2012). Anti-nociceptive (Dewan, Sangraula and Kumar, 2000), anti-

histaminic (Shivkar and Kumar, 2003) and anti-oxidative effect (Roy et al., 2005; Kumar et al., 2006; Freitas et al., 2007) have constantly been highlighted in literature concerned with latex of *Calotropis procera.* Latex is also known to have a protective potential to avoid sepsis. The study presented LP to down regulate IL-1β for anti-inflammatory actions(Lima-Filho *et al.*, 2010). Platelet degranulation activates macrophages. Mediators released from platelets accelerates the inflammatory response of the body. (Seddek *et al.*, 2009). LP-treated septic mice had longer clotting time and higher platelet count (Lima-Filho *et al.*, 2010).

Invitro studies explored superoxide dismutase (SOD) (Freitas *et al.*, 2007) and ascorbate peroxidase (to a lesser extent) in latex helps to reduce formation of reactive oxygen species (ROS) that causes tissue damage. In-vivo studies of Calotropis procera latex validate the protective anti-inflammatory effect of soluble high molecular weight latex to reduce edema and MPO levels (Kumar *et al.*, 2015). Various sections of latex separated by ion exchange chromatography were examined for leucocytes infiltrations (Ramos et al., 2009; (Lima-Filho et al., 2010a; Oliveira et al., 2012. In-vivo studies of LPP_I fraction initiate expression of iNOS activating macrophages which are bactericidal. (Ramos *et al.*, 2020). The findings were supported by the previous similar findings of latex protein section to inhibit neutrophil infiltrations through Nitrous oxide (NO) production (Ramos et al., 2009; Araújo Viana et al., 2017). Inhibition of the pro-inflammatory cytokines TNF-α, IL-1β, COX-2 and iNOS were found in study on latex proteins (Freitas *et al.*, 2012). Later, it was found that the inhibitory activity on cytokines was due to sub-fractions LPP_{II}-IAA sub-section of the latex (Ramos *et al.*, 2020). LPP_{III} also downregulates IL-1β and serves an anti-inflammatory action(Lima-Filho et al., 2010a; Sousa et al., 2020). This section of latex causes release of IL-10, an immunoregulator cytokine from healthy and infected cells (Sousa *et al.*, 2020). Recent researches validate Latex administration increases mRNA expression of TNFα and IL-1. IL-6 and iNOS participate for microbial clearance (Nascimento *et al.*, 2016).

2.10.5 Copper sulphate

Copper is a redox active transition metal that is required in living systems. It is needed in optimum amount to thrive whereas the deviated amount is dangerous to living system. A d- block element found in Siberia, America, Jharkhand and Assam states of

India. having three significant states of oxidation as 0, +1, and +2. Metallic copper oxidises soon in presence of air to Cu_2O. while at a high temperature > 200^0C further oxidises to CuO though effectivity of both (Cu_2O and CuO) is considered same(Hans *et al.*, 2013). Important ores are malachite, azurite, chalcocite, copper pyrites. Moist air makes green coating of copper sulphate. After zinc and iron, copper is the common trace element. It has been used since ancient Indian culture (2800-1000 B.C) in general formulations. From centuries in Hindu culture the holy ganga river water used to be kept in copper utensils. Use of copper and copper compounds is prevalent in medical industry and medical related applications. Copper is used in IUD's, utensils, dental fillings, copper based ointments (upto20% of elemental copper) are safe for dermal contact(Hostýnek and Maibach, 2006) Copper pillow cases are used for reducing wrinkles and improving skin well-being. Use of copper impregnated socks for redness, fissure cuts, skin rashes, burning or itching problems(Borkow and Gabbay, 2009). In anthroposophical medicines, copper is been used via oral, sub-cutaneous or in ointments to activate the body to utilise its healing forces. Use of copper is seen throughout the traditional and modern civilisations as elemental copper, Copper oxide, copper silicates, copper sulphate and copper chlorides(DollDollwet, H.H., & Sorenson, 1985).

2.10.5.1 Role of copper in human physiology Copper, an important trace element in human body that exists in tissues. Liver stores most part of it and is available in less amounts in brain tissues, muscles and kidneys. It plays the role of an anti-oxidant and pro-oxidant. Apart from these essential roles, copper is an important metal that signals to transfer the information between and within living cells. A copper deficient human body loses the capacity to use iron stored in liver and leads to anaemia. Copper plays a role to convert the iron in diet to haemoglobin. Deficiency of copper in melanin and myelin producing enzymes leads to a disturbed balance between oxidant and anti-oxidant defence system of human body. Physiology and metabolism of copper is associated with various enzyme systems of human body (metalloproteinase, vitamin-c, ascorbate oxidase, galactose oxidase, amino-oxidases, cytochrome oxidase, lysine oxidase, dopamine hydroxylase and tyrosinase and anti-oxidant enzymes superoxidase dismutase). It is an effective anti-bacterial, anti-fungal and biocidal and so has a

remarkable role in topical applications prepared for wound healing. Herbal ingredients when reduced in particle size provides efficient bioavailability and increased stability that enhances its pharmacological activity. At the same time herbal ingredients due to their anti-oxidant action can help to reduce size of metal ions Topical penetration of copper will be better when it is reduced to a nanoparticle size. Reduction of drug molecules to nanoparticle size enhances drug penetration tendency and future bioavailability.

2.10.5.2 Daily body requirement of copper and toxicity levels Copper is found in many dietary sources as oysters, meat, chocolates, cow milk, and whole grains (Olivares and Uauy, 1996). As per the food and nutrition board, institute of medicine, D.C, Washington and academy press optimum human requirement of copper in diet is about 4000-5000 µg daily with a bioavailability of 65-70% and a half-life of 13-33 days(Barceloux, 1999). Copper by oral intake in human body gets stored in hepatocytes and is secreted in plasma (Luza, nutrition and 1996, 1996). The amount above 80,000 µg to 100,000 µg is toxic(Borkow, 2015). Normal serum concentration ranges from 74-122 µgm/dl in women to 79-131µgms/dl in males. Copper in urine is 10-60 µgm/dl and in hair is 130 µgm/dl. Gastrointestinal symptoms arise when blood concentration of copper rises above 3mg/l(Gorter, Butorac and Cobian, 2004). When toxic, it damages the anti-oxidant enzyme systems and redox sensitive genes are also activated while it suppresses the zinc consumptions and iron transport in human body(Mustafa and AlSharif, 2018). Copper deficiency leads to menkes syndrome while the copper toxicity leads to Wilson disease(Turnlund *et al.*, 2005),(Brewer, 2012). Copper is relatively non-toxic compared to other metals on dermal contact so is at a low risk of antagonistic reactions. Till date safety exists while using CuO containing products with elemental copper upto 20%(Gorter, Butorac and Cobian, 2004).

2.10.5.3 Role of copper in skin physiology Copper is one among the nine important essential micronutrients for human skin. Out of the required amount of 110mg in a 70kg adult human body, 15 % proportion exists in human skin.

1) It promotes the proliferation of dermal fibroblasts and the upregulation of the fibres of elastin and Collagen.

2) It serves as a co-factor for lysyl oxidase needed for ECM protein linking(Rucker *et al.*, 2018).

3) Copper stabilises the skin ECM as increased cross-linking occur in a particular dose(Sajithlal, Chithra and Chandrakasan, 1999).

4) Copper serves as a co-factor of superoxide dismutase, an anti-oxidant enzyme in skin.

5) It constrains membrane damage and lipid peroxidation and serves as a co-factor of an

essential enzyme (tyrosinase) for melanin skin pigment synthesis(Olivares and Solano, 2009),(Hearing and Jiménez, 1987).

6) Copper binding peptide Cu-GHK in human plasma helps in angiogenesis, enhances the collagen and elastin fibres and glycosaminoglycan synthesis and withstands dermal stem cells and fibroblasts that further help in expression of integrins(Pickart and Margolina, 2018), (Kang *et al.*, 2009).

2.10.6 Egg yolk oil

Evidence based research exists for use of egg yolk oil in traditional methods(Vujanovic and Vujanovic, 2013), (Rastegar *et al.*, 2011), (Yenilmez *et al.*, 2015) Products containing egg yolks are acknowledged in pharmacies and cosmetics(Xiao *et al.*, 2020). Egg yolk oil is a liquid extracted from egg yolk. Composed of phospholipids, fatty acids and cholesterol (Salari *et al.*, 2016). Used as a popular traditional Chinese medicines for a treatment against heat, muscle soreness and is known for its moisturizing effect. Traditionally extracted by application of direct heat or organic solvent-assisted extraction. Modern techniques include enzyme-assisted extractions or supercritical CO2-assisted extraction. Each method has its benefits and drawbacks. (Wu *et al.*, 2016). Recent Research has established EYO to possess anti-inflammatory and wound healing activity. (Shen *et al.*, 2015)(Rastegar *et al.*, 2011)

2.10.6.1 Anti-inflammatory inflammation is a natural immune reaction of body to different types of tissue damage due to external factors. Uncontrolled inflammation leads to chronic inflammatory ailments. Components of the egg is found to possess property to act against inflammation in a minimal dose of 2ml. studies have revealed EYO to reduce oxidative stress and increase vascular penetrability(Xiao *et al.*, 2020).

the therapeutic efficacy of egg oil is irrespective of egg species but differs with extraction utilised in another study, where the eczema symptoms were were flared in 3 days and worsened in 5 days in group where egg oil extracted by reduced pressure distillation technique was used.(Wu *et al.*, 2016) . the fact was reverified in another study with EYO extracted from three different sources resulted results at a therapeutic dose between 100-300mg/kg had a noteworthy difference in effect with direct heat-extracted oil compared to the organic extraction technique. The study also highlighted the effect of an organic diet on the effectivity of EYO. (Mahmoudi *et al.*, 2013). The anti-inflammatory activity of egg yolk oil is attributed to signal transduction of NF- κB with reduced expression of target genesQ(Shen *et al.*, 2015). To claim clinically the therapeutic efficacy of EYO, research is required with different models for proper explication regarding the molecular mechanism and anti-inflammatory activity of EYO.

2.10.6.2 Wound healing effect Egg yolk oil has an accelerating effect in wound healing. Recent research studies highlight the use of EYO in traditional burn treatments. as it magically repairs the damaged wound surface, promotes re-epithelialization and due to its traditional beliefs, it is used in acute cases. (Rastegar *et al.*, 2011), (Salari *et al.*, 2016). hydrophobic property of the egg yolk oil can be explored due to their hydrophobic property. Formulations with egg yolk oil and chitosan are used to accelerate wound healing(Yenilmez *et al.*, 2015). fatty acids from yolk of an egg possess antimicrobial activity. Egg yolk oil consists of both lipoproteins as HDL and LDL. HDL and phospholipids has anti-oxidant and anti-inflammatory property. Anti-oxidant property of egg yolk have been inversely related to degree of fatty acid saturations(Sugino *et al.*, 1997).

2.11 Nanoemulsions

They constitute the heterogeneous collection of colloidal substances to form an isotropic system stabilized with the help of surfactants and co-surfactants that act as emulsifiers and co-emulsifiers at the interface of two liquids to form transparent or translucent mixtures of oil-in water droplets with size <100 nm(McClements, 2012). However literature supports, other particle sizes can be in between the droplets range from 200 to 500 nm in size (Shaker *et al.*, 2019), stabilized by surfactants and co-

surfactants. As per the authorized definition given by united states food and Drug Administration (Administration, 2011) and European Commission (European Commission, 2009) nanoparticles and nanocarrier colloidal systems are considered different with diameters below 100 nm (McClements, 2012). and up to 500nm size (Shaker *et al.*, 2019) respectively. Various types of nanocarrier systems popular in the past few years include Dendrimers, liposomes, solid lipid nanoparticles, polymeric nanoparticles, micelles, nanospheres, carbon nanotubes and nanoemulsion etc (Alshawwa *et al.*, 2022). Entrapping the active pharmaceutical ingredient into a nanocarrier system has presented very hopeful results in the field of percutaneous and transdermal delivery(Elmowafy, 2021). According to the Brick-and-mortar model available for the skin. It acts as an efficient biological barrier and low permeability is attributed to its unique structure. API's that are lipid soluble show enhanced permeability compared to hydrophilic API's.(Modamio, Lastra and Mariño, 2000) 3-5 mm deep dermis provides a connection to systemic circulation. through available capillary network elasticity to the skin. Skin enzymes that contribute to the metabolic capacity of skin(Strolin, Whomsley and Baltes, 2005) are capable of converting the active molecular forms into inactive forms that can decrease the efficacy of topically applied therapeutic drugs (Steinsträsser and Merkle, 1995). Drugs with smaller molecular weight are easier to penetrate compared to smaller molecular weight products(Baroli, 2010). Despite of the existence of various routes for drug penetration, lack of drug permeability (Review, 2015) leads to poor bioavailability. This can be considered as a cause for lack of desired in-vivo activity against claimed prior in-vitro activity. Presence of an efficient drug delivery carrier system provides potential for bioavailability (Khogta *et al.*, 2020) of API's. Nanotechnology, a latest capable field of science with high potential in the field of traditional and modern medicines. Originated in 1950s with the proposal of a physicist Richard feynman with his proposal in research of matter to possesses unique properties at nanoscales. Potential of Ayurveda is being explored in recent years with the novel drug delivery system. Novel drug delivery systems combine pharmaceutics, chemistry and molecular biology. When applied in herbal medicines, helps in increasing efficacy and the nanosized particles reduce the drug to very low doses. Novelty in drug delivery system of herbal and artificial drugs will help to significantly impart therapeutic efficacy for a single

and group of drugs administered together (Harwansh, Deshmukh and Rahman, 2019). Nanoemulsions are one among the various nano carrier systems available for effective delivery of drug. They are isotropic sub-micron thermodynamically stable emulsions. Available as oil in water, water in oil and bicontinuous biphasic systems. When diluted in water they produce no change. Small size allows easy dispersibility of nanoemulsion(Shaker *et al.*, 2019). Small size and large surface area of skin helpful for semi-solid formulations used for topical applications are creams, ointments and gels.

Extensive use of plants and plant products exist in comparison to the synthetic drugs. There exists vast and literature for their traditional use(Fahimi *et al.*, 2016). Modern scientific tools need to be used to claim the therapeutic effects of plants. Use of the natural resources for treatment is very popular these days. Moreover, depleting natural resources makes nanotechnology as the need of the hour to be incorporated in the traditional system of medicine for effective bioavailability, successful reduction of dose and efficient use of depleting natural flora. Traditionally medicinal plants are frequently used to treat a wide range of skin-related illness including burn wound healing. Herbal remedies help control burn wounds by cleaning, debriding and creating an environment that is conducive to healing naturally.

The main working apparatus in nanoemulsion includes the oil phase, the emulsification agents and the aqueous phase. The oil phase can be formed with castor oil, corn oil, coconut oil, linseed oil, mineral oil, olive oil, peanut oil, evening primrose oil or herbal oil. When oil and water are mixed it leads to a crude emulsion formation, where the two media can separate themselves from each other on long standing referred to as the phenomenon of coalescence. Here emulsification agents can support this medium generating stability for nanoemulsion. Surfactant helps to decrease the surface tension between the two liquids (Tadros *et al.*, 2004) These emulsifying agents are classified as spans and tweens. A surfactant should be fairly harmless with compatible taste, odor and chemical stability with that specific product. The surfactant should have the property to reduce the surface tension in a low concentration, should prevent coalescence and should help in increasing polydispersity index and viscosity. (Khanna *et al.*, 2018)

2.11.1 Types of nanoemulsion Depending upon their constituents and average droplet the nanoemulsions are classified under three categories(Khanna *et al.*, 2018):

2.11.1.1 *Oil in water nanoemulsion* (o/w) oil droplets dispersed in aqueous phase.

2.11.1.2 *Water in oil nanoemulsion* (w/o) water droplets dispersed in oil phase

2.11.1.3 *Bicontinuous* It is formed when oil is dispersed in an aqueous system W/O/W or water is dispersed in oil system.

2.11.2 Types of surfactants & co-surfactants

2.11.2.1 *Cationic ions* – primary, secondary or tertiary amines

2.11.2.2 *Anionic ions* –examples are carboxylates, sulphates, phosphates, etc.

2.11.2.3 *Zwitterions*- possess both anionic & cationic groups.

2.11.2.4 *Non-ionic*- stearyl alcohol, ethyl alcohol, oleyl alcohol, etc.

2.11.3 Difference Between Emulsions and Nanoemulsion droplet size in nanoemulsion is 1-200 nm, while that of emulsions is 1-20 micrometre. Nanoemulsions are translucent, stable, and clear while emulsions on the other hand are cloudy, unstable, and undergo creaming, sedimentation and ostwals ripening, coalescence and flocculation. Molecular diffusions arising from emulsions can be considered as the chief mechanism which hampers the stability of nanoemulsion. Similarly, microemulsions possess thermodynamic stability while nanoemulsions are not only kinetically stable but also have the property to separate into their constituent phases.

2.11.4 Preparations Of Nanoemulsion (Khanna *et al.*, 2018)

2.11.4.1 High energy methods Formation of nanometric scale droplets, stability, rheology and color of nanoemulsion depends upon the duration of high energy method of production, properties of the sample and even its composition (Khanna *et al.*, 2018). Inspite of the fact that this method of production has a nanoemulsion output with preferred properties and they are even suitable to be produced on a large scale this type is not preferred for production of nanoemulsion of proteins, nucleic acids and enzymes and the drugs that are thermodynamically unstable like retinoids and macromolecules. They make available the strong forces that dismantle the large-sized molecules to small droplets of nanosize. Alternatively low energy method (Kumar *et al.*, 2019)has been researched to promote the production of ultra-small droplets. For this method, the

system's stored energy is used which changes the HLB (hydrophilic-lipophilic balance) and this is used to form small droplets. High energy methods include high pressure homogenisation, microfluidization and ultrasonication.

2.11.4.1.1 High pressure homogenization they are extensively utilized to provide an increased force of high energy and constant flow to create small-sized particles of 1nm size. The emulsion is made to pass through a small hole with intense pressure. Type of sample, homogenizer used, duration and temperature along with the power of high energy used to control the size of nanoemulsions. On the other hand, particles tend to combine (coalescence) in solutions with large phase volume ratios. this can be overtaken by reducing interfacial tension with the help of the use of large quantities of surfactants or mixing the surfactant in a dispersed phase rather than in a continuous phase. the mixture of surfactants or the surfactant cosurfactant mixture also helps to overcome coalescence. (Lovelyn and Attama, 2011). They are more effective than biopolymers for the formation of nanoemulsions(Azeem *et al.*, no date)

2.11.4.1.2 Microfluidization Microfluidization is a patented mixing technology, which makes use of a device called microfluidizer. This device uses a high-pressure positive displacement pump (500 - 20,000 psi), which forces the product through the interaction chamber, consisting of small channels called "microchannels". The product flows through the micro-channels onto an impingement area resulting in very fine particles of submicron range. The two solutions (aqueous phase and oily phase) are combined and processed in an inline homogenizer to yield a coarse emulsion(Khanna *et al.*, 2018). The coarse emulsion is introduced into a microfluidizer where it is further processed to obtain a stable nanoemulsion. The coarse emulsion is passed through the interaction chamber of the microfluidizer repeatedly until the desired particle size is obtained. The bulk emulsion is then filtered through a filter under nitrogen to remove large droplets resulting in a uniform nanoemulsion. High-pressure homogenization and microfluidization can be used for the fabrication of nanoemulsions at laboratory and industrial scale, whereas ultrasonic emulsification is mainly used at laboratory scale.(Khanna *et al.*, 2018)

2.11.4.1.3 Ultrasonication method Uses mechanical or electrically generated ultrasonic acoustic frequency waves that break the emulsion droplets. The probe of the

ultrasonicator emits ultrasonic waves by changing the energy input and time. This method is employed for the formation of oil in oil emulsions with sizes in the microns size range. When the microbubbles collapse by the change in acoustic waves, high turbulent forces break the droplets to nanosize. This technique is helpful in the formation of nanosized emulsions without the use of surfactants. The efficiency depends upon the intensity of ultrasonication and the time of sonication(Kumar *et al.*, 2019).

2.11.4.2 Low energy methods they require less energy for the formation of nanoemulsions. The efficiency of this method lies in the utilisation of internal chemical energy of the system. the concentration of surfactant required is comparatively more(Kumar *et al.*, 2019). there are two categories under it. Isothermal method that is suitable for thermal sensitive compounds. Spontaneous emulsification, Phase inversion composition are classified under this method. On the other hand the thermal methods are utilized for the formation of solid lipid nanoparticles where the heat is required to keep the phase in liquid form(Safaya and Rotliwala, 2020).

2.11.4.2.1 Phase Inversion temperature when size of the droplet reduces it increases its stability against sedimentation, creaming and Ostwald ripening favours it as the favorable mechanism of nanoemulsion formation with the help of various surfactant mixtures. The phase inversion temperature method uses oil water and non-ionic surfactants together with modified affinities for each other. Nanoemulsion prepared by this method is of two types transitional inversion & catastrophic inversion(Khanna *et al.*, 2018) the former is results from a change in ionic content or warmth of the system while the later brings a change by changing surfactant concerning the HLB value. The temperature-dependent phenomenon of surfactants used in this process helps to fabricate the nanoemulsions. the affinity towards lipids increases on heating owing to the dehydration process. (Lovelyn and Attama, 2011) This reduces the degradation of drugs that are not thermodynamically stable eg: tretinoin and peptides. O/W nanoemulsion gets converted to W/O emulsion in phase inversion. (Khanna *et al.*, 2018)

2.11.4.2.2 Phase Inversion Composition Method: This method is used to prepare nanoemulsions of droplet size around 50nm at room temperature without use of any

organic solvent or heat and high energy methods. A solution of surfactant in oil is prepared and water is added stepwise by gentle stirring at a constant temperature(Khanna *et al.*, 2018). At initial stage w/o emulsion is generated which is later followed by increase in dispersed phase volume leading to w/o emulsion without any extra energy consumption. This is also termed as a catastrophic inversion (Safaya and Rotliwala, 2020)

2.11.4.2.3 Spontaneous Emulsification method this method is a two-stage process that involves the preparation of a homogeneous organic phase by mixing oil phase with surfactant with an affinity towards lipid and aqueous phase with co-surfactant having an affinity towards water. with continuous magnetic stirring in the next phase, the oil and aqueous phase are mixed this reduces interfacial tension between the two phases and reduces the size of oil droplets. Solvents The third and final stage involves the removal of the aqueous phase. the mixing order does not influence the spontaneous formation of nanoemulsions(Safaya and Rotliwala, 2020)

2.11.5 Advantages of Nanoemulsions as a Drug Delivery System(Khanna *et al.*, 2018).

1. The small size of particles in nanoemulsions reduces the gravity effect that avoids creaming or sedimentation effect when stored for long times.
2. Formation of nanoemulsion prevents flocculation and coalescence effect. Small size of droplets and elasticity of droplets has a contributing effect in this.
3. Nanoemulsion is a very efficient drug delivery system for transdermal drug delivery which allows their rapid penetration through the skin
4. Properties of fluidity at an optimum concentration of oil and they are optically transparent behavior gives the subject a pleasant feel when applied to skin
5. In comparison to microemulsions Nanoemulsion preparation is feasible with a comparatively low surfactant concentration that is approved for human consumption for internal administration.
6. Even physical properties like spreadability, moisturizing, and easy skin penetration are contributing factors to small droplet size in nanoemulsion

7. Alcohol base in perfumes can be avoided moreover the fragrance enhancers are easily

administered through nanoemulsion formation because of their easy formulations

8. They can be used instead of liposomal vesicles owing to their higher stability

2.11.6 Disadvantages of Nanoemulsions In Drug Delivery Systems

1.Special instruments that are employed in nanoemulsion formation are now available. This facility has not been there in past years Its production is expensive to the industry

2.The role of surfactants and co-surfactants in the formation of submicron droplets has to be thoroughly studied.

3.The interfacial chemistry of the substances has to be very well understood before formulating a nanoemulsion from it.

Chapter-3

Rational of the study

Numerous available therapeutic herbs for wound healing are mentioned in traditional texts of Ayurvedic science. Researchers suggest that polyherbal with diverse potency yields better results and the prolific combinations are convenient to patients as they reduce the need of multiple drug intake. Since burn wounds destroy the immunological barrier, they are highly susceptible to progressive infections so they need to be covered with suitable medication for a period of two to three weeks so that this first line of defense system of the human body is rapidly restored. Antimicrobial and wound healing ointment, gel, powder, medicated ghrita and taila individually or in polyherbal combinations are used to cover second-degree burn wounds. Herbal extracts are preferred in polyherbal formulations as they contain a concentrated form of desired phytoconstituents. Depending upon the extractive values mentioned in standard official compendium they can provide therapeutic dose for proper wound healing. But, the major limitation of these formulations is lack of bioavailability, penetration power, solubility, stability (related to dosage form development) and drug permeability for therapeutic efficacy since, skin favors penetration of smaller sized, non-irritating lipophilic or amphiphilic molecules. This impedes efficient and fast delivery of conventional and traditional formulations to target site. Drug delivery to skin using these routine Ayurvedic formulations is challenging. Further, larger surface area of burns increases the cost of herbal medications to the patient. Hence, it is important to find polyherbal combinations with novel dosage forms that need to be therapeutically effective and affordable at low doses.

The approach of translational research is utilized to prepare innovative formulations by merging the polyherbal combinations into advanced technology of nanocarrier systems for topical herbal remedies. One of the most intriguing possibilities is the use of NDDS. It is a promising strategy for transdermal drug penetration through the skin and provides efficient drug delivery that outdoes the drawbacks of standard conventional formulations with reduced use of amount of drug extracts and entices the consumers by making the drug more effective & safe.

Wound healing drugs were gathered from traditional references of drugs from plant animal and mineral origin to prepare a PHMN (Polyherbomineral nanoemulsion).

Textual and Published references exist regarding the anti-microbial anti-oxidant and burn wound healing activity of Curcuma longa, Aloe vera and Calotropis procera latex extract and Neem seed oil from plant origin, egg yolk oil from animal origin and copper sulphate/tuttha (T) as the mineral origin. Pharmacodynamics (rasa, guna, veerya and vipaka) and multiple phytoconstituents like alkaloids, flavonoids, polyphenols, tannins, sterols, triterpenoids and volatile oils in this various drug combinations have a capacity to work in a synergistic way depending upon the combinations of active principles to result with anti-inflammatory, anti-oxidant, anti-microbial, collagen formation for fast epithelialization with angiogenesis effect. Copper is reported to be effective antimicrobial and promotes angiogenesis.

Curcuma longa extract is mixed with Neem seed oil and Egg yolk oil used as an oil phase mixed with non-ionic surfactants and co-surfactants Labrasol ALF and transcutol (S_{mix}) was homogenized with high-speed homogenization to prepare a homogeneous system. Aqueous soluble extracts were later loaded in this nanoemulsion system.

In vitro wound healing studies of prepared PHM nanoemulsion on HUVEC cells reported no toxicity, keratinocyte mobilization and marked angiogenesis. The zone of inhibition was measured to mark the antimicrobial activity of the formulation. HPTLC analysis of formulation reported the presence of mono-unsaturated fatty acids, alkaloids, bioflavonoids and polyphenols (oleic acid, curcumin, gallic acid, rutin and berberine) that provide the desired pharmacological activity. Developing a nanoemulsion with nanosized particles will offer a large interfacial area of droplets to combine herbal oil phase with oil and aqueous soluble herbal drug extracts together to improve their solubility, absorption, bioavailability and efficacy in reduced drug dose of poorly soluble drugs and overcome the before mentioned drawbacks of the conventional topical formulations. Additionally, This will also provide a mixture of the oil phase and aqueous phase along with herbal extracts to travel across the skin barrier by trapping the herbal drug extracts in emulsion droplets.

Aim

Development and Evaluation of Herbomineral Nanoemulsion for the treatment of second-degree Burns

Objectives

- To Authenticate and Evaluate the quality of raw materials as per the standard protocol.
- To develop and Characterize the Nanoemulsion.
- To conduct the Stability study of the Optimized Formulation.
- To evaluate the Efficacy of optimized Formulation by using in-vitro and in-vivo study

Chapter-4

Experimental work

Curcuma longa (Turmeric), Aloe vera (Ghrit kumari), and Calotropis procera latex (Ark ksheera), Neem seed oil (Nimba taila), Egg yolk oil (Roghan baiza murgh) and Copper sulphate (Tuttha) are well known herbs used in wound healing. The present study describes the extraction procedures, standardization of the prepared extracts of these herbs using parameters mentioned in Ayurvedic Pharmacopoeia of India (API), nanoemulsion preparation, characterisation and invitro and In vivo evaluation methods.

4.1 Materials and methods

4.1.1 Materials

The list of materials used in the research work study are listed in table 9 along with their respective sources

Table 9 List of various materials used in the research work

Material(s)	Source (s)
Absolute alcohol	Loba Chemie Pvt. Ltd. India
Ammonia solution	
Acetone	
Bontrager's reagent	
Burchard's reagent	
Benedicts reagent	
Berberine	
Betadine	WinMedicare Pvt.Ltd.
Conc. Sulphuric acid (H_2SO_4)	Loba Chemie Pvt. Ltd. India
Chloroform	Loba Chemie Pvt. Ltd. India
Copper sulphate	Qualikem's pvt. Ltd.
Carbon Tetrachloride (CCL_4)	Loba Chemie Pvt. Ltd. India
Curcumin	
Disposable Syringes	Dispovan Hindustan Ltd
Dragendroff's reagent	Loba Chemie Pvt. Ltd. India
DDW	Bio-Age, Equipments and services, Punjab
Ethyl acetate	Loba Chemie Pvt. Ltd. India
Egg Yolk Oil	Hamdard Laboratories (India)
Fehling's solution	Loba Chemie Pvt. Ltd. India
Ferric chloride	
Gallic Acid	
Glacial Acetic Acid	
Hydrochloric acid	
Hager's reagent	
Iodine	Central drug house (p) Ltd.

Ketamine	**Empower pharma Pvt. Ltd.**
Lead acetate solution	**Loba Chemie Pvt. Ltd. India**
Liquid Paraffin	**Loba Chemie Pvt. Ltd. India**
Labrafac	**Gattefosse India Pvt. Ltd.**
Neem Seed Oil	**Unjha Pharmacy Gujrat.**
n- Butanol	**Loba Chemie Pvt. Ltd. India**
Precoated silica plates	**Sigma- Aldrich, Merck Pharma Ltd.**
Phenolphthalein	**Central drug house (p) Ltd.**
Pyridine	**Loba Chemie Pvt. Ltd. India**
Potassium hydroxide (KOH)	
Potassium iodide (KI)	
PEG 200	
PEG 400	
Proplylene Glycol	
Sodium Thiosulphate ($Na_2S_2O_3$)	**Central Drug House (p) Ltd**
Rutin Trihydrate	**Loba Chemie Pvt. Ltd. India**
Silver Sulphadiazine	**Green stone brand**
Span 20	**Loba Chemie Pvt. Ltd. India**
Span 80	
Tween 80	
Tween 20	
Transcutol	**Gattefosse India Pvt. Ltd.**
Toluene	**Loba Chemie Pvt. Ltd. India**
Veet Hair removal cream	**Re kitt Benckiser (India) Ltd.**
Wagner's reagent	**Loba Chemie Pvt. Ltd. India**
Xylocaine	**Zydus Healthcare Ltd.**

4.1.2 Equipments & Instruments- List of equipments and instruments used in various stages of research process were enlisted in Table 10 along with its model/manufacturer details.

Table 10 List of instruments and equipment used in various stages of work

Equipments/ Instruments	Source (s)
Abbe's Refractometer	
Brookfield Viscometer	**Model no. LVDV-E viscometer, B**
Chest freezer	**Model no. CHF 200B**
Digital pH meter	**Model LT-111, Labtronics, India Ltd.**
Hot air oven	**Q-5297, Navyug india by labtherm**
Hot plate with magnetic stirrer	**2-MLH , Remi electrotechnik Ltd.**
Lyophilizer	**EBT-12N**
Muffle furnance	**Bionics scientific, India**
Rotary Evaporator	**RV8599, IKA India pvt. Ltd.**
Remi centrifuge – for ependroff's	**RW-12C, Remi electrotechnik Ltd.**

Remi centrifuge- for test tubes	**RW-8C, Remi electrotechnik Ltd.**
Remi centrifuge – table top	**RM-02plus, ZICN-12346**
REMI humidity chamber	**CHM-10 plus, Remi electrotechnik Ltd.**
Rectangular Water Bath	**Ambala house**
REMI emulsifier mixer	**ROT-127, Remi electrotechnik Ltd.**
Soxhlet apparatus	**Ambala manufacturers**
Samsung refrigerator	**Model no. RR22k272ZS8/NL/2016**
Sonicator (Digital Ultrasonic Cleaner)	**LMUC-4, LABMA**
Tissue Homogeniser	**REMI, RQ-1, India**
UV Spectrophotometer	**UV1800-240V, Shimadzu, Japan**
U.V. Cabinet	**Ambala manufacturers**
Water bath shaker	**Labfit**
Weighing balance	**8CE-12, Shimadzu ana**
Water purification system	**TUVF-S, direct ultra Bio-AGE, pvt.Ltd**
Weighing Electronic Balance	**PGB 630, wensar Ltd.**

4.1.3 Methods

Collection of the Whole herbal plant & mineral drugs dried and raw turmeric rhizomes (Curcuma longa) were collected from Lugani traders, Amritsar. Aloe vera was collected from the local garden in Amritsar and and Calotropis procera was collected from the local wild growth around DAV college Jalandhar. The three plants were identified by Dr. Rohit Johari, Associate Professor in the Department of Dravya guna, Dayanand Ayurvedic college, Jalandhar, Punjab, India. A voucher specimen no. R.S-198, 199 & 200 were deposited into the Herbal Health Research consortium, Amritsar, India Neem seed oil and egg yolk oil was purchased from Unjha pharmacy, Gujrat & Hamdard pharmaceuticals, New Delhi respectively. they were available at local chemist in district, Jalandhar.

4.2 Characterization, organoleptic evaluation and standardization of Curcuma longa, Aloe vera and Calotropis procera Latex

physicochemical standardization of the herbal drugs was carried out to identify and assess the quality of phytoconstituents. All the parameters (mentioned as per section 4.2.1- 4.2.8) were evaluated in triplicate which is described in the following subsections (Khanna *et al.*, 2024) (Chandel, Pathak and Tailang, 2011) (Kunle, 2012)

4.2.1 Morphological and Organoleptic Evaluation the samples were evaluated based on method described by API standards and as mentioned in the research paper by Khanna et al(Khanna *et al.*, 2024). The parts used from the raw drugs were collected and subjected for morphological evaluations.

4.2.2 Moisture content / Loss on drying (LOD) 10 grams of drug (without preliminary drying) were placed in an evaporating plate, dried at 105°C for 5 hours and then weighed until the difference between the result of two consecutive measurements did not exceed 0.25%. Weight is considered constant when there is less than 0.01g difference between the two weight.(Khanna *et al.*, 2024)

4.2.3 Total Ash Value (TA) total ash value was calculated as the Residue remaining after incineration of the 2gm of an air-dried sample of powdered and dried drugs(Khanna *et al.*, 2024).

4.2.4 Acid Insoluble ash (AIA) total Ash recovered from the ash value is mixed with 25 ml dilute HCl and boiled for 5 minutes. Then, the mixture was filtered through the ashless filter paper (Whatman filter paper no.41). The filtrate was subjected for washing with hot water to make it chloride-free and again kept in muffled furnace to maintain same weight. After weighing the ash, the percentage of acid-insoluble ash was calculated using the formula(Khanna *et al.*, 2024) .

$$\text{Acid Insoluble Ash} = \frac{\text{Weight of Ash residue}}{\text{Wt. of sample}} \text{x}100$$

4.2.5 Water Soluble ash (WSA) ash obtained from the above method (Acid insoluble ash) was mixed with 25ml water and kept to boil for 5 minutes. Filter the mixture through ashless filter paper (Whatmann filter paper no.41), the filtrate was then washed with hot water. Weighed the obtained residue to Calculate the Percentage using the formula as below(Anonymous, no date).

$$\text{Water Insoluble Ash} = \frac{\text{Weight of Ash residue}}{\text{Wt. of sample}} \text{x}100$$

$$\text{Water Soluble} = \text{Total Ash} - \text{Water Insoluble Ash}$$

4.2.6 Alcohol soluble extractive (ASE) measured 5gm sample (coarse powder) and added in a closed conical flask with 100ml of alcohol. The conical flask was shaken frequently for 6 hours and kept undisturbed for 18 hours and filtered 25 ml of filtrate was taken in the evaporating dish and allowed the content to evaporate. The percentage was calculated after weighing the residue (Khanna *et al.*, 2024)

$$\text{Water Soluble Extractive Value} = \frac{\text{Weight of Residue}}{\text{Wt. of sample}} \text{x} 100$$

4.2.7 Water-soluble extractive (WSE) measure 5gm of the sample (coarse powder) and added into closed conical flask with 100ml of water. The conical flask was shaken frequently for 6 hours and kept undisturbed for 18 hours and then filtered. 25 ml of filtrate was taken in the China dish and allowed the contents to evaporate. The percentage was calculated after weighing the residue (Khanna *et al.*, 2024).

$$\text{Water soluble Extractive value} = \frac{\text{Weight of Residue}}{\text{Wt. of Sample}} \text{x} 100$$

4.2.8 Determination of pH value determine pH value of aqueous solution of drug by preparing a 1% solution of herbal drug w/v with the help of a glass electrode. (Khanna *et al.*, 2024)

4.2.9 Determination of trace and heavy metals in the plant materials the analysis was performed according to flame atomic absorption spectroscopy in three herbal drugs. Calibration curves prepared from Standard Working solutions in range of 1ppm to 10 ppm from stock solutions of 1000 ppm of all standards of Hg, Cd, As and Pb purchased from merck, Germany for reference purpose. 3gm of Accurately dried samples were treated with 3ml nitric acid for about 5 hr duration. Later proportionate to HNO_3, half in amount of $HCLO_4$ was added and heated for about 6 hrs till solution becomes clear and flames stop coming out of it. Then add milli-Q water and boil for 15min to reduce to half the prior volume. Cool and filter with whatmann filter paper no.42. Make the volume upto 50ml with milli-Q water. Prepare blank in similar way and aspirate each was assessed for AAS. (Kulhari *et al.*, 2013)

4.2.10 Microbiological evaluation- prepare two petri dishes by adding 15ml of liquified casein soyabean digest agar and 1ml of prepared sample is poured on this medium of not more then 45^0C temperature is kept on petri dish (dilute pretreated

material if needed) On obtaining the colony count of near about 300. Incubate them for 48-72hrs. colony numbers are counted in colony counter Upto 300 colonies are considered for good evaluation. For fungal count sabult dextrose agar is taken with chloramphenicol is used and a reliable count upto a maximum of 100 colonies are considered for good evaluation.

4.2.11 specific pathogens 1 ml of sample is transferred in 50 ml of nutrient broth and incubated. This is used for examination of E. Coli, salmonella typhi, pseudomonas aerigunosa and staphylococcus aureus.

Escherichia coli 1ml of the prepared subculture is takne with 5ml of Mc. Conkey agar and incubated for 37^0C. Red coloured growth is indicative of the presence of E. coli.

Pseudomonas aeruginosa 1 ml of prepared subculture is taken with cetrimide agar and incubated at 37^0C for 24hrs. the plates are detected for growth.

Staphylococcus aureus inoculate subculture on Baird-parker agar, incubate at 37^0C for 48 hrs. check for growth for the presence of microorganisms.

4.3 Physicochemical standardization of oil drugs

Standardized Oil drugs were purchased from market and subjected to authentication procedures of measuring specific gravity, viscosity, density, refractive index, saponification value, free fatty acids, acid value and iodine value.

4.3.1 Specific gravity oven dried; empty specific gravity bottle was weighed. Then the bottle is weighed after filling with water. Later the oven dried water is weighed after filling with neem seed oil and egg yolk oil separately (Jibril *et al.*, 2012). The specific gravity is calculated by the formula mentioned below

$$\text{Specific gravity} = \frac{W3 - W2}{W1}$$

W3= weight of bottle with oil

W2= weight of empty bottle

W1= weight of bottle with water

4.3.2 Density - density of oils is related to oil saturation and molecular weight. It is directly proportional to unsaturation and it is inversely related in proportion to molecular weight of the oil. Weight of the empty cylinder is taken, followed by weight of cylinder with separately with both oils is taken. The difference of weight is divided individually by volume occupied for Neem seed oil and Egg yolk oil. (Bhandare and Naik, 2015)

4.3.3 Viscosity Brookfield viscometer was used to measure the viscosity of oil. The oil is placed in a glass container and temperature is maintained at 35 ± 10^0C. spindle number 62s with attached helipath and was used to apply shear force on the prepared formulation to assess the flowability measure the internal friction of oil and measure the force needed to turn the spindle at the provided rate. (Aremu, 2009)

4.3.4 Determination of Saponification value the amount of KOH needed to neutralise the free fatty acids in accurate measures 1gm of oil sample is calculated as the saponification value. Two 250 ml RBF are taken. Both are filled with 25ml of alcoholic 0.5N KOH. 2ml oil is added in the first test flask (a) and the other is used as a blank (b). Both the flask undergoes reflux condensation for 30 minutes. The solutions are kept to cool for 30 min and after adding 2-3 drops of indicator solution (phenolphthalein) titration is performed with 0.5N Hcl in a burette. The end point is marked from pink to colourless. The amount of Hcl required equation is mentioned below(Thangarasu and Anand, 2019)

$$\text{saponification value} = \frac{(\text{b} - \text{a})\ \text{X}\ 0.02805\ \text{X}\ 1.000}{\text{Wt. of Sample}}$$

M.W- molecular weight of KOH, (g/mol)

b- Volume of HCl for blank sample (ml)

a- Volume of HCl for test sample (ml)

4.3.5 Determination of Iodine value two iodine flasks are taken and filled with 10ml of CCL_4. A sample of oil is added only in the test flask labelled 'a' and the other flask is labelled as 'b'. 20ml of iodine monochloride is added to both the KI-moistened flasks. The mixture was kept in a dark chamber for 30 min. The solution in both flasks

is titrated with 0.1N sodium thiosulphate ($Na_2S_2O_3$). The starch powder is used as an indicator with endpoint marked from purple to colourless. The iodine value of the oil with the amount of sodium thiosulphate needed to reach the endpoint. The iodine value is further calculated with the formula mentioned below

$$\text{Iodine value} = \frac{(b - a) \text{ X } 0.01269 \text{ X } 100}{\text{Wt. of Sample}}$$

b- Volume of $Na_2S_2O_3$ for blank sample (ml)

a- Volume of $Na_2S_2O_3$ for test sample (ml)

4.3.6 Estimation of free fatty acids (Acid value) dissolve 1 gm of oil sample in 50ml ethanol and diethyl ether to prepare a solution. Titration is performed with 0.1N KOH in a burette using phenolphthalein as an indicator. End point is marked from colourless to pink colour. The acid value is calculated as mentioned below

$$\text{Acid value} = \frac{\text{M. W X N X V KOH}}{\text{Wt. of Sample}}$$

M.W- molecular weight of KOH, g/mol

V KOH- Volume of KOH (ml)

N- Normality of KOH, (mol/ml)

4.3.7 Peroxide value add 30ml of 3:2 ratio of glacial acetic acid and chloroform respectively in two conical flasks. Pour precisely measured 5gm of the oil sample in only one flask. Label this as 'a' and another one as 'b'. Further add 0.5ml of a saturated solution of potassium iodide (KI) and 30ml water is added after 1 minute time gap. Solution is titrated with sodium thiosulphate. The disappearance of yellow colour is followed by addition of another 0.5ml of starch solution considered as the end point. Starch solution is used as an indicator substance.

$$\text{Peroxide value} = \frac{10\ (a - b)}{\text{Wt. of Sample}}$$

4.4 Standardisation process for mineral drug

Copper sulphate was purchased standardised from the market and was compared with general physical and chemical properties mentioned in a standard official compendium of Ayurveda like morphological structure, colour, transparency, hardness and specific gravity. Chemical properties like the effect of heat, solubility heavy metal examination and the copper assay were determined for comparison.

4.4.1 Solubility 1 gm of tuttha (copper sulphate powder) was poured in 20ml of warm water. 0.1ml of methyl orange solution is mixed and the colour change is noted.

4.4.2 Determination of Iron mix 25ml of water to 5g of powdered copper sulphate. Boil by adding 2 ml of concentrated nitric acid. On cooling add excess ammonia solution. Wash the precipitate with ammonia water solution (1:4). Wash the filter paper with solution HCl mixed in water. Filter and wash the residue. Calculate the weight of the residue after ignition.

4.4.3 X-ray diffraction studies Powder X-ray Diffraction (XRD) is one of the primary techniques used by mineralogists to examine the physico-chemical makeup of unknown materials. XRD is an easy tool to determine the size and shape of any compound for identification. Powder Diffraction technique was used to determine the crystallographic structure of the material.

4.4.4 Copper assay- 1.0g of sodium fluoride is added to the aliquot is taken from the prepared stock solution (10µg/ml) of prepared sample and mixed with ammonia solution for precipitation to occur. After dissolving the precipitate with acetic acid solution. 1.0g of potassium iodide is added and then titration is performed with 0.1N sodium thiosulphate solution with starch solution indicator. Colour change from brown to white is an indicator of end point. Copper amount is then calculated with the given formula

$$\% \text{ Copper} = \frac{0.06354 \text{ X } 0.1\text{N X ml of sod. thiosulphate used X aliquot x}100}{\text{Wt. of Sample x total volume}}$$

4.5 Preparation of herbal drug extracts

4.5.1 Ethanolic extract of Curcuma longa curcuma longa extract is extracted using solid-liquid extraction of biologically active compounds in the Soxhlet apparatus. Braga et al explored in his work that the conventional method of curcumin extraction by Soxhlet technique yield is better than other solid liquid extraction methods, hydro distillation or ultrasound assisted extraction (UAE). This extraction process is considered as the benchmark (Braga *et al.*, 2003) (Manasa, Kamble and Chilakamarthi, 2023). The method was followed as described by cuica and racovita. The process involves dry raw coarsely powdered curcuma longa poured in prepared thimble and extracted with ethanol (1:100) as an efficient solvent(Shirsath *et al.*, 2017). The solvent on reaching the overflow level in the extracting apparatus is taken inside the syphon tube and then it returns to the distillation flask. The extraction cycle is made to repeat until the desired concentration of the extract is reached. The solvent is then evaporated in a rotary evaporator at 65 rpm and at 78.3^{0}C ethanol was separated by distillation process. After this it was dried in hot air oven, to receive the dry extract (Ciuca and Racovita, 2023). The process is shown in figure 6

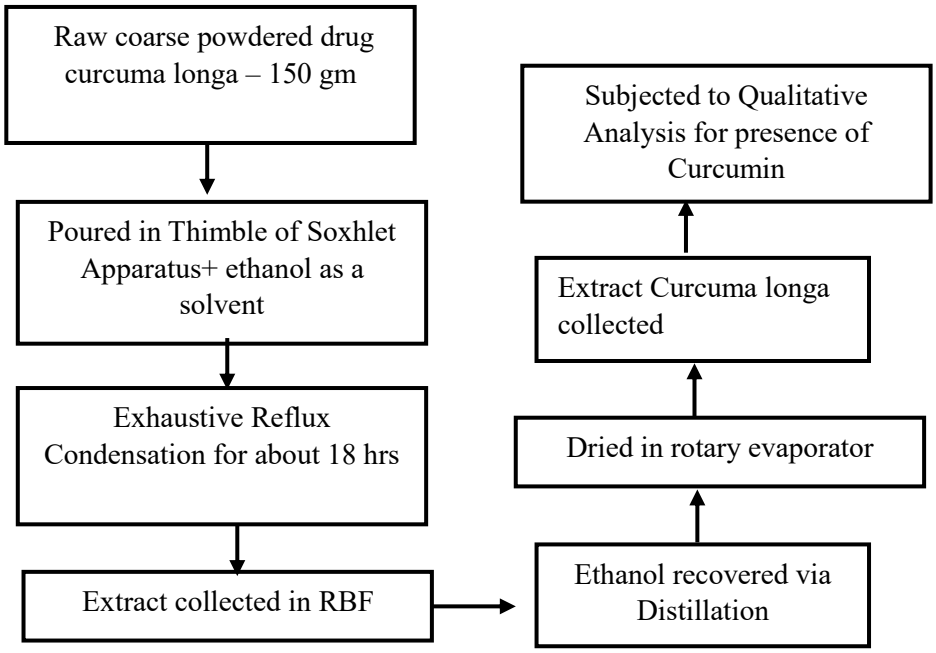


Figure 6 Graphical representation of the preparation of curcuma longa extract from rhizomes of curcuma longa

4.5.2 Aqueous extract of Aloe vera freshly cut Aloe vera was taken and 1kg of peeled leaves were mixed with 300 mL of water and crushed in a mechanical grinder. The method was adapted as mentioned in singh et al. Dispersion mixture was agitated on a mechanical shaker for 12hrs and filtered through whatmann filter paper no.1. The filtrate was then lyophilized in a lyophilizer. The dried extract was stored in dark packed in tightly sealed container at 4^0C(Singh *et al.*, 2017). The process is described in figure 7

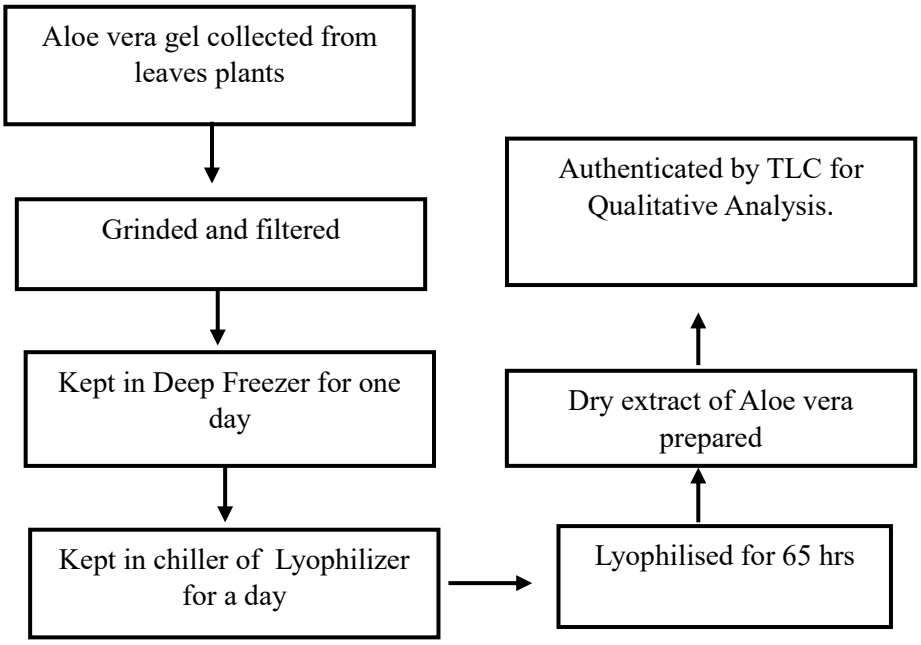


Figure 7 Graphical representation of the formation of Aloe vera extract from fresh Aloe vera gel

4.5.3 Calotropis procera latex extract

The method for preparation of Calotropis procera latex extract is as stated in alencar et al. Fresh Latex from the leaves is collected from plants aerial portions in Eppendorf tubes with a 1:1 ratio of distilled water at a temperature of around 25^0C to 28^0C. After being centrifugated at 5000 rpm (25^0C for 10 min) the rubber fraction (RL) is separated and a clear supernatant is obtained. This is then lyophilized for 45 hrs to be used for further process(Alencar *et al.*, 2006). The process is described in figure 8

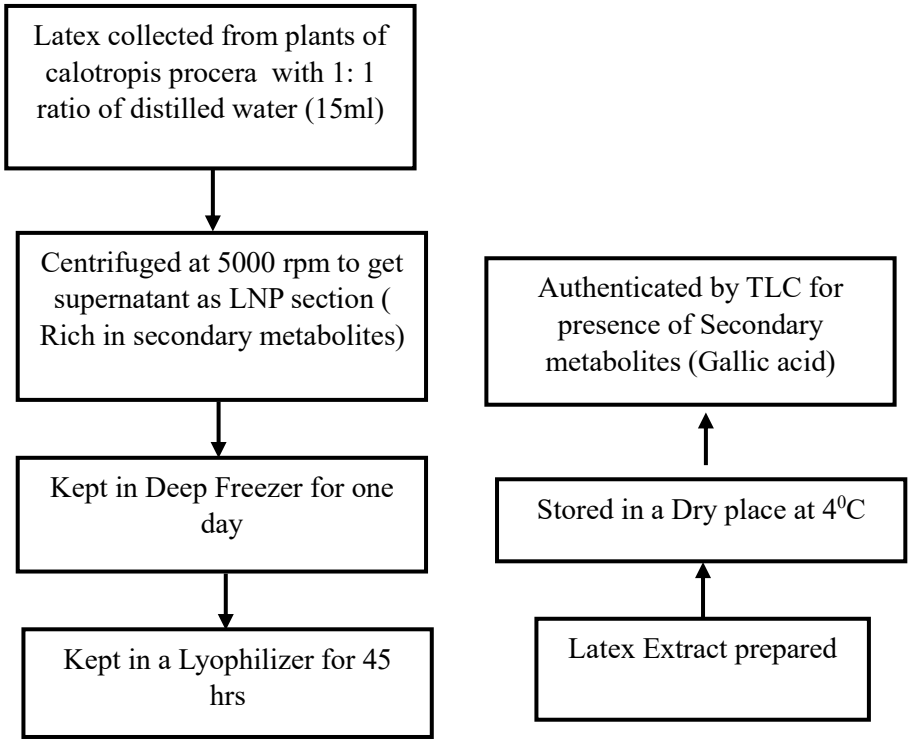


Figure 8 Graphical representation of the formation of Calotropis procera Latex extract from fresh latex

4.6 Physicochemical standardization of herbal drug extracts

Physicochemical standardisation of the herbal extracts was performed according to the parameters and their procedures mentioned from 4.2.2- 4.2.8

4.7 Preliminary phytochemical screening of the prepared extracts

4.7.1 Alkaloids

Dragendroff's test - herbal extracts are mixed with a few ml of dragendroff's reagent. Presence of yellow colour is indicative of the presence of alkaloids (Kashyap and Hait, 2021)

Mayer's test- appearance of cream coloured precipitate on addition of Mayer's reagent is indicative of the presence of alkaloids (Nortjie *et al.*, 2022)(Adejoh, Alli and Okoh, 2021).

Wagner's test appearance of reddish brown colour precipitates on addition of wagner's reagent is indicative for the presence of alkaloids (Nortjie *et al.*, 2022).

Hager's test appearance of yellow precipitate on addition of hager's reagent is indicative for the presence of alkaloids(Gopinath *et al.*, 2012)

4.7.2 flavonoids

Ammonia test yellow precipitates on addition of 5ml of ammonia solution followed by 1ml of sulphuric acid indicative of flavonoids presence in herbal extracts(Adejoh, Alli and Okoh, 2021)

Pew's test- Appearance of red colour on addition of water soluble solution of extract with zinc metal (0.1mg) with about 8 drops of sulpfuric acid solution is indicative of presence of flavonoids(Auwal *et al.*, 2014).

4.7.3 Saponins

Suspension is prepared by mixing herbal extracts with distilled water. This is filled in a cylinder with graduated markings and the Shaked for 15min. development of a foam layer of 2 cm is indicative of the presence of saponins(Pawar, Patil and Nagrik, 2015) (Kashyap and Hait, 2021)(Nortjie *et al.*, 2022)

4.7.4 phenols

Ferric chloride test 10ml of the herbal extracts on mixing with few drops of ferric chloride solution show the presence of bluish black colour is indicative of presence of phenols(Nortjie *et al.*, 2022).

Lead acetate test 10ml of herbal extracts on mixing with lead acetate solution. Presence of yellow colour is indicative for the presence of phenols(Nortjie *et al.*, 2022)

4.7.5 carbohydrates

Fehlings test Boil extract with 1ml of fehling solution A & B. Appearance of red precipitates indicates the formation of cuprous oxide and hence the presence of carbohydrates(Nortjie *et al.*, 2022)

Benedict's test Boil extract with benedict's reagent (2ml). appearance of yellow to orange colour is *indicative* for the presence of carbohydrates

Molisch's test Dissolve drug extract was mixed with water. Alcoholic α-naphol and sulfuric acid was added one after another. Violet purple colour ring at mixing boundary of two solutions is indicative for the presence of carbohydrates(Gopinath *et al.*, 2012).

4.7.6 Glycosides

Libermann- Burchard's test Mix the herbal extracts with some drops of aceitic acid in chloroform. Mix well and then concentrated sulfuric acid is added to this mixture along the test tube sides, Presence green colour in various samples indicates the presence of glycosides (Gopinath *et al.*, 2012) (Kashyap and Hait, 2021).

Bontrager's test- take 5 minutes to boil the extract of herbal drug with 2ml of sulfuric acid for sometime. After cooling and filtration, add an equal amount of chloroform with a few drops of 10% NH3 and heated again. Rose-pink to red colour appearance in ammonia layer is indicative of glysosides (Gopinath *et al.*, 2012)(Kashyap and Hait, 2021)

Keller-kiliani test – Mix drug extract with glacial aceitic acid. Then addition of one drop each from ferric chloride and concentrated sulfuric acid solution. Red brown colour formed at the interface of the 2 layers followed by blue green colour in the upper layer is indicative for the presence of glycosides(Gopinath *et al.*, 2012)

Salkowski test Mix the herbal extracts with chloroform and concentrated sulfuric acid in 2:3 ratio. The presence of reddish brown colour at the junction of the solutions is the positive test for the presence of glycosides (Gopinath *et al.*, 2012) (Pawar, Patil and Nagrik, 2015).

4.7.7 Protein

Biuret test appearance of violet colour on addition of biuret reagent to the drug extract is indicative for the presence of amino acids

Ninhydrin test appearance of violet colour on boiling of 5% ninhydrin solution with herbal extracts is indicative for the presence of amino acids

4.8 Chromatographic Evaluation (TLC) of Herbal Drug Extracts

4.8.1 TLC for Qualitative Analysis of Phytochemicals in Curcuma Longa Extract

Qualitative analysis of Phytochemicals- "Curcumin" from the 1% ethanolic solution of curcuma longa was separated by TLC.

Mobile phase for Curcumin Chloroform : Toluene : Methanol (5 : 4 : 1 v/v)Toluene: Ethyl acetate: Formic acid (5 : 1.5 : 0.5)

Stationary phase Precoated 60F- 254, silica plates from Merck pharma, sigma-Aldrich of 20 x 20 cm size were taken. They were cut into 6x10 cm size. Prepared by establishing a 1 cm distance from origin and 1 cm distance from solvent front with 8 cm as a solvent travel distance.

Procedure Plates were activated by keeping at 90^0C, 5 minutes prior to the analysis. Elution was performed in a previously saturated glass beaker, 30 min before placing the plate. approximately 10µl of sample concentrate of solute was separately spotted on the TLC plates using capillary glass tubes with an approximately 1 cm distance between each band of standard and samples. The plates were then developed in glass chambers via mobile phase elution. Bands generated in the visible region were then visualised in a UV light for short wavelength (254nm) and long wavelength (366 nm) analyses. The Rf value of the powder was obtained respectively. This procedure was followed for 1 % Concentrated solution of curcuma longa in ethanol.

4.8.2 TLC for Qualitative Analysis of Phytochemicals in Calotropis latex extract

Precoated 60F- 254, silica plates from Merck pharma, sigma- Aldrich of 20 x 20 cm size were taken. They were cut into 6x10 cm size. Prepared by establishing a 1 cm distance from origin and 1 cm distance from solvent front with 8 cm as a solvent travel distance. Mobile phase Prepared solution of Lyophilized ark ksheera extract in 1% ethanol was evaluated for the qualitative analysis of marker compounds 'Rutin and gallic acid' as secondary metabolites by TLC to confirm their presence. (Parihar and Balekar, 2016) (Cavalcante *et al.*, 2020).

Qualitative analysis of Phytochemicals- "Rutin trihydrate" and Gallic acid were analysed from calotropis procera latex (LNP section) separated by TLC.

Mobile phase for Rutin Toluene : Ethyl acetate : Formic acid : Methanol (2 : 3 : 1 : 3 v/v)

Mobile phase for Gallic Acid Toulene: Ethyl acetate: Formic acid: (10: 7: 1 v/v)

Stationary phase Precoated 60F- 254, silica plates from Merck pharma, sigma-Aldrich of 20 x 20 cm size were taken. They were cut into 6x10 cm size. Prepared by establishing a 1 cm distance from origin and 1 cm distance from solvent front with 8 cm as a solvent travel distance. *Procedure*: Plates were activated by keeping at 90^0C, 5 minutes prior to the analysis. Elution was performed in a previously saturated glass beaker, 30 min before placing the plate. approximately 10µl of sample concentrate of solute was separately spotted on the TLC plates using capillary glass tubes with an approximately 1 cm distance between each band of standard and samples. The plates were then developed in glass chambers via mobile phase elution. Bands generated in the visible region were then visualised in a UV light for short wavelength (254nm) and long wavelength (366 nm) analyses. The Rf value of the powder and extract obtained respectively. Quantitative analysis was undertaken further by HPTLC method.

4.8.3 TLC for Qualitative Analysis of Phytochemicals in Aloe Vera Extract - "Berberine" & "Rutin" were analysed from aqueous extracts of Aloe vera

- *Mobile phase for Berberine* Butanol: Ethyl acetate: Acetic acid: Water (6: 10: 2: 2 v/v)

Mobile phase for Rutin Toluene: Ethyl acetate: Formic acid: Methanol (2: 3: 1: 3 v/v)

Stationary phase Precoated 60F- 254, silica plates from Merck pharma, sigma-Aldrich of 20 x 20 cm size were taken. They were cut into 6x10 cm size. Prepared by establishing a 1 cm distance from origin and 1 cm distance from solvent front with 8 cm as a solvent travel distance.

Procedure Plates were activated by keeping at 90^0C, 5 minutes prior to the analysis. Elution was performed in a previously saturated glass beaker, 30 min before placing the plate. approximately 10µl of sample concentrate of solute was separately spotted on the TLC plates using capillary glass tubes with an approximately 1 cm distance between each band of standard and samples. The plates were then developed in glass

chambers via mobile phase elution. Bands generated in the visible region were then visualised in a UV light for short wavelength (254nm) and long wavelength (366 nm) analyses. The Rf value of the sample was obtained.

4.9 Preformulation studies

Preformulation studies guarantee the formulation developing process ends in a stable, safe and effective dosage format. The healthcare provider thoroughly describes the physical and chemical properties of all the drug constituents and their possible interactions with various excipients. Preformulation studies help to formulate the necessary physical and chemical parameters of the newly prepared formulation and suggest the incompatibility of the drug ingredients with the excipients(Jamadar and Husen Shaikh, 2017).

4.9.1 Fourier transform infrared spectroscopy (FTIR) This technique helps to determine the atomic vibrations in the molecules by either absorbing or transmitting the IR rays(Nandiyanto, Oktiani and Ragadhita, 2019). FTIR studies were evaluated based on absorption wavenumbers. It was assessed based on several absorption waves in the sample and the presence of single, double and triple bonds in the samples. 10mg of the dry extract was used to prepare 100mg KBr disc. The disc was placed in the spectrometer and the mid-IR spectrum was obtained in the range of 400-4000cm^{-1} (Fatima *et al.,* 2020)

4.9.2 Calculation of peak absorbance wavelength (λmax) for the herbal drugs stock solutions of Curcuma longa extract (CE) was prepared in ethanol and methanol distinctly while Aloe vera extract (AE) and Calotropis procera latex extract (CAE) were dissolved in different solvent systems (water, ethanol and methanol) to get peak absorbance wavelength (λmax)

4.9.3 Preparation of calibration curves after getting the peak absorbance wavelength. Desired amount of extract was mixed with suitable solvent to prepare stock solution and different aliquots of varying concentrations were prepared with ethanol for CE and in an aqueous medium for AE and CAE.

4.9.4 Screening of components for the nanoemulsion First step in the development process is to screen the surfactants and co-surfactants for the herbal oils based on solubility testing studies

4.9.4.1 Screening of Excipients for oils Since herbal medicines possess unidentified phytochemicals, non-ionic surfactant possesses the benefit to reduce the chances of interactions between the respective ions (Disch, Drewe and Fricker, 2017). Ease of miscibility was criteria for visual observation for the selection of the surfactant. 8 non-ionic surfactants (Tween 20, Tween 80, Span20, Span 80 Propylene glycol and PEG, Labrasol ALF and Transcutol) were screened 1 ml of surfactant was added to the 1 ml to each of oil separately (Neem Seed Oil and Egg Yolk oil). The mixture was then heated for 30 seconds between 40 and 45 degrees Celsius to help in mixing properly. The selection of surfactant phase compatible with oil phase was carried out by visual examination for a clearness of the mixtures. The mixture was observed for the phase separation after 2,4,6 and 24 hrs. Miscibility and phase separation was kept as screening criteria for the selection of surfactant and co-surfactant. Different excipients and oils were taken in ratio 1:1 individually or in combinations as presented below in table 11 and shown in figure 9 Labrasol ALF and Transcutol-P (S_{mix}) was found to be miscible with both oils taken together (Oil_{mix}) but, phase separation was seen after 24 hrs.

Table 11. Screening of various surfactants and co-surfactants for oil phase

Sr. no.	Surfactant/ co-surfactant	Ratio	Sr. no.	Surfactant/ co-surfactant	Ratio
1	EYO+NSO	1:1	**10**	NSO+ Tween 80	1:1
2	EYO+ Tween 80	1:1	**11**	NSO + Tween 20	1:1
3	EYO + Tween 20	1:1	**12**	NSO + PG	1:1
4	EYO + PG	1:1	**13**	NSO + PEG 400	1:1
5	EYO+ PEG 400	1:1	**14**	NSO + Span 80	1:1
6	EYO + Span 80	1:1	**15**	NSO+ Labrasol ALF	1:1
7	EYO + Labrasol ALF	1:1	**16**	NSO+ Transcutol-P	1:1
8	EYO+ Transcutol-P	1:1	**17**	NSO+ EYO+ Labrasol ALF + Transcutol	1:1

Among all the surfactants used for visual screening process. A mixture of NSO and EYO (oil_{mix}) was most compatible with the mixture of Labrasol ALF and Transcutol-P (S_{mix}) Further screening for various ratios of oil_{mix} with various ratios of Labrasol ALF and Transcutol-P were made.

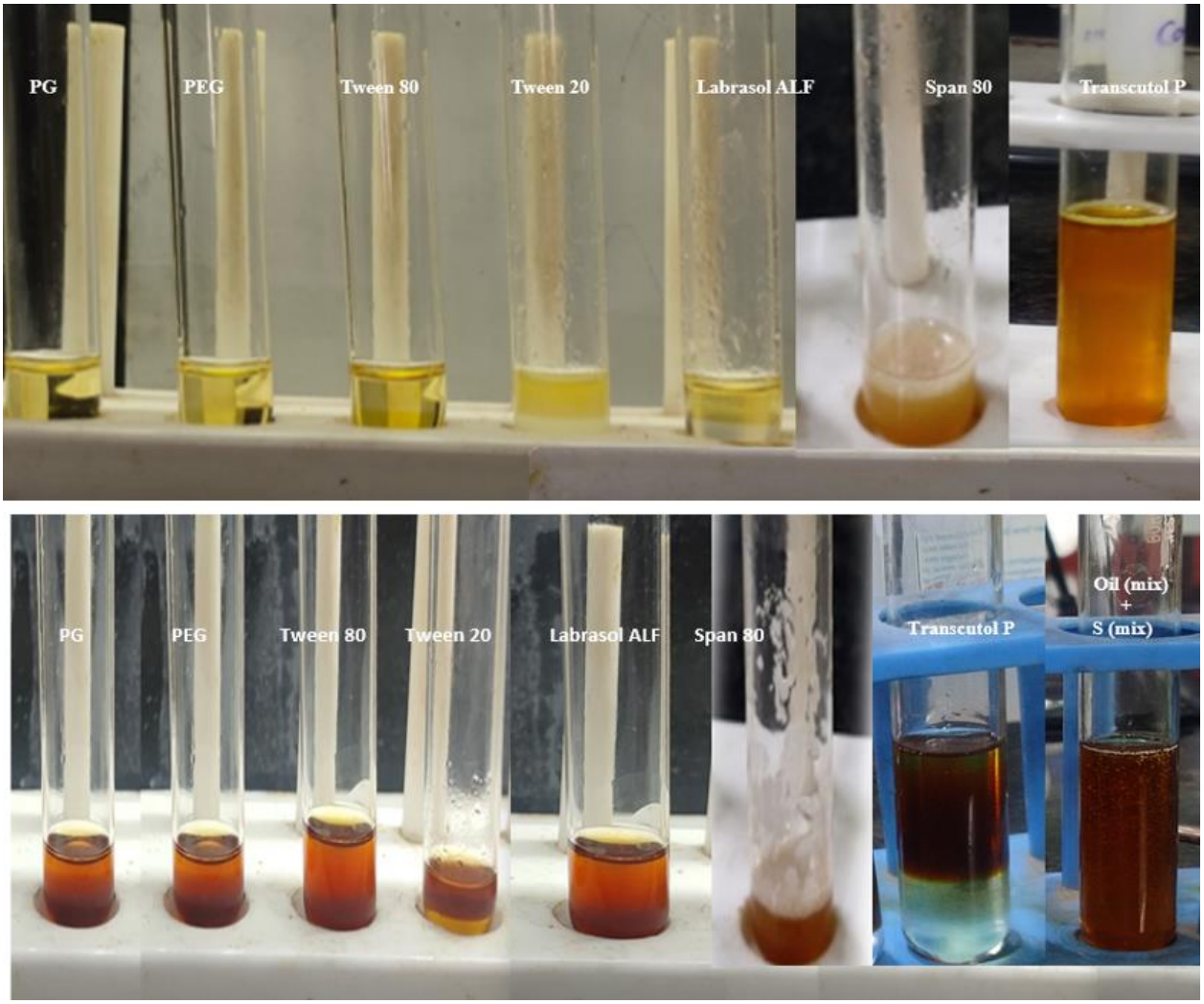


Figure 9 Screening process of various excipients for oil phase

The 1:1, 1:2, 2:1 and 3:2 ratio oil(mix) was individually mixed with 1:1, 1:2, 2:1 and 3:2 ratio of S(mix) in various combinations as mentioned in table 12. Visual inspection revealed that 3:2 ratio of each Oil(mix) and S(mix) yielded the oil mixture without any phase separation for more than 72hrs.

Table 12 Various combination ratios prepared for surfactant and co-surfactants

Oil_{mix}	Labrasol ALF	Transcutol	Oil_{mix}	Labrasol ALF	Transcutol
1:1	1	1	2:1	1	1
1:1	1	2	2:1	1	2
1:1	2	1	2:1	2	1
1:1	3	2	2:1	3	2
1:2	1	1	3:2	1	1
1:2	1	2	3:2	1	2
1:2	2	1	3:2	2	1
1:2	3	2	3:2	3	2

was the oil soluble drug. Aloe vera extract (AE) and Calotropis latex extract (CAE) were aqueous soluble drugs used in this formulation. Since Curcuma longa extract (CE) was less soluble in Neem seed oil and Egg yolk oil. Since the oils were the part of drugs selected for formation of Nanoemulsion so, the surfactants were screened for the better solubility of turmeric extract. A fixed quantity of curcuma longa, Aloe vera and Calotropis procera latex extract (100mg) is mixed with 1 ml of Tween 20, Tween 80, PEG200, PEG400 and PEG600, Labrasol ALF and Transcutol-P, and water separately in Eppendorf tubes. Supernatants with undissolved matter in all mixtures were achieved by vortexing for 2 minutes and then the mixtures were centrifuged at 3000 x g for 15 minutes. To quantify the undissolved matter, 10 µl of supernatant was taken and mixed in 10ml ethanol as a solvent. The absorbance was taken in UV spectrophotometer at 421nm, 278nm and 268nm respectively. The concentrations were determined from their respective calibration curves on dilutions.

4.10 Formulation of CAACET (Calotropis latex, Aloe vera, Curcuma longa extracts and tuttha) loaded Nano emulsion system. CAACET loaded nanoemulsion system was prepared by spontaneous emulsification and high speed homogenisation method (Sharma, Singh and Harikumar, 2020) (Mazonde, Khamanga and Walker, 2020). In brief meticulously 0.545 gm of the Curcuma longa extract (CE) was mixed thoroughly with heated herbal oil mixture at 45^0C to 50^0C, followed by surfactant and co-surfactant. The mixture was kept on a REMI mixer with hot plate at 300 rpm for about 5 minutes to get an isotropic mixture. The homogeneous phase was diluted to a measured amount of aqueous phase with the help of high-speed homogenisation at

6500 rpm to form a nano emulsion system. Aqueous soluble drugs (CAE, AE & T) are then serially loaded dropwise in this nanoemulsion system followed by ultrasonication in a bath sonicator for a period of 5 minutes to formulate nanoemulsion.

4.10.1 Preliminary screening of formulation based on emulsification

Preformulation solubility studies have shown that herbal drugs were having good solubility in selected surfactant and co-surfactant. The emulsification tendency of these excipients was further considered to emulsify the selected oil phase and later their compatibility with the other selected Herbo mineral components in aqueous phase, so that it results in a homogeneous single phase. preliminary initial screening was carried out with surfactants and oil phase and later oil soluble herbal drugs in oil and surfactant mixed phase followed with addition of aqueous soluble drugs in the prepared single-phase system. The formulation was prepared in batches using CE, NSO and EYO as the oil_{mix} phase, Labrasol-Alf and Transcutol-p denoted as S_{mix} and CAE, AE & tuttha as the aqueous phase. Finding the emulsification tendency of surfactants and co-surfactants compatible with the herbal drugs, oil and mineral phase. Several compositions were prepared by this method with various 3:2 ratio of oil_{mix} and S_{mix} and varying S_{mix} and aqueous phase in 1:1, 1:2 and 2:1 concentration. The ratio of oil_{mix} phase was taken in 3:2 ratio and the oil (mix) were varied with $S_{(mix)}$ by taking oil from 1 to 9 parts with respect to Smix in 3:2 ratios (Table 13). Preliminary emulsions were prepared in three batches (F1- F27) with water as an aqueous phase. F13, F25 & F26 Compositions in mixture resulted in stable emulsions. Physical tests were performed with these two emulsion mixtures.

4.10.2 Phase behaviour by Pseudo-ternary Phase Diagram (Ptpd): Based on observations from solubility study and preliminary screening process, the surfactants and co-surfactants were selected. Concentration of oil phase, surfactant and the co-surfactant phase was validated with the help of pseudo ternary phase diagram. The pseudo-ternary phase diagram was constructed to determine the changes in ratio of $oil_{(mix)}$ phase and $S_{(mix)}$ on transparency or turbidity of nano emulsions. Pseudo ternary phase diagram was plotted accordingly by selecting the increasing and decreasing concentration of oil(mix) concerning different ratios of S(mix). Different combinations of NSO: EYO (oil(mix) were taken (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) with 3:2

combinations of S(mix). They were mixed in glass test tubes and vortexed to cover the maximum boundaries of three phases. For each weight ratio of oil(mix) and S(mix) were mixed with a specified ratio of aqueous phase (de-ionised water). Three ends of the triangle were labelled as oil(mix), S(mix) and aqueous phase and P-TPDs were plotted using Todd Thompson Triplot software of 4.1.2 version with data as shown in table 13 The emulsion samples prepared were specified as stable or unstable (phase separated) the samples were examined for phase separation, flowability with transparency by visual inspection. Area in the plot covered helped to decide the appropriate concentration range of selected component for physicochemical properties the stable and transparent emulsions in the plot to screen for the ratio for effective formation of nanocarrier system. further studies are undertaken with consideration of DOE.

Table 13 preliminary screening of nanoemulsion based on emulsification

Formulation code	Oil_{mix} (3:2)	S_{mix} (3:2)	Aqueous phase	Formulation code	Oil_{mix} (3:2)	S_{mix} (3:2)	Aqueous phase	Formulation code	Oil_{mix} (3:2)	S_{mix} (3:2)	Aqueous phase
F1	90	3:2	5	**F10**	90	3.9:2.6	3.3	**F19**	90	1.9:1.3	6.6
F2	80	6:4	10	**F11**	80	7.8:5.2	6.6	**F20**	80	3.9:2.6	13.3
F3	70	9:6	15	**F12**	70	12:8	10	**F21**	70	06:04	20.0
F4	60	12:8	20	**F13**	60	15.9:10.6	13.3	**F22**	60	7.8:5.2	26.6
F5	50	15:10	25	**F14**	50	19.8:13.2	16.6	**F23**	50	9.9:6.6	33.3
F6	40	18:12	30	**F15**	40	24:16	20.0	**F24**	40	12:08	40.0
F7	30	21:14	35	**F16**	30	27.9:18.6	23.3	**F25**	30	13.8:9.2	46.6
F8	20	24:16	40	**F17**	20	31.8:21.2	26.6	**F26**	20	15.9:10.6	53.3
F9	10	27:18	45	**F18**	10	36:24	30.0	**F27**	10	18:12	60.0

4.10.3 Experimental design for the optimisation of Nanoemulsion: Depending upon the pseudo ternary phase diagram. Design of expert software was applied to further optimise the best formulations that present desired features of nanoemulsion. The composition mixtures can result in nanoemulsion by using BBD. Formulation was optimised by applying 3 factor and 3 levels (High and Low values). The volume was changed to obtain the robust formulation with longer stability and effective size to help in skin permeability. Upper and lower limits of the oil phase, aqueous phase surfactant and co-surfactant for optimisation of the nanoemulsion are presented in table no.13. A three level and 3 factor box behnken design was applied (Ferreira *et al.*, 2007). In this design the independent variables with varying ratios of oil phase (A), Surfactant (mix)

phase (B) and aqueous phase (C) were used. These components were represented in the design at a high (+) level and low (-) levels as presented in table 14 for optimisation of the composition for Nanoemulsion. The effect of each of the selected factor was evaluated to the variables selected as response variables (Dependent variables) such as droplet size (1), PDI (2) and zeta potential (3). Based on the independent variables and the levels, 17 experimental runs were designed using BBD. Based upon the interactions of the independent variables on the dependent variables, linear polynomial equations representing the effect of independent variables on dependent variables analysed through ANOVA were generated using the design of expert software.

The linear equations and quadratic models were generated by the Design Expert software (version 11). These equations represented the effect of each independent variables on selected dependent variables (i.e. response), which were analysed statistically through analysis of variance (ANOVA)

Table 14 Experimental levels of independent variables (Aqueous phase, surfactant phase and oil phase) in Design of Experiments (DOE)

Oil Phase	**Low**	**High**
NSO	60	6000
EYO	40	4000
Aqueous phase	4000	90000
Surfactant Ratio	**Low**	**High**
Transcutol	480	4000
Labrafil	640	6000

(Levels are expressed as µl)

4.11 Characterisation Parameters:

4.11.1 Physical parameters optimised Polyherbomineral nanoemulsion (PHMN) was determined physically by colour, odour touch viscosity, refractive index and spreadibility time.

4.11.1.1 Colour odour and touch optimised PHM nanoemulsion was evaluated for colour and odour and touch with physical senses.

4.11.1.2 Refractive index and Viscosity Nanoemulsion was dropped on prism, refractive index was measured using abbe's refractometer(Ahmad *et al.*, 2019) and viscosity was measured using Brookfield rotational viscometer. The formulation was placed in a glass container and temperature maintained at 35±10°C. spindle number 62 was used to apply shear force at 70 rpm on the prepared formulation to assess the flowability (Gull *et al.*, 2020).

4.11.1.3 Spreadability Spreadability of the nanoemulsion was measured in triplicate using the glass plate. 100 µl of the nanoemulsion was dropped in a diameter of 10mm, at the centre of the glass plate (48mm in length and 146mm in length. Above this plate another plate is placed on this plate. A 50gm weight is placed on the top plate for 1minute. Spreadability of the formulation was measured in mm, using following formula.(Donthi *et al.*, 2023)

$$\text{Spreadability} = \frac{\text{mass X length}}{\text{Time (seconds)}}$$

Where,

M= weight tied to the upper slide

L= length of the glass slide

T= time taken for complete detachment of both slides from each other

4.11.2 Chemical parameters

4.11.2.1 Size PDI and Zeta potential Size and polydispersity index (PDI), zeta potential and pH of Polyherbomineral nanoemulsion were evaluated for chemical parameters. size and PDI using the zeta sizer. Samples were filled in the disposable cuvettes and dynamic light scattering was measured at an angle of 90 degrees with a temperature maintained around 25^0C. The samples were diluted using the de-ionised water and accustomed with 200V. pH was measured using the glass electrode dipped

in the optimised nanoemulsion by digital pH-meter at 25±0.5^{0}C (Sharma, Singh and Harikumar, 2020). De-ionised water was used to dilute the formulation. The samples were analysed in triplicate for size, PDI and zetapotential and pH and mean data was recorded in triplicate with standard deviations.

4.11.2.2 HPTLC for Curcuma longa extract (CE) and prepared formulation with standard

4.11.2.2.1 ***Preparation of Standard Solution***: Accurately weighed 2mg of standard curcumin is taken in a 2 ml volumetric flask. The solution is sonicated after adding 1ml of methanol to the standard. After complete dissolution of standard, the volume is made up to 2ml using methanol as a solvent. This standard solution is used for fingerprinting on HPTLC plates.

Table 15 Chromatographic details used in HPTLC of Curcuma longa extract and prepared formulation in comparison with standard

Application mode	CAMAG Linomat 5- Applicator
Applicator filtering system	Whatmann filter paper no.1
Stationary phase	MERCK-TLC/HPTLC silica gel 60F254 on aluminium sheets
Application (Y axis) start position	10mm
Development end position	80mm from plate base
Standard application volume	5.0 µL
Sample application volume	5.0 µL(Extract) and 25.0 µL (Formulation)
Distance between tracks	15mm
Development mode	CAMAG TLC Twin Trough Chamber
Chamber saturation time	30 minutes
Mobile Phase (MP)- Curcumin	Chloroform: Toluene: Methanol (5:4:1 v/v)
Visualization	@254 nm
Quatification	@425nm
Drying Mode, Temp & Time	TLC Plate Heater Preheated at 100±5^{0}C for 3 minutes

4.11.2.2.2 ***Preparation of Test Solution (Extract):*** Accurately weighed 100 mg of sample is taken in a 250ml volumetric flask. The solution is mixed with 10ml of Toluene: Diethyl ether (1:1 v/v). the solution is cooled, filtered using Whatman filter paper and concentrated in an evaporating dish to 5ml on a water bath after reflux heating in a flask on a water bath for 30 minutes. This 5ml concentrate (or make up the volume with 1:1 Toluene and diethyl ether) is poured in a volumetric flask. The test solution is is used for fingerprinting in HPTLC procedure.

4.11.2.2.3 ***Preparation of Test Solution (Formulation)***: Add 5ml toluene: Diethyl ether mixture (1:1 v/v) to 1gm of accurately weighed sample. The volume is made up to 5ml after filtration of the above solution with Whatman filter paper and anhydrous sodium sulphate**.**

The details about the chromatographic conditions during HPTLC of curcuma longa extract are tabulated in Table 15

4.11.2.3 HPTLC details for Calotropis latex extract (CAE) with prepared formulation and standard

4.11.2.3.1 ***Preparation of Standard Solution***: Accurately weighed 2mg of standard gallic acid and 5mg of rutin each is taken separately in a 2 ml volumetric flask. The solutions are sonicated after adding 1ml of methanol to the standards. After complete dissolution of standards, the volume of both flasks is made up to 2ml using methanol as a solvent. This standard solution is used for fingerprinting on HPTLC plates.

4.11.2.3.2 ***Preparation of Test Solution (Calotropis procera latex Extract):*** Accurately weighed 500 mg of sample is taken in a 250ml volumetric flask. The solution is mixed with 10ml of methanol. The solution is cooled, filtered using Whatman filter paper no.1 and concentrated in an evaporating dish to 10ml on a water bath after reflux heating in the reflux flask on a water bath for 30 minutes. This 10ml concentrate (or make up to 10ml with methanol) is poured in a volumetric flask. The test solution is is used for fingerprinting in HPTLC procedure.

4.11.2.3.3 ***Preparation of Test Solution (Formulation)***: Add 10ml methanol to a 1gm of accurately weighed sample. Cool the solution after heating the above mixture for

30minutes in a 250ml reflux flask. The volume is made up to 10ml after filtration of the above solution with whatmann filter paper. The test solution is is used for spot development in the HPTLC procedure. The details about the chromatographic conditions during HPTLC of Calotropis procera latex extract are tabulated in Table 16

Table 16 Chromatographic details of HPTLC of Calotropis Procera latex extract and prepared formulation with the standard

Application mode	CAMAG Linomat 5- Applicator
Applicator filtering system	Whatmann filter paper no.1
Stationary phase	MERCK-TLC/HPTLC silica gel 60 F254 on aluminium sheets
Application (Y axis) start position	10mm
Development end position	80mm from plate base
Standard application volume	5.0 µL
Sample application volume	5.0 µL(Extract) and 20.0 µL (Formualtion)
Distance between tracks- gallic acid	12.5 mm
Distance between tracks- Rutin	13.3 mm
Development mode	CAMAG TLC Twin Trough Chamber
Chamber saturation time	30 minutes
Mobile Phase (MP)- gallic acid	Toluene: Ethyl acetate: formic acid (10:7:1 v/v)
Mobile phase (MP)- Rutin	Toluene: Ethyl acetate: formic acid: Methanol (2: 3: 1: 3 v/v)
Visualization (gallic acid & Rutin)	@254 nm
Quatification-Gallic acid	@278 nm
Quatification- Rutin	@257 nm
Drying Mode, Temp & Time	TLC Plate Heater Preheated at 100±5^{0}C for 3 minutes

4.11.2.4 HPTLC details for Aloe vera extract with prepared formulation and standard

4.11.2.4.1 ***Preparation of Standard Solution***: Accurately weighed 2mg of standard Berberine and 5mg of Rutin is taken in a 2 ml & 5ml volumetric flasks respectively. The solutions are sonicated after adding 1ml of methanol to the standards. After complete dissolution of standard, the volume is made up to 2ml & 5ml for the respective standards using methanol as a solvent. This standard solution is used for fingerprinting on HPTLC plates.

4.11.2.4.2 ***Preparation of Test Solution (Aloe vera Extract):*** Accurately weighed 500 mg of sample is taken in a 250ml volumetric flask. The solution is mixed with 10ml of Methanol. The solution is cooled, filtered using Whatman filter paper no.1 after reflux heating in a flask on a water bath for 30 minutes. This 10ml (or make up the volume with methanol) is poured in a volumetric flask. The test solution is is used for fingerprinting in HPTLC procedure.

4.11.2.4.3 ***Preparation of Test Solution (Formulation)***: Add 10ml methanol to a 1gm of accurately weighed sample. Cool the solution after heating the above mixture for 30minutes in a 250ml reflux flask. The volume is made up to 10ml after filtration of the above solution with whatmann filter paper no.1. The test solution is is used for spot development in the HPTLC procedure.

The details about the chromatographic conditions during HPTLC of Aloe vera extract are tabulated in Table 17

Table 17 Chromatographic details of HPTLC of Aloe vera extract and prepared formulation with the standard

Application mode	CAMAG Linomat 5- Applicator
Applicator filtering system	Whatman filter paper no.1
Stationary phase	MERCK-TLC/HPTLC silica gel 60 F254 on aluminium sheets
Application (Y axis) start position	10mm
Development end position	80mm from plate base
Standard application volume	5.0 µL
Sample application volume	20.0 µL
Distance between tracks- Berberine	15 mm
Distance between tracks- Rutin	13.3 mm
Development mode	CAMAG TLC Twin Trough Chamber
Chamber saturation time	30 minutes
Mobile Phase (MP)- Berberine	Butanol: Ethyl acetate: Acetic acid: water (6: 10: 2: 2 v/v)
Mobile phase (MP)- Rutin	Toluene : Ethyl acetate : formic acid : Methanol (2: 3: 1: 3 v/v)
Visualization- Berberine	@366 nm
Visualization- Rutin	@254 nm
Quatification-Berberine	@366 nm
Quatification- Rutin	@257 nm
Drying Mode, Temp & Time	TLC Plate Heater Preheated at 100±5^{0}C for 3 minutes

4.12 In-Vitro Studies of the Optimised Formulation

4.12.1 Anti-microbial activity Anti-microbial activity of the formulation was tested against gram positive and gram-negative bacteria by agar well diffusion method. Staphylococcus aureus (ATCC4827), pseudomonas aeruginosa (ATCC25923) and Escherichia coli (ATCC5922) (Nayak, Raju and Ramsubhag, 2008). by determining the zone of inhibition. Gram positive and gram-negative bacteria were collected from the biochemistry lab of the pharmacy department, lovely professional university. The

bacteria were cultured in the nutrient agar media (Venkataraman and Nagarsenker, 2013). Agar well diffusion method was adapted for the process. 2.8 gm of nutrient agar media is dissolved in 90ml of water. Sterilised petri plates are taken and each of the bacteria is pipetted at the centre of the separate plate. The nutrient agar mixture was sterilised and cooled. The mixture was poured in the petri plates. Upon solidification 6mm diameter bores were made in the petri plates. Formulation will be added. Plates will be incubated at 370C for 18 hours. Anti-microbial activity will be calculated by measuring the zone of inhibition after the incubation period (Balouiri, Sadiki and Ibnsouda, 2016).

4.13.2 Cell Line Studies

4.13.2.1 Cell Culture and Cytotoxicity Determination HUVEC cells were cultured at 37 °C with 5% CO_2 in high-glucose DMEM with 10% foetal bovine serum. MTT assay was used to assess the cytotoxicity of Polyherbomineral nanoemulsion on HUVEC cells. HUVEC cells were plated on 96 well plates and incubated overnight at 37°C with 5% CO_2. Cells were treated with Polyherbomineral nanoemulsion for 24, 48 and 72 hours. 10 µL of 5mg/ml MTT was applied to HUVEC cells at 37 °C to assess cell viability. The yellow tetrazolium salt of MTT is changed to purple coloured formazan. This is dissolved in the soluble solvent and the absorbance of the coloured solution is taken at 500-600nm.

4.13.2.2 VEGF-A and PECAM expression assay $1x10^5$ of HUVEC cells were seeded and cultured in each well of 12 well plates; treated with PHM nanoemulsion. After incubation, cells were collected in a FACS tube, fixed with 4% formaldehyde, followed by permeabilization with buffer (0.1% triton X) and stained with 5µl of anti-VEGF (Invitrogen, MA5-13182), and Anti-PECAM-1 (Invitrogen-11-0311-82) antibody, followed by 20 minutes of incubation. Samples were centrifuged once again for 5 minutes at 1500×g, and washed with 1X PBS thrice. The supernatant was discarded and resuspended in 1X FBS. VEGF-A and PECAM-1 were quantified with BD BD accuriTM C6 SORP Flow Cytometer, and the procured data was analyzed with Flow jo V10 software. The cellular levels of VEGF-A and PECAM-1 were evaluated by ELISA. Culture supernatant of HUVEC cells after treatment with was collected 100 µg/mL and 200 µg/mL of a Polyherbomineral nanoemulsion was performed by

following the manufacturer's instructions. Samples were read at 450 nm using a microplate reader (Synergy H1 microplate reader, Biotek)

4.13.2.3 ELISA to evaluate for cellular levels of VEGF and PECAM assays, cell culture of treated HUVEC cells with PHM nanoemulsion was collected and analysed by ELISA at 450nm using ELISA microplate reader

4.13.2.4 Wound Healing assay the assay was performed according to the protocol decided by Yogesh et al(Talekar *et al.*, 2017) . After seeding HUVEC cells in a 12-well plate, we let them grow to 80-90% confluence before doing the wound healing (scratch) experiment. Using a sterile 2.5 L pipette tip, a uniform scratch was produced across the monolayer in each well. Following a 24hour treatment with 100 g/mL or 200 g/mL of a Polyherbomineral nano formulation, the cells were washed with sterile 1X PBS to remove any remaining debris. ImageJ (Software 1.53s, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) was used to quantify the percentage of wound closure following image acquisition using an inverted microscope (Nikon-SC600) at 10x magnification. % wound closure = [(Area of wound at 0 h − Area of wound at 24 h)/Area of wound at 0 h] × 1es00 (Ishika.Roy et al, 2023)

4.14 In vivo study of the optimised formulation on second-degree burn wounds in rats the study of the prepared nanoemulsion was performed on IEAC guidelines with approval no. LPU/SOP/89/230912/15619002. Adult healthy male rats of approximately 250-300gm weight were bought from national institute of pharmaceutical education and research (NIPER), Mohali. Rats were acclimatised to laboratory conditions by providing cages with rice husk bedding at a temperature of 25±2^{0}C & 45% RH at a 12h light and 12h dark cycles were sustained in the animal house with help of artificial control of lightning. The animals were provided a standard pellet diet and water ad libitum. The animals were divided into 5 groups having 6 animals in each group. All animals were tagged as identification of different groups. The rats of group I to V were considered as control group standard group, blank group, low dose and high dose formulation groups. Low and high dose is decided depending upon the invitro study and literature review. The animals will be weighed and anaesthetised by intraperitoneal injection of ketamine 75mg/kg and xylazine 15mg/kg. Dorsum of the anaesthetized rats will be shaved with a commercial depilator cream and

antisepsis with 1 % polyvinylpyrrolidone. Rats were depilated with depilator cream and antisepsis with 1% polyvinylpyrrolidone and a deep second-degree burn were induced by applying a 70^0C previously heated, spherical aluminium metal seal (2cmx2cm) adhering to the wooden handle on the dorsum of the rats. The contact of metal seal is maintained for 10 sec (Guo *et al.*, 2017) After induction of burns, animals were kept in a cage for 4 hrs to closely observing the extent of burn and providing time for the general condition to recover. The development of the wound was confirmed by epithelial debridement. After induction of burns, the wound will be debrided and rinsed with 0.9% normal saline. The standard test and blank formulations were administered twice a day for 21 days. Until the burn wound gets epithelialized. Radius of the burn wound was measured using the digital vernier callipers and burn wound pictures were recorded using a digital camera on 0, 3rd, 7th, 14th and 21st day. The average food intake and body weight changes of the rats will be monitored during the experiment. The average food intake and body weight changes of the rats will be monitored during the experiment. Rats will be sacrificed as per the Committee for the Purpose of Control and Supervision of Experiments on Animals. (CPCSEA) guidelines Pharmacological evaluation of treatment efficacy will be assessed by studying wound healing parameters. Skin healing was assessed and evaluated by physical measurement of wound contraction was assessed by measuring wound area on 0, 3rd, 7th, 14th & 21st day. pro-inflammatory (MPO, TNF- α) & anti-inflammatory (IL-10) cytokines. Biomarkers for anti-oxidants assays include catalase and glutathione assays. Collagen synthesis was assessed by hydroxyproline and hexosamine assays along with histopathological examination Biochemical parameters and Histopathological examinations were undertaken after 21 days of treatment was undertaken to evaluate healing parameters. The study plan is presented diagrammatically in figure10. Granulation tissue was collected from the dorsal parts of animals and preserved in 10% formalin for further examinations.

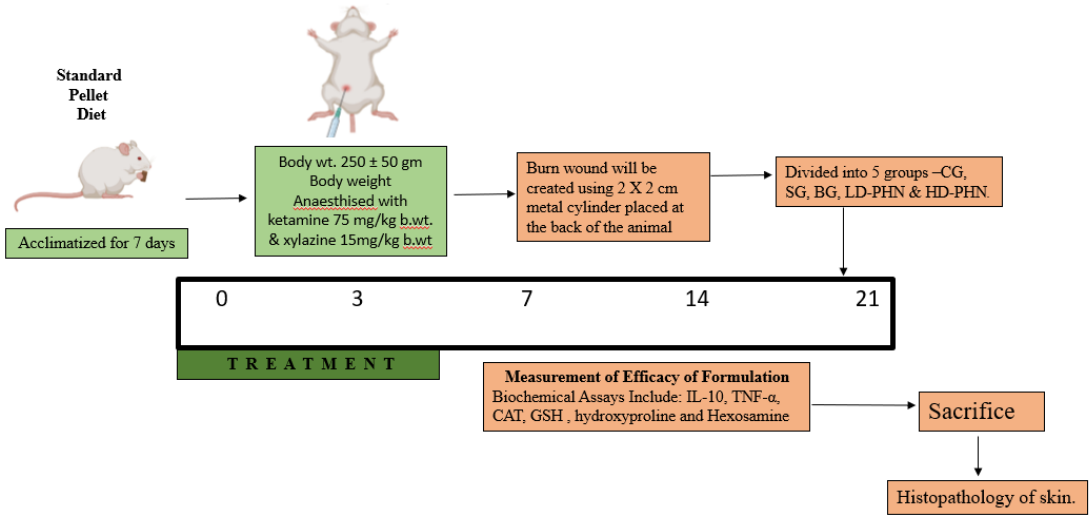


Figure 10 Diagrammatic representation of study design

4.14.1 Evaluation parameters for wound healing activity

4.14.1.1 Measurement of wound area

Measurement of wound diameter was a main criterion for indication of progressive healing where burn edge contraction was expressed as a decrease of the original burn diameter. All measurements were recorded from the day of burn wound induction till the completion of treatment course. Burn edge diameter was measured using a digital calliper every day before the application of medicine and will be expressed as the decrease of original burn diameter(Guo *et al.*, 2017). This is recorded for further analysis.

4.14.1.2 Detection of neutrophil sequestration (*Myeloperoxidase assay*).

A sample of skin tissue (100 mg) will be physically homogenized in 1 ml ice-cold potassium phosphate buffer consisting of 115 mM monobasic potassium phosphate. Homogenates were centrifuged at 3,000g for 10 minutes at 4^0C, the supernatants removed, and the pellets were resuspended in 1 mL CTAB buffer consisting of dibasic potassium phosphate, cetyltrimethylammonium bromide, and acetic acid. The suspensions were sonicated on ice for 40 seconds. Supernatants were incubated for 2 hours in a water bath shaker at 60^0C. Samples will be either stored at - 80^0C, if assays are not performed immediately. Aliquots of 20 mL of standards or samples were added to a 96- well immunosorbent microplate, followed by the addition of 155 mL of 20 mM TMB/DMF (3,39,5,59-tetramethylbenzidine/ N,N-dimethylformamide) substrate in 115 mM potassium phosphate buffer to each well. The samples were mixed well, after

which 20 mL of 3 mM H2O2 was rapidly added to each well. The reaction was stopped immediately by adding 50 mL/well of 0.061 mg/mL catalase. The plates were read at 620 nm. The final concentrations were expressed as micrograms per microliter (mg/mL)(Hemmila *et al.*, 2010)

4.14.1.3 Cytokine analysis *(IL10 & TNF-α)*

A 100-mg sample of dorsal skin was homogenized in 1 mL ice-cold lysis buffer consisting of 50 mL phosphate buffer saline and protease inhibitor and 50 mL Triton X. Homogenates were centrifuged at 3000g for 5 minutes, and the supernatants were collected and stored frozen at -80^0 C until use. Rat interleukin10, Tumour necrosis factor (Hemmila *et al.*, 2010) were measured by sandwich enzyme-linked immunosorbent assay (ELISA) using antibodies and reagents from R&D Systems. Results were adjusted for previous dilution and expressed as picograms per millilitre (Pg/mL)(Hemmila *et al.*, 2010).

4.14.1.4 Anti-oxidant activity

4.14.1.4.1 Catalase Activity (CAT)

Samples of 200 mg were homogenized with a mortar and pestle in 4 ml of ice-cold extraction buffer (100 mM potassium phosphate buffer (pH 7.0) + 0.1 mM EDTA). The homogenate was filtered through a muslin cloth and centrifuged at 12,000 X g for 15 min. The supernatant fraction was used as a crude extract for enzyme activity assays and all the procedures above were performed at 4^0C. Total catalase activity was measured according to the Beers and Sizer's method. The reaction mixture (1.5 ml) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 20 mM H_2O_2 and 50 ml enzyme extract. The reaction was started by adding the enzyme extract, and the decrease in H_2O_2 was monitored at 240 nm and quantified by its molar extinction coefficient (36 M^{-1} cm^{-1}). The results were expressed as mMol H_2O_2 $min^{-1}g^{-1}$ DM.

4.14.1.4.2 Glutathione Peroxidase activity

This helps to scavenge free radicals. A 10% homogenate of tissue was prepared in 5% trichloroacetic acid and centrifuged at 3000 rpm for 10 min. The supernatant was separated and to 0.1 ml of supernatant, 4 ml of 0.3 M phosphate buffer and 0.5 ml of 5, 5'-dithiobis-(2-nitrobenzoic acid) were added. Absorbance of the reaction mixture was read at 412 nm. The standard curve was plotted, and the tissue GSH level was determined and expressed as μg/g tissue(Kumar *et al.*, 2015)

4.14.1.5 Collagen activity

4.14.1.5.1 Determination of Hydroxyproline

Healed tissue was excised and dried in glass vials in a 110^0C oven for 48 h. 5 mg of lyophilized sample was hydrolysed with 5 ml of 6 N HCl at 110^0C for 18-20 h in a sealed tube for estimation of hydroxyproline(Talekar *et al.*, 2017)

4.14.1.5.2 Determination of Hexosamine

5 mg of healed tissue was lyophilized tissue sample was hydrolysed with 5 ml of 2 N HCl at 110^0C for 6-7 hrs., evaporated to dryness and the residue was dissolved in known amount of water. The solution was treated with 1 ml of freshly prepared 2% acetyl acetone in 0.5 M sodium carbonate and boiled for 15 min. After cooling 5 ml of 95% ethanol, 1 ml of Ehrlich's reagent was added and mixed thoroughly. The purple red color developed was read after 30 min at 530 nm spectrophotometrically(Talekar *et al.*, 2017).

4.15 Histopathological studies

Autopsy samples were taken from the skin of mice in all groups. The samples were fixed in 10% formalin solution for 24 h. Tap water was used to wash the samples then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used to dehydrate the samples. Xylene was used to clear the specimens then embedded in paraffin at 56 °C in hot air oven for 24 h. Tissue blocks in paraffin bees wax were prepared for sectioning at 4 microns thickness using a sledge microtome. Prepared tissue sections were collected on glass slides, de-paraffinized and stained by haematoxylin & eosin stain (H&E) for examination through the light electric microscope to assess for morphological changes. Digital photomicrographs were captured at representative locations and the burns will be evaluated for the extent of acanthosis, new blood capillaries formation and regeneration.

4.16 Statistical analysis

The data obtained from all the experimental work was analysed statistically the obtained data is expressed in form of graphs and tables. The data is analysed using one wayAnova test followed by Bonferroni test using SPSS software. The results obtained were compared with normal controls. The results are expressed in terms of P factor at 1% or 5% values (Pvalue <0.001 or P value < 0.05)

Chapter-5 Results and Discussion

5.1 Authentication of the Raw Materials

Herbal drugs submitted to HHRC were authenticated and verified by microscopic, organoleptic and pharmacognostic details. These studies have been accomplished in consultation with literature available in Quality standard of Indian Medicinal Plant and with submitted herbarium species no. R.S- 198,199 and 200. and were listed with botanical names Curcuma longa, Aloe vera and Calotropis procera.The details are mentioned in table 18

Table 18 Authentication details of raw drugs used in the formulation

Name of the drug	Biological /Chemical name	Article Reference number
Turmeric	Curcuma Longa	10/2021/TP/040
Aloe vera	Aloe vera	10/2021/TP/038
Ark ksheera	Calotropis procera	10/2021/TP/039

5.2 Morphological description of the drug

The samples were evaluated based on method described by API and mentioned in the research paper standard by Singh et al(Singh *et al.*, 2017). The morphological details are mentioned in table 19

5.3 Physico-chemical standardization of the procured raw materials the procured raw materials were tested for physicochemical parameters, microbiological details and limit tests the results are mentioned in table 20-23

Table 19 Morphological Details of Curcuma longa, Aloe vera, Calotropis procera and Tuttha

Sr. no.	Name of the drug	Shape	Texture	Colour	Odour	Taste
1.	**Aloe vera (AV)**	Spike shaped, (start wide and taper to point)	Serrated leaves with distinct teethed spikes on its margins	Vibrant green	Characteristics	Nauseous & bitter
2.	**Calotropis procera latex (CPL)**	Liquid on collection	Smooth on collection and when kept, achieves a brownish covering above it.	Milky white in colour when collected and granular appearance in due course of time	Pungent	An unpleasant bitter taste gives a burning sensation in the mouth
3.	**Curcuma Longa (CL)**	Pyriform in shape	Rough to touch	Pale yellow from outside to brownish orange inside	Aromatic	Characteristic & sense of taste similar to black pepper.
4.	**Copper sulphate**	Crystal lumps	Translucent, brittle	Sky blue	Pleasing smell	Metallic taste

Table 20 Physico-chemical standardisation parameters of procured raw materials for quality control

Parameters	***Curcuma Longa rhizomes***	***Aloe vera leaf gel***	***Calotropis procera latex***
F M% W/W	0	---------	---------
LOD%W/W	1±0.1	95.36±0.1	10.02±0.2
T A%W/W	9.16±0.7	1.18 ±0.2	1.53±0.1
AIA%W/W	0.2 ±0.3	0±0.0	0.20±0.0
WSE%W/W	12.53±0.1	0.68±0.2	18.78±0.05
ASE%W/W	8.4 ±0.2	2.01±0.0	0.14±0.0
VO%V/W	4±0.4	1.65 ±0.0	-------
All values are expressed as mean±S.D. (n=3)			

Table 21 Microbiological Parameters and Limit Test of Curcuma Longa (Turmeric)

Parameters	**Curcuma Longa rhizomes**	**Standard values in API-II**
Total microbial count	250cfu/g	$1x10^5$cfu/g
Total yeast and moulds	40cfu/g	$1x10^3$cfu/g
E. coli	Absent	Absent
S. aureus	Absent	Absent
p. aeruginosa	Absent	Absent
S.typhi	Absent	Absent
Heavy metal analysis		
Arsenic	Not detected	3ppm
Cadmium	Not detected	0.3ppm
Mercury	Not detected	1ppm
Lead	Not detected	10ppm
Aflatoxins analysis		
B1,B2 and G1, G2	Not detected Not detected	B1 &G1- NMT 0.5ppm B2&G2- NMT 0.1ppm
Pesticide residue	Not detected	Absent

Table 22 Microbiological Parameters and Limit Test of Calotropis procera (Ark)

Parameters	**Calotropis procera**	**Required**
Total microbial count	250cfu/g	$1x10^5$cfu/g
Total yeast and moulds	40cfu/g	$1x10^3$cfu/g
E. coli	Absent	Absent
S. aureus	Absent	Absent
p. aeruginosa	Absent	Absent
S.typhi	Absent	Absent
Heavy metal analysis		
Arsenic	Not detected	3ppm
Cadmium	Not detected	0.3ppm
Mercury	Not detected	1ppm
Lead	Not detected	10ppm
Aflatoxins analysis		
B1,B2 and G1, G2	Not detected Not detected	B1 &G1- NMT 0.5ppm B2&G2- NMT 0.1ppm
Pesticide residue	Not detected	Absent

Table 23 Microbiological Parameters and Limit Test of Aloe vera (Kumari)

Microbiological Parameters	**Aloe vera**	**Required**
Total microbial count	300cfu/g	$1x10^5$cfu/g
Total yeast and moulds	50cfu/g	$1x10^3$cfu/g
E. coli	Absent	Absent
S. aureus	Absent	Absent
p. aeruginosa	Absent	Absent
S. typhi	Absent	Absent
Heavy metal analysis		
Arsenic	Not detected	3ppm
Cadmium	Not detected	0.3ppm
Mercury	Not detected	1ppm
Lead	Not detected	10ppm
Aflatoxins analysis		
B, B2 and G1, G2	Not detected Not detected	B1 &G1- NMT 0.5ppm B2&G2- NMT 0.1ppm
Pesticide residue	Not detected	Absent

Morphological details were spotted based on details mentioned in the API. Organoleptic details establish the identification of the drugs depending on qualitative parameters. Physicochemical parameters provide quantitative details that are used to hit the standard parameters for the quality of raw and finished drugs(Gupta, Lakshmi and Jha, 2015). These parameters are presented in table no.2. Extractive values were helpful in the determination of the number of soluble constituents in the herbs. The values of all parameters in the raw sample of Curcuma longa confirm with the standard values prescribed in the monographs. Foreign matter (FM), total ash (TA), AIA (acid insoluble ash), water soluble extractives (WSE) and alcohol soluble (ASE) and volatile oil (VO) are NMT 2%, 9%,1%,12% and 8% respectively. The standard values for the raw Aloe vera leaf gel and the Calotropis Procera latex have not been mentioned in the standard compendium. The drugs were subjected to heavy metal, aflatoxins, Pesticide residue and microbiological analysis. The herbal drugs were detected with a total bacterial and fungal plate count in C. longa (200cfu/g, 30cfu/g), A.vera (300cfu/g,50cfu/g) and C.procera (250cfu/g, 40cfu/g). The drugs were also screened for the absence of specific pathogens (E.coli, staphylococcus aureus, salmonella typhi and pseudomonas aeruginosa), aflatoxins, pesticide residue and heavy metals. The observed and the standard values are given in table 21-23. It was observed that the organoleptic and physicochemical analysis along with the limit tests of the raw herbal drugs were done and the observations were compared with the available standard value in the Ayurvedic Pharmacopeia of India. All the physico-chemical parameters were performed in triplicates with the mean data being recorded. The observations are tabulated in table 20

5.4 Quality control parameter for Standardisation of mineral and oil drugs

Copper sulphate was purchased authenticated from Qualikem's pharma ltd. It was examined for physico-chemical parameters and subjected to Heavy metal analysis.

5.4.1 XRD analysis of copper sulphate

The phase polarity of pure copper sulphate crystals was examined by XRD techniques. The crystal structure was triclinic. First three Sharp peaks were identified at 16.522^0, 19.361^0 and 27.289^0 are identification for crystalline structure (figure 11). The results were matched with JCPDS file that confirms the taken raw material is copper sulphate.

XRD study shows the peaks identified in the raw material on comparing with JCPDS data.

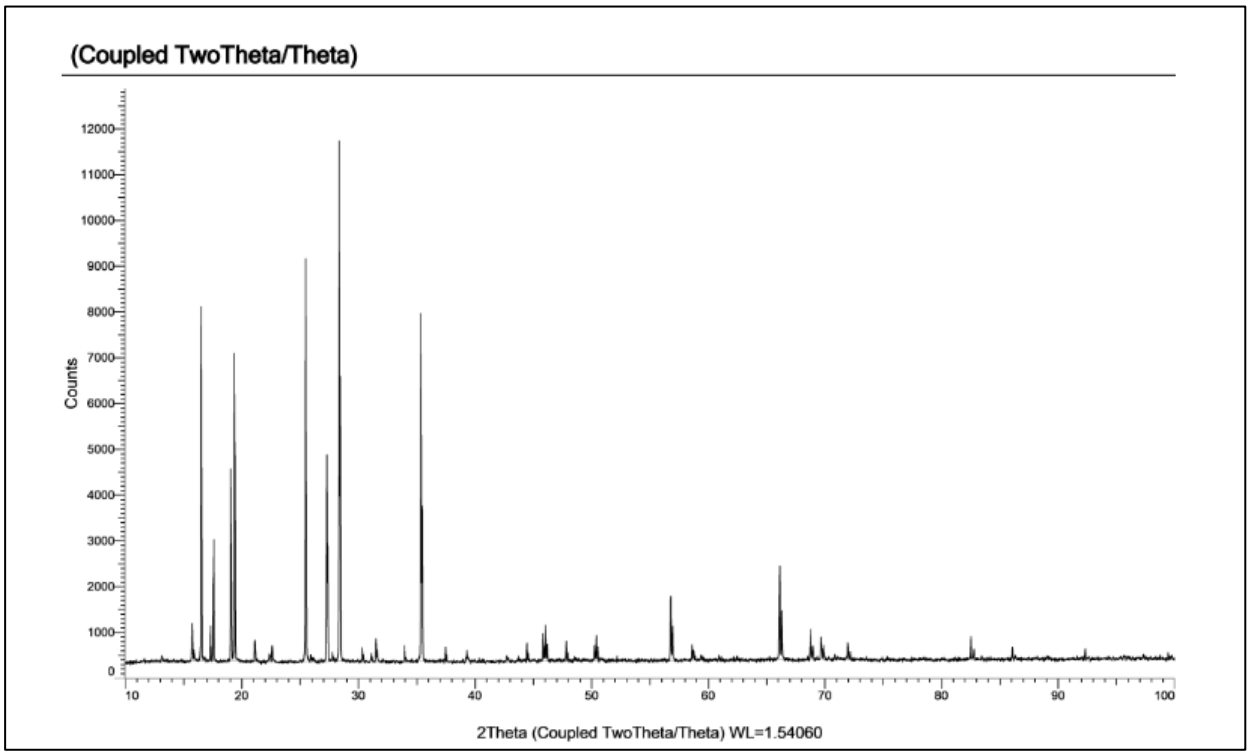


Figure 11 X-Rd analysis of copper sulphate

5.4.2 Physical parameters and heavy metal analysis of herbal oils and mineral drug the herbal oils and mineral drugs was assessed for physical and chemical parameters mentioned in Ayurvedic pharmacopoeia(table25). Neem seed oil and Egg yolk oil (herbal oils) were also checked for physico-chemical parameters and heavy metal levels (limit tests) as mentioned in table 26,27

5.5 Preparation of the herbal plant extracts

5.5.1 Ethanolic extract of curcuma longa

Rhizomes of curcuma longa was collected from the local market in Amritsar, Punjab, drug authentication was done from HHRC, Amritsar. Observation table of raw rhizomes used and ethanol consumed along with the obtained yield are presented in table 27. The yield of ethanolic extract obtained were 8.96%.

Table 24 physical parameters of herbal oils and mineral drug

Physical Parameters	**Neem seed oil**	**Egg yolk oil**	**Copper sulphate**
Colour	Greenish brown	Transparent with a light yellow hue	Blue coloured
Odour	Unpleasant, characteristic	Unpleasant	Odourless
Taste	Bitter	Tasteless	Bitter
Solubility	Soluble in methanol, ethanol, ethylacetate and DMSO	Soluble in methanol, & DMSO	Soluble in water
Specific gravity	0.927±0.001	1.296±0.36	2.13±0.2
Refractive index	1.46 0.015	1.71±0.68	-------
All values are expressed mean ±S.D (n=3)			

Table 25 Heavy metal analysis (limit test) for herbal oils and mineral drug

Heavy metals	Neem seed oil (NSO)	Egg yolk oil (EYO)	Tuttha (Copper sulphate)	Requirement
Arsenic	Not detected	Not detected	Not detected	3ppm
Cadmium	Not detected	Not detected	Not detected	0.3ppm
Mercury	Not detected	Not detected	Not detected	1ppm
Lead	Not detected	Not detected	Not detected	10ppm

Table 26 Chemical standardization parameters for herbal oils for quality control

Parameters	**Neem Seed Oil (NSO)**	**Egg Yolk Oil (EYO)**
Saponification value (%w/w)	194.7±1.6	237.19±2.9
Acid value (mg/KoH)	25.22±0.52	5.01±0.4
Peroxide value (meq/Kg)	2.69±0.1	1.78±0.1
All values are expressed as mean ±S.D (n=3)		

Table 27 Observations and yield of Curcuma longa Extract obtained from powdered Curcuma longa

Sr.No.	Observation	I	II	III	Total
1	Weight of the Powdered dry curcuma longa	150gm	150gm	150gm	450 gm
2.	Quantity of Ethanol used	1000ml	1000ml	1000ml	1000 ml
3.	Quantity of the extract obtained	13.44gm	13.56gm	14.0gm	41.0gm

5.5.2 Aqueous extract of Aloe Vera

Aloe vera was collected from the local market in Jalandhar after authentication from HHRC. After washing and peeling. Aloe vera extract was formed after peeling and grinded the aloe vera pulp. The extract was then subjected to lyophilisation. Observation table of weight of raw Aloe vera pulp and water consumed along with the obtained yield are presented in table 28 The yield of aqueous extracts obtained were (1.48%).

Table 28 Observations and yield of Aloe vera Extract obtained from Aloe vera pulp

Sr. No.	Observation	I	II	III	Total
1	Weight of the Aloe vera pulp	615 gms	483 gms	705 gms	1803 gm
2.	Quantity of water used	1000ml	1000ml	1000ml	3000ml
3.	Quantity of the extract obtained	9.10 gms	7.14 gms	10.4 gms	26.64 gm

5.5.3 Aqueous extract of Calotropis Procera Latex

The plant was collected from Jalandhar after authentication from HHRC. After extracting its latex, it was packed in eppendorf's for later centrifugation and lyophilisation. The observation table of raw latex collected and water consumed during

the process along with the obtained yield are presented in table 29 The yield of aqueous extracts obtained were (4.9%).

Table 29 Observations and yield of Calotropis procera Latex Extract obtained from Calotropis procera latex

Sr. No.	Observation	I	II	III	Total
1	Weight of the Calotropis procera Latex	205 ml	210 ml	215 ml	630 ml
2.	Quantity of water used	205 ml	210 ml	215 ml	630 ml
3.	Quantity of the extract obtained	10.0gm	10.3gm	10.5gm	30.8gms

5.6 Standardization of Prepared Herbal Extracts

The prepared extracts of herbal drugs were subjected to physico-chemical analysis for quality control and the results were compared with the available references. The results are tabulated in table no.30

Table 30 Physicochemical standardisation of Herbal extracts

Parameters	***Curcuma Longa rhizomes***	***Aloe vera leaf gel***	***Calotropis procera latex***
F M% W/W	0	---------	---------
LOD%W/W	1±0.1	95.36±0.1	10.02±0.2
T A%W/W	9.16±0.7	1.18 ±0.2	1.53±0.1
AIA%W/W	0.2 ±0.3	0±0.0	0.20±0.0
WSE%W/W	12.53±0.1	0.68±0.2	18.78±0.05
ASE%W/W	8.4 ±0.2	2.01±0.0	0.14±0.0
VO%V/W	4±0.4	1.65 ±0.0	-------
All values are expressed as mean±S.D. (n=3)			

5.7 Phytochemical Screening of Prepared Extracts of Herbal Drugs

The prepared extracts were subjected to preliminary phytochemical screening and Thin layer chromatography analysis and the results are tabulated in table 31 & 32, Figure 12.

Table 31 Preliminary Phytochemical screening of prepared extracts of herbal drugs

Sr.No.	Qualitative Test	Phytochemical Constituent	Observation		
			CE	AE	CAE
1.	Alkaloids	Dragendroff's test	+	+	+
		Mayer's test	+	-	+
		Wagner's test	+	-	+
		Hager's test	+	-	+
2.	Flavonoids	Ammonia test	+	-	+
		Pew's test	-	+	+
3.	Saponins	Foam test	-	-	+
4.	Phenols	Ferric chloride test	+	-	-
		Lead acetate test	+	+	-
5.	Carbohydrates	Fehling's test	+	-	+
		Benedict's test	+	+	+
		Molisch's test	+	+	+
6.	Glycosides	Libermann Test	-	+	-
		Bontrager's Test	+	-	-
		Keller-kiliani test	+	+	-
		Salkowski Test	+	-	+
7.	Proteins	Biuret test	_	+	+
		Ninhydrin Test	+	+	+

(+) Indicates the presence of the compound (-) indicates the absence of the compound

Table 32 Thin layer chromatographic details of herbal drug extracts

Curcuma longa extract	
Curcumin visualization	@356nm
Mobile Phase	Chloroform: Toluene: Methanol (5: 4: 1 v/v)
Rf value	0.63
Aloe vera extract	
Rutin visualisation	@254nm
Mobile Phase	Toluene: Ethyl acetate: Formic acid: Methanol (2: 3: 1: 3 v/v)
Rf values	0.86,0.62,0.42,0.40,0.37
Berberine visualisation	@356nm
Mobile Phase	Butanol: Ethyl acetate: Acetic acid: Water (6: 10: 2: 2 v/v)
Rf values	0.86, 0.46 and 0.28
Calotropis procera latex extract	
Gallic acid visualisation	**@254nm**
Mobile Phase	Toluene: Ethyl acetate: Formic acid (10: 7: 1 v/v)
Rf value	0.91

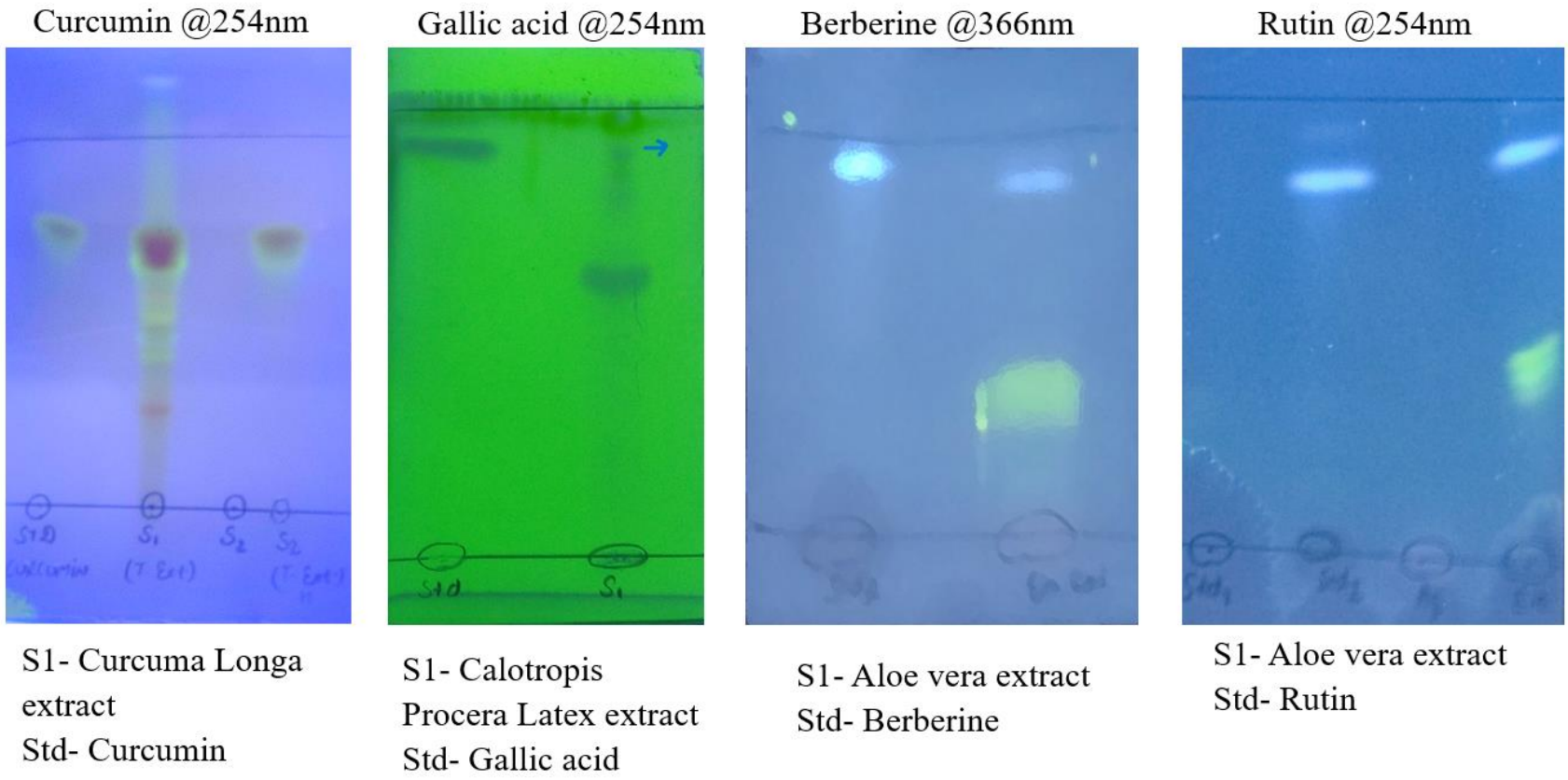


Figure 12 Pictorial presentation of Thin Layer Chromatography of prepared Herbal Extracts

5.8 Preformulation studies

5.8.1 Drug incompatibility studies FTIR spectra were analyzed in the mid-infrared region of 4000-400cm-1. The study is analyzed by identifying absorption bands in entire IR spectra. Bands In this Range indicate the complex compound. All the available peaks of the extracts singly and in combinations show that there is no physical interaction among them and the ingredients are compatible with each other. The details of the peaks received are given in table 33 &34

Table 33 FTIR spectrum assigned to Calotropis procera latex extract, Aloe vera extract and Copper sulphate.

Wave number in the test sample cm^{-1}	Reference wave number (cm^{-1})	Functional group
Calotropis procera latex (Aqueous extract)		
3211	3550-3200	O-H stretching
1638	1650-2000	C-H bending
1395	1330-1420	O-H bending
1062	1020-1250	C-N stretch
Aloe vera (Aqueous extract)		
3269	3200-3550	-OH stretching
2919	2840-3000	C-H stretching
1549	1500-1550	N-O stretching
1404	1330-1420	O-H bending
1242,1030	1020-1250	C-N stretching
Copper sulphate (Aqueous extract)		
3190	3550-3200	-OH stretching
1639	1566-1650	C-C Stretching
1393	1330-1420	-OH bending
865	790-860	C=C bending
Curcuma longa (Ethanolic extract)		
3321	3200-3550	O-H stretching
2924	2840-3000	C-H stretching
1676	1650-2000	C-H bending
1511	1500-1550	N-O stretching
1581	1566-1650	C=C Stretching
1376	1330-1420	O-H bending

Table 34. FTIR spectrum assigned to Curcuma longa ethanolic extract with individual aqueous extracts and all the aqueous extracts together

Ethanolic and Aqueous extracts		
Wave number in the test sample (cm^{-1})	**Reference wave number (cm-1)**	**Functional group**
Curcuma longa + Calotropis procera		
3212	3550-3200	O-H stretching
2924	2500-3300	O-H stretching
1637	1600-1650	C=C stretching
1395	1330-1420	O-H bending
1062	1085-1150	C-O Stretching
Curcuma longa extract and Aloe vera		
3334	3550-3200	O-H stretching
2923	2840-3000	C-H stretching
1676	2000-1650	C-H bending
1581	1566-1650	C=C Stretching
1511	1500-1550	N-O stretching
1376	1330-1420	OH bending
Curcuma longa + Copper sulphate		
3307	3550-3200	O-H stretching
2924	3000-2840	C-H stretching
1674	2000-1650	C-H bending
1598	1566-1650	C=C Stretching
1511	1500-1550	N-O stretching
Calotropis procera+ Aloe vera + copper sulphate (Aqueous extracts)		
3292	3550-3200	O-H stretching
2228	2120-250	C≡C bonds
1637	1566-1650	C-C Stretching
1078	1020-1250	C-N stretch

5.9 Screening of excipients for components of the nanoemulsion

5.9.1 Calculating the λmax for herbal drugs by UV spectrophotometric method different concentrations of the herbal drugs are taken with ethanol and aqueous medium for CE, AE and CAE by photometric method between 200-600nm. Peak absorbance wavelength for CE, AE and CAE was found to be 422nm, 278nm and 268nm respectively.

5.9.2 Preparation of the calibration curves for herbal drugs: to prepare the calibration curve a stock solution of 1000 µg/ml (CE) was dissolved in ethanol. Aliquots of 2 µg/ml, 4 µg/ml, 6µg/ml, 8µg/ml and 10µg/ml were prepared in 10ml of ethanol and absorbance was recorded at 421nm by withdrawing 20 µl, 40µl, 60 µl, 80 µl and 100 µl from the prepared stock stolutions. A stock solution of 4000µg/ml of Aloe vera extract (AE) was dissolved in aqueous medium and aliquots of 0.05ml, 0.2ml, 0.75ml, 1ml and 1.25ml were withdrawn from this stock solution to prepare the dilutions with 200µl, 400µl, 600µl, 800 µl and 1000 µl in 10ml of aqueous medium with absorbance taken at 278nm. For CAE Stock solution of 1000 µg/ml was prepared and dilutions of 2 µg/ml, 4 µg/ml, 6µg/ml, 8µg/ml and 10µg/ml were prepared in 10ml of aqueous medium by withdrawing 20 µl, 40 µl,60 µl,80 µl and 100 µl from the stock solution and dissolving in 10ml of aqueous medium with absorbance taken at 268nm. The prepared calibration curves are shown in figure 13

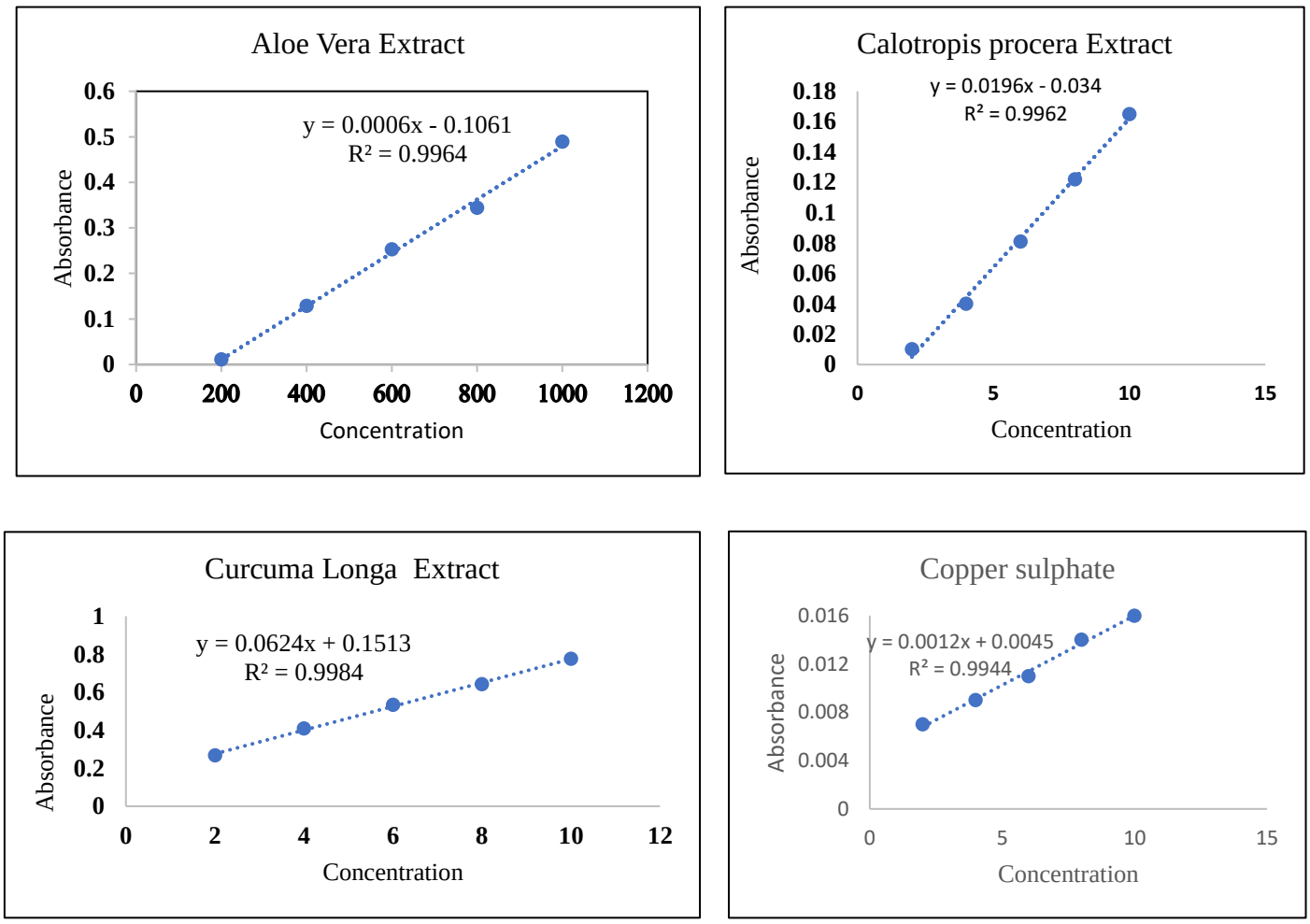


Figure 13 Calibration curve of herbal drugs for preformulation studies.

5.9.3 Screening of excipients for oils

Selection of surfactant phase for oil(mix): Ease of miscibility were criteria for visual observation for the selection of the surfactant. Non-ionic surfactants (Tween 20, Tween 80, Propylene glycol and PEG, Labrafil and Transcutol, Span 20 and Span 80) were screened. Labrafil and Transcutol was found to be compatible with the both oils on the basis of visual inspection. Phase separation after 24 hrs was used as a tool for selection for surfactants.

5.9.4 Screening of the herbal components of the nanoemulsion solubility of Curcuma longa, Aloe vera extract and Calotropis procera latex extract in selected components and excipients of Nanoemulsion such as oil, surfactant, and co-surfactant was carried out to choose the best

suitable component based on the amount of oil and water soluble drugs solubilized in each component figure 14 which was analyzed by developed calibration curve for herbal components.

solubility studies of curcuma longa extract

Solubility studies of Aloevera extract

Solubility studies of calotropis procera latex extract

Figure 14 Solubility studies of herbal drugs for preformulation studies.

of curcuma longa extract was mixed in 7 non-ionic surfactants (tween 20, tween 80, PEG 200, PEG 400, PEG 600 labrafil, and transcutol) separately. Solubility was calculated from Absorbance taken in UV spectrophotometer for the various surfactants and co-surfactants. The results in descending order were as follows. Labrasol ALF (33.78 µg/ml, transcutol P (25.60 µg/ml), tween 80 (20.62 µg/ml) PEG 200 (25.121 µg/ml), PEG 400 (8.9 µg/ml) tween 20 (4.57 µg/ml) and PEG 600 (1.39 µg/ml). Based on above results labrasol and transcutol were found to be the effective surfactants for the solubility of Curcuma longa. Graphical representation is presented in figure 15

5.9.6 Screening of the excipients for the other herbal components of nanoemulsion (AE, CAE) A fixed quantity of AE and CAE was mixed in 7 non-ionic surfactants and water separately. Absorbance of CAE taken in UV spectrophotometer for the various surfactants and co-surfactants and observed concentrations were calculated as given below in descending order

Labrasol ALF (43.6 mg/ml), transcutol (12.4 mg/ml), water (12.4 mg/ml), tween 20 (9.85mg/ml) PEG 400 (9.75mg/ml), PEG 600 (8.75 mg/ml), tween 80 (7.3mg/ml) and PEG 200 (4.05mg/ml) Based on above results labrasol, transcutol and tween 80 were found to be

the effective surfactants for the solubility of AV and CAE. The solubility data of CAE are presented in figure 16

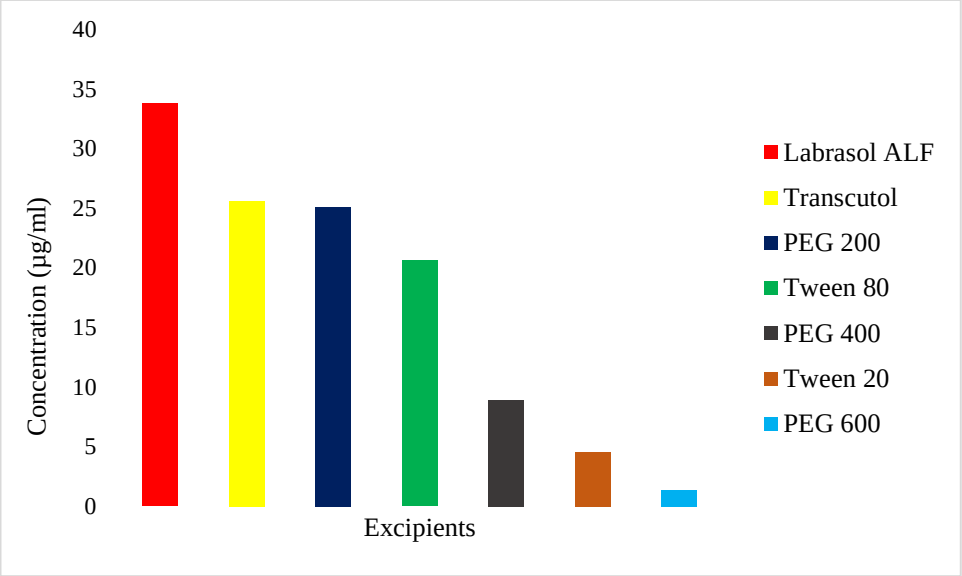


Figure 15 Solubility study of curcuma longa extract

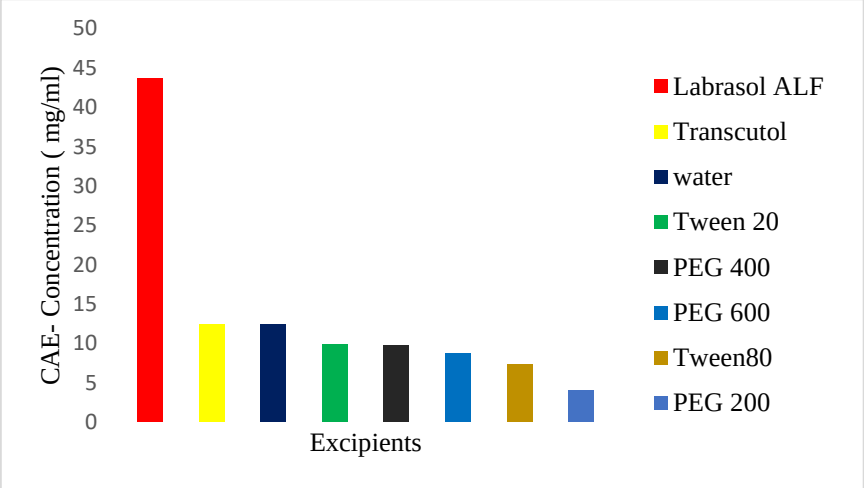


Figure 18 Solubility study of calotropis procera latex extract

Absorbance of AE in different surfactants, co-surfactants and water was recorded in UV spectrophotometer and concentrations were calculated in descending order as labrasol ALF (187µg/ml), PEG 400 (60 µg/ml), transcutol (58 µg/ml), PEG 200 (53.4 µg/ml), tween20(55 µg/ml), tween 80 (42 µg/ml), PEG 600 (42 µg/ml) and water (41.3 µg/ml). Values are represented in figure 17

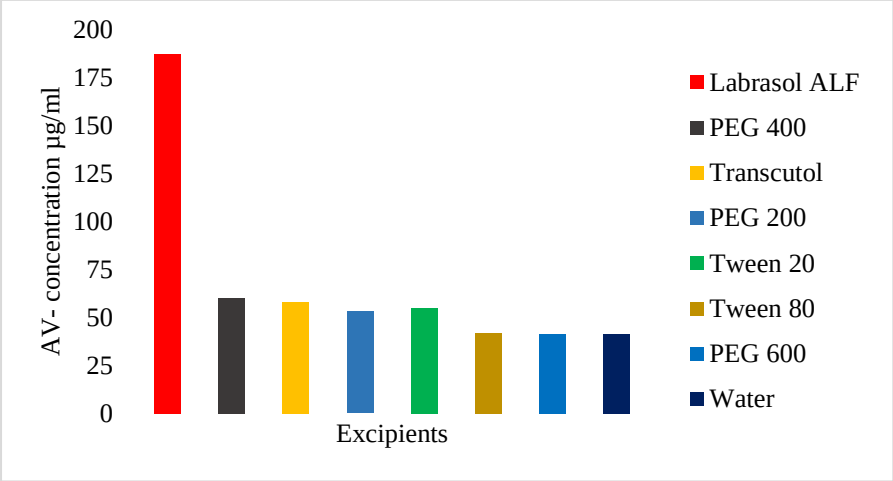


Figure 17 Solubility study of Aloe vera extract

Above data was informative about the solubility of components of nanoemulsion, Calotropis, Aloe vera and Curcuma longa extracts (CAACE). Maximum solubility was observed in surfactant labrasol ALF followed with co-surfactant transcutol P and water in Calotropis procera and Aloe vera extracts while solubility of Curcuma longa was assisted with these surfactants and co-surfactants since the drug was oil soluble. Further the CAACET loaded nanoemulsions were prepared with the help of these surfactants and co-surfactants

5.10 Formulation of CAACET loaded nanoemulsion system spontaneous nano emulsification and high speed homogenisation method was adapted for preparation of CAACET loaded nanoemulsion system(Sharma, Singh and Harikumar, 2020)(Mazonde, Khamanga and Walker, 2020). In brief meticulously 0.54 gm of the turmeric extract was mixed thoroughly with heated herbal oil mixture at 45^0C to 50^0C, followed by the selected surfactant and co-surfactant. The mixture was kept on a REMI mixer with hot plate at 300 rpm for about 5 minutes to get an isotropic mixture. The homogeneous phase was diluted to a measured amount of aqueous phase. The process was followed with high-speed homogenisation at 6500 rpm to form a nano emulsion system. Aqueous soluble drugs are then serially loaded dropwise in this nanoemulsion system. The size of nanoemulsion can be accustomed by changing the ratio of oil phase and water phase and the surfactant and co-surfactant phase in the oil phase(Ferreira *et al.*, 2007). A particular sequence of method was adapted for emulsion

formation. CE among the other herbal drug components was initially mixed with the organic phase using a vortex mixture. Later the aqueous phase was added drop wise followed by the aqueous soluble extracts and mineral drug at last. ultrasonication in a bath sonicator was used as a last step for a period of 5 minutes to remove trapped air during homogenisation process and reduce the droplet size to formulate efficient nanoemulsion

5.10.1 Screening the emulsification efficiency

Efficiency of selected surfactants and co-surfactants to solubilize herbal extracts and tutha were further considered by preparing the emulsion compositions by the described method in section 4.10.1 the emulsification tendency of the selected S(mix) were selected on the basis of formed single phase system. initial formulations prepared during the screening process were prepared with oil(mix) taken in ratio 1:1 with S(mix) in ratio of 1:2, 2:1 & 3:2. The formulations prepared with smix ratio 3: 2 were stable for 24 hrs accompanied with phase separation after 72hrs. depending upon this indication the next formulations were prepared with oil (mix) ratio of 3:2 with s(mix) ratio of 1:2, 2:1 & 3:2 respectively (F1 -F27) as seen in figure 18

Among the 27 preliminary emulsions F25, F-26 were found as a one phase system with S(mix) ratio of 3:2 others underwent phase separation. Hence these ratios well-thought-out for further studies.

zeta potential and PDI was further studied with DOE. This is diagrammatically represented figure 19 a pseudo ternary phase diagram was constructed with these 27 preliminary formulations prepared with changing ratio of oil(mix) and S(mix). The compositions of these formulations are shown in table 13 The results divulge that preliminary formulations prepared with 1:1 oil(mix) and 1:1 & 2:1 S(mix) ratio have shown complete phase separation. F25 &F26 formulations with S(mix) ratio of 3:2 have shown stability without phase separation. In the p-TPD. This led to the information that the particular combination of oil(mix) and S (mix) in 3:2 ratio had good impact on stability of the preliminary emulsion system. influence of oil mix and S mix on droplet size.

Figure 18 preliminary screening of the formulation based on emulsification efficiency

Due to immisibility of the both oils together there was a need for using the surfactants and co-surfactants for effective emulsification of oils. Labrafil and transcutol was selected as the surfactant and co-surfactant respectively since they presented proper emulsification at a comparatively low concentration. All input factors of DOE table have shown quadratic model. Formulations was optimised based on response factors-1,2 and 3 for size, zeta potential and PDI. ANOVA presented that model for size is quadratic and significant.

5.11 Construction of P-TPD

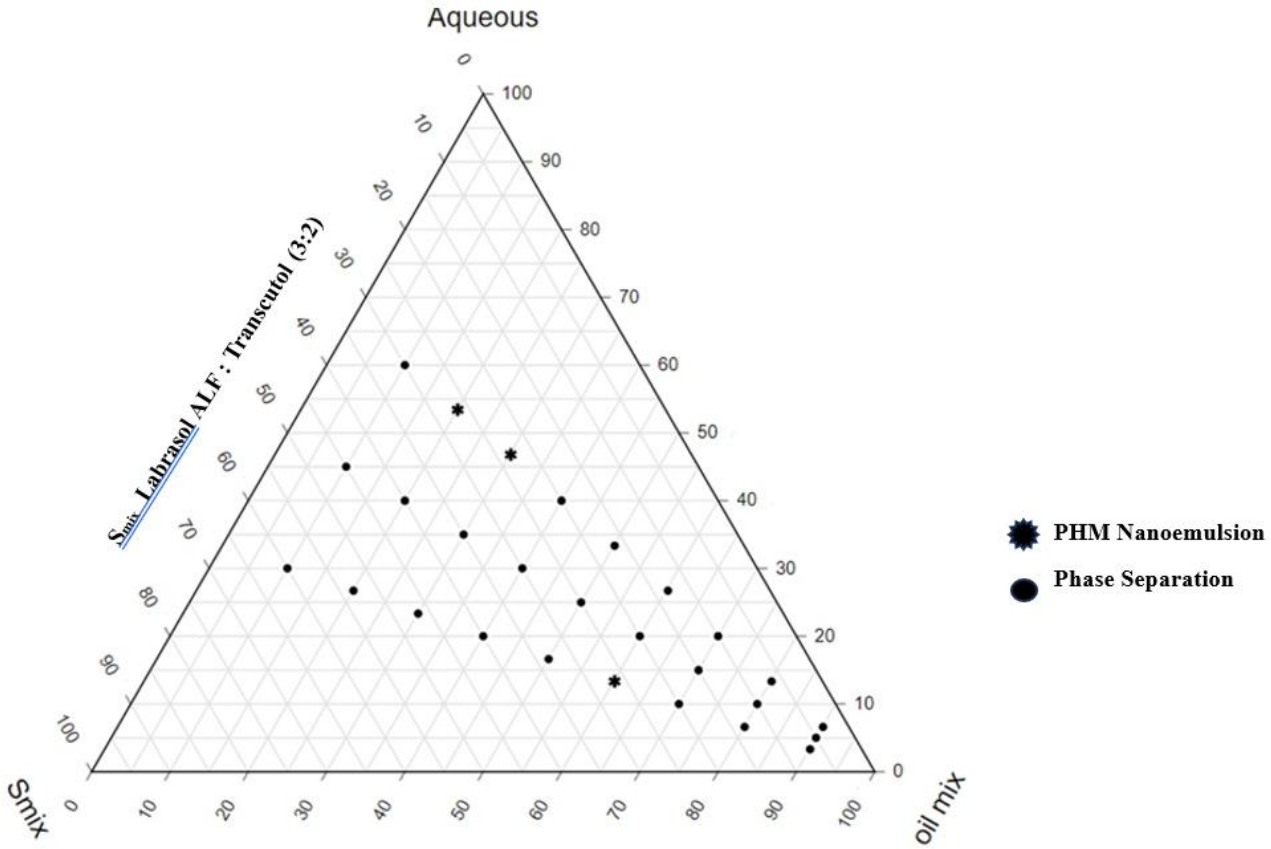


Figure 19 Construction of pseudo ternary phase diagram

Table 35 Input and Output variables selected for Design of experiments

Sr.No.	Factor 1 A: Oil Phase	Factor 2 B: Aqueous Phase	Factor 3 C: (Smix)	Response 1 (Size)	Response 2 (PDI)	Response 3 (Zeta)
1	-1	-1	0	453.3	0.668	-3.60
2	1	-1	0	281.8	0.125	-21.4
3	-1	1	0	221.4	0.905	-6.1
4	1	1	0	208	0.789	-5.8
5	-1	0	-1	456.5	0.283	-29.4
6	1	0	-1	469.6	0.411	-20.7
7	-1	0	1	134	0.411	-20.7
8	1	0	1	239.4	0.281	-3.31
9	0	-1	-1	351.36	0.281	-2.64
10	0	1	-1	168.7	0.212	-2.77
11	0	-1	1	165.0	0.212	-2.77
12	0	0	0	461.1	0.442	-5.83
13	0	0	0	326.46	0.307	-23.8
14	0	0	0	203.7	0.236	-18.43
15	0	0	0	291.8	0.088	-28.8
16	0	0	0	164.4	0.207	-33.2
17	0	0	0	277.5	0.265	-29.06

1.12 Optimization of CAACET loaded nanoemulsion

5.12.1 ***DOE*** application of DOE was carried out using BBD. 17 experimental runs to assess the influence of variables like 'A' for oil(mix),'B' for aqueous phase and 'c" for S(mix) and on responses 1,2 &3 of droplet size, PDI & zeta potential. 17 experimental runs with their input and output variables are presented in table 35. BBD proposes that the Response for factor-1 (droplet size) is altered between 134.0 to 469.6nm, factor-2 (PDI) between 0.125 to 0.905 and factor-3 (zeta potential) shows a range between -2.77 to -29.06. The DOE software depicted the results and expressed them as polynomial equations. ANOVA was conducted using this software to depict and estimate the consequence of nominated variable quantities their interactions and statistical significance. The results of ANOVA and fit summary for responses 1,2 and 3 are given in table 36,37 and 38. they interpreted that it was a quadratic model. Statistical evaluated result indicated P value less than 0.05, this indicated a significant relation of the response factors. Lack of fit presented P-value more than 0.05, indicating the insignificance and thus efficiency of the selected models. BBD presented the response 1,2 and 3 in mathematical equations presented as in equations 5.1 to 5.3. The co-efficient of various

factors in equations indicates the relation of factors on responses-1,2 and 3 for size, zeta potential and PDI respectively.

Table 36 ANOVA for Quadratic Model – Response factor-1 (Droplet Size)

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	1.945E+05	9	21614.13	27.20	0.0001	significant
A-Oil Phase	1197.32	1	1197.32	1.51	0.2593	
B-Aqueous phase	2203.48	1	2203.48	2.77	0.1398	
C-surfactant	1.730E+05	1	1.730E+05	217.66	< 0.0001	
AB	115.89	1	115.89	0.1458	0.7139	
AC	5821.69	1	5821.69	7.33	0.0303	
BC	394.02	1	394.02	0.4959	0.5041	
A^2	471.73	1	471.73	0.5937	0.4662	
B^2	326.79	1	326.79	0.4112	0.5418	
C^2	11395.23	1	11395.23	14.34	0.0068	
Residual	5562.32	7	794.62			
Lack of Fit	1676.59	3	558.86	0.5753	0.6611	not significant
Pure Error	3885.73	4	971.43			
Cor Total	2.001E+05	16				

According to the **Model F-value** of 27.20 indicates, the model is significant. High F-value has only only a 0.01% probability factor to be by noise.

Model terms consider that the **P-values** less than 0.0500 are significant. In this case C, AC, C^2 are significant model terms. Values more than 0.1000 indicate the model terms are not significant.

The **Lack of Fit F-value** of 0.58 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit is good. Final equation 5.1 is written in terms of coded factors and detects the relative impact of various factors by comparison with their different co-efficient.

+246.21 -12.23A-16.62B-147.04C-5.38AB+38.15AC-9.92BC-10.58A2 -8.81B2+52.02C2

(Eq:5.1)

Table 37 ANOVA for Quadratic Model – Response factor-2 (Zeta Potential)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2012.03	9	223.56	3.75	0.0476	significant
A-Oil Phase	433.95	1	433.95	7.28	0.0307	
B-Aqueous phase	221.97	1	221.97	3.72	0.0950	
C-surfactant	13.00	1	13.00	0.2182	0.6546	
AB	420.25	1	420.25	7.05	0.0327	
AC	85.75	1	85.75	1.44	0.2694	
BC	0.0000	1	0.0000	0.0000	1.0000	
A^2	2.39	1	2.39	0.0401	0.8470	
B^2	125.59	1	125.59	2.11	0.1899	
C^2	678.21	1	678.21	11.38	0.0119	
Residual	417.27	7	59.61			
Lack of Fit	287.42	3	95.81	2.95	0.1615	not significant
Pure Error	129.85	4	32.46			
Cor Total	2429.31	16				

The **Model F-value** of 3.75 implies the model is significant. There is a 4.76% chance that the large F-value could occur due to noise.

Model terms consider **P-values** less than 0.0500 to be significant. In this case, A, AB, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant The **Lack of Fit F-value** of 2.95 implies the Lack of Fit is not significant relative to the pure error. There is a 16.15% chance of this F-value to occur due to noise. Non-significant lack of fit is good to fit this model. The final equation 5.2 is written in terms of coded factors. This equation recognizes the relative impact of various factors by comparison with their different co-efficient.

$$\mathbf{-20.86 + 7.36A + 5.27B - 1.28C - 10.25AB + 4.63AC + 0.0000BC - 0.7535A^2 + 5.46B^2 + 12.69C^2} \quad \textbf{(Eq:5.2)}$$

Table 38 ANOVA for Quadratic Model – Response factor-3 (PDI measurement)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.7575	9	0.0842	3.87	0.0440	significant
A-Oil Phase	0.0609	1	0.0609	2.80	0.1380	
B-Aqueous phase	0.0611	1	0.0611	2.81	0.1376	
C-surfactant	0.0001	1	0.0001	0.0048	0.9465	
AB	0.0888	1	0.0888	4.09	0.0829	
AC	0.0001	1	0.0001	0.0037	0.9530	
BC	0.0239	1	0.0239	1.10	0.3294	
A^2	0.4403	1	0.4403	20.26	0.0028	
B^2	0.0405	1	0.0405	1.87	0.2142	
C^2	0.0441	1	0.0441	2.03	0.1972	
Residual	0.1521	7	0.0217			
Lack of Fit	0.0181	3	0.0060	0.1805	0.9044	not significant
Pure Error	0.1340	4	0.0335			
Cor Total	0.9096	16				

The **Model F-value** of 3.87 implies the model is significant. There is only a 4.40% chance that an F-value this large could occur due to chance.

P-values less than 0.0500 indicate model terms are significant. In this case A^2 is a significant model term. Values greater than 0.1000 indicate the model terms are not significant.

The **Lack of Fit F-value** of 0.18 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit is good to fit this model. The final equation 5.3 is written in terms of coded factors. This equation recognizes the relative impact of various factors by comparison with their different co-efficient.

$$+0.3280 + 0.0873A + 0.0874B - 0.0036C - 0.1490AB + 0.0045AC + 0.0773BC + 0.3234A^2 + 0.0981B^2 - 0.1024C^2 \quad \textbf{(Eq:5.3)}$$

5.13 Response Analysis for Optimisation the co-efficient of various factors of in the polynomial equations are able to determine the coactive and the opposed effect of three factors in different phases, oil_{mix}, S_{mix} and Aqueous phase factors on the nominated response variables of size, zeta potential and PDI identified as factors 1,2 &3.

5.13.1 Influence of variables on Droplet size

The globule size is an important factor for formation of nanoemulsion because it determines the absorption extent and effective permeability of drug into skin. It is required that the globule size should be less as shown in figure20. While plotting the same data to various models and ANOVA results (table 38) it was found that they are best defined with a quadratic polynomial model. The **equation 5.1** for the quadratic model for size shows the interaction of various variables on droplet size. When oil phase is increased with respect to aqueous there is not much significant change in size. When oil and aqueous phase is increased with respect to surfactant concentration it has a significant role because it decreases the surface tension and reduces the size. 3D-Graphical representation of the interaction of various on globule size is presented in figure 28 (A),(B) &(C)

5.13.2 Influence of variables on Zeta potential:

Zeta potential is used to evaluate the charge on the globules which is important for the stability of Nanoemulsion. It is an indicative of degree of electrostatic repulsion between the globules with similar charge in the dispersion medium. This factor has significant role on imparting the charge on the globule. The aqueous and oil phase has no significant role on imparting the charge on the globules. As the concentration of the surfactant is increased the zeta potential is showing negative mV potential. The ANOVA results presents the interaction of the various variables which was best described by the **equation 5.2** for the quadratic model and is presented with 3D graphs in figure 21 (A),(B) &(C)

5.13.3 Influence of variables on Poly-dispersity Index

Polydispersity index is playing an important factor for the stability of nanoemulsions. If the formulation has high polydispersity index, it is indicative of good size of droplets. ‘0’ PDI indicates uniformity (homogeneity) in the globules. It should be less than 0.5 which indicates uniformity of the globules. Higher PDI value shows coagulation or aggregation of the globules. As per the formulations prepared none of them presented the size more than 0.5 which is indicative of the uniformity of the size of the globules. As the concentration of the oil with respect to surfactant increases aqueous PDI value is also increasing. Similarly as the concentration of oil with respect to aqueous increases PDI value is decreasing. This is graphically represented by figure 22 (A),(B) &(C). Plotting the experimental data to quadratic models and ANOVA results, they are described efficiently with the quadratic model equation 5.3 as mentioned earlier.

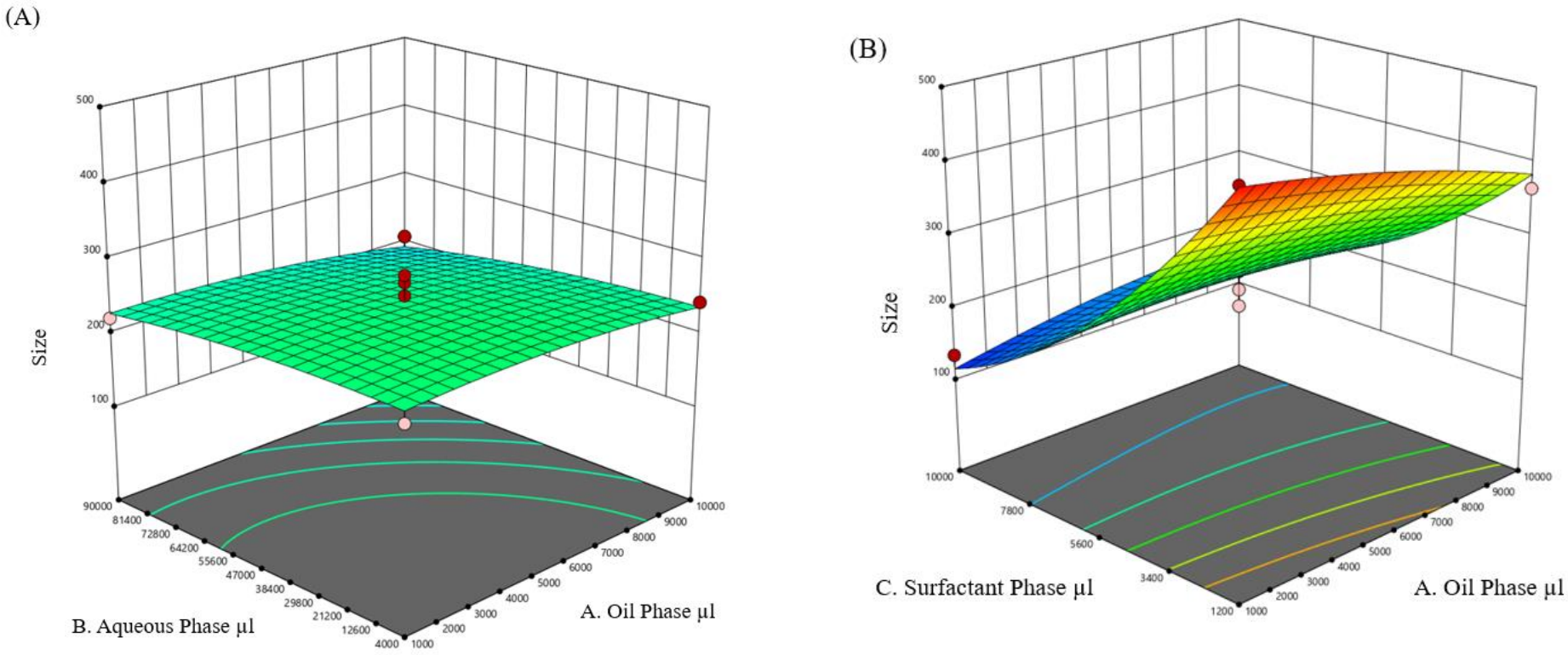


Figure 20 Response surface plot for the effect of (A) oil phase and aqueous phase, (B)Surfactant and oil phase, (C) Aqueous and surfactant phase on size of droplets.

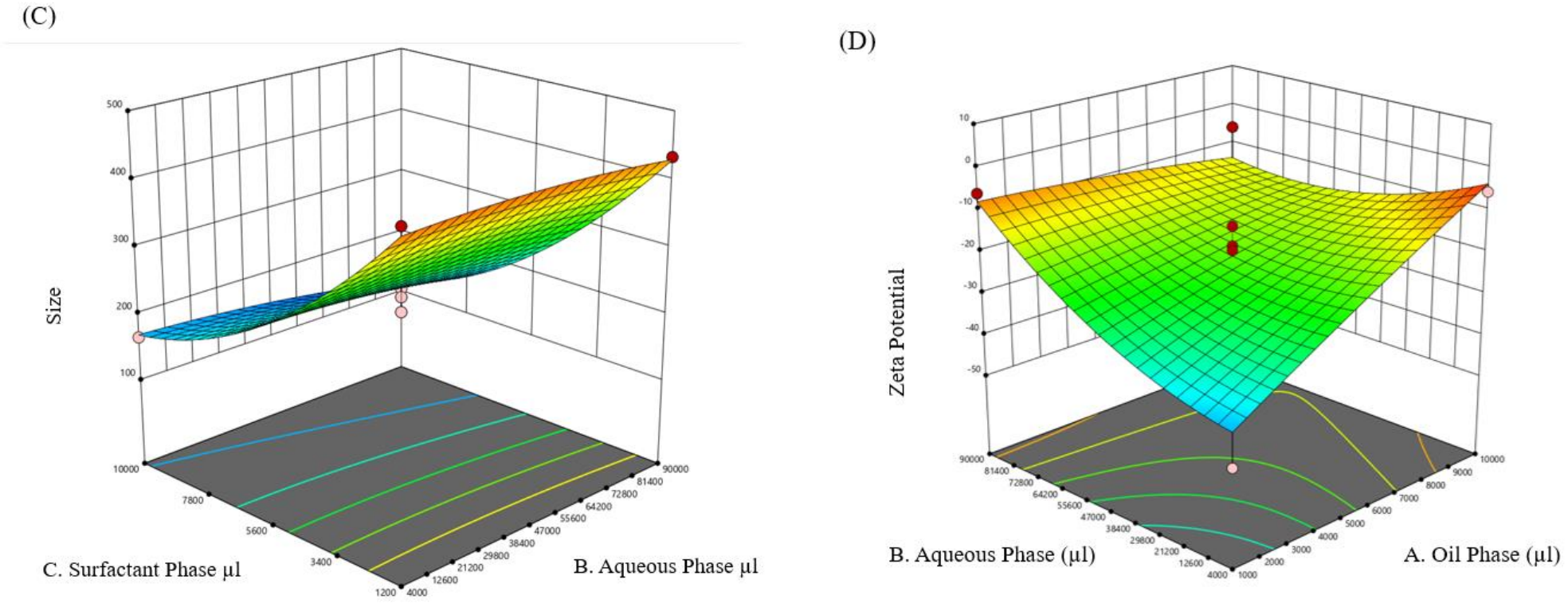
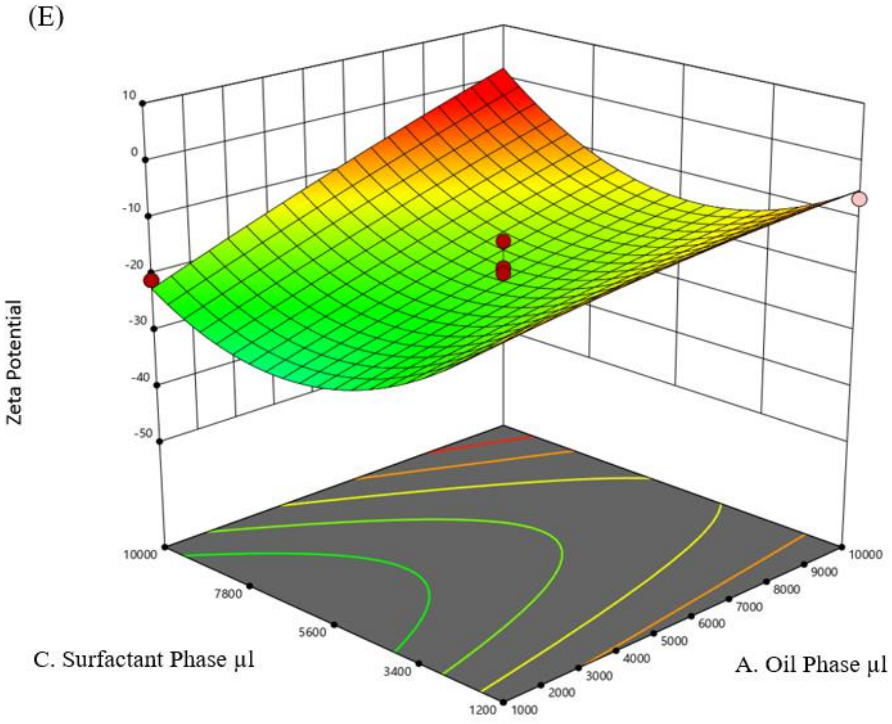
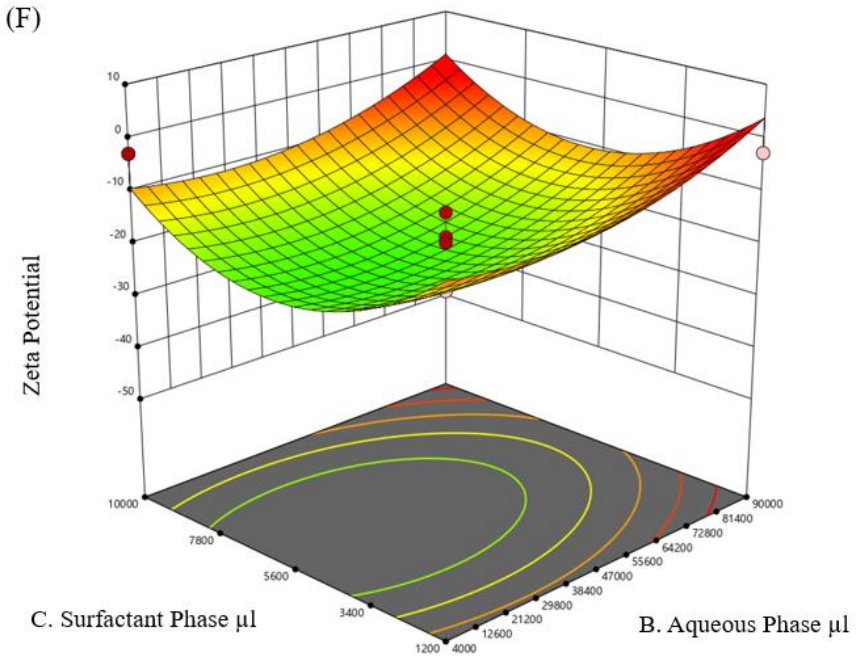


Figure 21 Response surface plot for the effect of (D) Aqueous phase and Oil phase, (E)Surfactant and oil phase, (F) Aqueous and surfactant phase on Zeta Potential.


(E)
Zeta Potential
10
0
-10
-20
-30
-40
-50
10000
7800
5600
3400
1200
C. Surfactant Phase µl
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
A. Oil Phase µl


(F)
Zeta Potential
10
0
-10
-20
-30
-40
-50
10000
7800
5600
3400
1200
C. Surfactant Phase µl
4000
12600
21200
29800
38400
47000
55600
64200
72800
81400
90000
B. Aqueous Phase µl

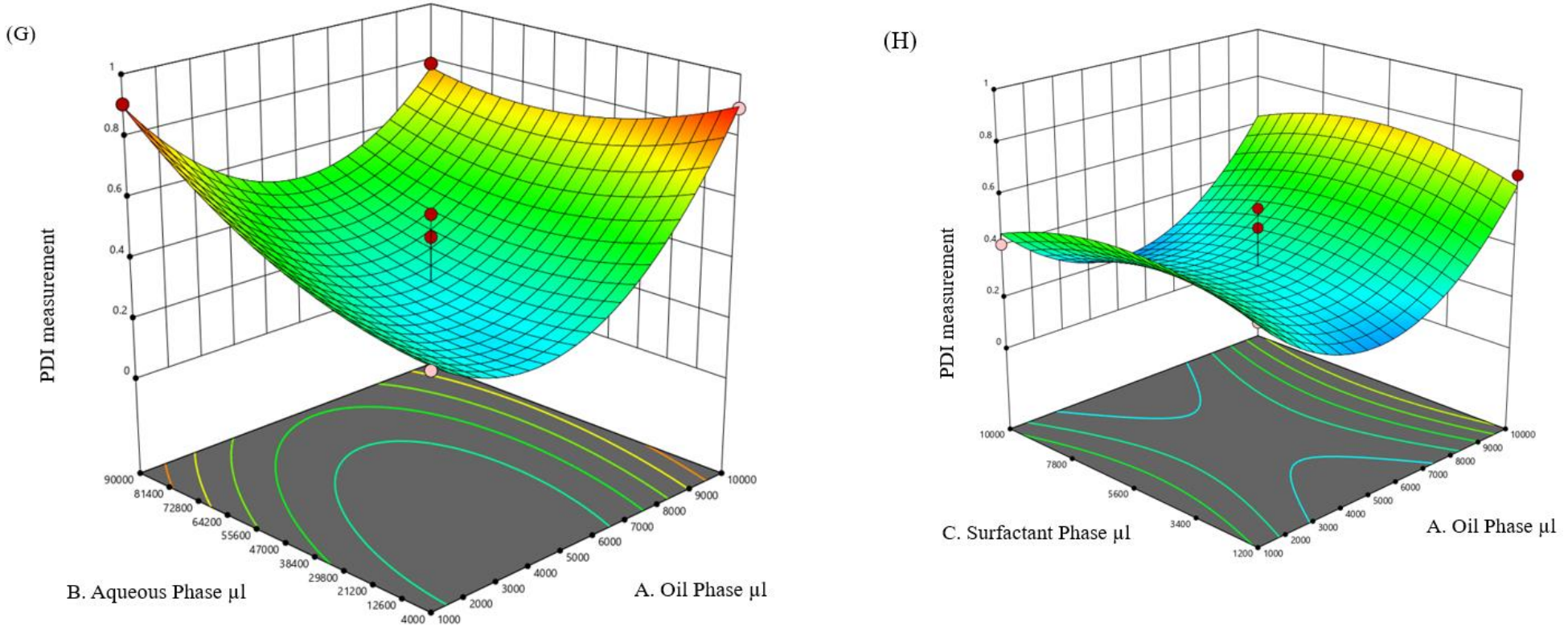


Figure 22 Response surface plot for the effect of (G) Aqueous phase and Oil phase, (H)Surfactant and oil phase, (I) Aqueous and surfactant phase on PDI measurement.

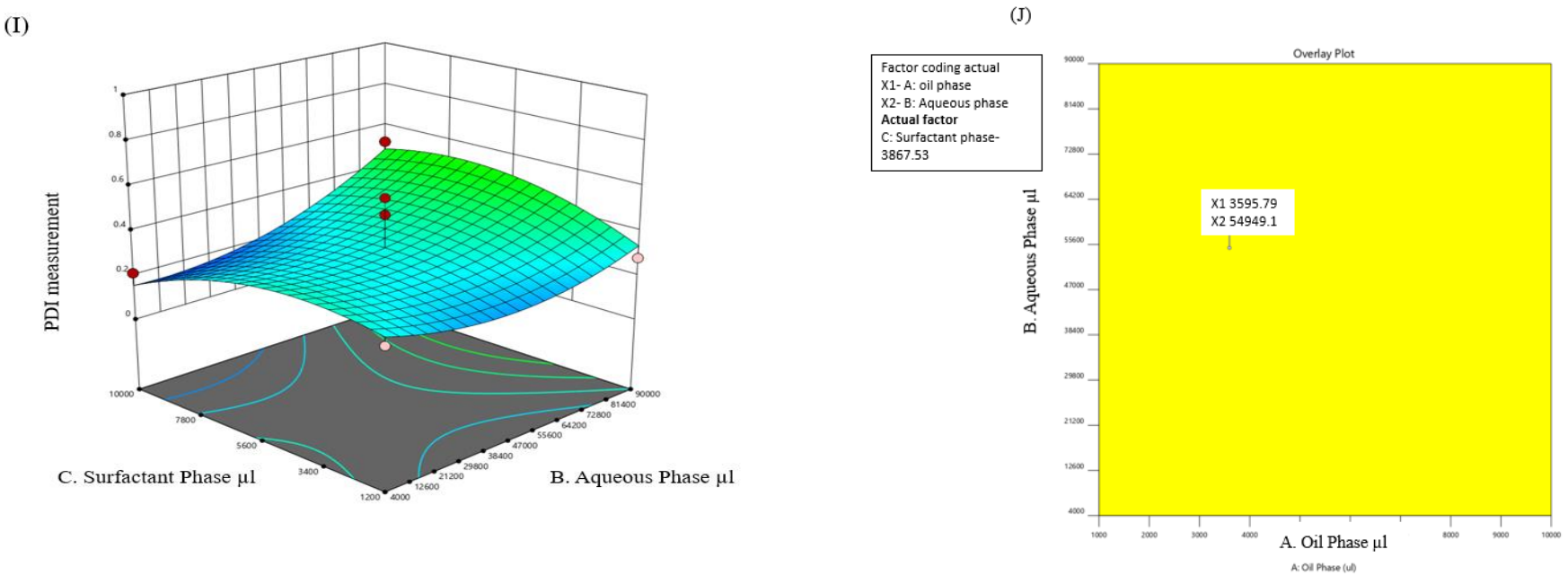


Figure 23 Overlay plot presenting optimized parameters (J) Aqueous phase and oil phase for obtaining Polyherbomineral Nanoemulsion

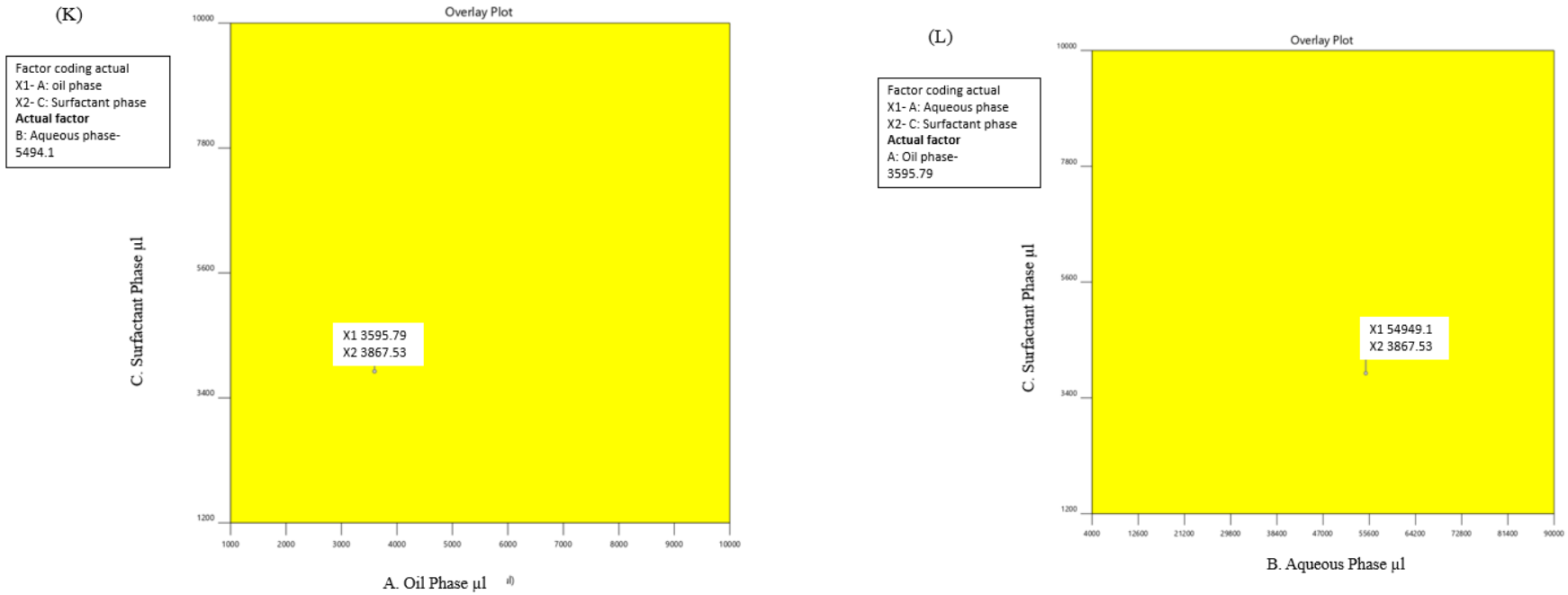


Figure 24 Overlay plot presenting optimized parameters (k) surfactant and oil phase (L) Surfactant and Aqueous phase for obtaining Polyherbomineral Nanoemulsion

Table39 Optimised formulation presenting predicted and observed values of Size, PDI & Zeta Potential in optimised formulation

1 of 100 Responses	Predicted Mean	Observed	Std Dev	95% CI low for mean	95% CI High for mean
Size	319.58	326.46	28.188	290.165	349.004
Zeta Potential	-18.90	-18.43	7.720	-26.963	-10.84
PDI measurement	0.360	0.307	0.147	0.206	0.514

5.14 Characterisation of the CAACET loaded nanoemulsion

5.14.1 Physical parameters

5.15.1.1 Colour, odour and touch upon physical observations the was canary yellow in colour with a faint characteristic odour and smooth in touch. The overall appearance of the nanoemulsion was good.

5.14.1.2 Refractive Index, pH and viscosity when the light rays pass from one medium to another it bends towards the normal. This bend was recorded as the refractive index After measuring Refractive index (RI) of each sample of nanoemulsion was separately measured three times, and mean value and standard deviation (SD) are calculated. As 1.39 ±0.5. pH was found to be 5.8 ±0.5 and viscosity measured at 25°C was found to be 98.5mpa.s. RI is also a tool that identifies uniformity of the nanoemulsion and the chemical collaboration of drug and the excipients used. pH of the PHM nanoemulsion expresses that it is suitable to be applied on skin. And viscosity determines the concentration of the solution So, from above observations it was was concluded that the nanoemulsion formed is one phase and uniform, appropriate to be applied on skin and not very concentrated in nature. (Kotta *et al.*, 2015)

5.14.1.3 Spreadability Spreadability of topical drugs are an important factor to determine the adherence of the drug. Optimised nanoemulsion was calculated by slip and drag method by the formula mentioned in section 3.12.1.3 Spreadability of formulated nanoemulsion was found to be 18.75gm/cm/sec. for topical drugs Spreadability significantly denotes its easy topical

application. Poor Spreadability eventually hampers the duration of drug residence on the skin and hence its bioavailability (Jogpal *et al.*, 2022)

5.14.2 Chemical parameters

5.14.2.1 Droplet size and Polydispersity index Developed nanoemulsion was characterized on the basis of particle size, PDI and droplet size of the particles. Particle size of the nanoemulsion without drug was found to be 156.7, 289.6nm, 281.1 nm and 353.3nm and the size in the optimized formulation was observed to be within the range between 164.4nm to 453.3nm. the size of particles in the PHM nanoemulsion is indicative of the nanometer range. (figure 25a)According to the earlier mentioned authorized definitions(Administration, 2011) (European Commission, 2009) nanoparticles upto 500nm(Shaker *et al.*, 2019) (Majeed *et al.*, 2019)size prepared in the nanocarrier colloidal system can actively entrap and transfer the active pharmaceutical ingredients to required site and thus assists in transdermal and percutaneous drug absorption (Alshawwa *et al.*, 2022) (Elmowafy, 2021) . thus the prepared nanoemulsion can penetrate through the outer layer of skin to promote burn wound healing. small particle size imparts physical stability to this colloidal system. The increase in shear stress by homogenisation and sonication leads to particle disintegration and increase in their amplitude thus decreasing particle size. This prevents aggregation of the particles (Van Zyl *et al.*, 2004) (Amaral *et al.*, 2006). (Yang *et al.*, 2021). Surfactants also prevent agglomeration process thus helping to reduce the particle size. Landfester et al demonstrated in his work that when ultrasonication time is kept constant the particle size is dependent solely on the amount of surfactants covering the area of droplets (Landfester *et al.*, 1999). Value of PDI in numerical oscillates between 0.0 (uniformly distributed particle size) to 1.0 (with multiple sized particles). The homogeneous distribution of the droplets indicated by the polydispersity index signifies the importance of surfactant mix to efficiently form the interface between the two immiscible liquids. This stabilises the prepared nanoemulsion figure 26

5.14.2.2 Zeta potential it denotes the charge on surface of the small droplets and the negative sign indicates repulsion forces between these charged droplets that reduces the coalescence effect on these droplets thus indicating the stability of the nanoemulsion system. zeta potential of the developed nanoemulsion was found to be -23.4. fatty acids present in the oil phase of the nanoemulsion system provides the surface charge on droplets. Figure.25 b

5.14.2.3 copper assay copper assay was found to be 0.10%w/w

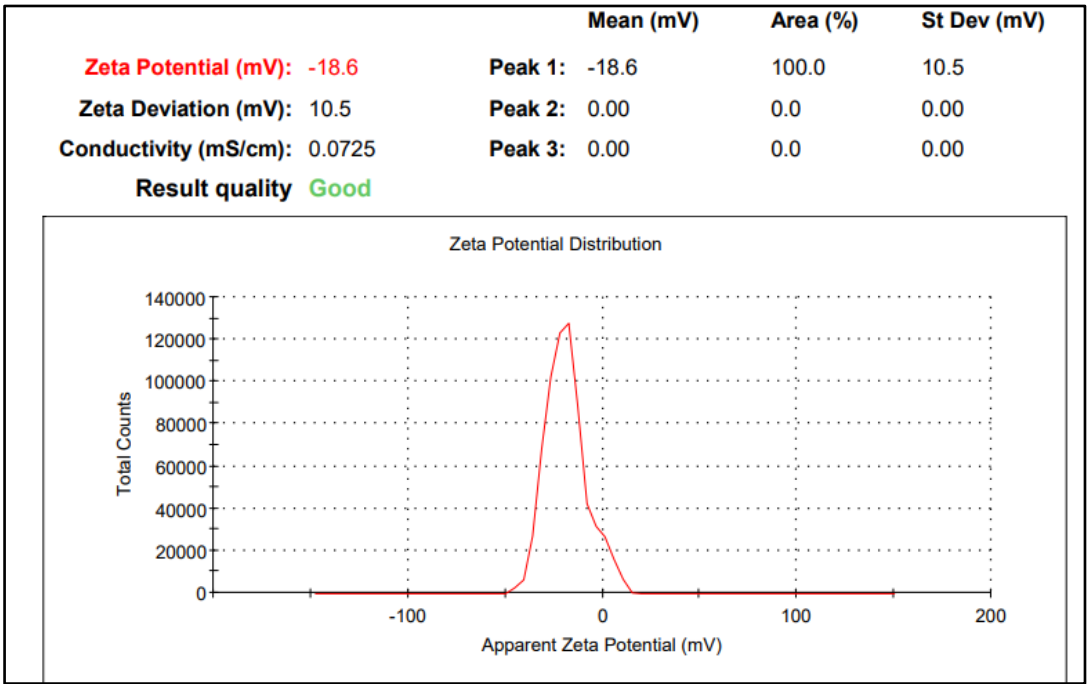


Figure 25 a Zeta potential of the optimized formulation

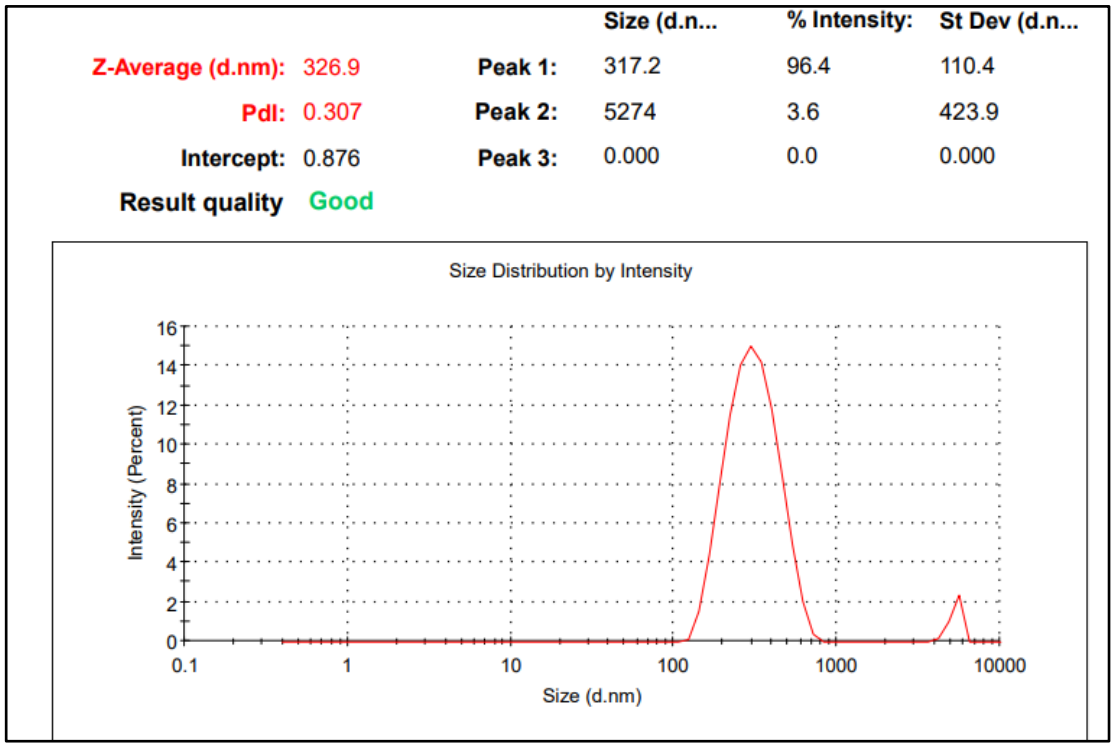


Figure 25 b Size of the optimized formulation

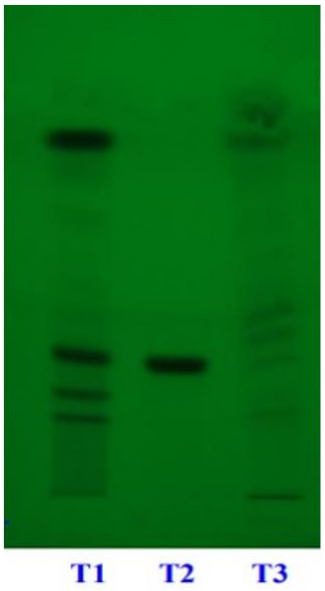
5.15 HPTLC Study for Aqueous and Ethanolic Herbal Extracts and prepared formulation with Standard HPTLC studies were performed in comparison with the phytochemical standards of respective herbal drugs extracts and with the prepared PHMN. The phytochemical standards used were curcumin in CE, Rutin and Berberine in AE, Gallic acid and Rutin in CAE extract and Oleic acid in NSO and EYO. The extracts were analysed at various suitable wavelengths.

Quantification of curcumin in curcuma longa extract was carried out @ 425nm. 10.32% yield was measured in curcuma longa ethanolic extract and 0.53% yield was measured in the PHMN. 2D and 3D densitogram of curcumin in curcuma longa extract, standard curcumin and in PHMN is shown in figure 28-a,b,c and figure 33 respectively. The curcumin was separated at Rf value of 0.21. Quantification of Rutin in AE and CAE was carried out @ 254 nm. Phytochemical standard of Rutin along with Rutin present in AE and CAE and prepared formulation was separated at Rf value of 0.61. yield of 0.40% and 0.62% was measured in AE and CAE respectively. Yield of Rutin in PHMN was measured to be 0.03%. 2D and 3D densitograms of Rutin in CAE and AE, standard Rutin and in PHMN is shown in figure 29-a,b,c,d and figure 34. Quantification of Gallic acid was performed @ 278nm in CAE, standard and in PHMN. Phytochemical standard of Gallic acid along with gallic acid present in CAE and the prepared formulation was separated at Rf value of 0.31. 0.72% yield was measured in CAE and 0.042% yield was measured in PHMN. 2D and 3D densitogram of Gallic acid in CAE, standard and PHMN is shown in figure 30-a,b,c and figure 35 respectively. Quantification of berberine was performed in Aloe vera extract@366nm. phytochemical standard of Berberine along with Berberine present in Aloe vera extract and the prepared formulation was separated at a Rf value of 0.26. The yield of 0.18% was measured in Aloe vera extract and 0.073% was the measured yield in PHMN. The details are shown in figure 31-a,b,c and figure 36. Quantification of oleic acid was performed @540nm. Oleic acid separated at Rf value of 0.20. Yield of oleic acid in NSO and EYO was found to be 78.3% and 64.19% respectively. yield of oleic acid in PHMN was found to be 4.39 %. The detail fingerprinting of oleic acid along with 2D & 3D densitograms obtained at various wavelengths are presented in figure 32 a,b,c,d and figure 38 respectively. -. Rf values of herbal extracts and the prepared formulation (PHMN) are mentioned in the table given in table 40. Photo documentation of the HPTLC study of herbal extracts and oils with phytochemicals is presented in figure 27.

Table 40 HPTLC Analysis of optimized formulation under UV at different wavelengths

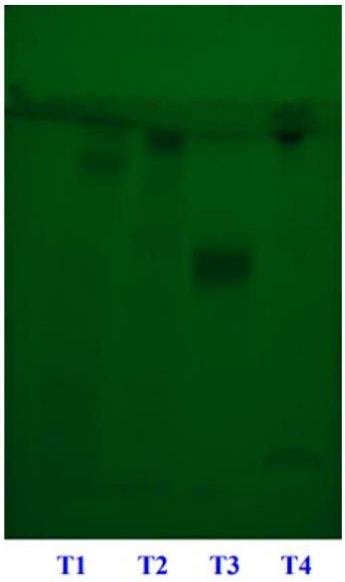
Herbal extracts	Herbal extracts			Formulation		
	Track ID	Max position on Rf	Quantity of marker compound (%age)	Track ID	Max position on Rf	Quantity of marker compound (%age)
Curcumin @425nm						
Curcuma longa extract	T1 T1 T1	0.21 0.27 **0.35**	**10.32**	T3 T3 **T3** T3	0.21 0.27 **0.35** 0.40	**0.53**
Berberine@366nm						
Aloe vera extract	T1 T1 T1 T1 T1	0.21 **0.26** 0.52 0.64 0.81	**1.8**	T3 **T3**	0.14 **0.26**	**0.73**
Rutin@257nm						
Aloe vera extract	T1 T1 T1 T1 T1 T1	0.16 0.26 0.30 0.38 **0.61** 0.86	**0.40**	T4 T4 T4 **T4**	0.30 0.38 0.40 **0.61**	**0.030**
Calotropis procera Latex extract	T2 T2 T2 T2 T2 T2 T2	0.12 0.16 0.18 0.26 **0.61** 0.79 0.79	**0.62**			
Gallic acid@278nm						
Calotropis procera latex extract	T1 T1 T1 T1	**0.31** 0.44 0.54 0.71	**0.72**	**T3** T3 T3 T3 T3 T3 T3 T3	**0.31** 0.44 0.54 0.59 0.61 0.71 0.78 0.84	**0.042**
Oleic acid@540nm						
Egg Yolk oil Neem seed oil	T1 T3 T3	**0.20** 0.13 **0.20**	**64.19** **78.30**	T2 T2	0.20 0.28	4.39

Curcumin @425nm



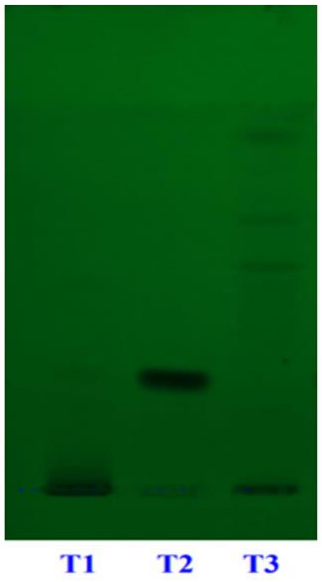
T1- Curcuma Longa extract
T2- Std Curcumin
T3- formulation

Rutin @278nm



T1-Aloe vera extract
T2-Calotropis Procera Latex extract
T2- Std Rutin
T3- formulation

Gallic acid @257nm



T1- Calotropis Procera Latex extract
T2- Std Gallic acid
T3- formulation

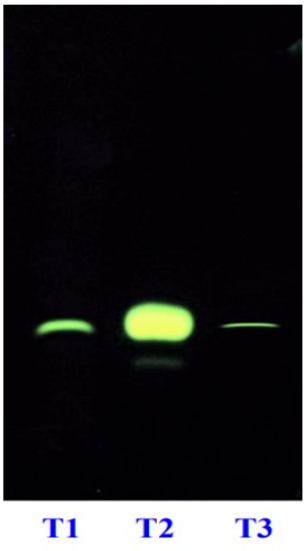
Oleic acid @540nm

T1- Egg Yolk oil
T2- Std Oleic acid
T3- Neem Seed oil

Oleic acid @540 nm

T1- Oleic acid standard
T2- Formulation

Berberine @366nm



T1- Aloe vera extract
T2- Std Berberine
T3- formulation

Figure 27 Photo documentation of the HPTLC study of herbal extracts and oils with phytochemical standards

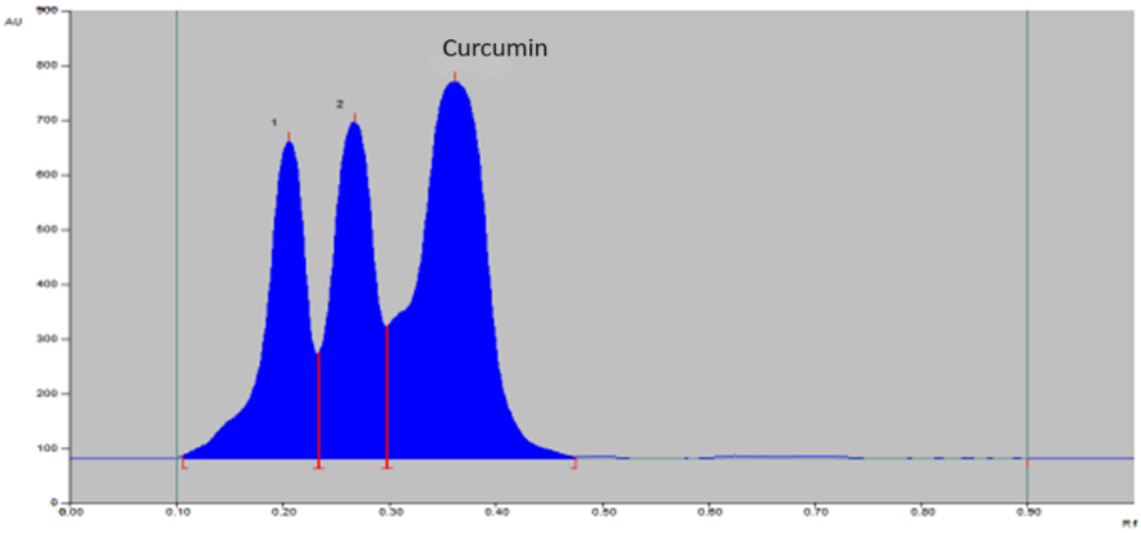


Figure 28 a 2D Chromatogram of Ethanolic extract of Curcuma Longa

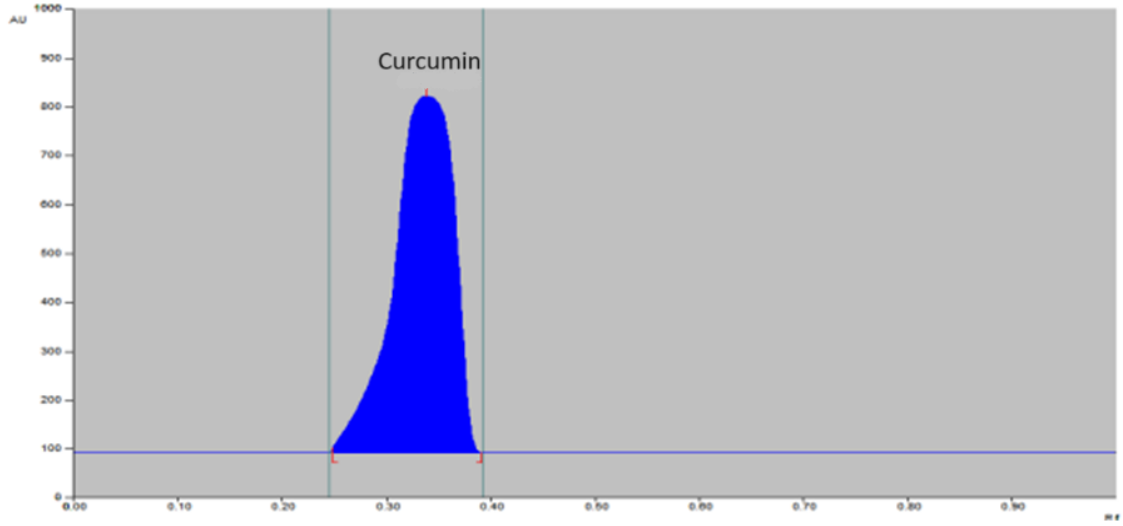


Figure 28 b Chromatogram of Standard Curcumin

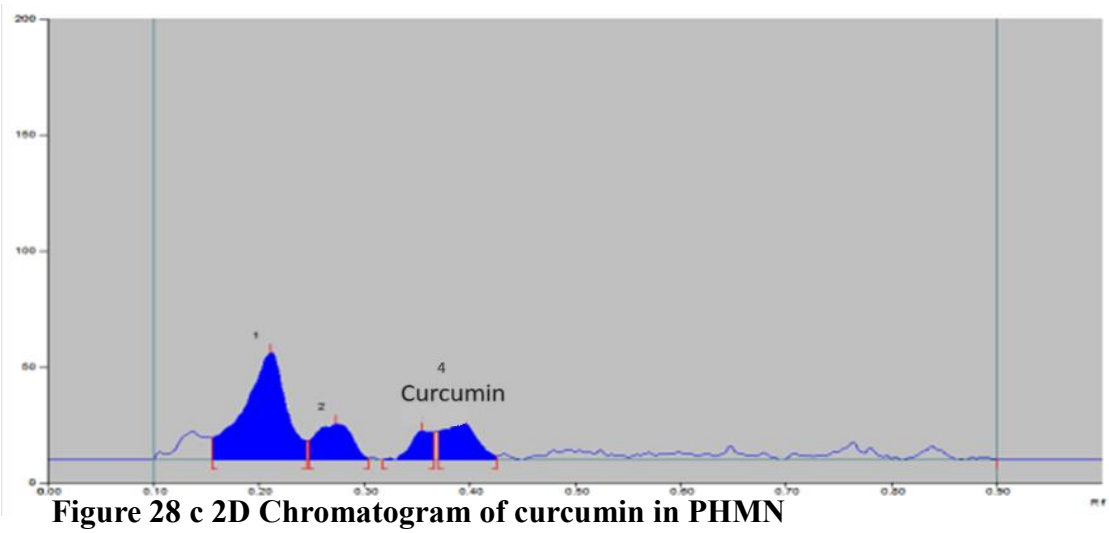


Figure 28 c 2D Chromatogram of curcumin in PHMN

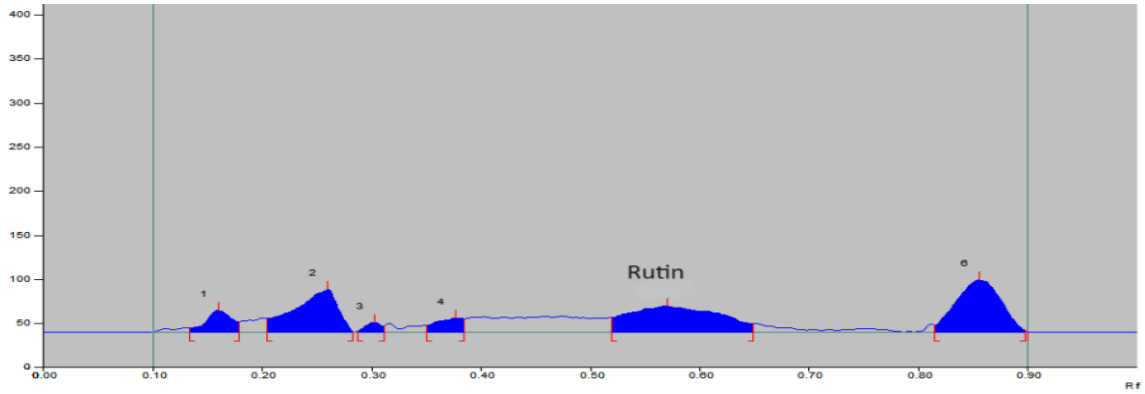


Figure 29 a 2D Chromatogram of Rutin in Aqueous extract of Aloe Vera

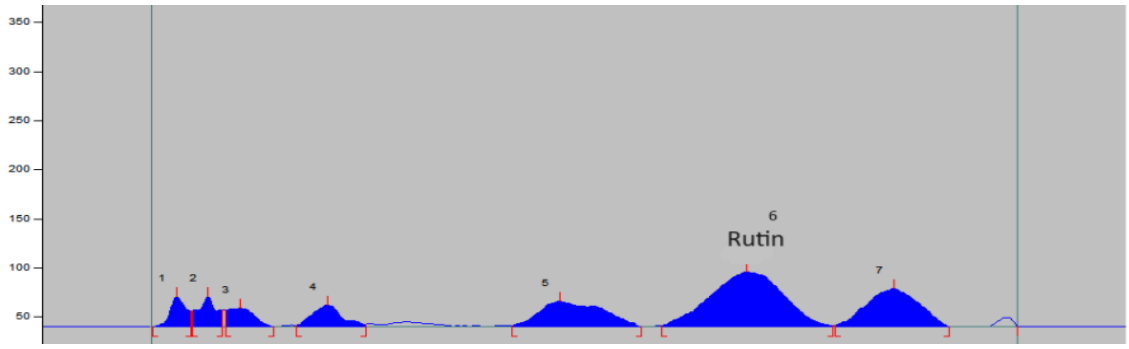


Figure 29 b 2D Chromatogram of Rutin in Aqueous extract of Calotropis Procera

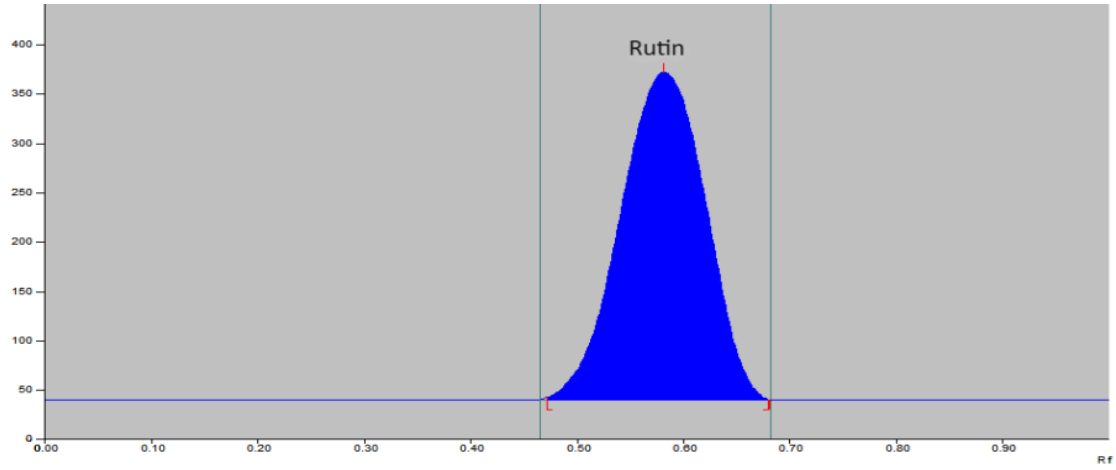


Figure 29 c 2D Chromatogram of Standard Rutin

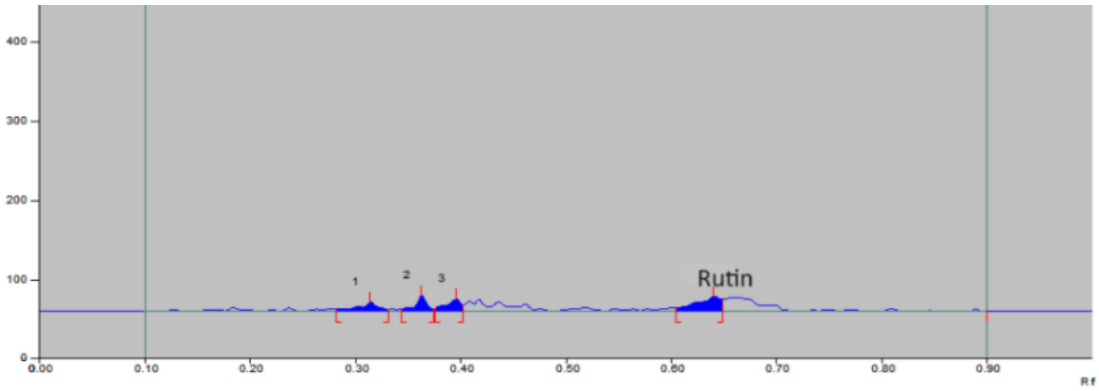


Figure 29 d 2D Chromatogram of Rutin in PHMN

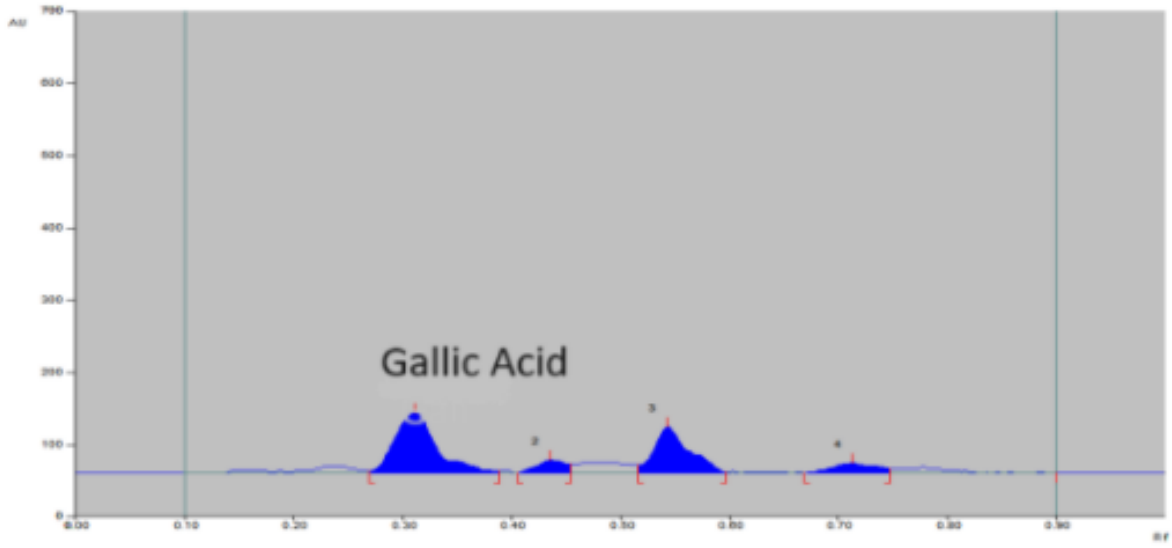


Figure 30a 2D Chromatogram of Gallic Acid in Aqueous extract of Calotropis Procera Latex

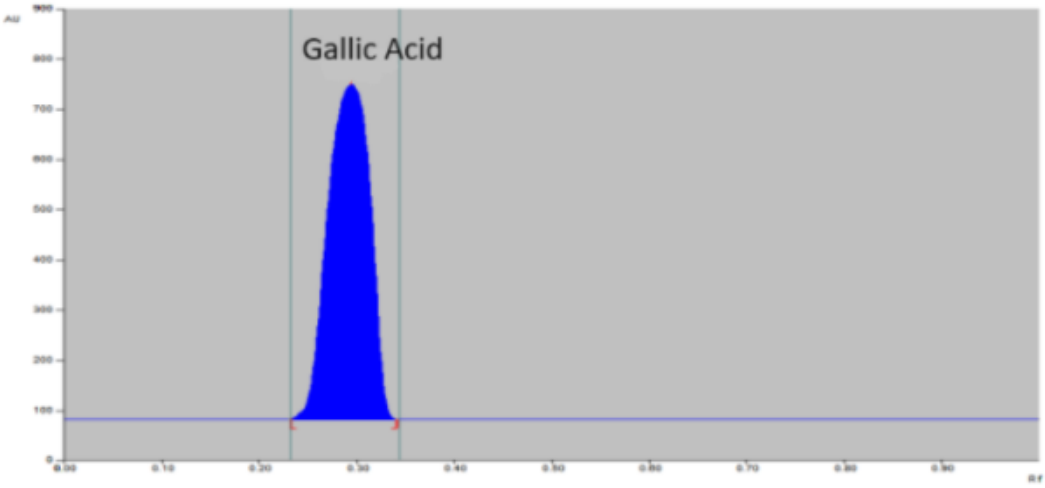


Figure 30b 2D Chromatogram of Standard Gallic Acid

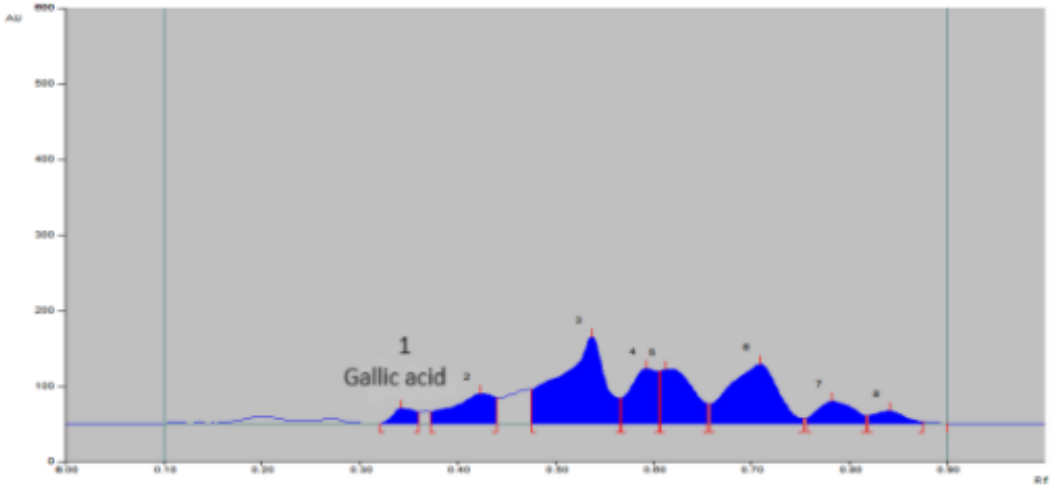


Figure 30 c 2D Chromatogram of Gallic Acid in PHMN

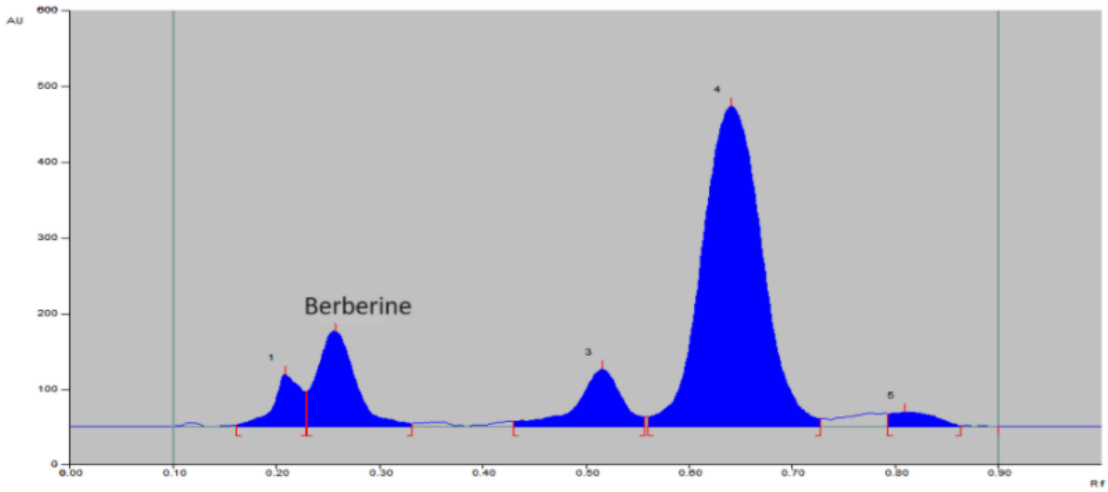


Figure 31a 2D Chromatogram of Berberine in Aloe Vera

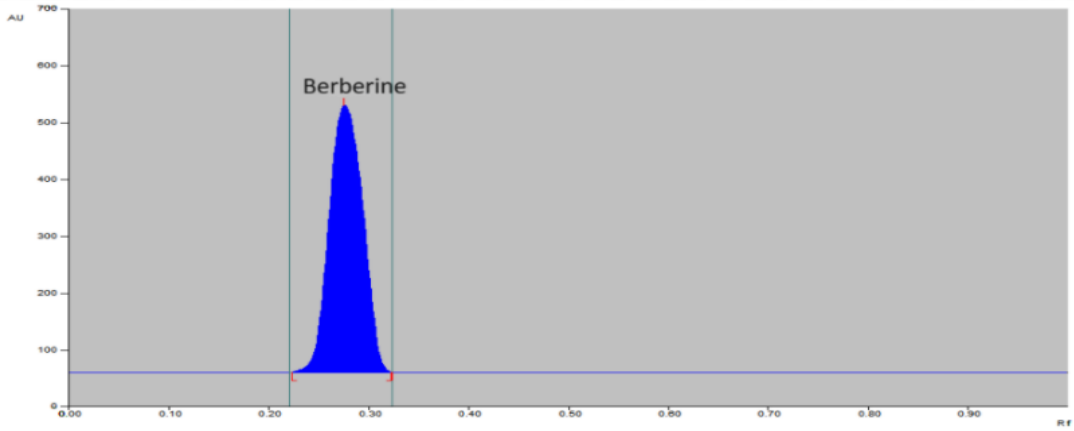


Figure 31b 2D Chromatogram of Standard Berberine

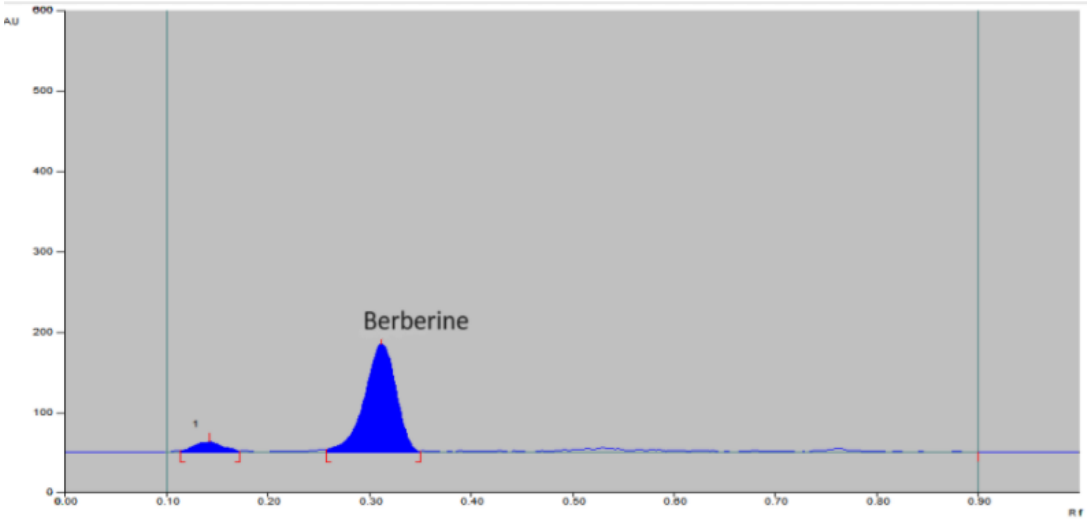


Figure 31c 2D Chromatogram of Berberine in PHMN

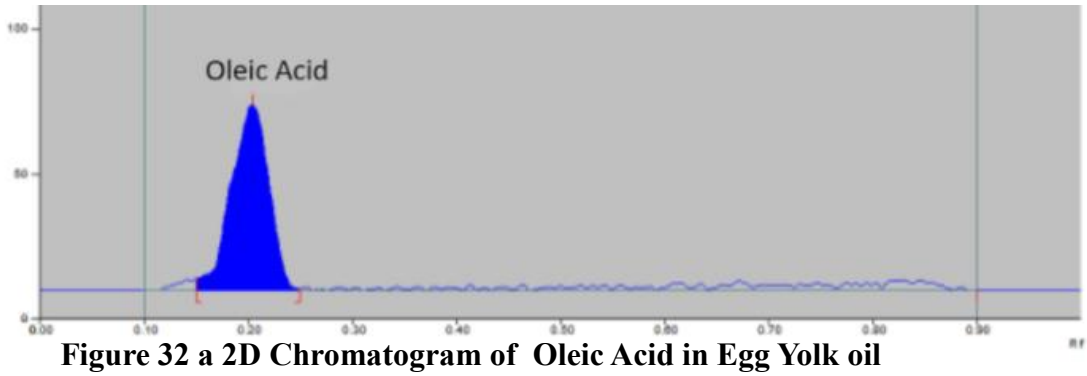


Figure 32 a 2D Chromatogram of Oleic Acid in Egg Yolk oil

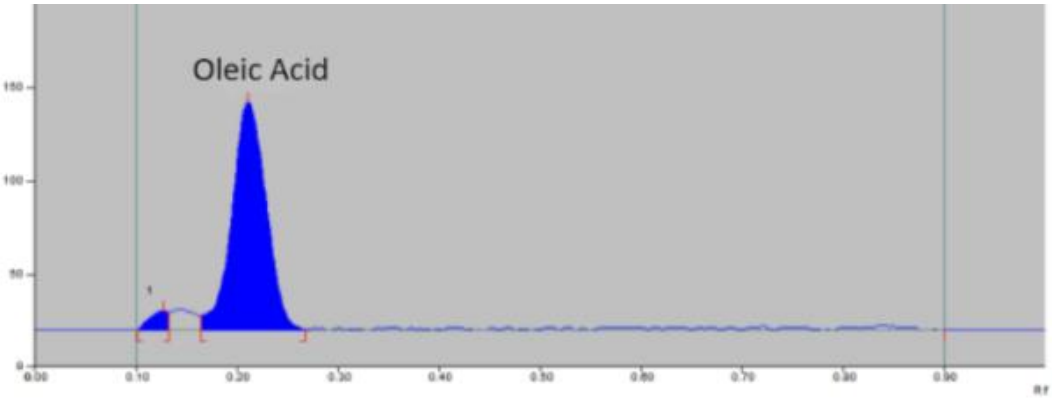


Figure 32 b 2D Chromatogram of Oleic Acid in Neem Seed oil

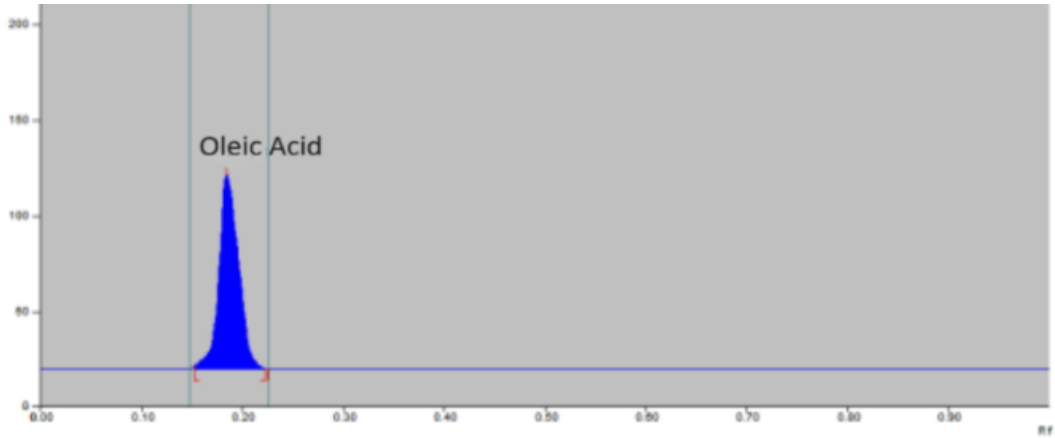


Figure 32 c 2D Chromatogram of Standard Oleic Acid

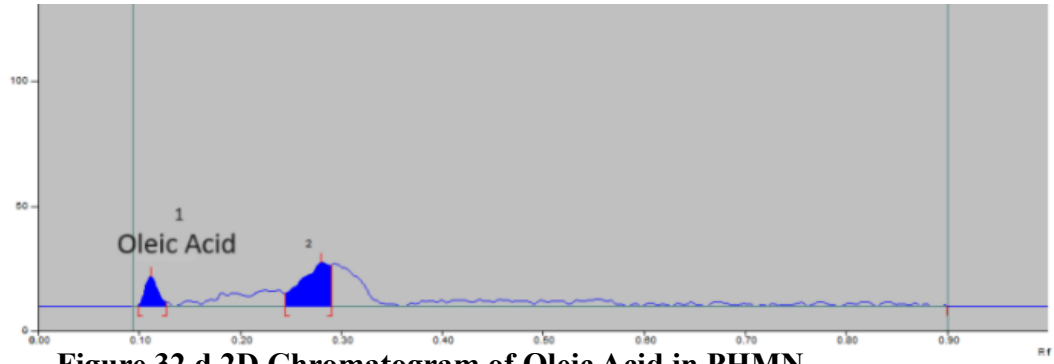


Figure 32 d 2D Chromatogram of Oleic Acid in PHMN

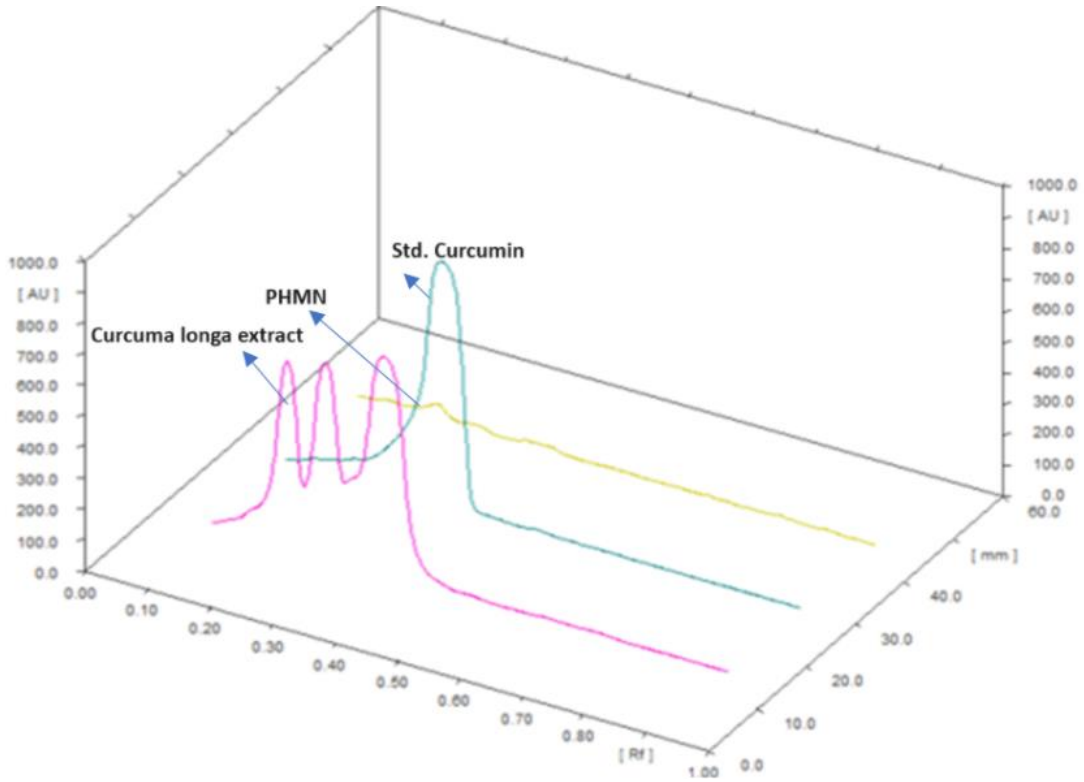


Figure 33 3D- Graphs of standard curcumin with prepared curcuma longa extract and PHMN

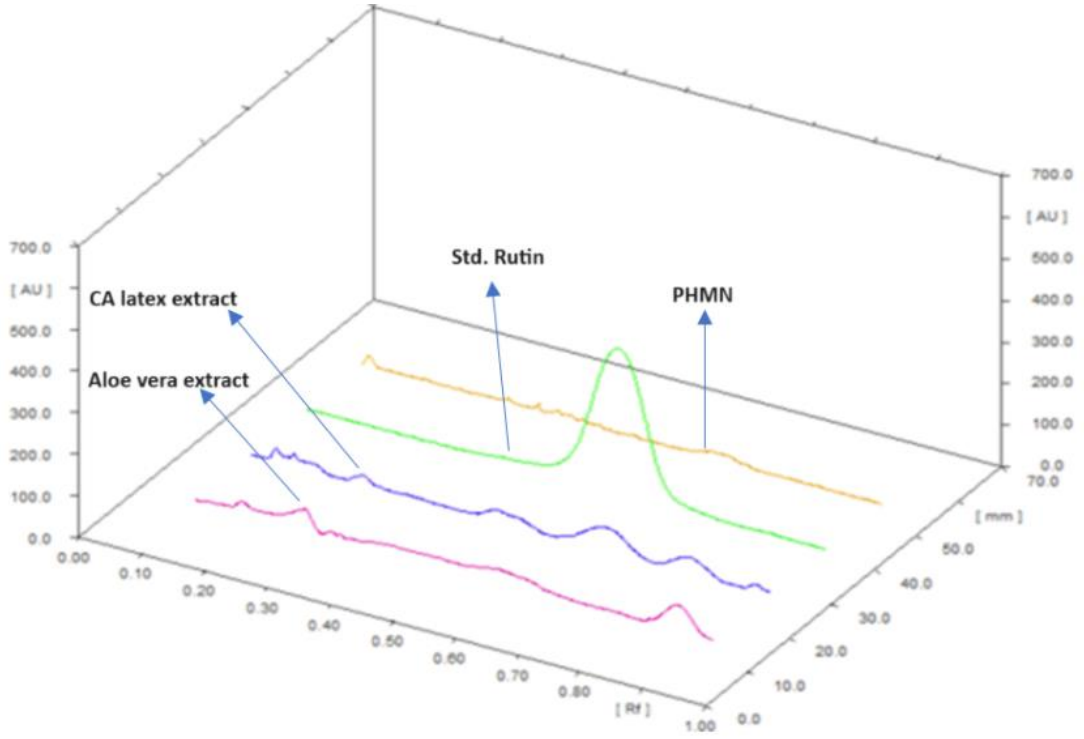


Figure 34 3D- Graphs of standard Rutin with prepared Aloe vera and calotropis procera latex extract and PHMN

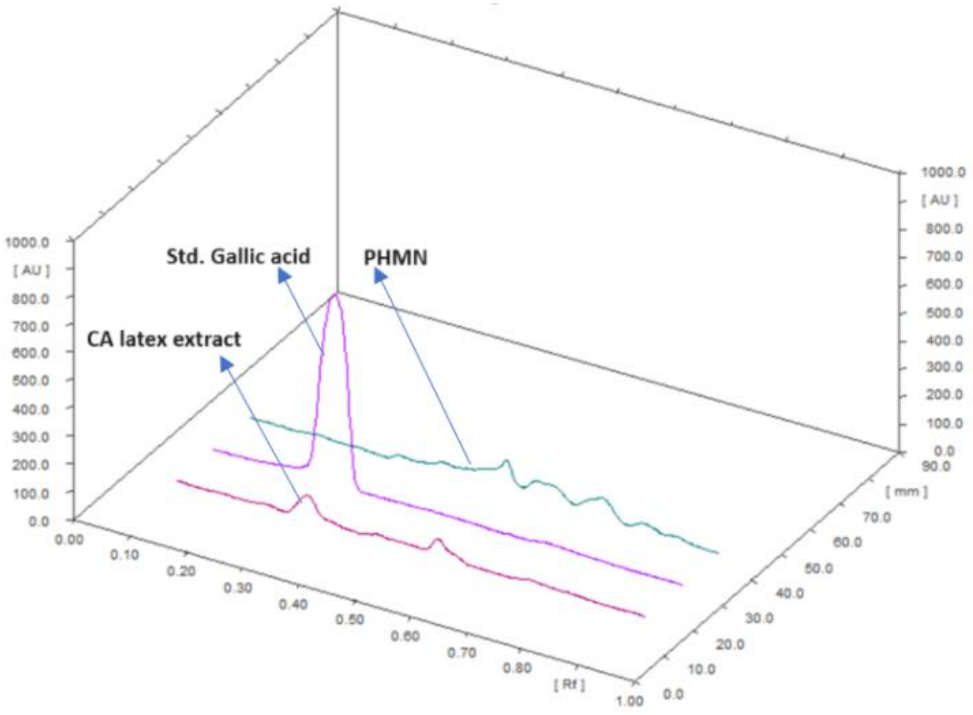


Figure 35 3D- graphs of standard Gallic acid with prepared calotropis procera latex extract and PHMN

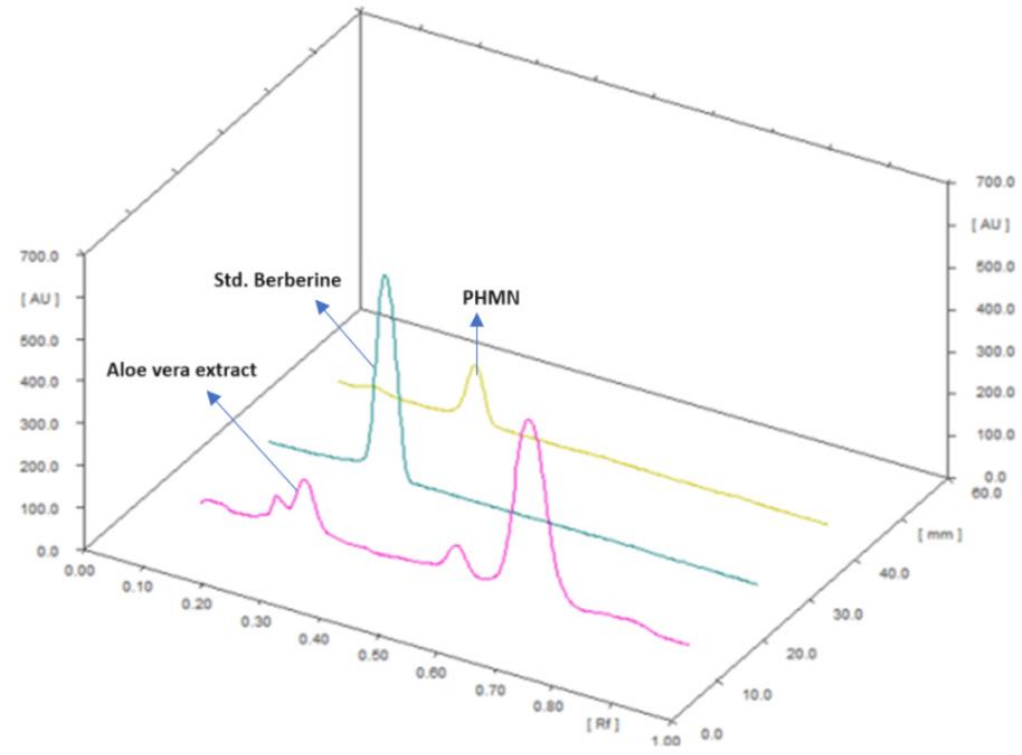


Figure 36 3D- graphs of standard Berberine with prepared Aloe vera extract and PHMN

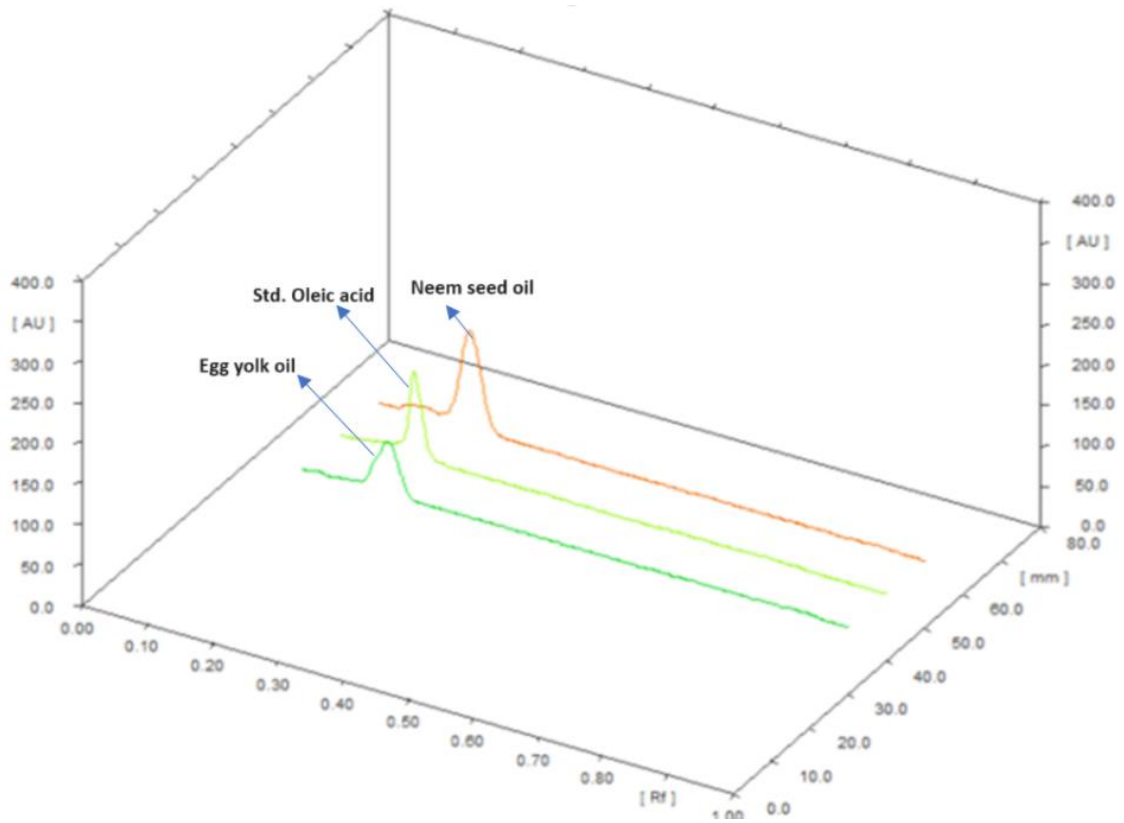


Figure 37 3D- graphs of standard Oleic acid with Neem seed oil and Egg Yolk oil

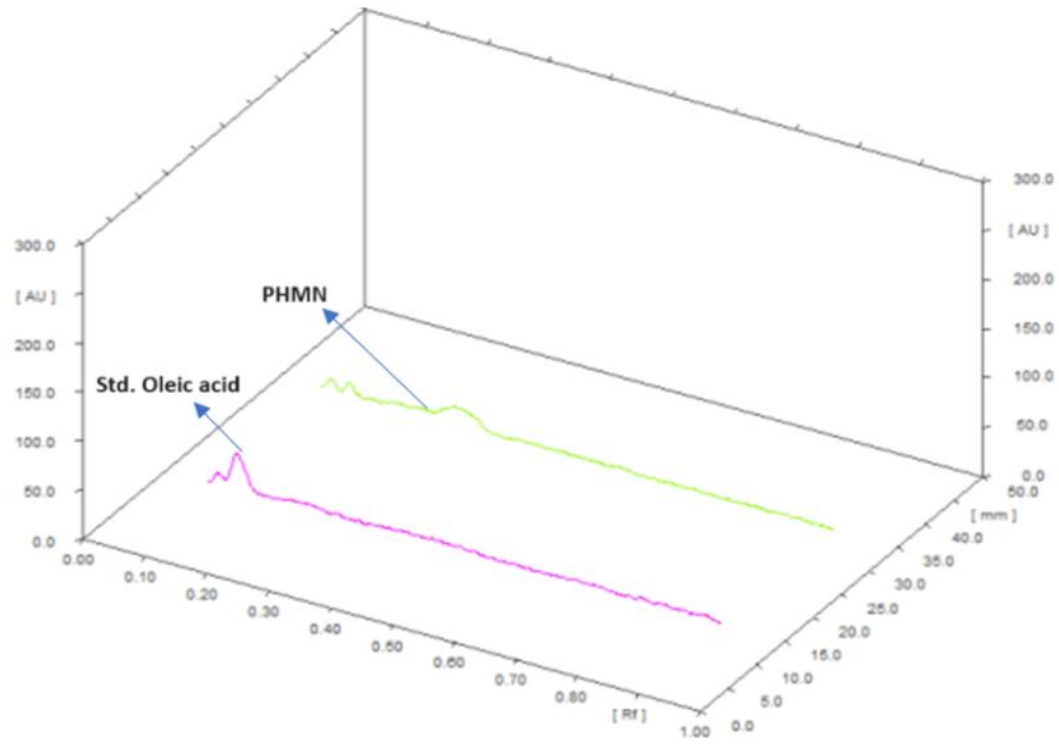


Figure 38 3D- graphs of Standard Oleic acid with PHMN

5.16 Stability studies

The stability of the optimized PHMN was studied for 6-month period. Color, odor, and viscosity were checked for physical parameters. size, zeta potential, pH and copper assay was checked for chemical parameters. All parameters were recorded at initial stage and after 3month and 6month duration period at varying relative humidity percentage. The results of samples stored in triplicates were carried out at varying relative humidity percentage at a period of 0, 3 & 6 months respectively. Physical and chemical parameters analyzed were found almost similar to their fresh samples. The results are tabulated in Table 41. The optimized formulation does not show any significant changes with the initial data. Hence, the prepared Optimized PHMN was considered stable at 40±0.2^{0}C/75±0.5%

Table 41 physical and chemical parameters for stability studies

STABILITY PARAMETERS			
Parameters	**0 Month**	**3 Month**	**6 Month**
Appearance (colour, odour and texture)	Canary yellow in colour, faint odour and uniform appearance	No significant changes	No significant changes
Size (nm)	326.46 ±15.45	350.3 ± 19.62	354.2±19.49
zeta potential (mv)	-18.43±1.75	-25.1±1.62	-25.6±1.15
PDI	0.307±0.03	0.402±0.03	0.411±0.01
Ph	5.85±0.04	5.85±0.04	5.9±0.1
Viscosity (cP)	104.9±2.51	110.9±1.52	110.1±0.66
Copper assay(%age)	0.1±0.001	0.10±0.001	0.099±0.001

5.17 In-Vitro studies Antimicrobial studies

Agar well diffusion method adapted to calculate the Zone of inhibition as a measure of the antibacterial activity after 18hours. Average values with standard deviations are listed in table 42. Zone of inhibition is an area of antibacterial growth where bacterial colonies are inhibited to grow. It is generally used to measure the vulnerability of bacteria

5.17.1 MTT assay HUVEC cells was cultured on 96 well plates. Treatment was given with PHM nanoemulsion at a concentration of 100 to 1000µg/ml at 3 different time intervals of 24hrs, 48hrs and 72hr. 10µL of 5mg/ml of MTT was applied to these cells for 3 hrs at 37^{0}C.

Table 42 Zone of inhibition of PHMN during anti-microbial study

Sr. No.	E.coli (mm)	S. Aureus (mm)	P. Aeruginosa (mm)
1	18	14	15
2	15	15	17
3	15	15	17
Average	16 ±1.73	14.6 ±0.57	16.3 ±1.15

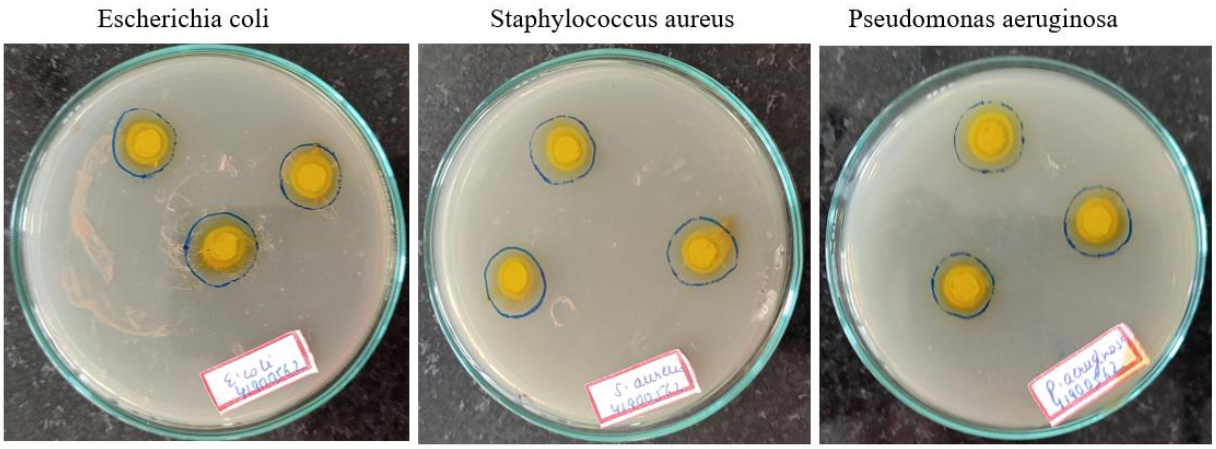


Figure 39 Antimicrobial study of PHMN for zone of inhibition

Absorbance of the coloured solution is taken. Dark colour of the solution denotes the mitochondrial activity by the viable cells. The data represents that the drug is non-toxic upto a concentration of 1000µg/ml. No toxicity was observed with increasing concentration of polyherbomineral Nanoemulsion till 72hrs. The results are presented graphically at different time intervals in figure 40

5.17.2 Wound Healing Assay The formulation was tested at a concentration of 100µg/ml and 200µg/ml. Efficient wound healing is seen from polyherbomineral Nanoemulsion at a concentration of 200µg/ml. Graph was plotted with percentage of wound closure against control, 100µg/ml and 200µg/ml of the PHM nanoemulsion. About 50% wound closure is seen at a concentration of 200µg/ml in 24 hrs (figure45). keratinocytes mobilisation can be an important factor for closing the wound gap.(Talekar *et al.*, 2017) Photographs representative of comparison between control, and different doses of PHM nanoemulsion are given in figure 43

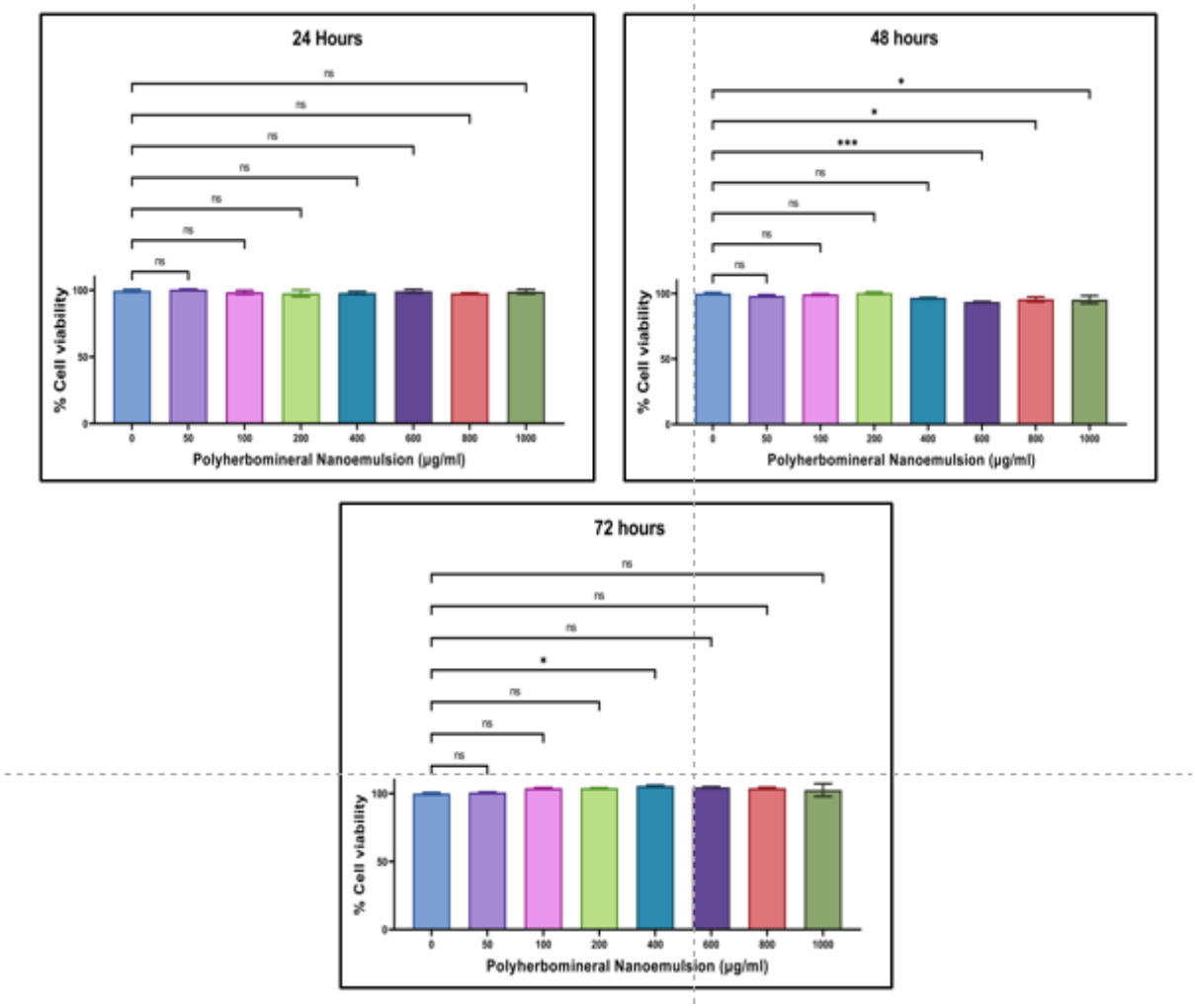


Figure 40 Graphical presentation of cytotoxicity studies from MTT assay

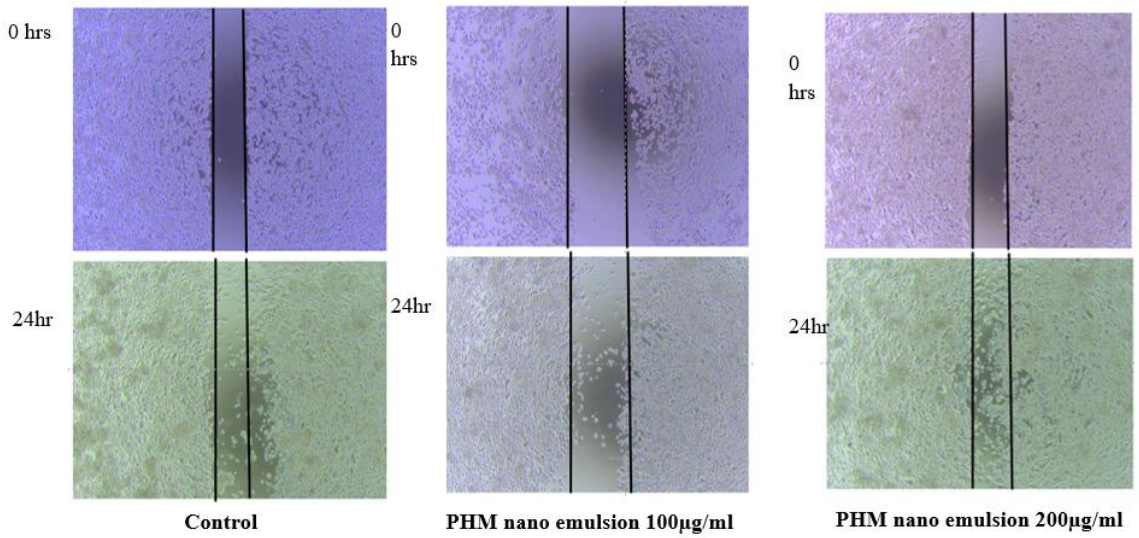


Figure 41 Representation of results from Wound Healing assay

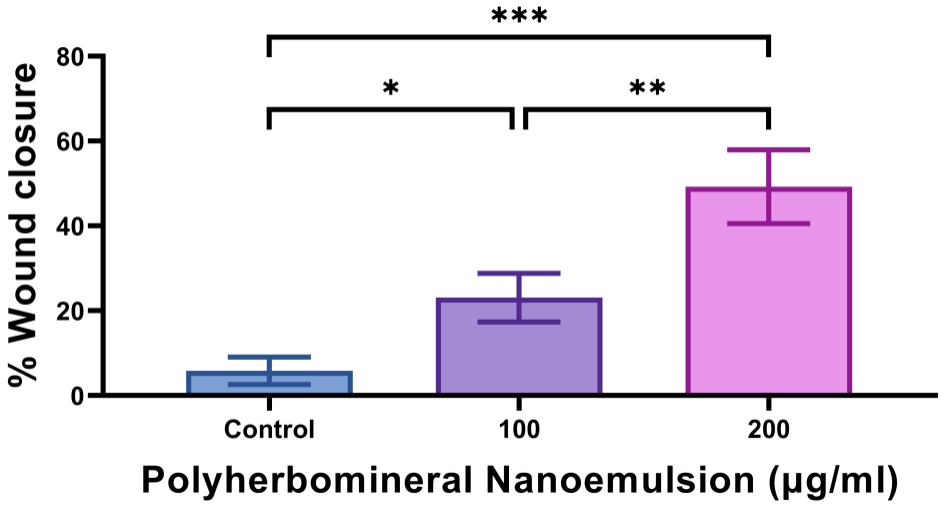
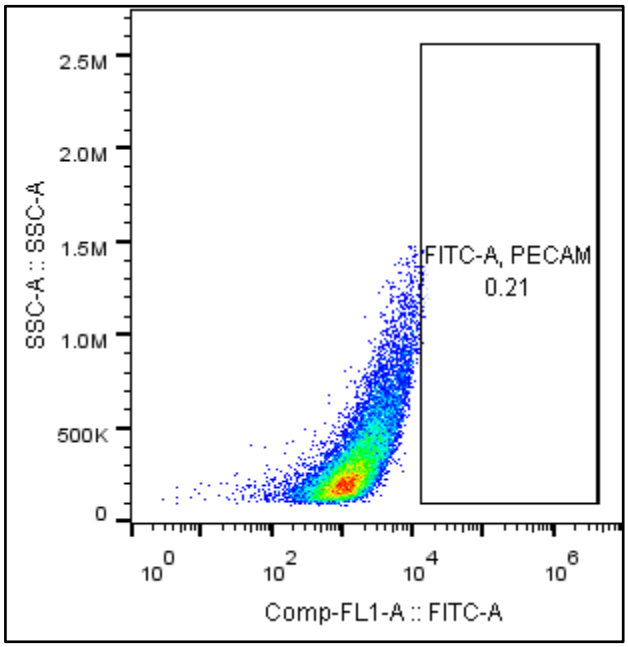


Figure 42 Graphical representation of wound healing from PHMN

5.17.3 VEGF-A & PECAM Assay HUVEC cells cultured in 12 well plates after treatment with PHM nanoemulsion at a concentration of 100 and 200µg/ml for 24 hrs. The angiogenesis activity of polyherbal formulation was evaluated by CAM assay. The quantification of VEGF-A and PECAM -1 was carried out in control and treated HUVEC cells by ELISA after treatment with anti- PECAM and anti-VEGF antibodies. The PECAM-1 expression is seen to be upregulated. It denotes marked angiogenesis marker at a concentration of 200µg/ml. this denotes angiogenesis activity of PHM nanoemulsion. (Figure 43 and table 44)

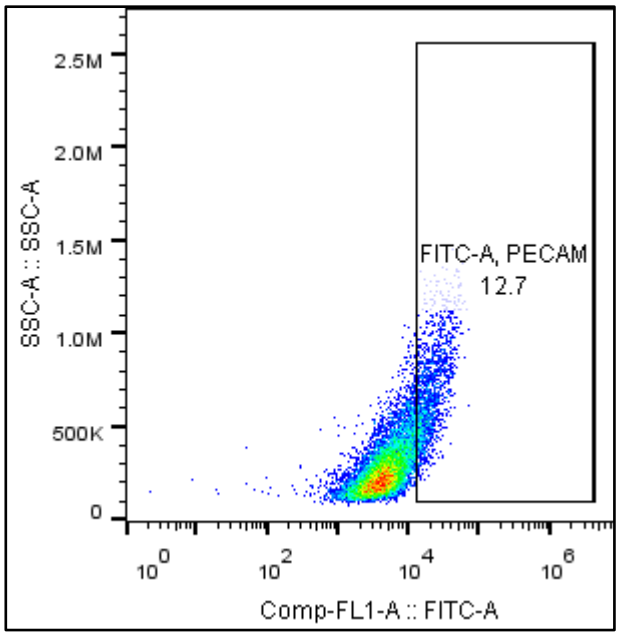
PECAM and VEGF assay denotes intracellular protein expression. The angiogenesis markers are seen on the blood vessels, leukocytes, platelets and between the junction of endothelial cells. It plays a role as an efficient signal for platelet functions, blood clot formation, leukocyte migration through blood vessels and formation of new blood vessels development (Woodfin, Voisin and Nourshargh, 2007). VEGF- A denotes the production and endurance of endothelial cells at lead to formation of new blood vessels(Arutyunyan *et al.*, 2016) figure 44 and table 45 represents the values in VEGF-A assays.



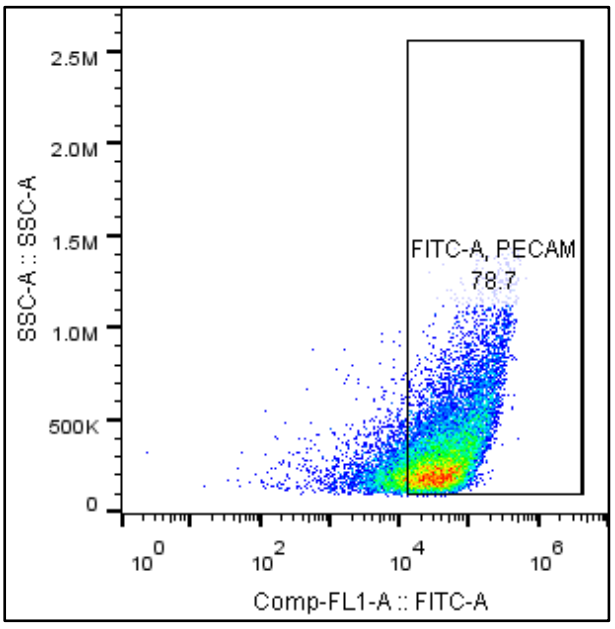
PECAM-1 assay in control

Table 43 Quantification of PECAM assay

Samples	MFI
Control	**1958**
PHMN (100 µg/ml)	**6842**
PHMN (200 µg/ml)	**51966**

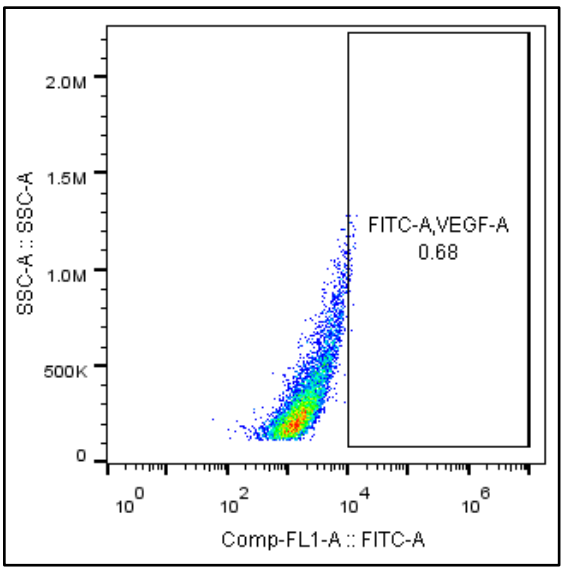


PECAM-1 assay at a concentration of 100µg/ml



PECAM-1 assay at a concentration of 200µg/ml

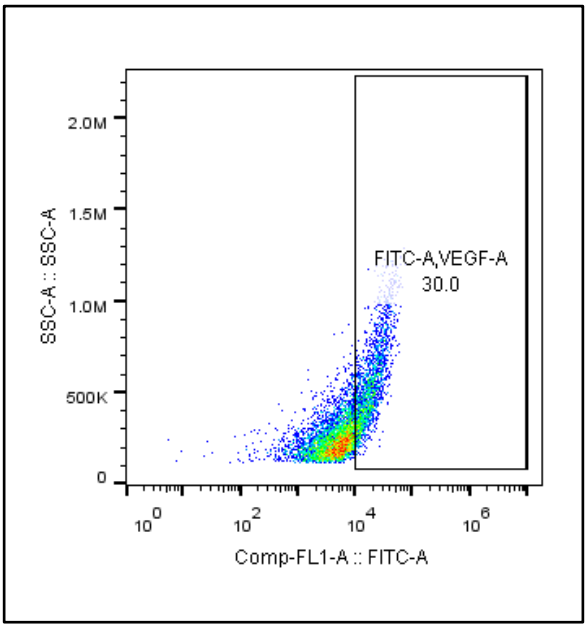
Figure 43 Representation of results from PECAM assay



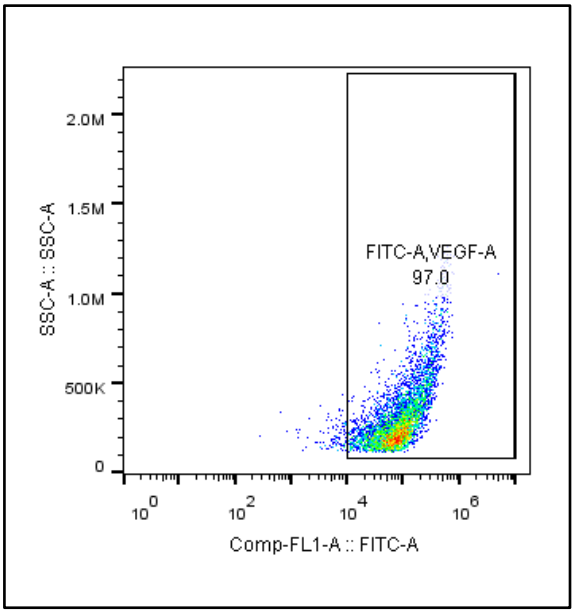
VEGF-A assay in control

Table 44 Quantification of VEGF-A assay

Samples	MFI
Control	**2174**
PHMN (100 µg/ml)	**9032**
PHMN (200 µg/ml)	**113964**



VEGF-A assay at a concentration of 100µg/ml



VEGF-A assay at a concentration of 200µg/ml

Figure 44 VEGF-A assay during in-vitro study of PHMN

5.17.4 ELISA HUVEC cells was treated with 100 µg/mL and 200 µg/mL of PHM nanoemulsion. The absorbance was taken at 450nm. VEGF-A and PECAM-1 values were quantified with these absorbance values using the calibration curve shown below (figure 45) Cellular VEGF-A and PECAM assay Values with dose of 200µg/ml is significant with p value <0.001 compared to PHM nanoemulsion at a dose of 100µg/ml and control.

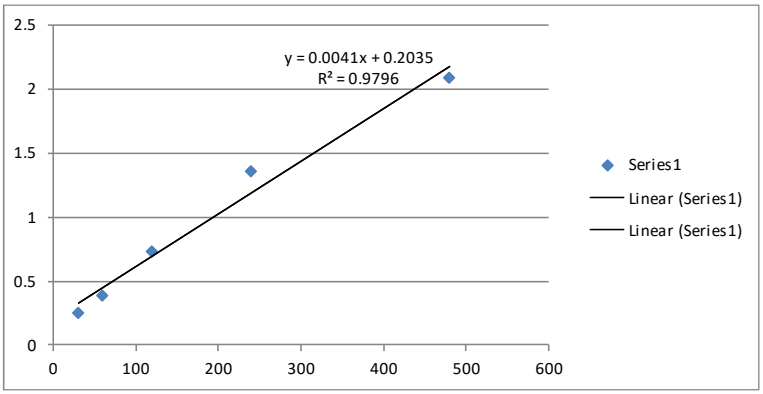
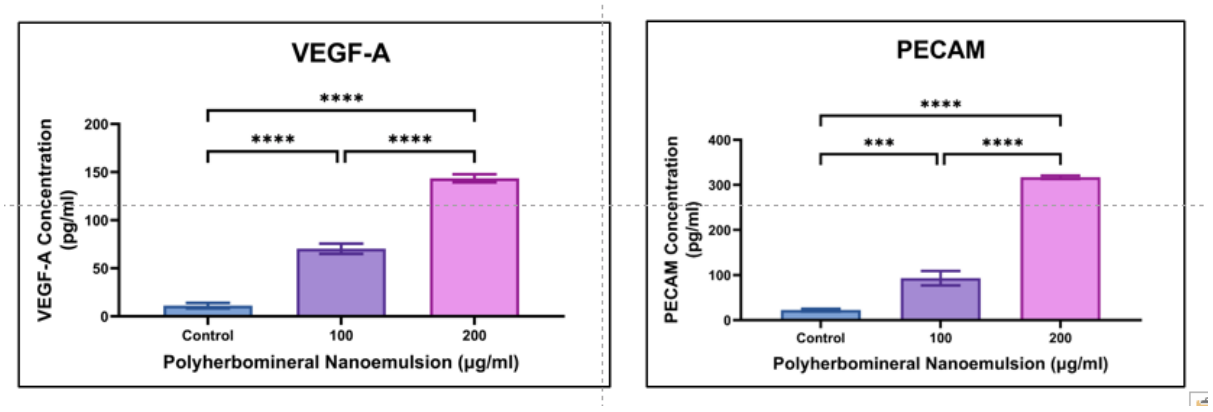


Figure 45 Calibration curve for ELISA assay during in vitro study



Pvalue < 0.05,** P value <0.01, ***P value <0.001

Figure 46 Graphical representation of VEGF-A and PECAM assay during in vitro study of PHMN

5.18 In vivo study of Burn wound healing

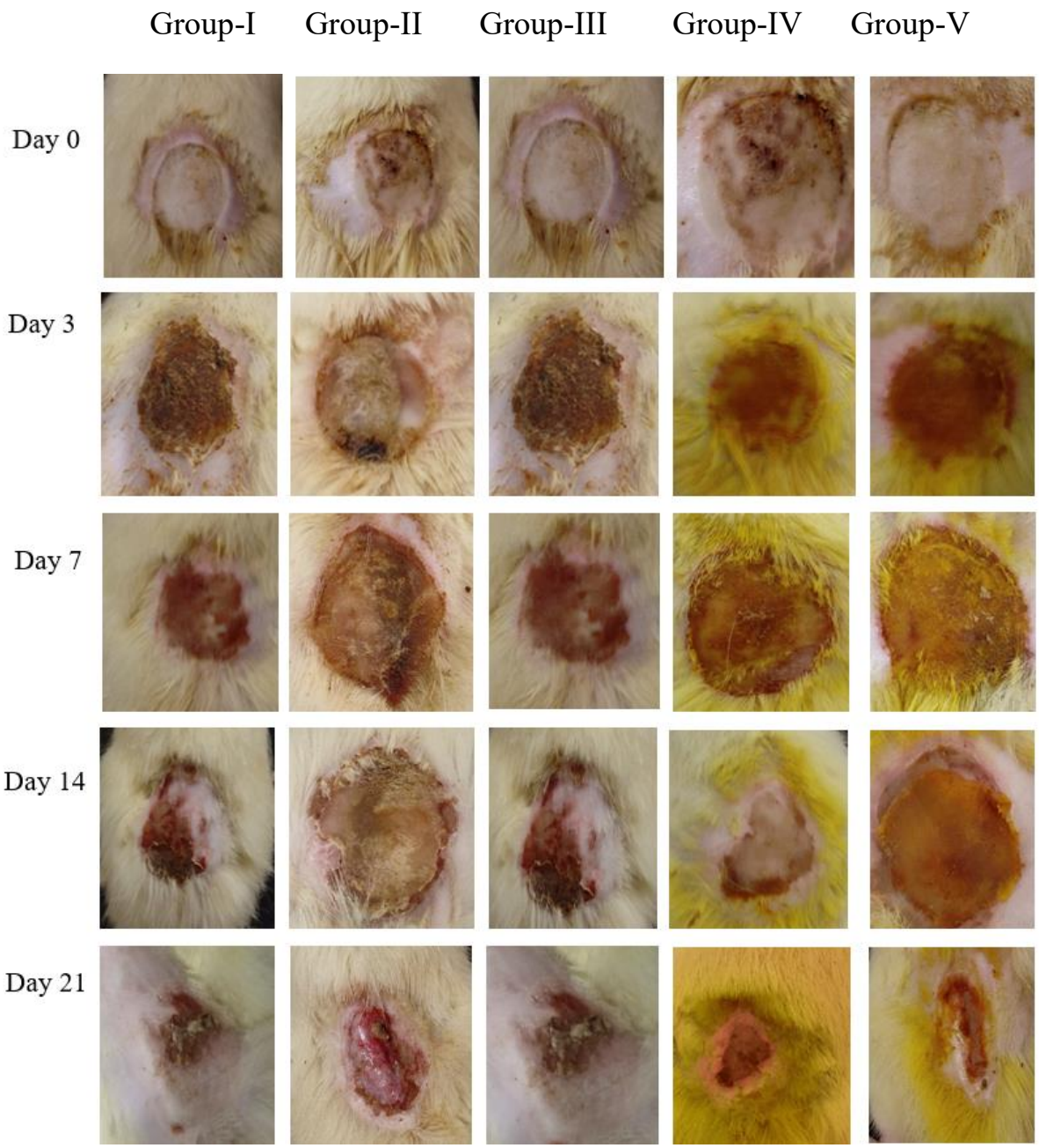


Figure 47 Burn wound healing activity in induced burn wound animal model

Pharmacological activity was assessed by Treatment on induced (2cmx2cm) second degree burn wound in Wistar rats was provided with local application of prepared PHMN and of the wound diameter (table 45) and wound contraction area was recorded and is presented in the figure 47. The statistical analysis of wound contraction study and biochemical assays performed have been mentioned in further sections.

5.18.1 Wound diameter (Cm^2)

Table no.45 Mean values of wound area (cm^2) in different groups

Wound Area	Day 0	Day 3	Day 7	Day 14	Day 21
Control	8.49±0.26	7.4±0.11	7.16±0.08	6.27±0.45	5.45±0.25
Standard	8.27±0.27	6.49±0.26	5.99±0.27	4.11*±0.10	2.33*±0.44
Blank	8.33±0.20	7.70±0.49	7.18±0.20	6.24±0.22	4.94±0.44
LDF	8.4±0.23	6.65±0.25	6.21±0.19	4.69**±0.27	3.67**±0.11
HDF	8.39±0.28	6.61±0.27	6.14±0.35	4.32*±0.32	2.85**±0.43

Values are expressed as mean (cm^2) ±SD

5.18.2 Wound contraction study

Table no.46 Mean values of wound contraction percentage in different groups

Wound Contraction	Day 0	Day 3	Day 7	Day 14	Day 21
Control	0	12.85±2.8	5.17±4.2	12.36±6.9	15.23±2.2
Standard	0	21.08±4.3	7.7±2.9	31.17±3.8	43.22±7.06
Blank	0	10.40±1.62	5.9±2.9	13.07±3.9	20.60±8.42
LDF	0	20.8±6.6	6.48±4.8	24.42±5.1	21.49±3.6
HDF	0	21.41±2.06	7.56±5.5	29.52±5.4	34.29±8.44

Values are expressed as Mean %age ±SD (n=6),

Statistical wound contraction was evaluated from wound diameters in different groups (table 46 and figure 48) by one-way ANOVA. Significant wound contraction was achieved in the treatment period of 21 days. To ascertain the effect in various groups, it was followed by the Bonferroni test at *P<0.001, **P<0.01. the results explored significant wound contraction was observed with standard and HDF 14^{th} day onwards (P value <0.001) as compared to the control group. On the 14^{th} day standard formulation exhibited better wound contraction compared to the control group (P-value <0.01) but no difference was seen in comparison to LDF & HDF (P value <0.001& P value <0.01). Low-dose formulation exhibited no difference in wound contraction in comparison to control, standard, and HDF. (P value <0.001, 0.01). HDF exhibited significant wound contraction in comparison to standard formulation but no difference in comparison to LDF (Pvalue <0.001).

On the last day of treatment (day 21) standard formulation showed significant wound contraction comparable to control and LDF (P value <0.001). Statistically significant results were found with LDF in comparison with standard formulation (P value <0.001). HDF exhibited significant differences in comparison to control but no difference was seen in comparison to the standard and LDF. (P value <0.001). [table 45]

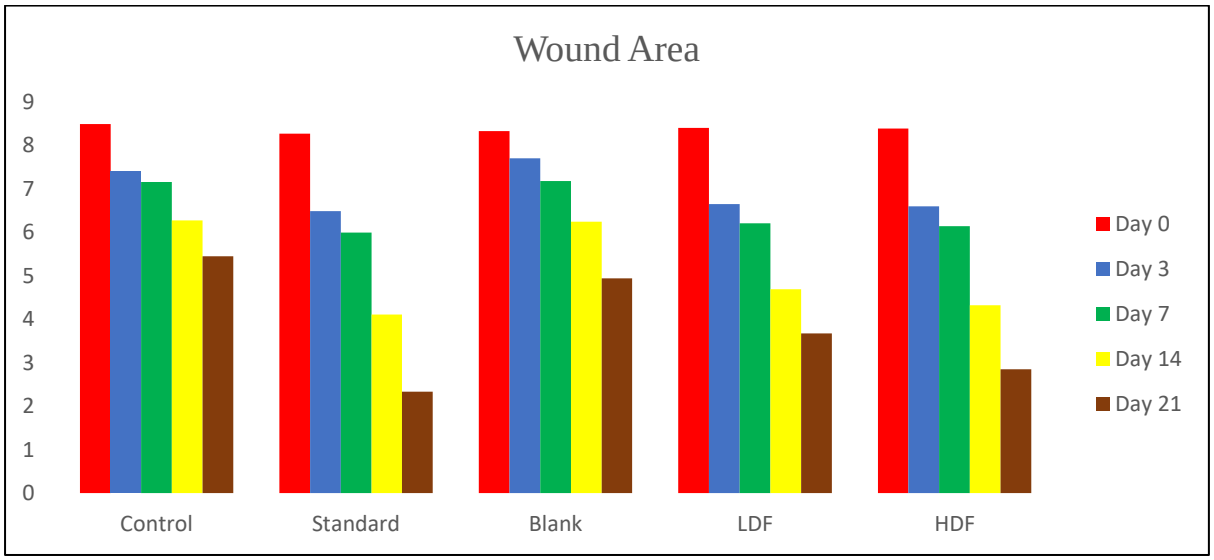


Figure 48. Representation of wound areas in different groups

wound size decreased due to the anti-inflammatory and anti-oxidant effect of PHMN in low and high doses that imparted wound healing with collagen formation activity. The presence of phytochemicals and tuttha contributes to new blood vessel formation. Silver sulphadiazine taken for standard formulation and prepared PHMN in low and high doses also showed the formation of granulation tissue in comparison to the control group which accelerates the healing process compared to the control group after the 14th day. The wound contraction data explored the wound-healing activity of the PHMN in a dose-dependent manner.

5.18.2 Cytokine analysis

5.18.2.1 Tissue necrosis factor- alpha (TNF-α)

The cytokine TNf- α was assessed from tissue homogenates. Inflammation is initiated within 24 hours of injury to lasts until complete healing. The local vascular dilatation process during inflammation brings neutrophils and macrophages to the injured site and starts the process of inflammation. These inflammatory cells release cytokines and chemokines during the process. It is the major inflammatory mediator involved in sepsis and death.(Jeschke *et al.*, 2020) The statistical ANOVA table no.47 presented below shows the F value highlights the significant

difference between different treatment groups at the level of significance 0.000 (P value < 0.001). This means that the application of PHMN significantly decreases the value of TNF-α, which is an inflammatory marker. However, the ANOVA does not depict that the decrease in inflammatory effect is pronounced in which group. To ascertain the specific effect of formulation post hoc Bonferroni test was conducted. The topical formulation applied in group II (Standard formulation) was the most effective (TNF-α value 51.54± 0.103). It showed a significant difference (P value <0.001) compared to groups IV &V. There exists a significant inflammatory difference between the LDF group and HDF group (P value <0.01). figure 49 presents the mean values of various groups along with their graphical representation. Table 48. highlights the mean values along with the significant differences obtained between various groups by the Bonferroni post hoc test.

Table 47 ANOVA table of TNF-α

ANOVA TABLE	Sum of squares	Degrees of freedom	Mean square	F – value	Significance
Between groups (TNF-α Values)	**5485.164**	**4**	**1371.291**	**8.443E4**	**0.000**
Within groups(error)	**0.406**	**25**	**0.016**		
Total	**5485.570**	**29**			

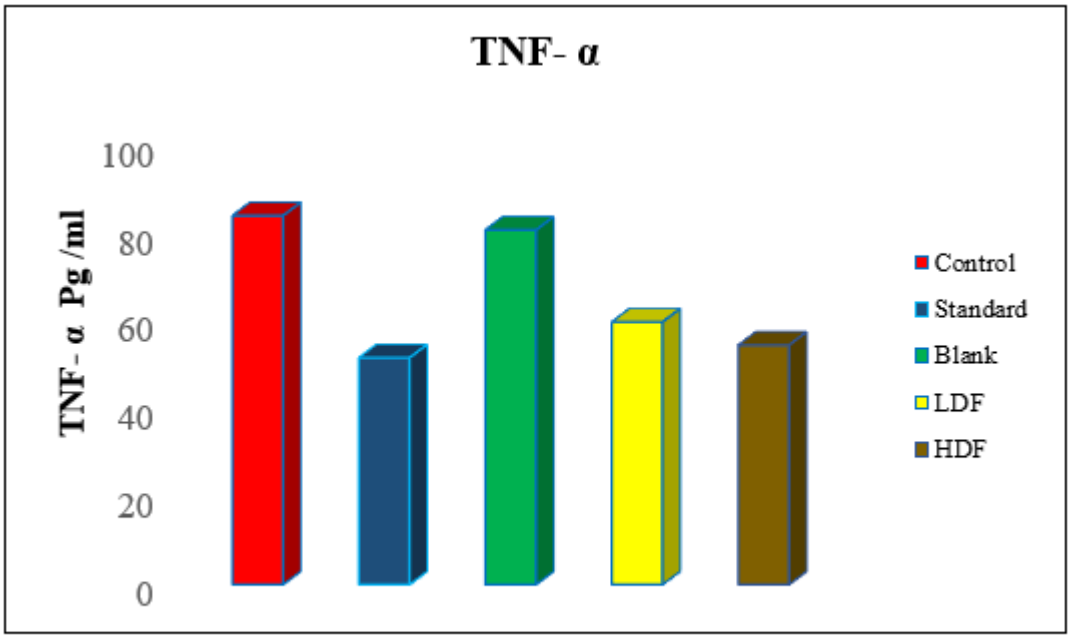


Figure 49 Graphical representation of mean values of TNF- in different groups

Table no. 48 Mean values and significance level of TNF-α in different groups

Sr. No.	Groups	No. of Rats (n)	Mean Values TNF-α Values (Pg/ml)	P value	P value for Bonferroni post hoc
I	Control	6	83.83±0.21		(0.000)1 Vs 2
II	Standard	6	51.54±0.103	0.000	(0.000) 2 Vs 4
III	Blank	6	80.58±0.107		(0.000) 2 Vs 5
IV	LDF	6	59.67±0.078		(0.000) 4 Vs 5
V	HDF	6	54.40±0.077		(0.000) 5 Vs 4

5.18.2.2 Interleukin-10 (IL-10)

It is a powerful anti-inflammatory cytokine that plays a centralized role in limiting the immune response of the host. It protects the damage to burn tissue and maintains host tissue homeostasis.(Mahung *et al.*, 2022). ANOVA Table 49 depicts that the application of PHMN and standard formulation significantly decreases the value of IL-10, which is an inflammatory marker that highlights the mean values along with the P values obtained from one-way ANOVA and post hoc Bonferroni test. but the ANOVA does not depict the decrease in inflammatory effect is pronounced in which group. To ascertain the specific effect of formulation post hoc Bonferroni test was conducted. The topical formulation applied in group II (Standard formulation) was the most effective (IL-10 value 98.20± 0.93). It showed a significant difference (P value <0.001) compared to groups IV &V compared to no treatment control group. Further, there exists a significant inflammatory difference between the LDF group and the HDF group (P value <0.01). figure 50 presents the mean values of various groups along with their graphical representation (Table50)

Table 49 ANOVA table of IL-10

ANOVA TABLE	Sum of squares	Degrees of freedom	Mean square	F – value	Significance
Between groups (IL-10)	5143787.425	4	1285946.856	1.263E6	0.000
Within groups(error)	25.459	25	1.018		
Total	5143812.884	29			

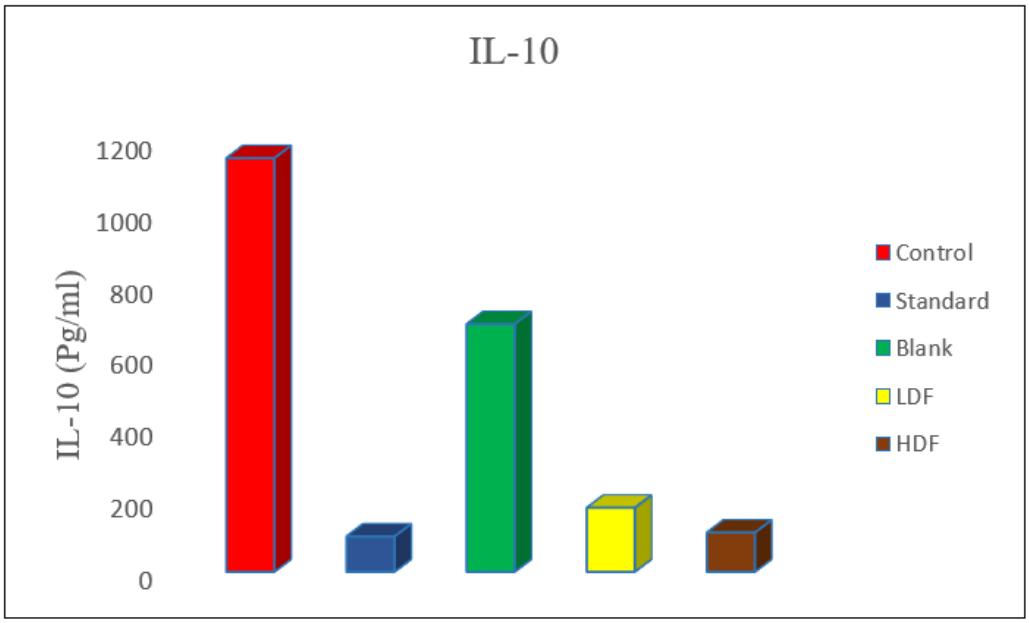


Figure 50 Graphical representation of mean values of IL-10 - in different groups

Effect on IL-10 is expressed as mean ± SD represented by bar graphs. P < 0.001 vs Standard. <0.001 vs LDF <0.001 vs HDF. Statistical analysis performed by one-way ANOVA followed by Bonferroni post hoc test

Table no. 50 Mean values and significance level of IL-10 in different groups

Sr. No.	Groups	No. of Rats (n)	Mean Values IL-10 values (Pg/ml)	P value	P value for Bonferroni post hoc
I	Control	6	1147±1.23	0.000	(0.000)1 Vs 2
II	Standard	6	98.20±0.93		(0.000) 2 Vs 4
III	Blank	6	687.305±1.06		(0.000) 2 Vs 5
IV	LDF	6	177.69±0.68		(0.000) 4 Vs 5
V	HDF	6	108.97±1.048		(0.000) 5 Vs 4

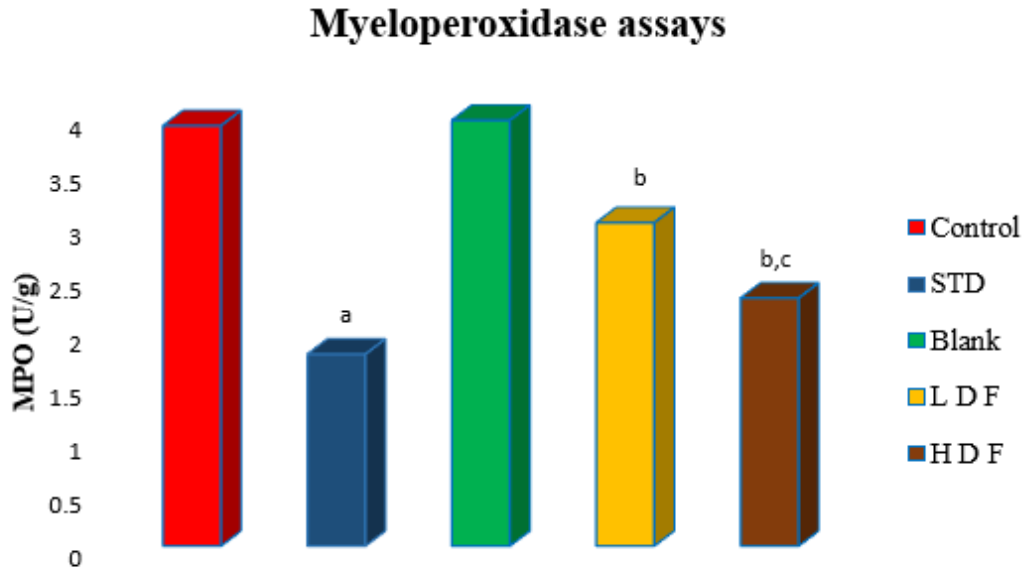
5.18.3 Myeloperoxidase assays (MPO)

The MPO assays represents the inflammatory activity induced by burns in rats. The mean values± SD of the MPO assays in different groups are mentioned in table 52 This enzyme is related with conventional inflammatory immune response of the immune system(Xiao *et al.*, 2014). The PMN cells play a crucial role in injury. It acts for the defence system of body's polymorphonuclear cell infiltrations and are known as the first line of defence. It is a marker of neutrophilic infiltrations to create a defence against the number of invading infections. MPO is known as body's physiologic response to destroy the invading organisms and protect the

body against infection and takes part in inflammation by catalysing the conversion of chloride and hydrogen peroxides to hypochlorite ($HCLO^-$) and oxidises tyrosine to tyrosyl radicals. By liberating other enzymes along with myeloperoxidase. MPO assay is a potential inflammatory marker. This assay is utilised to identify infection status in burn wouds. This assay was performed to determine the inflammatory response in wound after 21 days. Decreased MPO values indicate towards the decreased inflammation in burn wounds. (Hasmann et al., 2013). The statistical ANOVA table 51 highlights the significant difference between different treatment groups at the level of significance 0.01(P value < 0.001). But ANOVA does not

Table 51 ANOVA table for MPO values

ANOVA TABLE	Sum of squares	Degrees of freedom	Mean square	F – value	Significance
Between groups (MPO Values)	**22.165**	**4**	**5.541**	**166.240**	**0.000**
Within groups(error)	**0.833**	**25**	**0.033**	**----------**	**----------**
Total	**22.998**	**29**	**----------**	**----------**	**----------**



Effect on Myeloperoxidase. Data is expressed as mean ± SD represented by columns and bars. [a] $p < 0.0001$ vs Control; [b] $p < 0.0001$ vs SSD; c $p < 0.001$ vs Low Dose. Statistical analysis performed by one-way ANOVA followed by Bonferroni post hoc test.

Figure 51 Graphical representation of Myeloperoxidase assay

Table no. 52 Mean values and significance level of MPO in different groups

Sr. No.	Groups	No. of Rats (n)	Mean Values MPO values (mU/mg)	P value	P value for Bonferroni post hoc
1.	Control	6	3.9 ±0.17		(0.000)1 Vs 2
2.	Standard	6	1.78±0.17	0.000	(0.000) 2 Vs 4
3.	Blank	6	3.95±0.18		(0.092) 2 Vs 5
4.	LDF	6	3.06±0.18		(0.031) 4 Vs 5
5.	HDF	6	2.33±0.20		(0.015) 5 Vs 4

depicts the anti-inflammatory effect is pronounced in which group. To ascertain the specific effect of formulation post hoc Bonferroni test was conducted. The topical formulation applied in group II (MPO value 1.78± 0.17) outperformed all the treatment

groups followed by treatment given in group V (MPO mean value 2.3±0.20) at 0.01% level of significance (P value <0.01). compared to no treatment given in group I and III. This depicts that the application of HDF significantly decreased MPO values compared to control (MPO mean value 3.9±0.17) and therefore helps in relieving inflammation. The therapeutic efficacy of the HDF had significant near to the standard formulation given in group II. MPO assays in different groups are mentioned in table 52. The graphical representation of the mean values is shown in figure 51.

5.18.4 Anti-oxidant activity

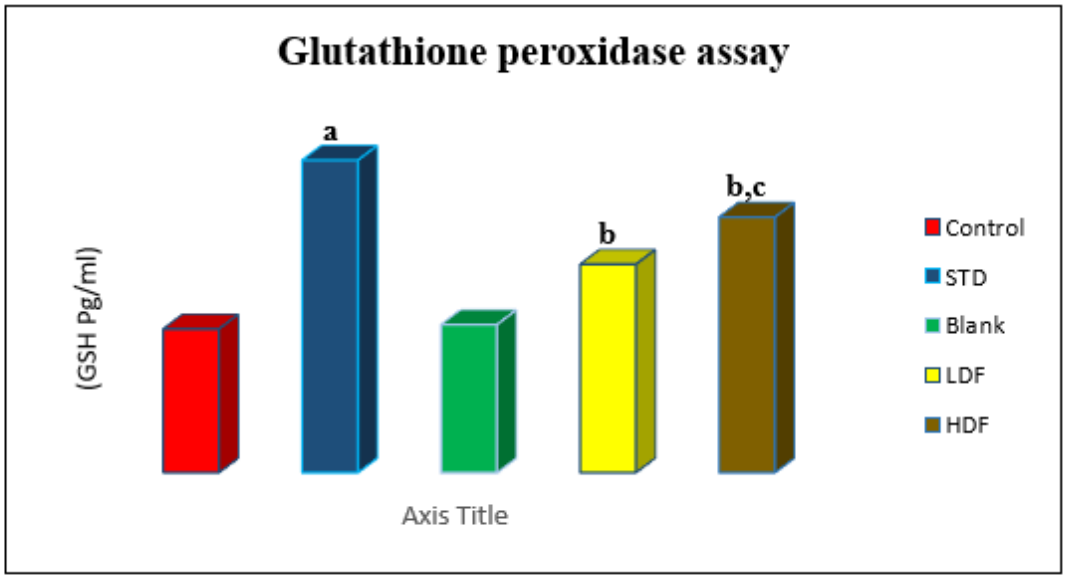
5.18.4.1 Glutathione peroxidase assays (GSH)

Antioxidant activity was assessed by glutathione peroxidase assays. The enzyme glutathione is an abundant intracellular antioxidant that protects the tissue from oxidative stress thus maintaining the antioxidants in the wound site. Burn wound injury damages the nearby tissue area. Glutathione reduces injury to the areas near the injured tissues (secondary tissue injury). It activates against oxidative stress caused in burn wounds and increases tissue survival (Margadant and Sonnenberg, 2010). ANOVA table 53 is mentioned, highlighting the significant difference between different treatment groups at the level of significance 0.01% (P value < 0.001). Table 54 represents the mean values± SD of the GSH assay in different groups This means that the application of PHMN significantly increases the GSH value which is an antioxidant marker. the graphical representation of the mean values is shown in figure 55 but the ANOVA does not depict the decrease in antioxidant effect is pronounced in which group

TABLE 53 ANOVA table for GSH values

ANOVA TABLE	Sum of squares	Degrees of freedom	Mean square	F – value	Significance
Between groups (GSH Values)	**13.073**	**4**	**3.268**	**46.076**	**0.000**
Within groups(error)	**1.773**	**25**	**0.071**	**----------**	**----------**
Total	**14.846**	**29**	**----------**	**----------**	**----------**

To ascertain the specific effect of formulation post hoc Bonferroni test was conducted. The topical formulation applied in group II (GSH value 2.85± 0.33) showed a significant difference (P value <0.01) compared to groups I, III, and IV. Group V (GSH value 2.33±0.71) had a significant difference (P value <0.01) compared to groups I, III, and IV at a 0.01% level of significance. This shows the therapeutic efficacy of the HDF had no significant difference from the standard formulation



Effect on GSH. Data is expressed as mean ± SD represented by line graphs. [a]p < 0.001 vs Control; [b]p < 0.05 vs SSD; [c]p < 0.05 vs Low Dose. Statistical analysis performed by one-way ANOVA followed by Bonferroni post hoc test.

Figure 52 Graphical representation of GSH values in different groups

Table no. 54 Mean values and significance level of GSH in different groups

Sr. No.	Groups	No. of Rats (n)	Mean Values GSH values (Pg/ml)	P value	P value for Bonferroni post hoc
1.	Control	6	1.31 ±0.11		(0.000)1 Vs 2
2.	Standard	6	2.85±0.33	0.000	(0.000) 2 Vs 4
3.	Blank	6	1.35±0.10		(0.092) 2 Vs 5
4.	LDF	6	1.9±0.20		(0.031) 4 Vs 5
5.	HDF	6	2.33±0.71		(0.015) 5 Vs 4

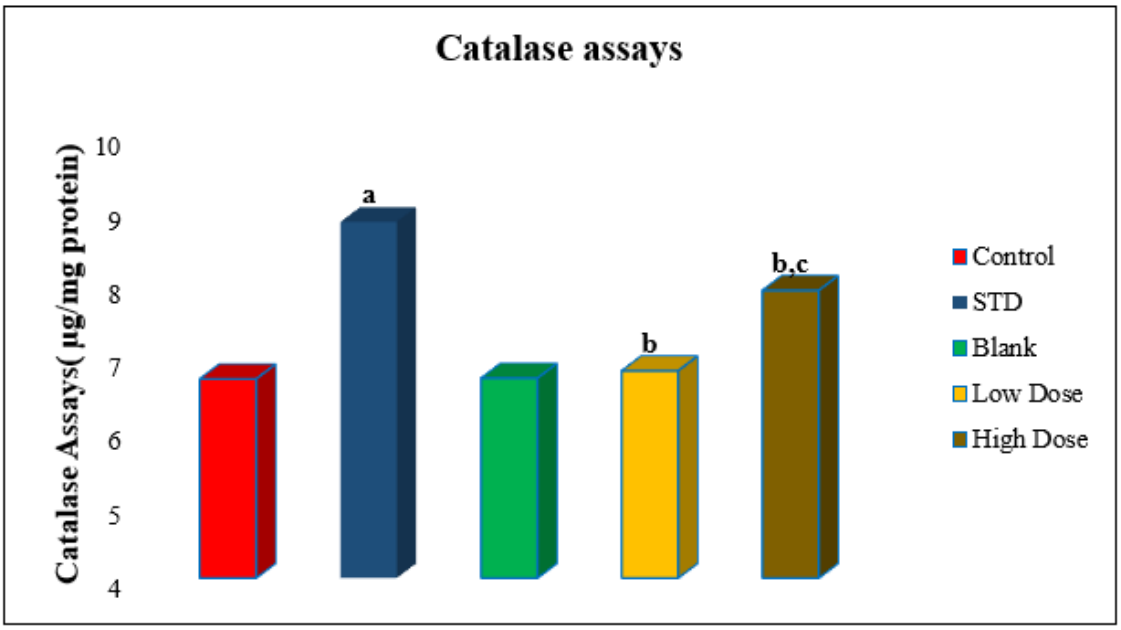
5.18.4.2 Catalase assay

Reactive oxygen species [superoxide (O_2^-), hydrogen peroxide (OH^-)] aggravates inflammation. Catalase enzyme is situated in peroxisomes that catalyzes the conversion of H_2O_2 to oxygen and water and prevents the tissue damage caused by peroxidases produced by frequent metabolic responses in burn-induced skin of rats. The protein level of catalases has an antioxidant effect that is required during the healing effect. The statistical ANOVA table 55 highlights the significant difference between different treatment groups at the level of significance 0.01% (P value < 0.001). This means that the application of PHMN significantly increases the Catalase assay value which is an antioxidant marker. but the ANOVA does not depict the increase in antioxidant effect is pronounced in which group. To determine the specific effect of formulation in specified groups post hoc Bonferroni test was conducted. The topical formulation applied in group II (CAT value 8.83± 0.40) was analysed statistically to have good performance with significant difference (P value <0.01) compared to group I, III and IV. Group V (CAT value 7.9±0.56) had significant difference (P value <0.01) compared to group I, III and IV at 0.01% level of significance. This shows the therapeutic efficacy of the HDF had no significant difference from the standard formulation. This means that the formulation significantly shows higher catalase enzyme assay, which is an anti-oxidant marker

The mean values± SD of the catalase assays in different groups are mentioned in table 56. the graphical representation of the mean values is shown in the figure 53

Table 55 ANOVA table for Catalase values

ANOVA TABLE	Sum of squares	Degrees of freedom	Mean square	F – value	Significance
Between groups (Catalase Values)	21.609	4	5.402	16.450	0.000
Within groups(error)	8.210	25	0.328	----------	----------
Total	29.819	29	----------	----------	----------



Data is expressed as mean ± SD represented by line graphs. [a]p < 0.001 vs Control; [b]p < 0.05 vs SSD; [c]p < 0.001 vs Low Dose. Statistical analysis performed by one-way ANOVA followed by Bonferroni post hoc test.

Figure 53 Graphical representation of catalase values in different groups

Table no. 56 Mean values and significance level of Catalase values in different groups

Sr. No.	Groups	No. of Rats (n)	Mean Values Catalase Values (µg/mg)	P value	P value for Bonferroni post hoc
1.	Control	6	6.7±0.52	0.000	(0.000)1 Vs 2
2.	Standard	6	8.83±0.40		(0.000) 2 Vs 4
3.	Blank	6	6.71±0.7		(0.092) 2 Vs 5
4.	LDF	6	6.81±0.62		(0.031) 4 Vs 5
5.	HDF	6	7.9±0.56		(0.015) 5 Vs 4

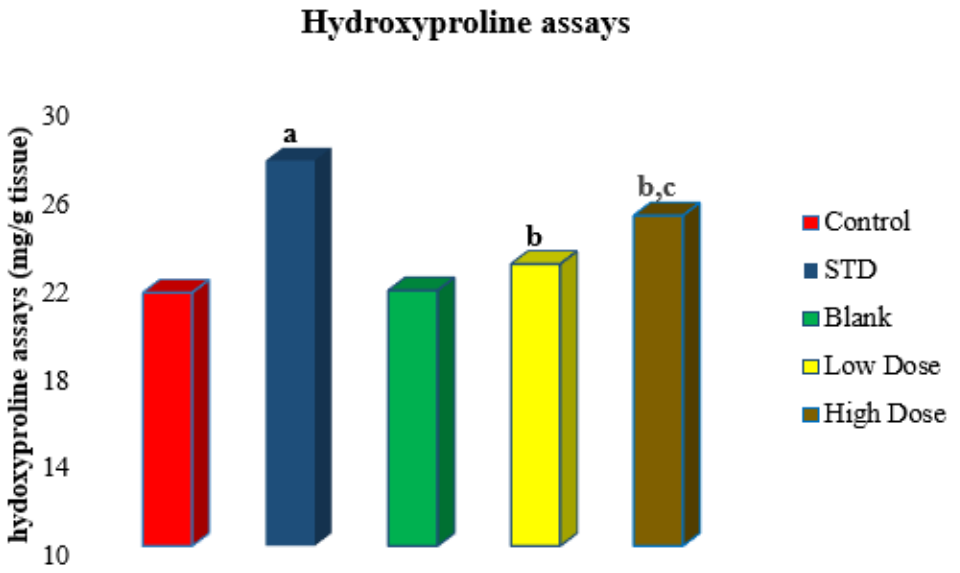
5.18.5 collagen assays

5.18.5.1 Hydroxyproline assays

Collagen is synthesized in growing cells during the healing process after burn injury. Hydroxyproline assays denote the formation of collagen in rats during the healing process(Dwivedi *et al.*, 2017). The mean values SD of the hydroxyproline assays in different groups are mentioned in table 58 The results showed increased hydroxyproline content that denotes increased cellular proliferation in groups where standard and HDF were applied. A graphical representation of the mean values is shown in figure 54. The above ANOVA table 57 represents p value is 0.000, which is less than 0.01. This means that the formulation significantly shows higher collagen formation, which is a biochemical marker for proper wound healing. To find out the specific effect of the formulation in particular groups post hoc Bonferroni test was conducted. The results were statistically higher ($p<0.01$) in group II (mean Hydroxyproline content 27.5± 1.87) compared to control (mean hydroxyproline content 21.5±1.87) HDF groups (mean hydroxyproline value 25±0.63) and group IV (mean hydroxyproline value 22.8±0.75) at 0.01% level of significance. Significantly higher values ($P<0.01$) in group V on application of HDF compared to I, II, and III group. This predicts the effect of HDF to be more effective then LDF and with comparable effect of standard drug. higher values ($P<0.01$) in group V compared to group III and group IV. HDF is comparatively effective in the standard formulation of statistical standards and positive control groups.

Table 57 ANOVA table for hydroxyproline values

ANOVA TABLE	Sum of squares	Degrees of freedom	Mean square	F – value	Significance
Between groups (Hydroxyproline Values)	155.133	4	38.783	23.553	0.000
Within groups(error)	41.167	25	1.647	----------	----------
Total	196.3	29	----------	----------	----------



Effect on Hydroxyproline. Data is expressed as mean ± SD represented by columns and bars. $^{a}p < 0.001$ vs Control; $^{b}p < 0.05$ vs SSD; $^{c}p < 0.05$ vs Low Dose. Statistical analysis performed by one-way ANOVA followed by Bonferroni post hoc test

Figure 54 Graphical representation of Hydroxyproline values in different groups

Table 58 Mean values and significance level of Hydroxyproline values in different groups

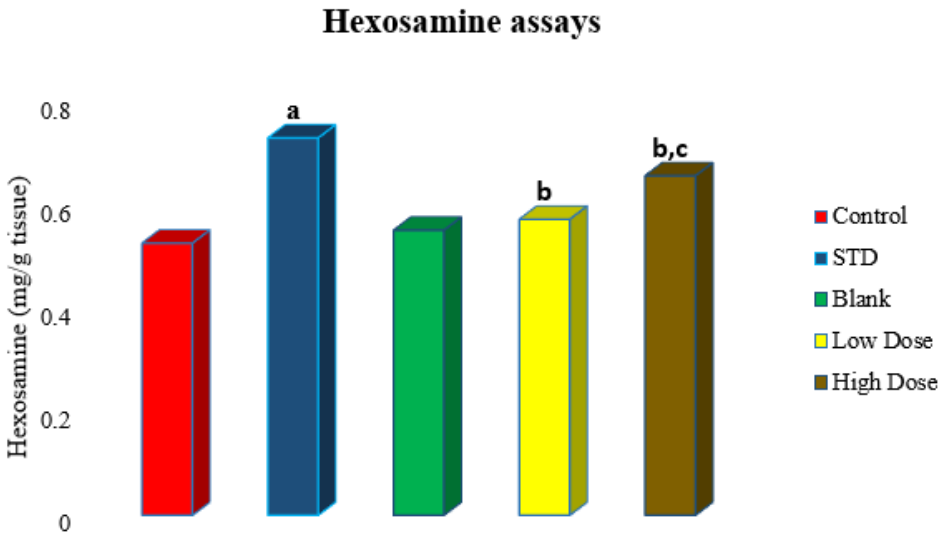
Sr. No.	Groups	No. of Rats (n)	Mean Values Hydroxyproline Assay Values (mU/mg)	P value	P value for Bonferroni post hoc
1.	Control	6	21.5±1.87		(0.000)1 Vs 2
2.	Standard	6	27.5±1.04		(0.000) 2 Vs 4
3.	Blank	6	21.6±1.63	0.000	(0.001) 2 Vs 5
4.	LDF	6	22.8±0.75		(0.072) 4 Vs 5
5.	HDF	6	25±0.63		(0.001) 5 Vs 4

5.18.5.2 Hexosamine assays

The Hexosamine assays denote the stabilization of collagen molecules. Its interactions between ions maintain the integrity of the tissue matrix and increase epithelialization in later post-burn phases of wound healing in rats. (Dwivedi *et al.*, 2017). The mean values ± SD of the Hexosamine assays in different groups are mentioned in Table 60. The graphical representation of the mean values is shown in figure 58The ANOVA table (Table 59.) represents a p-value (< 0.001). This means that the formulation significantly shows higher collagen formation, which is a dominant marker for proper wound healing. To ascertain the marked effect in particular groups Post hoc Bonferroni test was conducted for the purpose. The results were statistically higher ($p<0.001$) in group II (mean value 0.728±0.024) and group v (mean value 0.655±0.03) compared to control (mean value 0.525±0.05) and group III (mean value 0.55±0.06). Significantly

Table 59 ANOVA table for Hexosamine values

ANOVA TABLE	Sum of squares	Degrees of freedom	Mean square	F – value	Significance
Between groups (Hexosamine values)	**169**	**4**	**0.042**	**20.903**	**0.000**
Within groups(error)	**0.051**	**25**	**0.002**	**----------**	**----------**
Total	**169.05**	**29**	**----------**	**----------**	**----------**



Effect on Hexosamine. Data is expressed as mean ± SD represented by columns and bars. ap < 0.001 vs Control; bp < 0.05 vs SSD; cp < 0.05 vs Low Dose. Statistical analysis performed by one-way ANOVA followed by Bonferroni post hoc test.

Figure 55 Graphical representation of Hexosamine values in different groups

Table 60 Mean values and significance level of Hexosamine values in different groups

Sr. No.	Groups	No. of Rats (n)	Mean Values Hexosamine Assay Values (mU/mg)	P value	P value for Bonferroni post hoc
1.	Control	6	0.525±0.05	0.000	(0.000)1 Vs 2
2.	Standard	6	0.728±0.024		(0.000) 2 Vs 4
3.	Blank	6	0.55±0.06		(0.092) 2 Vs 5
4.	LDF	6	0.571±0.04		(0.037) 4 Vs 5
5.	HDF	6	0.655±0.03		(0.004) 5 Vs 4

5.19 Histopathological studies

After completing 21 days of the in-vivo study. The rats were sacrificed using cervical decortication and the skin of the animals were removed from their dorsum and stored. After H & E staining the specimens was observed at high power magnification (100x, 400x). changes observed related to skin pathology were recorded and shown figure 56 (I-V).

Group-I Control Group Granuation tissue consists of scar tissue. Fibroblastic cells are visible and a lot of inflammatory cell infiltrations are seen below the granulation tissue

Group-II Standard Formulation Less scar tissue is formed. Fibroblastic cells are visible Lot of inflammatory cell infiltrations are visible below the scar tissue

Blank formulation Thickness of scar tissue is more fibroblastic cells are visible. Fibrosis is developed. More inflammatory cells are visible below scar tissue.

Group-IV Low dose formulation Scar tissue is visible. Fibrosis is not formed. Lot of inflammatory cell filtrations are seen below squamous epithelium.

Group-V High dose formulation Scar tissue is very less visible. Inflammatory cells infiltrations are seen near dermis. Below squamous epithelium healing is satisfactory.

Histopathological studies reveal that in control group the wound bed showed excess inflammatory cells. Granulation tissue consists of scar tissue. Standard formulation presented satisfactory healing process but inflammatory reactions is not decreased. High dose formulation group presented a well organised wound healing process due to less inflammatory cells near epidermis and very less granulation tissue is formed. Improved histopathological picture of wound suggest a fast wound contraction rate and increase of hydroxyproline content is seen.

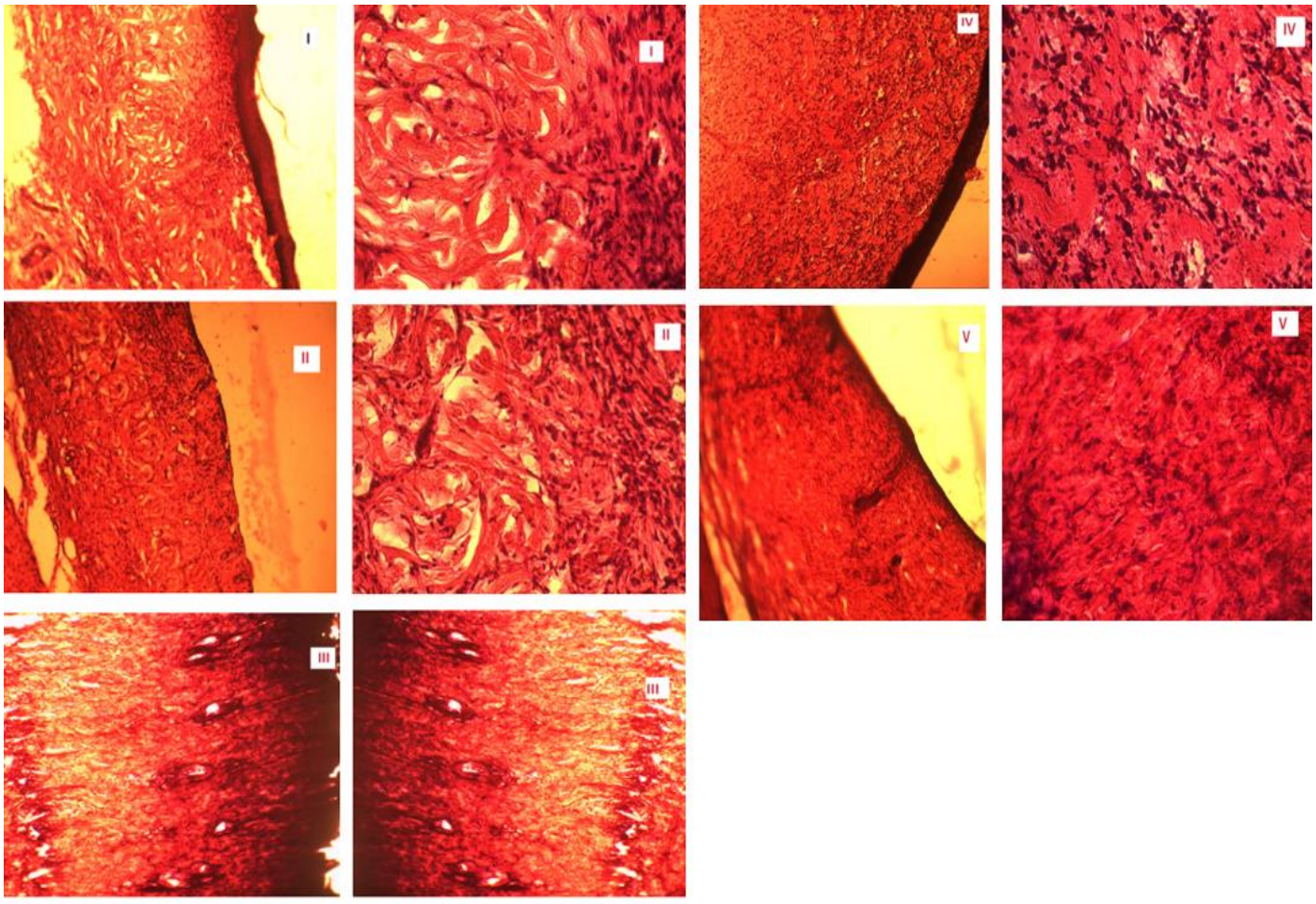


figure 56 Pictograph showing histopathological study of dorsum of skin of rats from group (I-V)

Chapter-6

Conclusions and future perspectives

Worldwide, herbal remedies are used as an alternative traditional form of therapy in healthcare system. Adverse effects of modern medicine have obligated public to review the use of herbals and provided strength to the traditional system of medicine. But the conventional methods of delivering herbal drugs are antiquated and has created a research gap. There is a need to revise the classical methods by novel drug delivery system. Nonetheless, a great deal of study is being done in this field. Nanoemulsions to administer and target herbal bioactives and extracts is a unique approach to medicine delivery. Enhanced Phytopharmaceuticals provide enhanced pharmacological activity at lower concentrations compared to that present in conventional herbs or herbal extracts. Selection of an appropriate nanocarrier system will be an innovative mode to provide cost-effective therapeutic dose through increased bioavailability. The simple technique of decreasing size to fabricate the herbal drugs in topical nanoemulsions has increased effectivity and stability of herbal drugs compared to conventional drug delivery system.

Curcuma longa, Aloe vera, Calotropis procera latex, Azadirachta indica seeds, egg yolk and Tuttha are found to have references in context of vrana management along with statistical research studies data for their anti-inflammatory, anti-microbial and wound healing activity either individually or in combinations with traditional and novel drug delivery techniques. The existing study was designed to examine the second degree burn wound healing efficacy of a polyherbomineral formulation that contained the above drugs in form of CE, AE, CAE, NSO, EYO and tuttha in an optimised composition. Since skin favours penetration of smaller sized non-irritating amphiphilic drug molecules. The drug delivery from surface burn tissue using leaves bandaging, wound fumigations or routine ghrita, oils, ointments, oils, creams, gel, lotions dosage form or dressings has been challenging. So, nanoemulsion was thought as an appropriate novel drug delivery technique to bind lipophilic and hydrophilic drugs in combinations for the preparation of polyherbomineral formulation.

WHO has allocated a specific set of guidelines for evaluating safety, effectiveness and quality of herbal medicines. To produce the quality oriented herbal drugs there is a

need to identify plants, season of collection, appropriate extraction techniques, standardization methods and to

Justify the combinations in polyherbal combinations. The procedure of preparation of formulation was followed with correct identification by organoleptic and pharmacognostic evaluation methods, physicochemical characterisation (ash values, extractive values, pH) of raw drugs and prepared extracts. Preliminary phytochemical, microbiological and heavy metal screening techniques. Phytochemical contour of the drug is used as a foundation because it determines the quality for efficacy of herbal and polyherbal combinations. In lieu of this, 5 marker compounds Curcumin, Rutin, Gallic acid, Berberine and Oleic acid served as the supplementary parameter to assess the quality of the sample drug individually and in the prepared formulation. Preformulating studies were conducted for visually screening the compatibility of the oil drugs with the combinations of various surfactants and co-surfactants. Calibration curves of the herbal drugs were formulated for screening the combinations of herbal drugs with the various surfactants and co-surfactants. After selection of labrasol ALF and transcutol as a compatible surfactant and co-surfactant for all herbal drugs and oils.

Significant healing can be achieved by polyherbomineral fomulation in burn wounds. New granulation tissue is formed followed by epithelialization process. The host immune system initiates the healing process. The process is augmented by the use of herbals that decrease inflammation and free radicals formation, hexosamine and hydroxyproline assays and early epithelialization denote efficient collagen formation(Becić *et al.*, 2005)(Fahimi *et al.*, 2015)(Jogpal *et al.*, 2022) hastened the healing process. (Jain *et al.*, 2007). Several research studies conducted with tracked literature and observed biocompatibility have demonstrated excellent healing activity with Polyherbal formulations prepared among the ingredients lead to a rapid wound contraction.

Chapter-7

Bibliography

1Ukaoma, A.A, 2Nwachuk (2019) 'Phytochemical And Antimicrobial Activity Of Neem Seed Oil (Azadirachta Indica) On Bacteria Isolates', *International Research journal of natural sciences*, 07(2), pp. 1–19.

Adejoh, J., Alli, L. A. and Okoh, M. P. (2021) 'Evaluation of the Anti-Plasmodial potential of Calotropis procera Latex in mice infected with Plasmodium berghei', *MethodsX*, 8(June), p. 101528. doi: 10.1016/j.mex.2021.101528.

Administration, D. (2011) 'Guidance for industry considering whether an FDA-regulated product involves the application of nanotechnology', *Biotechnology Law Report*, 30(5), pp. 613–616. doi: 10.1089/blr.2011.9814.

Ahmad, N. *et al.* (2019) 'Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation', *RSC Advances*, 9(35), pp. 20192–20206. doi: 10.1039/c9ra03102b.

Akbik, D. *et al.* (2014) 'Curcumin as a wound healing agent', *Life Sciences*, 116(1), pp. 1–7. doi: 10.1016/j.lfs.2014.08.016.

Akhoondinasab, M. R. *et al.* (2015) 'Assessing effect of three herbal medicines in second and third degree burns in rats and comparison with silver sulfadiazine ointment', *Burns*, 41(1), pp. 125–131. doi: 10.1016/j.burns.2014.04.001.

Akhoondinasab, M. R., Akhoondinasab, M. and Saberi, M. (2014) 'Comparison of healing effect of aloe vera extract and silver sulfadiazine in burn injuries in experimental rat model.', *World journal of plastic surgery*, 3(1), pp. 29–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25489521%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4236981.

Akhoondinasab, M. R., Akhoondinasab, M. and Saberi, M. (no date) *Comparison of Healing Effect of Aloe Vera Extract and Silver Sulfadiazine in Burn Injuries in Experimental Rat Model*. Available at: www.wjps.ir.

Al-Snafi, A. E. (2015) 'The constituents and pharmacological properties of *Calotropis Procera* - An Overview', *International Journal of Pharmacy Review & Research*, 5(3).

Alencar, N. M. N. *et al.* (2004) 'Anti-inflammatory effect of the latex from Calotropis procera in three different experimental models: Peritonitis, paw edema and hemorrhagic cystitis', *Planta Medica*, 70(12). doi: 10.1055/s-2004-835842.

Alencar, N. M. N. *et al.* (2006) 'Pro- and anti-inflammatory activities of the latex from Calotropis procera (Ait.) R.Br. are triggered by compounds fractionated by dialysis', *Inflammation Research*, 55(12), pp. 559–564. doi: 10.1007/s00011-006-6025-y.

Alshawwa, S. Z. *et al.* (2022) 'Nanocarrier Drug Delivery Systems: Characterization, Limitations, Future Perspectives and Implementation of Artificial Intelligence', *Pharmaceutics*, 14(4). doi: 10.3390/pharmaceutics14040883.

Alzohairy, M. A. (2016) 'Therapeutics role of azadirachta indica (Neem) and their active constituents in diseases prevention and treatment', *Evidence-based Complementary and Alternative Medicine*, 2016. doi: 10.1155/2016/7382506.

Amadeu, T. P. *et al.* (2003) 'Cutaneous Wound Healing: Myofibroblastic Differentiation and in Vitro Models', *The International Journal of Lower Extremity Wounds*, 2(2), pp. 60–68. doi: 10.1177/1534734603256155.

Amaral, R. *et al.* (2006) 'Microtensile bond strength of a resin cement to glass infiltrated zirconia-reinforced ceramic: The effect of surface conditioning', *Dental Materials*, 22(3), pp. 283–290. doi: 10.1016/j.dental.2005.04.021.

Amini, M. *et al.* (2010) 'Effect of Plantago major on burn wound healing in rat', *Journal of Applied Animal Research*, 37(1), pp. 53–56. doi: 10.1080/09712119.2010.9707093.

Anonymous (no date) *The Ayurvedic pharmacopoeia of India part-1, volume -ii, Govt. Of India, Ministry of Health and Family Welfare Department of AYUSH*.

Araújo Viana, C. *et al.* (2017) 'Cytotoxicity against tumor cell lines and anti-inflammatory properties of chitinases from Calotropis procera latex', 390, pp. 1005–1013. doi: 10.1007/s00210-017-1397-9.

Aremu, O. (2009) 'Physico-Chemical Stability Studies of Neem (*Azadirachta indica*) Seed Oil Cream', *African Research Review*, 3(3), pp. 1–11. doi: 10.4314/afrrev.v3i3.47509.

Argenta, L. C. *et al.* (2006) 'Vacuum-assisted closure: State of clinic art', *Plastic and Reconstructive Surgery*, 117(7 SUPPL.), pp. 127–142. doi: 10.1097/01.prs.0000222551.10793.51.

Arturson, G. (1996) 'Pathophysiology pharmacological lecture, 1995*', *Bu*, 22(4), pp. 255–274.

Arutyunyan, I. *et al.* (2016) 'Role of VEGF-A in angiogenesis promoted by umbilical cord-derived mesenchymal stromal/stem cells: In vitro study', *Stem Cell Research and Therapy*, 7(1), pp. 1–13. doi: 10.1186/s13287-016-0305-4.

Arya, S. and Kumar, V. L. (2005) 'Antiinflammatory efficacy of extracts of latex of Calotropis procera against different mediators of inflammation', *Mediators of Inflammation*, 2005(4), pp. 228–232. doi: 10.1155/MI.2005.228.

Atiyeh, B. S. *et al.* (2007) 'Effect of silver on burn wound infection control and healing: Review of the literature', *Burns*, 33(2), pp. 139–148. doi: 10.1016/j.burns.2006.06.010.

Auwal, M. S. *et al.* (2014) 'Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa).', *Veterinary research forum : an international quarterly journal*, 5(2), pp. 95–100. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25568701%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4279630.

Azeem, A. *et al.* (no date) 'Brief/Technical Note Nanoemulsion Components Screening and Selection: a Technical Note'. doi: 10.1208/s12249-008-9178-x.

Balouiri, M., Sadiki, M. and Ibnsouda, S. K. (2016) 'Methods for in vitro evaluating antimicrobial activity: A review', *Journal of Pharmaceutical Analysis*, 6(2), pp. 71–79. doi: 10.1016/j.jpha.2015.11.005.

Barajas-Nava, L. A. *et al.* (2013) 'Antibiotic prophylaxis for preventing burn wound infection', *Cochrane Database of Systematic Reviews*, 2013(6). doi: 10.1002/14651858.CD008738.pub2.

Barceloux, D. G. (1999) 'Donald G. Barceloux', 37(2), pp. 217–230.

Barillo, D. J., Pozza, M. and Margaret-Brandt, M. (2014) 'A literature review of the military uses of silver-nylon dressings with emphasis on wartime operations', *Burns*, 40(S1), pp. S24–S29. doi: 10.1016/j.burns.2014.09.017.

Baroli, B. (2010) 'Penetration of nanoparticles and nanomaterials in the skin: Fiction or reality?', *Journal of Pharmaceutical Sciences*, 99(1), pp. 21–50. doi: 10.1002/jps.21817.

Barret, J. P. and Herndon, D. N. (2003) 'Effects of burn wound excision on bacterial colonization and invasion', *Plastic and Reconstructive Surgery*, pp. 744–750. doi: 10.1097/01.PRS.0000041445.76730.23.

Becić, F. *et al.* (2005) 'Topical treatment of standardised burns with herbal remedies in model rats.', *Bosnian journal of basic medical sciences / Udruzenje basicnih mediciniskih znanosti = Association of Basic Medical Sciences*, 5(4), pp. 50–57. doi: 10.17305/bjbms.2005.3233.

Benson, H. A. E. and Watkinson, A. C. (2012) *Transdermal and Topical Drug Delivery: Principles and Practice*, *Transdermal and Topical Drug Delivery: Principles and Practice*. doi: 10.1002/9781118140505.

Bezerra, C. F. *et al.* (2017) 'Latex proteins from Calotropis procera: Toxicity and immunological tolerance revisited', *Chemico-Biological Interactions*, 274, pp. 138–149. doi: 10.1016/j.cbi.2017.07.007.

Bhandare, P. and Naik, G. R. (2015) 'Functional Properties of Neem Oil as Potential Feedstock for Biodiesel Production', *International Letters of Natural Sciences*, 34(May), pp. 7–14. doi: 10.18052/www.scipress.com/ilns.34.7.

Biswas, T. K., Maity, L. N. and Mukherjee, B. (2004) 'Wound Healing Potential of Pterocarpus Santalinus Linn: A Pharmacological Evaluation', *International Journal of Lower Extremity Wounds*, 3(3), pp. 143–150. doi: 10.1177/1534734604268385.

Bitter, C. C. and Erickson, T. B. (2016) 'Management of Burn Injuries in the Wilderness: Lessons from Low-Resource Settings', *Wilderness and Environmental Medicine*, 27(4), pp. 519–525. doi: 10.1016/j.wem.2016.09.001.

Bjarnsholt, T. (2013) 'The role of bacterial biofilms in chronic infections.', *APMIS. Supplementum*, (136), pp. 1–51. doi: 10.1111/apm.12099.

Blakytny, R. and Jude, E. (2006) 'The molecular biology of chronic wounds and delayed healing in diabetes', *Diabetic Medicine*, 23(6), pp. 594–608. doi: 10.1111/j.1464-5491.2006.01773.x.

Borkow, G. (2015) 'Using Copper to Improve the Well-Being of the Skin', *Current Chemical Biology*, 8(2), pp. 89–102. doi: 10.2174/2212796809666150227223857.

Borkow, G. and Gabbay, J. (2009) 'Copper, An Ancient Remedy Returning to Fight Microbial, Fungal and Viral Infections', *Current Chemical Biology*, 3(3), pp. 272–278. doi: 10.2174/187231309789054887.

Bos, J. D. and Meinardi, M. M. H. M. (2000) 'EXPERIMENTAL DERMATOLOGY The 500 Dalton rule for the skin penetration of chemical compounds and drugs Bos JD, Meinardi MMHM. The 500 Dalton rule for the skin penetration', *Exp Dermatol*, 9(5), pp. 165–169. Available at: http://chemfinder.

Bouwstra, J. A. *et al.* (2000) 'The lipid organisation in the skin barrier', *Acta Dermato-Venereologica, Supplement*, (208), pp. 23–30. doi: 10.1080/000155500750042826.

Bouwstra, J. A. *et al.* (2001) 'Phase behavior of lipid mixtures based on human ceramides: Coexistence of crystalline and liquid phases', *Journal of Lipid Research*, 42(11), pp. 1759–1770. doi: 10.1016/s0022-2275(20)31502-9.

Braga, M. E. M. *et al.* (2003) 'Comparison of Yield, Composition, and Antioxidant Activity of Turmeric (Curcuma longa L.) Extracts Obtained Using Various Techniques', *Journal of Agricultural and Food Chemistry*, 51(22), pp. 6604–6611. doi: 10.1021/jf0345550.

Brewer, G. J. (2012) 'Copper toxicity in Alzheimer's disease: Cognitive loss from ingestion of inorganic copper', *Journal of Trace Elements in Medicine and Biology*, 26(2–3), pp. 89–92. doi: 10.1016/j.jtemb.2012.04.019.

Cambiaso-Daniel, J. *et al.* (2018) 'Topical Antimicrobials in Burn Care: Part 1—Topical Antiseptics', *Annals of Plastic Surgery*, p. 1.

Carmona, F. and Pereira, A. M. S. (2013) 'Herbal medicines: Old and new concepts, truths and misunderstandings', *Revista Brasileira de Farmacognosia*, 23(2), pp. 379–385. doi: 10.1590/S0102-695X2013005000018.

Casey, G. (2002) 'Physiology of the skin.', *Nursing standard (Royal College of Nursing (Great Britain) : 1987)*, 16(34). doi: 10.7748/ns2002.05.16.34.47.c3193.

Catalogus, T. S. *et al.* (no date) 'NEDERLANDSCH-INDIE'.

Cavalcante, G. S. *et al.* (2020) 'Chemical constituents of calotropis procera latex and ultrastructural effects on haemonchus contortus', *Revista Brasileira de Parasitologia Veterinaria*, 29(2), pp. 1–12. doi: 10.1590/S1984-29612020045.

Chan, E. W. C. *et al.* (2017) 'Cytotoxic cardenolides from calotropis species: A short review', *Records of Natural Products*. ACG Publications, pp. 334–344. doi: 10.25135/rnp.2017.1701.002.

Chandel, H. S., Pathak, A. K. and Tailang, M. (2011) 'Standardization of some herbal antidiabetic drugs in polyherbal formulation', *Pharmacognosy Research*, 3(1), pp. 49–56. doi: 10.4103/0974-8490.79116.

Chandran, P. K. and Kuttan, R. (2008) 'Effect of Calendula officinalis flower extract on acute phase proteins, antioxidant defense mechanism and granuloma formation during thermal burns', *Journal of Clinical Biochemistry and Nutrition*, 43(2), pp. 58–64. doi: 10.3164/jcbn.2008043.

Charles F.T. snelling, Allian R . Ronald, G. E. J. (1969) 'topical gentamycin therapy on burn wounds.pdf'. Boston, little brown, pp. 158–163. doi: 10.1097/00006534-197008000-00009.

Chaudhary preeti, A. S. A. K. N. (2017) 'A Review on Medical Utility of Calotropis Procera ', *WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH*, 3(1), pp. 335–342.

Cheppudira, B. *et al.* (2013) 'Curcumin: A novel therapeutic for burn pain and wound healing', *Expert Opinion on Investigational Drugs*, 22(10), pp. 1295–1303. doi: 10.1517/13543784.2013.825249.

Chundattu, S. J., Agrawal, V. K. and Ganesh, N. (2016) 'Phytochemical investigation of

Calotropis procera', *Arabian Journal of Chemistry*, 9, pp. S230–S234. doi: 10.1016/j.arabjc.2011.03.011.

Ciuca, M. D. and Racovita, R. C. (2023) 'Curcumin: Overview of Extraction Methods, Health Benefits, and Encapsulation and Delivery Using Microemulsions and Nanoemulsions', *International Journal of Molecular Sciences*, 24(10), pp. 1–29. doi: 10.3390/ijms24108874.

Cole, L. and Heard, C. (2007) 'Skin permeation enhancement potential of Aloe Vera and a proposed mechanism of action based upon size exclusion and pull effect', *International Journal of Pharmaceutics*, 333(1–2), pp. 10–16. doi: 10.1016/j.ijpharm.2006.09.047.

Cuttle, L. *et al.* (2006) 'A porcine deep dermal partial thickness burn model with hypertrophic scarring', *Burns*, 32(7), pp. 806–820. doi: 10.1016/j.burns.2006.02.023.

D., D. S. D. (2017) 'ARKA (Calotropis procera (Ait.) R.Br.): A potent Drug for Indian Materia Medica', *World Journal of Pharmacy and Pharmaceutical Sciences*, pp. 471–487. doi: 10.20959/wjpps20175-9138.

D.I.J. Morrow, P.A. McCarron, A. D. W. and R. F. D. (2007) 'lnnovative Strategies for Enhancing Topical and Transdermal Drug Delivery', *the open drug delivery journal*, 38, pp. 36–59. doi: 10.1144/GSL.SP.1988.038.01.04.

Dai, T. *et al.* (2010) 'Topical Antimicrobials for Burn Wound Infections', *Recent Patents on Anti-Infective Drug Discovery*, 5(2), pp. 124–151. doi: 10.2174/157489110791233522.

Dal'Belo, S. E., Rigo Gaspar, L. and Maia Campos, P. M. B. G. (2006) 'Moisturizing effect of cosmetic formulations containing Aloe vera extract in different concentrations assessed by skin bioengineering techniques', *Skin Research and Technology*, 12(4), pp. 241–246. doi: 10.1111/j.0909-752X.2006.00155.x.

Davies, J. (2006) 'Where have all the antibiotics gone?', *Canadian Journal of Infectious Diseases and Medical Microbiology*, 17(5), pp. 287–290.

Demling, R. H. (2005) 'The burn edema process: Current concepts', *Journal of Burn Care and Rehabilitation*, 26(3), pp. 207–227. doi: 10.1097/01.BCR.0000162151.71482.B3.

Dewan, S., Sangraula, H. and Kumar, V. L. (2000) 'Preliminary studies on the analgesic activity of latex of Calotropris procera', *Journal of Ethnopharmacology*, 73(1–2). doi: 10.1016/S0378-8741(00)00272-5.

Dhande, P. (2017) 'Burn wound healing potential of Jatyadi formulations in rats Research Journal of Pharmaceutical , Biological and Chemical Sciences Burn Wound Healing Potential of Jatyadi Formulations in Rats October – December', (March).

Disch, L., Drewe, J. and Fricker, G. (2017) 'Dissolution testing of herbal medicines: Challenges and regulatory standards in Europe, the United States, Canada, and Asia', *Dissolution Technologies*, 24(2), pp. 6–12. doi: 10.14227/DT240217P6.

Djerrou, Z. *et al.* (2010) 'Effect of virgin fatty oil of Pistacia lentiscus on experimental burn wound's healing in rabbits', *African Journal of Traditional, Complementary and Alternative Medicines*, 7(3), pp. 258–263. doi: 10.4314/ajtcam.v7i3.54788.

DollDollwet, H.H., & Sorenson, J. R. (1985) 'Historic uses of copper compounds in medicine. Trace elements in medicine', *Trace elements in medicine*, 2(2), pp. 80–87.

Donthi, M. R. *et al.* (2023) 'Nanoemulgel: A Novel Nano Carrier as a Tool for Topical Drug Delivery', *Pharmaceutics*, 15(1), pp. 1–28. doi: 10.3390/pharmaceutics15010164.

Dragicevic, N. and Maibach, H. I. (2015) 'Percutaneous penetration enhancers chemical methods in penetration enhancement: Drug manipulation strategies and vehicle effects', *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Drug Manipulation Strategies and Vehicle Effects*, (January), pp. 1–341. doi: 10.1007/978-3-662-45013-0.

Dursun, N. *et al.* (2003) 'Role of Thymus Oil in Burn Wound Healing', *Journal of Burn Care and Rehabilitation*, 24(6), pp. 395–399. doi: 10.1097/01.BCR.0000095513.67541.0F.

Dwivedi, D. *et al.* (2017) 'Evaluation of wound healing, anti-microbial and antioxidant potential of Pongamia pinnata in wistar rats', *Journal of Traditional and Complementary Medicine*, 7(1), pp. 79–85. doi: 10.1016/j.jtcme.2015.12.002.

Dwivedi, V. K. *et al.* (2010) 'Comparative efficacy of ampucare and silversulfadiazine against burn wound rat', *Journal of Applied Sciences Research*, 6(6), pp. 674–682.

Elias, P. M. (1983) 'Epidermal lipids, barrier function, and desquamation', *Journal of Investigative Dermatology*, 80(Suppl.). doi: 10.1111/1523-1747.ep12537108.

Elias, P. M. (2012) 'Structure and function of the stratum corneum extracellular matrix', *Journal of Investigative Dermatology*, 132(9), pp. 2131–2133. doi: 10.1038/jid.2012.246.

Elmowafy, M. (2021) 'Skin penetration/permeation success determinants of nanocarriers: Pursuit of a perfect formulation', *Colloids and Surfaces B: Biointerfaces*, 203(September 2020), p. 111748. doi: 10.1016/j.colsurfb.2021.111748.

European Commission (2009) 'Commission Recommendation on a Code of Conduct for Responsible Nanosciences and Nanotechnologies Research & Council Conclusions on Responsible Nanosciences and Nanotechnologies Research', p. 24. Available at: http://ec.europa.eu/research/science-society/document_library/pdf_06/nanocode-apr09_en.pdf.

Evers, L. H., Bhavsar, D. and Mailänder, P. (2010) 'The biology of burn injury', *Experimental Dermatology*, 19(9), pp. 777–783. doi: 10.1111/j.1600-0625.2010.01105.x.

Fahimi, S. *et al.* (2015) 'Wound healing activity of a traditionally used poly herbal product in a burn wound model in rats', *Iranian Red Crescent Medical Journal*, 17(9). doi: 10.5812/ircmj.19960.

Fahimi, S. *et al.* (2016) 'Formulation of a traditionally used polyherbal product for burn healing and HPTLC fingerprinting of its phenolic contents', *Iranian Journal of Pharmaceutical Research*, 15(1), pp. 95–105.

Fatima, K. *et al.* (2020) 'Authentication of various commercially available crude drugs using different quality control testing parameters', *Pakistan Journal of Pharmaceutical Sciences*, 33(4), pp. 1641–1657. doi: 10.36721/PJPS.2020.33.4.REG.1641-1657.1.

Ferreira, S. L. C. *et al.* (2007) 'Box-Behnken design: An alternative for the optimization of analytical methods', *Analytica Chimica Acta*, 597(2), pp. 179–186. doi: 10.1016/j.aca.2007.07.011.

Forslind, B. O. (1994) 'A domain mosaic model of the skin barrier', *Acta Dermato-Venereologica*, pp. 1–6. doi: 10.2340/00015555741214.

Freitas, A. P. F. *et al.* (2012) 'Protein fraction of Calotropis procera latex protects against 5-fluorouracil-induced oral mucositis associated with downregulation of pivotal pro-inflammatory mediators', *Naunyn-Schmiedeberg's Archives of Pharmacology*, 385(10), pp. 981–990. doi: 10.1007/s00210-012-0778-3.

Freitas, C. D. T. *et al.* (2007) 'Enzymatic activities and protein profile of latex from Calotropis procera', *Plant Physiology and Biochemistry*, 45(10–11). doi: 10.1016/j.plaphy.2007.07.020.

Friedl, H. P. *et al.* (1989) 'Roles of histamine, complement and xanthine oxidase in thermal injury of skin', *American Journal of Pathology*, 135(1), pp. 203–217.

Fumal, I. *et al.* (2002) 'The beneficial toxicity paradox of antimicrobials in leg ulcer healing impaired by a polymicrobial flora: A proof-of-concept study', *Dermatology*, 204(SUPPL. 1), pp. 70–74. doi: 10.1159/000057729.

G Y Sy 1, R. B. N. (2005) 'Healing activity of methanolic extract of the barks of Spathodea campanulata Beauv (Bignoniaceae) in rat experimental burn model]. .', *Dakar Med. 2005;*, 50(2), pp. 77–81.

Gabbiani, G. (2003) 'The myofibroblast in wound healing and fibrocontractive diseases', *Journal of Pathology*, 200(4), pp. 500–503. doi: 10.1002/path.1427.

Ganesh Babu, Sanjeeva, K. L. B. (2003) 'ffect of Tridax procumbens on burn wound healing', *indian drugs*, 40(8), pp. 488–491.

Gauglitz, G. G. *et al.* (2011) 'Hypertrophic scarring and keloids: Pathomechanisms and current and emerging treatment strategies', *Molecular Medicine*, 17(1–2), pp. 113–125. doi: 10.2119/molmed.2009.00153.

Ghasemi Pirbalouti, A., Azizi, S. and Koohpayeh, A. (2012) 'Healing potential of iranian traditional medicinal plants on burn wounds in alloxan-induced diabetic rats', *Revista Brasileira de Farmacognosia*, 22(2), pp. 397–403. doi: 10.1590/S0102-695X2011005000183.

Ghoneim, A. I. *et al.* (2002) 'Protective effects of curcumin against ischaemia/reperfusion insult in rat forebrain', *Pharmacological Research*, 46(3), pp. 273–279. doi: 10.1016/S1043-6618(02)00123-8.

Gomes, F. S. L. *et al.* (2010) 'Wound-healing activity of a proteolytic fraction from Carica candamarcensis on experimentally induced burn', *Burns*, 36(2), pp. 277–283. doi: 10.1016/j.burns.2009.04.007.

Gómez Castellanos, J. R., Prieto, J. M. and Heinrich, M. (2009) 'Red Lapacho (Tabebuia impetiginosa)-A global ethnopharmacological commodity?', *Journal of Ethnopharmacology*, 121(1), pp. 1–13. doi: 10.1016/j.jep.2008.10.004.

Gopinath, D. *et al.* (2004) 'Dermal wound healing processes with curcumin incorporated collagen films', *Biomaterials*, 25(10), pp. 1911–1917. doi: 10.1016/S0142-9612(03)00625-2.

Gopinath, S. M. *et al.* (2012) 'Preliminary phytochemical evaluation of leaf extracts of Gymnema sylvestre, Phyllanthus amarus, Phyllanthus reticulatus of Siddarabetta, Tumkur district, Karnataka', *International Journal of Pharmacognosy and Phytochemical Research*, 4(3), pp. 109–111.

Gorter, R. W., Butorac, M. and Cobian, E. P. (2004) 'Examination of the cutaneous

absorption of copper after the use of copper-containing ointments.', *American journal of therapeutics*, 11(6), pp. 453–458. doi: 10.1097/01.mjt.0000127148.83065.e5.

Greenhalgh, D. G. (2009) 'Topical Antimicrobial Agents for Burn Wounds', *Clinics in Plastic Surgery*, 36(4), pp. 597–606. doi: 10.1016/j.cps.2009.05.011.

guang Liang, X. L. (2015) 'synthesis and anti-inflammatory activities of monocarbonyl analogues of curcumin', *Bioorg Med Chem Lett.*, 18(4), pp. 1525–1529.

Gull, A. *et al.* (2020) 'Effect of polyherbal microemulsion on Staphylococcus epidermidis: Formulation development, CCD based optimization, characterization, and antibacterial activity by scanning electron microscopy', *Journal of Drug Delivery Science and Technology*, 57, p. 101641. doi: 10.1016/j.jddst.2020.101641.

Guo, H.-F. *et al.* (2017) 'A new model for studying deep partial-thickness burns in rats.', *International journal of burns and trauma*, 7(6), pp. 107–114. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29119063%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5665842.

Gupta, S. and Ravishankar, S. (2005) 'A Comparison of the Antimicrobial Activity of Garlic, Ginger, Carrot, and Turmeric Pastes Against', *FoodBorne Pathogens and Disease*, 2(4), pp. 330–340.

Gupta, R., Lakshmi, V. and Jha, C. (2015) 'X-Ray Diffraction of different samples of Swarna Makshika Bhasma', *AYU (An International Quarterly Journal of Research in Ayurveda)*, 36(2), p. 225. doi: 10.4103/0974-8520.175547.

Gupta, S. C., Kismali, G. and Aggarwal, B. B. (2013) 'Curcumin, a component of turmeric: From farm to pharmacy', *BioFactors*, 39(1), pp. 2–13. doi: 10.1002/biof.1079.

H.Hamman, J. (2008) 'composition and application of aloe vera leaf gel', (13), pp. 1599–1616. doi: DOI: 10.3390/molecules13081599.

Hafezi, F. *et al.* (2010) 'Actinidia deliciosa (kiwifruit), a new drug for enzymatic debridement of acute burn wounds', *Burns*, 36(3), pp. 352–355. doi: 10.1016/j.burns.2009.04.021.

Hallerstig, L. M. *et al.* (2017) 'Determination of silver in soft silicone wound dressings using dodecylbenzene sulfonic acid digestion and inductively coupled plasma optical emission spectroscopy', *Analytical Methods*, 9(1), pp. 149–153. doi: 10.1039/c6ay02230h.

Hamman, J. H. (2008) 'Composition and applications of Aloe vera leaf gel', *Molecules*, pp. 1599–1616. doi: 10.3390/molecules13081599.

Hamnett, N. *et al.* (2016) 'Improving and maintaining conformity of acticoat® dressings with shear reducing transfixion suture', *Journal of Burn Care and Research*, 37(4), pp. e398–e399. doi: 10.1097/BCR.0000000000000235.

Hans, M. *et al.* (2013) 'Role of copper oxides in contact killing of bacteria', *Langmuir*, 29(52), pp. 16160–16166. doi: 10.1021/la404091z.

Harikrishnan, R. and Balasundaram, C. (2008) 'In vitro and in vivo studies of the use of some medicinal herbals against the pathogen Aeromonas hydrophila in goldfish', *Journal of Aquatic Animal Health*, 20(3), pp. 165–176. doi: 10.1577/H05-035.1.

Harwansh, R. K., Deshmukh, R. and Rahman, M. A. (2019) 'Nanoemulsion: Promising

nanocarrier system for delivery of herbal bioactives', *Journal of Drug Delivery Science and Technology*, 51, pp. 224–233. doi: 10.1016/J.JDDST.2019.03.006.

Hashmi, D. L. S. and Haith, L. (2019) 'The Current State of Topical Burn Treatments: a Review', *Current Trauma Reports*, 5(3), pp. 160–168. doi: 10.1007/s40719-019-00170-w.

Hasmann, A. *et al.* (2013) 'Analysis of myeloperoxidase activity in wound fluids as a marker of infection', *Annals of Clinical Biochemistry*, 50(3), pp. 245–254. doi: 10.1258/acb.2011.010249.

Hassanzadeh, G. *et al.* (2013) 'Comparing effects of Silver sulfadiazine, Sucralfate and Brassica oleracea extract on burn wound healing', *Life Science Journal*, 10(SUPPL.3), pp. 104–113.

Hay, E. *et al.* (2019) 'Therapeutic effects of turmeric in several diseases: An overview', *Chemico-Biological Interactions*, 310, p. 108729. doi: 10.1016/j.cbi.2019.108729.

Hearing, V. J. and Jiménez, M. (1987) 'Mammalian tyrosinase-The critical regulatory control point in melanocyte pigmentation', *International Journal of Biochemistry*, 19(12), pp. 1141–1147. doi: 10.1016/0020-711X(87)90095-4.

Heggers, J. P. *et al.* (1996) 'Beneficial effect of Aloe on wound healing in an excisional wound model', *Journal of Alternative and Complementary Medicine*, 2(2), pp. 271–277. doi: 10.1089/acm.1996.2.271.

Hekmatpou, D. *et al.* (2019) 'The effect of aloe vera clinical trials on prevention and healing of skin wound: A systematic review', *Iranian Journal of Medical Sciences*, 44(1), pp. 1–9.

Hemmila, M. R. *et al.* (2010) 'Topical nanoemulsion therapy reduces bacterial wound infection and inflammation after burn injury', *Surgery*, 148(3), pp. 499–509. doi: 10.1016/j.surg.2010.01.001.

Hęś, M. and Gujska, & K. D. & D. G. & A. J.-G. & E. (2019) 'Aloe vera as a natural source of antioxidants- A Review', *plants foods and human nutrition*, 74(3), pp. 255–265. doi: 10.3166/phyto-2019-0213.

Hewlings, S. J. and Kalman, D. S. (2017) 'Curcumin: A review of its effects on human health', *Foods*, 6(10), pp. 1–11. doi: 10.3390/foods6100092.

Hill, J. R. and Wertz, P. W. (2003) 'Molecular models of the intercellular lipid lamellae from epidermal stratum corneum', *Biochimica et Biophysica Acta - Biomembranes*, 1616(2), pp. 121–126. doi: 10.1016/S0005-2736(03)00238-4.

Hostýnek, J. J. and Maibach, H. I. (2006) 'Copper hypersensitivity: Dermatologic aspects-overview', *Copper and the Skin*, pp. 115–148.

Hu, Y., Xu, J. and Hu, Q. (2003) 'Evaluation of Antioxidant Potential of Aloe vera (Aloe barbadensis Miller) Extracts', *Journal of Agricultural and Food Chemistry*, 51(26), pp. 7788–7791. doi: 10.1021/jf034255i.

Humbert, P. *et al.* (2004) *Agache ' s Measuring the Skin*.

Ikpeama, A., Onwuka, G. I. and Nwankwo, C. (2014) 'Nutritional composition of tumeric (Curcuma longa) and its antimicrobial properties', *International Journal of Scientific & Engineering Research*, 5(10), pp. 1085–1089.

Ishika.Roy et al (2023) 'Evaluation of Wound Healing Property of the Ethanolic Extract of

Glycyrrhiza Glabra on Vero Cell Lines Using In Vitro Scratch Assay Test', *Journal of Pharmacy and Bioallied Sciences*, 15(10), pp. S630–S635. doi: 10.4103/jpbs.JPBS_61_23.

Iwai, I. *et al.* (2012) 'The human akin barrier is organized as stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety', *Journal of Investigative Dermatology*, 132(9), pp. 2215–2225. doi: 10.1038/jid.2012.43.

Jagetia, G. C. and Rajanikant, G. K. (2012) 'Acceleration of wound repair by curcumin in the excision wound of mice exposed to different doses of fractionated γ radiation', *International Wound Journal*, 9(1), pp. 76–92. doi: 10.1111/j.1742-481X.2011.00848.x.

Jain, S. *et al.* (2007) 'Recent trends in Curcuma longa Linn.', *Pharmacognosy Reviews*, 1(1), pp. 119–128.

Jaiprakash, B. *et al.* (2006) 'Wound healing activity of Euphorbia hirta Linn.', *Indian Drugs*, 43(2), pp. 112–116.

Jalali, F. S. S. *et al.* (2009) 'Clinical and Microbiological Evaluations of Efficacy of Combination of Natural Honey and Yarrow on Repair Process of Experimental Burn Wound', *Journal of Animal and Veterinary Advances*, 8(8), pp. 907–911.

Jamadar, M. J. and Husen Shaikh, R. (2017) 'Preparation and Evaluation of Herbal Gel Formulation', *Research Article Journal of Pharmaceutical Research & Education Journal homepage*, 1(2), pp. 201–224. Available at: http://www.gyanvihar.org/researchjournals/.

Jarrahi, M. (2008) 'An experimental study of the effects of Matricaria chamomilla extract on cutaneous burn wound healing in albino rats', *Natural Product Research*, 22(5), pp. 422–427. doi: 10.1080/14786410701591713.

Jeschke, M. G. *et al.* (2020) 'Burn injury', *Nature Reviews Disease Primers*, 6(1). doi: 10.1038/s41572-020-0145-5.

Jibril, M. *et al.* (2012) 'Production and Characterization of Biodiesel from Jatropha Oil and Neem Oil', *International Journal of Emerging trends in Engineering and Development*, (March).

Joe, B., Vijaykumar, M. and Lokesh, B. R. (2004) 'Biological Properties of Curcumin-Cellular and Molecular Mechanisms of Action', *Critical Reviews in Food Science and Nutrition*, 44(2), pp. 97–111. doi: 10.1080/10408690490424702.

Jogpal, V. *et al.* (2022) 'Studies on Wound Healing Potential of Polyherbal Formulation using in vitro and in vivo Assays in Swiss Albino Rats', *Pharmacognosy Research*, 14(3), pp. 263–268. doi: 10.5530/pres.14.3.38.

Kang, Y. A. *et al.* (2009) 'Copper-GHK increases integrin expression and p63 positivity by keratinocytes', *Archives of Dermatological Research*, 301(4), pp. 301–306. doi: 10.1007/s00403-009-0942-x.

Karimi, M. *et al.* (2013) 'Effectsof Camellia sinensis ethanolic extract on histometric and histopathological healing process of burn wound in rat', *Middle East Journal of Scientific Research*, 13(1), pp. 14–19. doi: 10.5829/idosi.mejsr.2013.13.1.66118.

Kashibuchi, N. *et al.* (2002) 'Three-dimensional analyses of individual corneocytes with atomic force microscope: Morphological changes related to age, location and to the pathologic skin conditions', *Skin Research and Technology*, 8(4), pp. 203–211. doi:

10.1034/j.1600-0846.2002.00348.x.

Kashyap, N. K. and Hait, M. (2021) 'Preliminary Phytochemical Screening onRoot', 27(7), pp. 783–791.

Kaur, G., Alam, M. S. and Athar, M. (2004) 'Nimbidin suppresses functions of macrophages and neutrophils: Relevance to its antiinflammatory mechanisms', *Phytotherapy Research*, 18(5), pp. 419–424. doi: 10.1002/ptr.1474.

Kawahira, K. *et al.* (2008) 'Effects of ginsenoside Rb1 at low doses on histamine, substance P, and monocyte chemoattractant protein 1 in the burn wound areas during the process of acute burn wound repair', *Journal of Ethnopharmacology*, 117(2), pp. 278–284. doi: 10.1016/j.jep.2008.01.032.

Kesarwani, K. and Gupta, R. (2013) 'Bioavailability enhancers of herbal origin: An overview', *Asian Pacific Journal of Tropical Biomedicine*, 3(4), pp. 253–266. doi: 10.1016/S2221-1691(13)60060-X.

Khan, A. W. *et al.* (2013) 'Formulation development, optimization and evaluation of aloe vera gel for wound healing', *Pharmacognosy Magazine*, 9(36), pp. S6–S10. doi: 10.4103/0973-1296.117849.

Khanavi, Mahnaz *et al.* (2013) 'Antioxidant and burn healing potential of Galium odoratum extracts', *Research in Pharmaceutical Sciences*, 8(3), pp. 197–203.

Khanna, V. *et al.* (2018) 'NANOEMULSIONS – PRESENT AND FUTURE PERSPECTIVE - AN OVERVIEW', *JETIR*, 5(12), pp. 354–370.

Khanna, V. *et al.* (2024) 'Proportional analysis of powdered curcuma longa and curcuma longa extract', *14Th International Conference on Materials Processing and Characterization 2023*, 3007, p. 030027. doi: 10.1063/5.0192533.

Khogta, S. *et al.* (2020) 'Herbal nano-formulations for topical delivery', *Journal of Herbal Medicine*, 20(December 2017), p. 100300. doi: 10.1016/j.hermed.2019.100300.

Khorasani, G. *et al.* (2008) 'The effect of saffron (crocus sativus) extract for healing of second-degree burn wounds in rats', *Keio Journal of Medicine*, 57(4), pp. 190–195. doi: 10.2302/kjm.57.190.

Khosroshahi, A. F. *et al.* (2019) 'Skin burns: Review of molecular mechanisms and therapeutic approaches', *Wounds*, 31(12), pp. 308–315.

Khundkar, R., Malic, C. and Burge, T. (2010) 'Use of Acticoat^{TM} dressings in burns: What is the evidence?', *Burns*, 36(6), pp. 751–758. doi: 10.1016/j.burns.2009.04.008.

Kim, D. C. *et al.* (2005) 'Curcuma longa extract protects against gastric ulcers by blocking H 2 histamine receptors', *Biological and Pharmaceutical Bulletin*, 28(12), pp. 2220–2224. doi: 10.1248/bpb.28.2220.

Kiran, K. and Asad, M. (2008) 'Wound healing activity of sesamum indicum L seed and oil in rats', *Indian Journal of Experimental Biology*, 46(11), pp. 777–782.

Kollef, M. H. *et al.* (1999) 'Inadequate antimicrobial treatment of infections: A risk factor for hospital mortality among critically III patients', *Chest*, 115(2), pp. 462–474. doi: 10.1378/chest.115.2.462.

Kotian, S. *et al.* (2018) 'The Role of Natural Medicines on Wound Healing: A

Biomechanical, Histological, Biochemical and Molecular Study', *Ethiopian journal of health sciences*, 28(6), pp. 759–770. doi: 10.4314/ejhs.v28i6.11.

Kotta, S. *et al.* (2015) 'Formulation of nanoemulsion: A comparison between phase inversion composition method and high-pressure homogenization method', *Drug Delivery*, 22(4), pp. 455–466. doi: 10.3109/10717544.2013.866992.

Krokida, M., Pappa, A. and Agalioti, M. (2011) 'Effect of drying on Aloe's functional components', *Procedia Food Science*, 1, pp. 1523–1527. doi: 10.1016/j.profoo.2011.09.225.

Kulac, M. *et al.* (2013) 'The effects of topical treatment with curcumin on burn wound healing in rats', *Journal of Molecular Histology*, 44(1), pp. 83–90. doi: 10.1007/s10735-012-9452-9.

Kulhari, A. *et al.* (2013) 'Investigation of heavy metals in frequently utilized medicinal plants collected from environmentally diverse locations of north western India', *SpringerPlus*, 2(1), pp. 1–9. doi: 10.1186/2193-1801-2-676.

Kumar Dubey, V. and Jagannadham, M. V. (2003) *Procerain, a stable cysteine protease from the latex of Calotropis procera*, *Phytochemistry*. doi: 10.1016/S0031-9422(02)00676-3.

Kumar, M. *et al.* (2019) 'Techniques for formulation of nanoemulsion drug delivery system: A review', *Preventive Nutrition and Food Science*, 24(3), pp. 225–234. doi: 10.3746/pnf.2019.24.3.225.

Kumar, V. *et al.* (2015) 'Antiedematogenic and antioxidant properties of high molecular weight protein sub-fraction of Calotropis procera latex in rat', *journal of basic and clinical pharmacy*, 6(2), p. 69. doi: 10.4103/0976-0105.152098.

Kumar, V. *et al.* (2021) ' Pharmacodynamic appraisal of wound-healing herbs of Sushruta Samhita ', *AYU (An international quarterly journal of research in Ayurveda)*, 42(1), p. 1. doi: 10.4103/ayu.ayu_34_20.

Kumar, V. L. *et al.* (2006) 'A comparative study on the efficacy of rofecoxib in monoarticular arthritis induced by latex of Calotropis procera and Freund's complete adjuvant', *Inflammopharmacology*, 14(1–2), pp. 17–21. doi: 10.1007/s10787-006-1512-x.

Kumar, V. L. and Basu, N. (1994) *Anti-inflammatory activity of the latex of Calotropis procera*, *Journal of Ethnopharmacology*.

Kunle (2012) 'Standardization of herbal medicines - A review', *International Journal of Biodiversity and Conservation*, 4(3), pp. 101–112. doi: 10.5897/ijbc11.163.

Lachiewicz, A. M. *et al.* (2017) 'Bacterial Infections after Burn Injuries: Impact of Multidrug Resistance', *Clinical Infectious Diseases*, 65(12), pp. 2130–2136. doi: 10.1093/cid/cix682.

Lai, F. *et al.* (2020) 'What's new in the field of phospholipid vesicular nanocarriers for skin drug delivery', *International Journal of Pharmaceutics*, 583, p. 119398. doi: 10.1016/j.ijpharm.2020.119398.

Landfester, K. *et al.* (1999) 'Formulation and stability mechanisms of polymerizable miniemulsions', *Macromolecules*, 32(16), pp. 5222–5228. doi: 10.1021/ma990299+.

Lantz, R. C. *et al.* (2005) 'The effect of turmeric extracts on inflammatory mediator production', *Phytomedicine*, 12(6–7), pp. 445–452. doi: 10.1016/j.phymed.2003.12.011.

Lazarus, G. S. *et al.* (1994) 'Definitions and Guidelines for Assessment of Wounds and

Evaluation of Healing', *Archives of Dermatology*, 130(4), pp. 489–493. doi: 10.1001/archderm.1994.01690040093015.

Lijnen H.R (2002) 'Matrix Metalloproteinases and Cellular Fibrinolytic Activity', *BIOCHEMISTRY* , pp. 107–115. doi: 10.1023/a:1013908332232.

Lima-Filho, J. V. *et al.* (2010) 'Proteins from latex of Calotropis procera prevent septic shock due to lethal infection by Salmonella enterica serovar Typhimurium', *Journal of Ethnopharmacology*, 129(3). doi: 10.1016/j.jep.2010.03.038.

Linares, H. A. (1996) 'From wound to scar', *Burns*, 22(5), pp. 339–352. doi: 10.1016/0305-4179(95)00164-6.

Lisa C. Zaba, James G Krueger, and M. A. L. (2009) 'Resident AND "inflammatory" dendritic cells in human skin', *Journal of inves*, 2(129), pp. 1–14. doi: 10.1038/jid.2008.225.Resident.

Lissoni, P. *et al.* (1998) 'Biotherapy with the pineal immunomodulating hormone melatonin versus melatonin plus Aloe vera in untreatable advanced solid neoplasms', *Natural Immunity*, 16(1), pp. 27–33. doi: 10.1159/000069427.

Liu, M. *et al.* (2008) 'Madecassoside isolated from Centella asiatica herbs facilitates burn wound healing in mice', *Planta Medica*, 74(8), pp. 809–815. doi: 10.1055/s-2008-1074533.

Lodhi, S. *et al.* (2010) 'Effect of Tephrosia purpurea (L) pers. On partial thickness and full thickness burn wounds in rats', *Journal of Complementary and Integrative Medicine*, 7(1). doi: 10.2202/1553-3840.1344.

Lovelyn, C. and Attama, A. A. (2011) 'Current State of Nanoemulsions in Drug Delivery', *Journal of Biomaterials and Nanobiotechnology*, 02(05), pp. 626–639. doi: 10.4236/jbnb.2011.225075.

Luza, S., nutrition, H. S.-T. A. journal of clinical and 1996, undefined (1996) 'Liver copper storage and transport during development: implications for yt'2', *Academic.Oup.Com*, (March). Available at: https://academic.oup.com/ajcn/article/63/5/812S/4651475.

Madan Ranjit, P. and Rao, E. G. (2012) 'AN OVERVIEW OF PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF CALOTROPIS PROCERA', *FS J Pharm Res* |, 1(2), pp. 18–25. Available at: www.fonscientia.com/jpharmres.

Maenthaisong, R. *et al.* (2007) 'The efficacy of aloe vera used for burn wound healing: A systematic review', *Burns*, 33(6), pp. 713–718. doi: 10.1016/j.burns.2006.10.384.

Mahmoudi, M. *et al.* (2013) 'Anti-inflammatory and analgesic effects of egg yolk: A comparison between organic and machine made', *European Review for Medical and Pharmacological Sciences*, 17(4), pp. 472–476.

Mahung, C. *et al.* (2022) 'Early expression of IL-10, IL-12, ARG1, and NOS2 genes in peripheral blood mononuclear cells synergistically correlate with patient outcome after burn injury', *Journal of Trauma and Acute Care Surgery*, 93(5), pp. 702–711. doi: 10.1097/TA.0000000000003602.

Majeed, A. *et al.* (2019) 'Preparation, Characterization and Applications of Nanoemulsions: An Insight', *Journal of Drug Delivery and Therapeutics*, 9(2), pp. 520–527. doi: 10.22270/jddt.v9i2.2410.

Majno, G. and Pas-, L. (2000) 'Past, Present, and Future of Antimicrobial Agents', 99, pp. 11–18.

Manafi, A. *et al.* (2008) 'Enhancing drugs absorption through third-degree burn wound eschar', *Burns*, 34(5), pp. 698–702. doi: 10.1016/j.burns.2007.07.018.

Manasa, P. S. L., Kamble, A. D. and Chilakamarthi, U. (2023) 'Various Extraction Techniques of Curcumin─A Comprehensive Review', *ACS Omega*, 8(38), pp. 34868–34878. doi: 10.1021/acsomega.3c04205.

Margadant, C. and Sonnenberg, A. (2010) 'and Wound Healing', 11(2), pp. 97–105.

Maver, T. *et al.* (2015) 'A review of herbal medicines in wound healing', *International Journal of Dermatology*, 54(7), pp. 740–751. doi: 10.1111/ijd.12766.

Mazonde, P., Khamanga, S. M. M. and Walker, R. B. (2020) 'Design, optimization, manufacture and characterization of Efavirenz-loaded flaxseed oil nanoemulsions', *Pharmaceutics*, 12(9), pp. 1–22. doi: 10.3390/pharmaceutics12090797.

McClements, D. J. (2012) 'Nanoemulsions versus microemulsions: Terminology, differences, and similarities', *Soft Matter*, 8(6), pp. 1719–1729. doi: 10.1039/c2sm06903b.

McIntosh, T. J. (2003) 'Organization of skin stratum corneum extracellular lamellae: Diffraction evidence for asymmetric distribution of cholesterol', *Biophysical Journal*, 85(3), pp. 1675–1681. doi: 10.1016/S0006-3495(03)74597-4.

McQuillan, R. F. *et al.* (2012) 'A randomized controlled trial comparing mupirocin and polysporin triple ointments in peritoneal dialysis patients: The MP 3 study', *Clinical Journal of the American Society of Nephrology*, 7(2), pp. 297–303. doi: 10.2215/CJN.07970811.

Merzaia, A. B. *et al.* (2017) 'Isolation of Bioactive components from Calotropis procera Plant Latex-A Review', *Ijcbs*, 11(January), pp. 95–101. Available at: www.iscientific.org/Journal.html.

Michaels, A. S., Chandrasekaran, S. K. and Shaw, J. E. (1975) 'Drug permeation through human skin: Theory and invitro experimental measurement', *AIChE Journal*, 21(5), pp. 985–996. doi: 10.1002/aic.690210522.

Mishra, S. *et al.* (2022) 'Potential of Polyherbal Formulation in Burn Wound Model', *Scholars International Journal of Traditional and Complementary Medicine*, 5(1), pp. 19–23. doi: 10.36348/sijtcm.2022.v05i01.003.

Modamio, P., Lastra, C. F. and Mariño, E. L. (2000) 'A comparative in vitro study of percutaneous penetration of β-blockers in human skin', *International Journal of Pharmaceutics*, 194(2), pp. 249–259. doi: 10.1016/S0378-5173(99)00380-4.

Mohanty, C., Das, M. and Sahoo, S. K. (2012) 'Chadana_2012_Sustained wound healing activity of curcumin.pdf'.

Mohanty, C. and Sahoo, S. K. (2017) 'Curcumin and its topical formulations for wound healing applications', *Drug Discovery Today*, 22(10), pp. 1582–1592. doi: 10.1016/j.drudis.2017.07.001.

Moniruzzaman, M. *et al.* (2012) 'In vitro antioxidant effects of aloe barbadensis miller extracts and the potential role of these extracts as antidiabetic and antilipidemic agents on streptozotocin-induced type 2 diabetic model rats', *Molecules*, 17(11), pp. 12851–12867. doi:

10.3390/molecules171112851.

Morganti, P. *et al.* (2001) 'Percutaneous absorption and delivery systems', *Clinics in Dermatology*, 19(4), pp. 489–501. doi: 10.1016/S0738-081X(01)00183-3.

Moser, K. *et al.* (2001) 'Passive skin penetration enhancement and its quantification in vitro', *European Journal of Pharmaceutics and Biopharmaceutics*, 52(2), pp. 103–112. doi: 10.1016/S0939-6411(01)00166-7.

Mueen Ahmed, K. R. A.-D. V (1999) 'Calotropis species (Asclepediaceae) A comprehensive Review', 2(1), pp. 48–52. Available at: http://www.phcog.com/article.asp?issn=0973-1296;year=2005;volume=1;issue=2;spage=48;epage=52;aulast=Mueen;type=0 4100-http://www.phcog.com/article.asp?issn=0973-1296;year=2005;volume=1;issue=2;spage=48;epage=52;aulast=Mueen (Accessed: 19 November 2021).

Müller, G. *et al.* (2002) 'Interleukin-10-treated dendritic cells modulate immune responses of naive and sensitized T cells In vivo', *Journal of Investigative Dermatology*, 119(4). doi: 10.1046/j.1523-1747.2002.00496.x.

Mustafa, S. K. and AlSharif, M. A. (2018) 'Copper (Cu) an Essential Redox-Active Transition Metal in Living System—A Review Article', *American Journal of Analytical Chemistry*, 09(01), pp. 15–26. doi: 10.4236/ajac.2018.91002.

N.Niamsa and Sittiwet, C. (2009) 'antimicrobial activity of curcuma longa aqueous extract'. mahasankhara 44150 thialand: academic journals, pp. 173–177. doi: 10.3923/jpt.2009.173.177.

Nafisi, S. and Maibach, H. I. (2018) *Skin penetration of nanoparticles*, *Emerging Nanotechnologies in Immunology: The Design, Applications and Toxicology of Nanopharmaceuticals and Nanovaccines*. Elsevier Inc. doi: 10.1016/B978-0-323-40016-9.00003-8.

Naik, M. *et al.* (2014) 'Study of anti-inflammatory effect of neem seed oil (Azadirachta indica) on infected albino rats', *Journal of Health Research and Reviews*, 1(3), p. 66. doi: 10.4103/2394-2010.153880.

Nandiyanto, A. B. D., Oktiani, R. and Ragadhita, R. (2019) 'How to read and interpret ftir spectroscope of organic material', *Indonesian Journal of Science and Technology*, 4(1), pp. 97–118. doi: 10.17509/ijost.v4i1.15806.

Nascimento, D. C. D. O. *et al.* (2016) 'Latex protein extracts from Calotropis procera with immunomodulatory properties protect against experimental infections with Listeria monocytogenes', *Phytomedicine*, 23(7), pp. 745–753. doi: 10.1016/j.phymed.2016.03.012.

Nayak, B. S., Raju, S. S. and Ramsubhag, A. (2008) 'Investigation of wound healing activity of Lantana camara L. in Sprague dawley rats using a burn wound model', *International Journal of Applied Research in Natural Products*, 1(1), pp. 15–19.

Nenaah, G. E. (2013) 'Potential of using flavonoids, latex and extracts from Calotropis procera (Ait.) as grain protectants against two coleopteran pests of stored rice', *Industrial Crops and Products*, 45, pp. 327–334. doi: 10.1016/j.indcrop.2012.12.043.

Ng, K. W. and Lau, and W. M. (2015) 'Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Drug Manipulation Strategies and Vehicle Effects', in Nina Dragicevic, H. I. M. (ed.) *Springer, Berlin, Heidelberg*. Berlin, Heidelberg: Springer,

pp. 3–11. doi: 10.1007/978-3-662-45013-0.

Noorbakhsh, S. I. *et al.* (2021) 'Educational Case: Burn Injury—Pathophysiology, Classification, and Treatment', *Academic Pathology*, 8. doi: 10.1177/23742895211057239.

Norlén, L. (2001) 'Skin barrier structure and function: The single gel phase model', *Journal of Investigative Dermatology*, 117(4), pp. 830–836. doi: 10.1046/j.1523-1747.2001.01463.x.

Nortjie, E. *et al.* (2022) 'Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review', *Plants*, 11(15). doi: 10.3390/plants11152011.

Ofoegbu, O. and Uzo Anya, A. (2012) 'optimised reduction of free fatty acid content from neem seed oil for biodiesel production. Sustainable Eco-construction View project Food Chemistry and sustainability View project', (September). Available at: www.textroad.com.

Olivares, C. and Solano, F. (2009) 'New insights into the active site structure and catalytic mechanism of tyrosinase and its related proteins', *Pigment Cell and Melanoma Research*, 22(6), pp. 750–760. doi: 10.1111/j.1755-148X.2009.00636.x.

Olivares, M. and Uauy, R. (1996) 'Copper as an essential nutrient', *American Journal of Clinical Nutrition*, 63(5). doi: 10.1093/ajcn/63.5.791.

Oliveira, R. S. B. *et al.* (2012) 'Inflammation induced by phytomodulatory proteins from the latex of Calotropis procera (Asclepiadaceae) protects against Salmonella infection in a murine model of typhoid fever', *Inflammation Research*, 61(7). doi: 10.1007/s00011-012-0460-8.

Omidbakhsh, N. and Sattar, S. A. (2006) 'Broad-spectrum microbicidal activity, toxicologic assessment, and materials compatibility of a new generation of accelerated hydrogen peroxide-based environmental surface disinfectant', *American Journal of Infection Control*, 34(5), pp. 251–257. doi: 10.1016/j.ajic.2005.06.002.

Oryan, A. *et al.* (2010) 'Effect of aqueous extract of Aloe vera on experimental cutaneous wound healing in rat', *Veterinarski Arhiv*, 80(4), pp. 509–522.

Oryan, A., Alemzadeh, E. and Moshiri, A. (2017) 'Burn wound healing: Present concepts, treatment strategies and future directions', *Journal of Wound Care*, 26(1), pp. 5–19. doi: 10.12968/jowc.2017.26.1.5.

Pallavi Srivastava 1, S. D. (2008) 'Burn wound healing property of Cocos nucifera: An appraisal', *Indian Journal of Pharmacology*, 40(4), pp. 144–6. doi: 10.4103/0253-7613.43159.

Panchatcharam, M. *et al.* (2006) 'Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species', *Molecular and Cellular Biochemistry*, 290(1–2), pp. 87–96. doi: 10.1007/s11010-006-9170-2.

Paramasivam, M. *et al.* (2009) 'High-performance thin layer chromatographic method for quantitative determination of curcuminoids in Curcuma longa germplasm', *Food Chemistry*, 113(2), pp. 640–644. doi: 10.1016/j.foodchem.2008.07.051.

Parasuraman, S., Thing, G. S. and Dhanaraj, S. A. (2014) 'Polyherbal formulation: Concept of ayurveda', *Pharmacognosy Reviews*, 8(16), pp. 73–80. doi: 10.4103/0973-7847.134229.

Parihar, G. and Balekar, N. (2016) 'Calotropis procera: A phytochemical and

pharmacological review', *Thai Journal of Pharmaceutical Sciences*.

Parks, S. E. G. and W. C. (2009) 'Metalloproteinases and Their Inhibitors: Regulators of Wound Healing', *Int j Biochem Cell Biol*, 40(6–7), pp. 1344–1347. doi: 10.1016/j.biocel.2007.10.024.Metalloproteinases.

Patel, D. K., Patel, K. and Dhanabal, S. (2012) 'Phytochemical standardization of Aloe vera extract by HPTLC techniques', *Journal of Acute Disease*, 1(1), pp. 47–50. doi: 10.1016/s2221-6189(13)60011-6.

Pawar, M. A., Patil, S. S. and Nagrik, D. M. (2015) 'Phytochemical and Physicochemical Investigation of Curcuma Longa Linn Rhizome', *International Journal of Chemical and Physical Sciences*, 4, pp. 2319–6602. Available at: www.ijcps.org.

Payne, W. G. *et al.* (2008) 'Enzymatic debriding agents are safe in wounds with high bacterial bioburdens and stimulate healing.', *Eplasty*, 8, p. e17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18461120%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2311452.

Peñaloza, H. F. *et al.* (2016) 'Opposing roles of IL-10 in acute bacterial infection', *Cytokine and Growth Factor Reviews*. doi: 10.1016/j.cytogfr.2016.07.003.

Pickart, L. and Margolina, A. (2018) 'Regenerative and protective actions of the GHK-Cu peptide in the light of the new gene data', *International Journal of Molecular Sciences*, 19(7). doi: 10.3390/ijms19071987.

Pillai, N. R. and Santhakumari, G. (1981) 'Anti-arthritic and anti-inflammatory actions of nimbidin', *Planta Medica*, 43(1), pp. 59–63. doi: 10.1055/s-2007-971474.

Poole, M. D. (1993) 'Are we facing the end of the antibiotic era?', *Ear, Nose and Throat Journal*, 72(6), p. 433. doi: 10.1177/014556139307200614.

Poon, V. K. M. and Burd, A. (2004) 'In vitro cytotoxity of silver: Implication for clinical wound care', *Burns*, 30(2), pp. 140–147. doi: 10.1016/j.burns.2003.09.030.

Prajakta N. Dongare *et al.* (2021) 'Recent development in novel drug delivery systems for delivery of herbal drugs: An updates', *GSC Advanced Research and Reviews*, 8(2), pp. 008–018. doi: 10.30574/gscarr.2021.8.2.0158.

Prasad, S. *et al.* (2014) 'Curcumin, a component of golden spice: From bedside to bench and back', *Biotechnology Advances*, 32(6), pp. 1053–1064. doi: 10.1016/j.biotechadv.2014.04.004.

Priya, K. S. *et al.* (2002) 'Healing potential of Datura alba on burn wounds in albino rats', *Journal of Ethnopharmacology*, 83(3), pp. 193–199. doi: 10.1016/S0378-8741(02)00195-2.

R.S.Vardanyan and V.J.Hruby (2006) 'Sulfonamide drugs and trimethoprim', *Synthesis of Essential Drugs*, pp. 499–523.

R H Davis, K Y Rosenthal, L R Cesario, G. A. R. (1989) 'Processed Aloe vera administered topically inhibits inflammation', *J Am Podiatr Med Assoc* ., 79(8), pp. 395–97. doi: 10.7547/87507315-79-8-395.

Radha, M. H. and Laxmipriya, N. P. (2015) 'Evaluation of biological properties and clinical effectiveness of Aloe vera: A systematic review', *Journal of Traditional and Complementary Medicine*, 5(1), pp. 21–26. doi: 10.1016/j.jtcme.2014.10.006.

Raina, N. *et al.* (2022) 'Biopolymers and treatment strategies for wound healing: an insight view', *International Journal of Polymeric Materials and Polymeric Biomaterials*, 71(5), pp. 359–375. doi: 10.1080/00914037.2020.1838518.

Raina, N. *et al.* (2023) 'New Insights in Topical Drug Delivery for Skin Disorders: From a Nanotechnological Perspective', *ACS Omega*, 8(22), pp. 19145–19167. doi: 10.1021/acsomega.2c08016.

Ramos, M. V. *et al.* (2006) 'Latex proteins from the plant Calotropis procera are partially digested upon in vitro enzymatic action and are not immunologically detected in fecal material', *Fitoterapia*, 77(4). doi: 10.1016/j.fitote.2006.02.010.

Ramos, M. V. *et al.* (2007) 'Immunological and allergenic responses induced by latex fractions of Calotropis procera (Ait.) R.Br.', *Journal of Ethnopharmacology*, 111(1). doi: 10.1016/j.jep.2006.10.034.

Ramos, M. V. *et al.* (2009) 'Involvement of NO in the inhibitory effect of Calotropis procera latex protein fractions on leukocyte rolling, adhesion and infiltration in rat peritonitis model', *Journal of Ethnopharmacology*, 125(3). doi: 10.1016/j.jep.2009.07.030.

Ramos, M. V. *et al.* (2012) 'Proteins derived from latex of C. procera maintain coagulation homeostasis in septic mice and exhibit thrombin- and plasmin-like activities', *Naunyn-Schmiedeberg's Archives of Pharmacology*, 385(5), pp. 455–463. doi: 10.1007/s00210-012-0733-3.

Ramos, M. V. *et al.* (2013) 'New insights into the complex mixture of latex cysteine peptidases in calotropis procera', *International Journal of Biological Macromolecules*, 58, pp. 211–219. doi: 10.1016/j.ijbiomac.2013.04.001.

Ramos, M. V. *et al.* (2020) 'Anti-inflammatory latex proteins of the medicinal plant Calotropis procera: a promising alternative for oral mucositis treatment', *Inflammation Research*, 69(9), pp. 951–966. doi: 10.1007/s00011-020-01365-7.

Rasik, A. M. *et al.* (1999) 'Healing potential of Calotropis procera on dermal wounds in Guinea pigs', *Journal of Ethnopharmacology*, 68(1–3). doi: 10.1016/S0378-8741(99)00118-X.

Rastegar, F. *et al.* (2011) 'The effect of egg yolk oil in the healing of third degree burn wound in rats', *Iranian Red Crescent Medical Journal*, 13(10), pp. 739–743.

Ravanti, L. and Kähäri, V.-M. (2000) *Matrix metalloproteinases in wound repair (Review)*, *INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE*.

Ren, K. and Torres, R. (2009) 'Role of interleukin-1β during pain and inflammation', *Brain Research Reviews*, pp. 57–64. doi: 10.1016/j.brainresrev.2008.12.020.

Reuter, J. *et al.* (2008) 'Investigation of the anti-inflammatory potential of Aloe vera gel (97.5%) in the ultraviolet erythema test', *karger.com*, 21, pp. 106–110. doi: 10.1159/000114871.

Review, M. (2015) 'Research and Reviews : Research Journal of Biology NANOEMULSION : An Effective Therapy for Transdermal Drug', 3(1).

Roberts, M.S. and Walters, K. A. (2008) 'Dermal Absorption and Toxicity Assessment', in Roberts, M.S. and Walters, K. A. (ed.). USA, pp. 1–16. Available at: https://espace.library.uq.edu.au/view/UQ:172967.

Roy, S. *et al.* (2005) 'Antioxidant and protective effect of latex of Calotropis procera against alloxan-induced diabetes in rats', *Journal of Ethnopharmacology*, 102(3). doi: 10.1016/j.jep.2005.06.026.

Rucker, R. B. *et al.* (2018) 'Rucker1998', 67(February), pp. 996–1002.

Safaya, M. and Rotliwala, Y. C. (2020) 'Nanoemulsions: A review on low energy formulation methods, characterization, applications and optimization technique', *Materials Today: Proceedings*, 27(xxxx), pp. 454–459. doi: 10.1016/j.matpr.2019.11.267.

Saikaly, S. K. and Khachemoune, A. (2017) 'Honey and Wound Healing: An Update', *American Journal of Clinical Dermatology*, 18(2), pp. 237–251. doi: 10.1007/s40257-016-0247-8.

Sajithlal, G. B., Chithra, P. and Chandrakasan, G. (1999) 'An in vitro study on the role of metal catalyzed oxidation in glycation and crosslinking of collagen', *Molecular and Cellular Biochemistry*, 194(1–2), pp. 257–263. doi: 10.1023/A:1006988719374.

Sakarcan, A. *et al.* (2005) 'Ginkgo biloba extract improves oxidative organ damage in a rat model of thermal trauma', *Journal of Burn Care and Rehabilitation*, 26(6), pp. 515–524. doi: 10.1097/01.bcr.0000185115.17261.50.

Salari, M. *et al.* (2016) 'Efficacy of egg yolk and nitroglycerin ointment as treatments for acute anal fissures: A randomized clinical trial study', *Electronic physician*, 8(10), pp. 3035–3041. doi: 10.19082/3035.

Sarkar, S., Singh, R. P. and Bhattacharya, G. (2021) 'Exploring the role of Azadirachta indica (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach', *3 Biotech*, 11(4), pp. 1–12. doi: 10.1007/s13205-021-02745-4.

Scheuplein, R. J. (1978) 'Permeability of the skin: a review of major concepts.', *Current problems in dermatology*, pp. 172–186. doi: 10.1159/000401285.

Schröter, A. *et al.* (2009) 'Basic nanostructure of stratum corneum lipid matrices based on ceramides [EOS] and [AP]: A neutron diffraction study', *Biophysical Journal*, 97(4), pp. 1104–1114. doi: 10.1016/j.bpj.2009.05.041.

Schultz, G. S. *et al.* (2011) 'Principles of wound healing', in *Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists*. University of Adelaide Press, pp. 423–450. doi: 10.1017/UPO9781922064004.024.

Seddek, A. L. S. *et al.* (2009) 'Extract from Calotropis procera latex activates murine macrophages', *Journal of Natural Medicines*, 63(3). doi: 10.1007/s11418-009-0335-7.

Seyed Jalal Hosseinimehr 1, Ghasemali Khorasani (2010) 'Effect of aloe cream versus silver sulfadiazine for healing burn wounds in rats', *Acta Dermatovenerol Croat* ., 18(1), pp. 2–7.

Shaker, D. S. *et al.* (2019) 'Nanoemulsion: A review on mechanisms for the transdermal delivery of hydrophobic and hydrophilic drugs', *Scientia Pharmaceutica*, 87(3). doi: 10.3390/scipharm87030017.

Shanbhag, T. *et al.* (2010) 'Effect of Phyllanthus niruri. Linn on burn wound in rats', *Asian Pacific Journal of Tropical Medicine*, 3(2), pp. 105–108. doi: 10.1016/S1995-7645(10)60045-4.

Sharma, A., Singh, A. P. and Harikumar, S. L. (2020) 'Development and optimization of

nanoemulsion based gel for enhanced transdermal delivery of nitrendipine using box-behnken statistical design', *Drug Development and Industrial Pharmacy*, 46(2), pp. 329–342. doi: 10.1080/03639045.2020.1721527.

Sharma, K., Mittal, A. and Chauhan, N. (2015) 'Aloe vera as penetration enhancer', *International Journal of Drug Development and Research*, 7(1), pp. 31–43.

Sharma, P., Verma, S. and Misri, P. (2016) 'Global need for novel herbal drug formulations', *International Journal of Pharmacognosy and Phytochemical Research*, 8(9), pp. 1535–1544.

Shefali, K. and Sumeet, D. (2010) *INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES Indigeneous ayurvedic knowledge of some species in the treatment of human disease and disorders*, *Kaul & Dwivedi*.

shehan Hettiaratchy, peter D. (2004) 'Pathophysiology of burns', *Point Veterinaire*, 328, pp. 1427–9. doi: 10.1055/b-0042-186541.

Shen, Q. *et al.* (2015) 'Egg yolks inhibit activation of NF-κB and expression of its target genes in adipocytes after partial delipidation', *Journal of Agricultural and Food Chemistry*, 63(7), pp. 2013–2025. doi: 10.1021/jf5056584.

Shenoy, K. R. and Shenoy (Nileshwar), A. (2014) 'Manipal Manual of Surgery', (With CD), p. 1193. Available at: http://www.cbspd.co.in/manipal-manual-of-surgery-4ed-9788123924168-k-r-shenoy.html#product-tabs2.

Shindhe, P. S. *et al.* (2023) 'An integrated management (Ayurveda and Modern medicine) of accidental burn injury: A case study', *Journal of Ayurveda and Integrative Medicine*, 14(2), p. 100691. doi: 10.1016/j.jaim.2023.100691.

Shirsath, S. R. *et al.* (2017) 'Intensification of extraction of curcumin from Curcuma amada using ultrasound assisted approach: Effect of different operating parameters', *Ultrasonics Sonochemistry*, 38, pp. 437–445. doi: 10.1016/j.ultsonch.2017.03.040.

Shivaprasad, H. V. *et al.* (2009) 'Cysteine proteases from the Asclepiadaceae plants latex exhibited thrombin and plasmin like activities', *Journal of Thrombosis and Thrombolysis*, 28(3), pp. 304–308. doi: 10.1007/s11239-008-0290-2.

Shivkar, Y. M. and Kumar, V. L. (2003) 'Histamine mediates the pro-inflammatory effect of latex of Calotropis procera in rats', *Mediators of Inflammation*, 12(5). doi: 10.1080/09629350331000161970 8.

Shupp, J. W. *et al.* (2010) 'A review of the local pathophysiologic bases of burn wound progression', *Journal of Burn Care and Research*, 31(6), pp. 849–873. doi: 10.1097/BCR.0b013e3181f93571.

Sidhu, G. S. *et al.* (1998) 'Enhancement of wound healing by curcumin in animals', *Wound Repair and Regeneration*, 6(2), pp. 167–177. doi: 10.1046/j.1524-475X.1998.60211.x.

Singh, A. K. (2016) *Nanoparticle Pharmacokinetics and Toxicokinetics*, *Engineered Nanoparticles*. doi: 10.1016/b978-0-12-801406-6.00006-6.

Singh, A. N. *et al.* (2010) 'Purification of a novel cysteine protease, procerain B, from Calotropis procera with distinct characteristics compared to procerain', *Process Biochemistry*, 45(3), pp. 399–406. doi: 10.1016/j.procbio.2009.10.014.

Singh, Anjali *et al.* (2014) 'Effect of Neem oil and Haridra on non-healing wounds', *AYU (An*

International Quarterly Journal of Research in Ayurveda), 35(4), p. 398. doi: 10.4103/0974-8520.158998.

Singh, S. *et al.* (2017) 'Characterization, organoleptic evaluation and standardization of aqueous extracts of antidiabetic herbs Trigonella foenum, Allium sativum, Aloe vera, Phyllanthus niruri', *Journal of Pharmacy Research*, 11(11), pp. 1370–1375. Available at: https://www.embase.com/search/results?subaction=viewrecord&id=L619909719&from=export.

Singh, S. (2017) *Singh Sukhbir , Singh P . R ., Singh A . K . and Rajendra Gupta . 2017 . Comparative Performance Evaluation of Sugarcane Cutter Planters . Agricultural Engineering*.

Singh, S. P. and Singh, D. (2010) 'Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: A review', *Renewable and Sustainable Energy Reviews*, 14(1), pp. 200–216. doi: 10.1016/j.rser.2009.07.017.

Singh, V. *et al.* (2007) 'The pathogenesis of burn wound conversion', *Annals of Plastic Surgery*, 59(1), pp. 109–115. doi: 10.1097/01.sap.0000252065.90759.e6.

Sloan, K. B. (1989) 'Prodrugs for dermal delivery', *Advanced Drug Delivery Reviews*, 3(1), pp. 67–101. doi: 10.1016/0169-409X(89)90005-7.

Somboonwong, J. *et al.* (2012) 'Wound healing activities of different extracts of Centella asiatica in incision and burn wound models: an experimental animal study', *BMC Complementary and Alternative Medicine*, 12. doi: 10.1186/1472-6882-12-103.

Sousa, B. F. *et al.* (2020) 'Latex proteins downregulate inflammation and restores blood-coagulation homeostasis in acute salmonella infection', *Memorias do Instituto Oswaldo Cruz*, 115(11), pp. 1–12. doi: 10.1590/0074-02760200458.

Souto, E. B. *et al.* (2020) 'New nanotechnologies for the treatment and repair of skin burns infections', *International Journal of Molecular Sciences*, 21(2). doi: 10.3390/ijms21020393.

SSehgal Rajesh *, Chaudhary Manu (2010) 'EFFICACY EVALUATION OF AMPUCARE IN COMPARISON WITH SILVER SULFADIAZINE IN BURN WOUND HEALING IN RATS.', 2(1), pp. 85–88.

Steinsträsser, I. and Merkle, H. P. (1995) 'Dermal metabolism of topically applied drugs: Pathways and models reconsidered', *Pharmaceutica Acta Helvetiae*, 70(1), pp. 3–24. doi: 10.1016/0031-6865(94)00047-Y.

Strolin, M., Whomsley, R. and Baltes, E. L. (2005) 'Differences in absorption, distribution, metabolism and excretion of xenobiotics between the paediatric and adult populations', *Expert Opinion on Drug Metabolism and Toxicology*, 1(3), pp. 447–471. doi: 10.1517/17425255.1.3.447.

Sugino, H. *et al.* (1997) 'Antioxidative Activity of Egg Yolk Phospholipids', *Journal of Agricultural and Food Chemistry*, 45(3), pp. 551–554. doi: 10.1021/jf960416p.

Tadros, T. *et al.* (2004) 'Formation and stability of nano-emulsions', *Advances in Colloid and Interface Science*, 108–109, pp. 303–318. doi: 10.1016/j.cis.2003.10.023.

Taifa Rozy, Barman Kumar Pankaj and Medhi Champak (2020) 'a Critical Study of Dagdha Vrana (Burn Injury) in Ayurvedic System of Medicine', *International Journal of Ayurveda and Pharma Research*, 8(7), pp. 84–88. doi: 10.47070/ijapr.v8i7.1492.

Talekar, Y. P. *et al.* (2017) 'Studies on wound healing potential of polyherbal formulation using in vitro and in vivo assays', *Journal of Ayurveda and Integrative Medicine*, 8(2), pp. 73–81. doi: 10.1016/j.jaim.2016.11.007.

Teot, L. *et al.* (2012) 'Burn wound healing: Pathophysiology', *Handbook of Burns: Reconstruction and Rehabilitation Volume 2*, pp. 47–54. doi: 10.1007/978-3-7091-0315-9_4.

Thangapazham, R. L., Sharad, S. and Maheshwari, R. K. (2013) 'Skin regenerative potentials of curcumin', *BioFactors*, 39(1), pp. 141–149. doi: 10.1002/biof.1078.

Thangarasu, V. and Anand, R. (2019) *Comparative evaluation of corrosion behavior of aegle marmelos correa diesel, biodiesel, and their blends on aluminum and mild steel metals*, *Advanced Biofuels: Applications, Technologies and Environmental Sustainability*. Elsevier Ltd. doi: 10.1016/B978-0-08-102791-2.00017-9.

Thom, D. (2017) 'Appraising current methods for preclinical calculation of burn size – A pre-hospital perspective', *Burns*, 43(1), pp. 127–136. doi: 10.1016/j.burns.2016.07.003.

Tiwari, V. K. (2012) 'Burn wound: How it differs from other wounds', *Indian Journal of Plastic Surgery*, 45(2), pp. 364–373. doi: 10.4103/0970-0358.101319.

Toklu, H. Z. *et al.* (2007) 'Silymarin, the antioxidant component of Silybum marianum, protects against burn-induced oxidative skin injury', *Burns*, 33(7), pp. 908–916. doi: 10.1016/j.burns.2006.10.407.

Toll, R. *et al.* (2004) 'Penetration profile of microspheres in follicular targeting of terminal hair follicles', *Journal of Investigative Dermatology*, 123(1), pp. 168–176. doi: 10.1111/j.0022-202X.2004.22717.x.

Tonnesen, Feng and Clark (2000) 'Angiogenesis in Wound Healing (Supplement)', *Contemporary Surgery*, 5(1), pp. 40–46.

Topman, G., Lin, F. H. and Gefen, A. (2013) 'The natural medications for wound healing - Curcumin, Aloe-Vera and Ginger - do not induce a significant effect on the migration kinematics of cultured fibroblasts', *Journal of Biomechanics*, 46(1), pp. 170–174. doi: 10.1016/j.jbiomech.2012.09.015.

Toriseva, M. and Kähäri, V. M. (2009) 'Proteinases in cutaneous wound healing', *Cellular and Molecular Life Sciences*, pp. 203–224. doi: 10.1007/s00018-008-8388-4.

Ts, A., Sweta, K. M. and Surendranath, D. (2022) 'A critical study on Burn Injury (Dagda Vrana) with special reference to Sushruta Samhita', *Journal of Ayurveda and Integrated Medical Sciences*, 7, p. 86. Available at: www.jaims.in.

Turnlund, J. R. *et al.* (2005) 'Long-term high copper intake: Effects on copper absorption, retention, and homeostasis in men', *American Journal of Clinical Nutrition*, 81(4), pp. 822–828. doi: 10.1093/ajcn/81.4.822.

Upadhyay, N. K. *et al.* (2009) 'Safety and healing efficacy of Sea buckthorn (Hippophae rhamnoides L.) seed oil on burn wounds in rats', *Food and Chemical Toxicology*, 47(6), pp. 1146–1153. doi: 10.1016/j.fct.2009.02.002.

Upadhyay, N. K. *et al.* (2011) 'Mechanism of wound-healing activity of hippophae rhamnoides L. leaf extract in experimental burns', *Evidence-based Complementary and Alternative Medicine*, 2011. doi: 10.1093/ecam/nep189.

Venkataraman, M. and Nagarsenker, M. (2013) 'Silver sulfadiazine nanosystems for burn therapy', *AAPS PharmSciTech*, 14(1), pp. 254–264. doi: 10.1208/s12249-012-9914-0.

Venkatesha, S. H. (2016) *Hemostatic Interference of Plant Latex Proteases*, *SM J Clin Pathol*. Available at: https://www.researchgate.net/publication/308725379.

Venus, M., Waterman, J. and McNab, I. (2010) 'Basic physiology of the skin', *Surgery*, 28(10), pp. 469–472. doi: 10.1016/j.mpsur.2010.07.011.

Verbelen, J. *et al.* (2014) 'Aquacel® Ag dressing versus Acticoat™ dressing in partial thickness burns: A prospective, randomized, controlled study in 100 patients. Part 1: Burn wound healing', *Burns*, 40(3), pp. 416–427. doi: 10.1016/j.burns.2013.07.008.

Vogler, B. K. and Ernst, E. (1999) 'Aloe vera: A systematic review of its clinical effectiveness', *British Journal of General Practice*, 49(447), pp. 823–828.

Vujanovic, S. and Vujanovic, J. (2013) 'Bioresources in the pharmacotherapy and healing of burns: A mini-review', *Burns*, 39(6), pp. 1031–1038. doi: 10.1016/j.burns.2013.03.016.

Wang, W. *et al.* (2019) 'Nano-drug delivery systems in wound treatment and skin regeneration', *Journal of Nanobiotechnology*, 17(1), pp. 1–15. doi: 10.1186/s12951-019-0514-y.

Wang, X. W. *et al.* (1985) 'Tissue deposition of silver following topical use of silver sulphadiazine in extensive burns', *Burns*, 11(3), pp. 197–201. doi: 10.1016/0305-4179(85)90070-1.

Warriner, R. and Burrell, R. (2005) 'Infection and the Chronic Wound', *Advances in Skin & Wound Care*, 18(Supplement 1), pp. 2–12. doi: 10.1097/00129334-200510001-00001.

Wesley J, Christina A, C. N. et al (2009) 'Wound healing activity of the leaves of Tribulus terrestris (Linn) aqueous extract in rats.', *Journal of Pharmacy Research*, 2(5), pp. 841–843. doi: 10.4103/0253-7613.43159.

Williams, A. A. (2003) *Transdermal and Topical Drug Delivery from Theory to Clinical Practice*. Illustrate. pharmaceutical press. Available at: https://books.google.co.in/books/about/Transdermal_and_Topical_Drug_Delivery_fr.html?id=hWwpR2MtF2MC&redir_esc=y.

Woodfin, A., Voisin, M. B. and Nourshargh, S. (2007) 'PECAM-1: A multi-functional molecule in inflammation and vascular biology', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 27(12), pp. 2514–2523. doi: 10.1161/ATVBAHA.107.151456.

World Health Organization (2021) 'WHO model list of essential medicines - 22nd list, 2021', *Technical document*, p. 2021. Available at: https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2021.02.

Wu, P. *et al.* (2016) 'Assessment of egg yolk oil extraction methods of for shizhenkang oil by pharmacodynamic index evaluation', *Molecules*, 21(1). doi: 10.3390/molecules21010106.

Xiao, M. *et al.* (2014) 'Role of autophagy and apoptosis in wound tissue of deep second-degree burn in rats', *Academic Emergency Medicine*, 21(4), pp. 383–391. doi: 10.1111/acem.12352.

Xiao, N. *et al.* (2020) 'Biological Activities of Egg Yolk Lipids: A Review', *Journal of Agricultural and Food Chemistry*, 68(7), pp. 1948–1957. doi: 10.1021/acs.jafc.9b06616.

Yam Mun F, Jamaludin Zainol, G. pillay and N. Y. (2002) 'Experimental Evaluation of Healing Process of Burn- wound Treated by Lyophilized Aloe vera Dressing', pp. 1–6. Available at: https://www.makingcosmetics.com/Aloe-Vera-Extract-in-Safflower-Oil_p_1149.html.

Yaman, I. *et al.* (2010) 'Effects of Nigella sativa and silver sulfadiazine on burn wound healing in rats', *Veterinarni Medicina*, 55(12), pp. 619–624. doi: 10.17221/2948-VETMED.

Yang, G. *et al.* (2021) 'Understanding the relationship between particle size and ultrasonic treatment during the synthesis of metal nanoparticles', *Ultrasonics Sonochemistry*, 73, p. 105497. doi: 10.1016/j.ultsonch.2021.105497.

Yastı, A. Ç. *et al.* (2015) 'Guideline and treatment algorithm for burn injuries', *Ulusal Travma ve Acil Cerrahi Dergisi*, 21(2), pp. 79–89. doi: 10.5505/tjtes.2015.88261.

Yenilmez, E. *et al.* (2015) 'Chitosan gel formulations containing egg yolk oil and epidermal growth factor for dermal burn treatment', *Pharmazie*, 70(2), pp. 67–73. doi: 10.1691/ph.2015.4126.

Yilmaz, E. and Andsoy, I. I. (2020) 'Traditional and modern practices in wounds and burn injuries in a population of North Western Turkey', *Burns*, 46(6), pp. 1436–1443. doi: 10.1016/j.burns.2020.01.009.

Yu, Y. Q. *et al.* (2021) 'Enhancing Permeation of Drug Molecules Across the Skin via Delivery in Nanocarriers: Novel Strategies for Effective Transdermal Applications', *Frontiers in Bioengineering and Biotechnology*, 9(March), pp. 1–17. doi: 10.3389/fbioe.2021.646554.

Zaidi, S. F. H. *et al.* (2009) 'Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against Helicobacter pylori', *Journal of Ethnopharmacology*, 121(2), pp. 286–291. doi: 10.1016/j.jep.2008.11.001.

Zhu, J. *et al.* (2017) 'Potential roles of chemical degradation in the biological activities of curcumin', *Food and Function*, 8(3), pp. 907–914. doi: 10.1039/c6fo01770c.

zothanpuii F*, R. R. (2018) 'A Review on Stability Studies of Pharmaceutical Products', *Asian journal of pharmaceutical and clinical research*, 13(10), pp. 3–9. doi: 10.31638/ijprs.v7.i1.00003.

Żwierełło, W. *et al.* (2023) 'Burns: Classification, Pathophysiology, and Treatment: A Review', *International Journal of Molecular Sciences*, 24(4). doi: 10.3390/ijms24043749.

Van Zyl, A. J. P. *et al.* (2004) 'The role of surfactant in controlling particle size and stability in the miniemulsion polymerization of polymeric nanocapsules', *European Polymer Journal*, 40(12), pp. 2717–2725. doi: 10.1016/j.eurpolymj.2004.07.021.

LOVELY PROFESSIONAL UNIVERSITY

Centre for

Research Degree Programmes

LPU/CRDP/PHD/EC/20210315/000870
Dated: 15 Jun 2021

Vibhu khanna
Registration Number: 41900562
Programme Name: Doctor of Philosophy (Ayurvedic Pharmacy)

Subject: Letter of Candidacy for Ph.D.

Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on 04 Dec 2020 by accepting your research proposal entitled: "DEVELOPMENT AND PHARMACOLOGICAL EVALUATION OF HERBOMINERAL NANOEMULSION FOR THE TREATMENT OF SECOND DEGREE BURNS." under the supervision of Dr. Saurabh Singh.

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

We wish you the very best!!

In case you have any query related to your programme, please contact Centre of Research Degree Programmes.

Head

Centre for Research Degree Programmes

Note:-This is a computer generated certificate and no signature is required. Please use the reference number generated on this certificate for future conversations.

RASĀYAN *J. Chem.*
Vol. 17 | No. 3 |957-963| July - September | 2024
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

GREEN SYNTHESIS OF COPPER NANOPARTICLES FROM ALOE VERA LEAF GEL CONCENTRATE AND ITS CHARACTERISATION

Vibhu Khanna[1], Saurabh Singh[1,✉], Kalvatala Sudhakar[1], Dileep Singh Baghel[1], Bimlesh Kumar[1] Sheetu Wadhawa[1] and Narendra Kumar Pandey[1]
School of Pharmaceutical Sciences, Lovely Professional University,
Phagwara-144402, (Punjab), India
✉Corresponding Author: saurabh.singh2514@gmail.com

ABSTRACT

Copper nanoparticles were synthesized from Aloe vera leaf gel extract concentrate from 0.1 Molar Copper sulphate pentahydrate solution. The Aloe vera gel concentrate and copper sulphate solution during the process were subjected to spectrophotometric absorption studies. Prepared nanoparticles were observed for size, zeta potential and polydispersity index, scanning electron microscopy, and energy dispersive X-ray analysis. The results showed that the size of elemental copper synthesized with the help of Aloe vera leaf gel extract concentrate was 272nm. SEM analysis confirmed the morphology of synthesized nanoparticles (spherical shapes). EDX studies further confirmed the presence of 67.21% elemental copper in newly synthesized nanoparticles prepared from Aloe vera leaf gel concentrate. The process of green synthesis uses eco-friendly resources to increase biological safety. The Aloe vera leaf gel extract concentrate efficiently served as a reducing and a capping agent to reduce the size of copper particles to the nanoparticle range. The method is low in cost and saves energy.

Keywords: Aloe Vera Leaves, Leaf Extract Concentrate, Copper Nanoparticles, Green Synthesis, Characterisation, Eco-Friendly, Particle Size.

RASĀYAN *J. Chem.*, Vol. 17, No.3, 2024

INTRODUCTION

Nanoparticles are used for electrical, optical, catalytic, biomedical and anti-bacterial applications. They can be prepared through green, chemical and physical methods.[1] The methodology for green synthesis for the synthesis of metal nanoparticles like gold, silver, iron zinc, palladium etc. is adapted considering the recent researches that contemplate the method with less pollution, eco-friendly behaviour[2], reduced human health hazards, operates at low temperatures and yields a mild reaction in contrast to another conventional chemical catalyst.[3,4] Plant-derived nanoparticles can be produced quickly, and gently, with non-toxic and environmentally friendly materials, a compatible method, and possess better bioavailability, stability and pharmacological activity to be used in medical and food applications.[5]

Various plant extracts of traditional Chinese plant extracts (Rosa Andeli, Gardenia jasminoides), cost-effective herbal extracts (piper, rose, cumin etc.) or other low-priced materials like tamarind, lemon juice, piper, milk, curd etc have been in use for this process.[6,7] Plant extracts encompass secondary metabolites (flavonoids, tannins, and terpenoids) that aid in nanoparticle synthesis. Thus, representing their catalytic and stabilizing properties.[5]

Green synthesis with Aloe vera gel concentrate can be used to minimize the size of copper particles. Reduction of drug molecules to nanoparticle size will enhance topical penetration of copper and provide ease to be used in nano formulations with better bioavailability. The Aloe vera concentrate reduces copper of copper sulphate pentahydrate ($CuSo_4.5H_20$) from Cu^{+2} oxidation state to Cu^{+1}. Abundant phenols and Flavonoids in Aloe vera pulp will provide anti-oxidant activity while proteins serve as the capping agent.[8] This article focuses on a short review of the uses of copper, the procedure employed to synthesise copper nanoparticles (CuNPs) along with its characterisation from Aloe vera leaf gel concentrate (Fig.-1).

http://doi.org/10.31788/RJC.2024.1738801

Proportional Analysis of Powdered Curcuma Longa and Curcuma Longa Extract

Vibhu Khanna[a)], Saurabh Singh[b)], Dileep Singh Baghel[c)], Bimlesh Kumar[d)]

School of Pharmaceutical sciences, Lovely Professional University, Phagwara, 144411, Punjab, India

[a)] drkhanna07@gmail.com
[b)] Corresponding author: saurabh.singh@lpu.co.in
[c)] dileep.15210@lpu.co.in
[d)] bimlesh.12474@lpu.co.in

Abstract: Ethnic medical practices recognise Curcuma longa, a multipurpose herb known since hundreds of years. It belongs to family zingiberaceae. Rhizomes of curcuma longa has traditional medicinal property, a popular spice in Indian food with known anti-microbial, anti-inflammatory, anti-allergic, anti-cancerous and anti-oxidant activities along with its hepatoprotective and hypolipidemic properties that make it fit for its traditional uses in Indian kitchen with known medicinal values. Quantitative estimation was performed for extracts obtained from powdered samples under continuous extraction with Soxhlet apparatus with 95% ethanol. Aim of this work is to evaluate the comparative proportion of potency of extract with physicochemical standardization parameters as ash content, moisture content, soluble & insoluble ashes in water along with chromatography. Chemical constituents as alkaloids, flavonoids, saponins and tannins were identified in extract of curcuma longa after pharmacognostic screening of raw powdered rhizomes of curcuma longa. This evidence will be helpful in standardisation of quality and purity of the extracts of curcuma longa

Keywords: *Pharmacognostic screening, Quantitative estimation, Haldi, Curcuma*

INTRODUCTION

Plants evolved along with the origin of life on the planet earth billions of years back. The ethnic plants have specified actions on human diseases and are widely used in human, animal and plant research. History divulges role of plants to have an important role in cure of diseases. pharmacological activity of these plants, have been exploited by pharmaceutical drug discovery and drug design. Phytoconstituents derived from the leaves, flowers, roots, fruits, seeds and bark of the plants contain bioactive constituents and are classified as alkaloids, tannins, flavonoids phenols etc. [1]. Apart from various modern treatments available for various diseased conditions, active pharmaceutical ingredient (API) in therapeutic agents formulated from herb curcuma longa helps in effective treatment. *Curcuma Longa* is one among the popular herb being used in complementary medicine.

Curcuma Longa with a traditional medicinal history dating 4000 years back. Among available 134 species of curcuma longa known by 53 synonyms in different languages of India. Synonyms of sanskrit are "Anestha, Bhadra, Bahula, Dhirgharajam Gandhaplashika, Gauri, Gharshani, Haridra, Harita, Hemaragi, Hemaragini, Hridayavilasini, Jayanti, Jawarantika, Kanchani, Kaveri, Kashpa, Kshamata, Laxmi, Mangalprada, Mangalya, Mehagni, Nisha, Nishakhya, Nishawa, Patawaluka, Pavitra, Pinja, Pita, Pitika, Rabhangavasa, Ranjani, Ratrimanika, Shifa, Shaobhna, Shiva Shyama, Umavara, Vairagi, Varavarnini, Varnadatri, Varnini, Vishagni, Yamini, Yoshitapriya and Yuvati". [2]

An Herbaceous perennial plant of family zingiberaceae Grows in different tropical regions with optimum temperature between 20-30 degrees needed for its growth with a good amount of rainfall or better irrigation conditions. Growth can be seen from sea level to 1500 m height [3]. Yellowish brownish rhizomes of 1-3 inches in size are manually gathered from below the soil. They are taken out of the ground, collected and powdered on drying.

3rd International Conference on Functional Materials, Manufacturing, and Performances
AIP Conf. Proc. 2986, 030027-1–030027-9; https://doi.org/10.1063/5.0192533
Published by AIP Publishing. 978-0-7354-4840-7/$30.00

NANOEMULSIONS – PRESENT AND FUTURE PERSPECTIVE - AN OVERVIEW

Vibhu Khanna, Saurabh Singh*, Dileep Singh Baghel, Barinder Kaur, Alka Nayar, Rajiv Ghimire
School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar - Delhi G.T. Road, Phagwara, Punjab (India)-144411

ABSTRACT: In view of the fact that in past decade the growing trend of interdisciplinary research in nanoemulsions have attracted awareness in formulations to design therapeutically effective drug because of its variety of uses in pharmaceutical industry. They are an efficient drug delivery system for transdermal, transocular, transnasal, drugs to brain and even for certain anticancer drugs When the size of droplets of this non-equillibrium systems is decreased they not only deliver drug in a sustained manner to increases rate of cure of patients and avoids repeated drug administration. Owing to some of its elite properties the nanoemulsions are considered as the effective and genuine novel drug delivey tool compared to conventional drug delivery system which include their thermodynamic stability, viscosity , bioavailability, optical clarity, easy to prepare moreover they are resistant to creaming, flocculation, coalescence and sedimentation. This research paper addresses for the types of nanoemulsions, their signifying features which differentiate them from emulsions and microemulsions, methods of preparations of stable nanoemulsions, their morphology and properties various routes of drug administration in this nanonized formulation. Specifically recent researches regarding practical applications in cosmetology and available patents.

Key words: nanoemulsions, transdermal, properties, drug delivery system.

1.INTRODUCTION

Nanoemulsions are multiphase colloidal dispersions of two non-equillibrium of structured immiscible liquids that are made to mix with each other so, when liquid is dispersed in another fluid. It is made stable by combination of surfactants and co-surfactants[1]. This part formed includes small size droplets with sizes between 5nm-200nm giving a transparent emulsion with decreased interfacial tension between oil & water phase. This availaibility of large interfacial area influences drug delivery or targeted drug delivery. Nanoemulsions dissolve large amount of oil substances and protects drug hydrolysis in body and degrading enzymes. Nanoemulsions provide sustained release of drug in controlled manner for long time. They are protected against flocculation, sedimentation, creaming effect . when droplet size is reduced to nano scale, it creates many physical properties like optically transparent and elastic behaviour. They provide promising behaviour of droplets after nanonization. Moreover the nanoemulsions on commercial scale can be prepared by a surfactant very less in amount. In this review paper we will try to concentrate on complete review of nanoemulsions, their properties, advantages, disadvantages related to the formation of this drug delivery system and challenges faced by nanoemulsions to commercialise this novel drug delivery system. They are known by various names as miniemulsions, ultrafine emulsions, micrometer emulsions[1,2] , milky emulsions, translucent emulsions [3]

THINK INDIA JOURNAL

ISSN:0971-1260
Vol-22-Issue-37-December -2019

NOVEL DRUG DELIVERY SYSTEM FOR HERBAL DRUGS- AND OVERVIEW

Vibhu Khanna[1], Pavani Sriram[2,] Amritpal Singh[3], Gurpal Singh[4], Kirti .S.Prabhu[5], Ashish Suttee[1]*

[1]School of Pharmaceutical Sciences, Lovely Professional University, Punjab-India 144411.
[2]Vaagdevi College of Pharmacy, Warangal, Telangana State
[3]Shri Dhanwantri Ayurvedic College, Chandigarh
[4]University Institute Of Pharmaceutical Sciences, Panjab University , Chandigarh
[5]Interim Translational Research Institute, Academic Health System, Doha, Qatar.
Corresponding Author: Dr. Ashish Suttee
Email ID: ashish7sattee@gmail.com

ABSTRACT

The basic idea behind is to exploit nanotechnology in drug delivery system where the drug is delivered in nanosized figure via non-toxic vehicles that trap and both hydrophilic and hydrophobic components of drugs are exploited for better results in traditional medicine. Modern pharmaceutical science has developed several novel drug delivery systems like liposomes, phytosomes, micropellets, transferosomes, ethosomes, niosomes, and proniosomes, etc. Mode of drug delivery will affect its efficacy significantly. At a certain concentration, the drugs give the optimum results and below which shows decreased therapeutics or no therapeutic efficacy at all. Drug delivery systems combine pharmaceutics, chemistry, and molecular biology. The conventional systems of medicines are having good therapeutic effects but their limitations fail to give desired concentration in blood. Thus NDDHS has the advantage to enhance the therapeutics by increases bioavailability, increased stability and many more. Novel drug delivery technique when assimilated in herbal active ingredients decreases the loss of therapeutic dosage via liver metabolism and reduces drug doses by converting the drug into nanosized form, which is more effective even at low doses, transfers drug to site of action with good bioavailability, sustained delivery and increased stability and Enhancement of pharmacological activity. Thus herbs can be used in a more advanced and scientific manner to change them into modern dosage formulations. This present review paper is to pen down the present novel drug herbal formulations with their active ingredients and application of novel drug formulations.

Keywords: *liposomes, transferosomes, NDDS, NHDDS.*

1. INTRODUCTION

As per WHO, Traditional medication is defined as " It is the sum of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness"[1]. Whereas other complementary medicines are defined as "broad set of health care practices that are not part of that country's tradition or conventional medicine and are not fully integrated into the dominant health-care system. They are used interchangeably with traditional medicine in some countries"[2].

There is a potential involvement where traditional medicine, as well as complimentary, can make to health care services and encourages member states to consider it an important part of the health system within the country's health systems, as it was introduced in Beijing's declaration in WHO congress of 2008. A new beginning is happening all over the globe with a comeback of

AN OVERVIEW ON ANTIMALARIAL DRUGS

Sakshi Sabharwal, Saurabh Singh*, Simranjeet Kaur, Nitika Anand, Dileep Singh Baghel, Vibhu Khanna

School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar - Delhi G.T. Road, Phagwara, Punjab (India)-144411

ABSTRACT:-

We all are well aware about the infectious disease malaria which is transmitted by the bite of female mosquito Anopheles carries the plasmodium parasite and when this mosquito suck blood, the parasite released into the blood vessels. There are four kinds of malarial parasites that can infect humans: "*Plasmodium vivax"*, "*P. ovale"*, "*P. Malariae"*, and "*P. Falciparum"*. *Plasmodium falciparum* is a very dangerous form of disease which enhances the death. An infected mother can also pass the disease to her baby at the time of birth known as "congenital malaria". From a very long time, significant antimalarial drugs comprised of natural herbs were used. Since 1930's these herbal drugs are extended more with a progession of many manufactured drugs used to treat malaria. Ayurvedic prescriptions were used to treat "jungle fever" (malaria) from thousands years which are the wellspring of two primary gatherings of present day antimalarial medications "artemisinin" and "qunine" subsidiaries. Per annum 1 million people died due to malaria. However no immunization has been created for "intestinal sickness" as the parasite continues to alter the collaboration of metabolic pathways during its life series. Thus, to overcome the deficiencies of manufactured formulations resisted by the malaria, we can trust that the plant inferred medications can end up being the wellspring of novel compounds to control malaria.

Keywords: - Malaria, Quinine, Artemisinin, Ayurveda, Phytocompounds

INTRODUCTION: -

Malaria is an irrestibile disease which is common in humid nations and the population depends on the utilization of natural herbs to fight against the ailment. According to the 2015 data of World Health Organization there is an estimation that 212 million cases of malaria and 4,29,000 deaths all over the world. In 2018, WHO revealed the information from 2015 to 2017 that there is no advancement in diminishing overall malaria cases. There are generally four types of mosquitoes which causes malaria are P. Falciparum, P. Malariae, P. Ovale & P. Vivax.

P. Vivax, P. Ovale and P. Malariae less prone than P. Falciparum and continue to live in the liver which can backfire its effect after numerous years or we can say again regenerate to cause malaria. P. Falciparum is the main host for occurring tertiary malaria which is very dangerous. For a long period of time quinine used as the most significant antimalarial drug. In 1930, quinine was having great extent and many synthetic compounds were composed with the help of quinine. The compound obtained was primaquine (8-aminoquinolines), chloroquine, amodiaquine (4-aminoquinolines) and proguanil, pyrimethamine (folic corrosive amalgamation inhibitors).

Some strains of P. Falciparum created resistance against chloroquine by the mid 1980. Some chemotherapeutic agents was also used to treat the malaria. So, to overcome this gap there is critical urge to find new drugs for malaria. For finding new drugs and to improve their efficacy it is required to develop reasonability and security for the traditional remedial plants from which the compound will be obtained which is going to be used to fight against ailment.

This article is written to give a small overview on the herbal plants that are used to treat malaria and to depict the gaps which are left unexplored and to consider the advancement related investigations.

AN OVERVIEW ON: APAMARGA KSHARA

Sakshi Sabharwal, Nitika Anand, Simranjeet Kaur, Vibhu Khanna, Dileep Singh Baghel, Saurabh Singh*, Amit Mittal
School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar - Delhi G.T. Road, Phagwara, Punjab (India)-144411

ABSTRACT:-

Apamarga (*Achyranthes aspera*, Amaranthaceae) is a vital healing dravya originate as a unwanted plant all over India. In conventional medication framework all pieces of this plant are utilized like beej, rmoola and shoots. In Atharvaveda, it is said to be kshetriya roga nasak and yatudhan Krimi nasak. In Yajurveda it is said to be used for hawan purpose due to its Rakshoghan Property. No other plant has better water removing property than Apamarga. Apamarga kshara is an alkaline Ayurvedic medicine, in powder form which is prepared from Apamarg- Prickly Chaff-Flower. Kshara is a caustic, alkaline in nature obtained from the ashes of Apamarga dravya. It is adaptable, because even in such places that are difficult in approach by ordinary measures can be treated by kshara. This alkaline preparation may be a amalgam of numerous dravyas or it can be a single dravya. It has a vast series of explanation about kshara explained through Acharaya Sushruta. Kshara has lots of therapeutic usages and also replace many surgical procedures. The large number of phytochemical elements have been extracted out from this plant which possess some properties like Arsha, Kusthaghana, paaproga nasak, Duhswapnanasana, unmade, Apasmara, Ashmari, Hikka-Swaas, Vish Chikitsa etc. The compacted form of herb is utilized in pneumonia and mixture of the root is utilized as gentle astringent in bowel disorders. Traditionally, it is used as anti-diabetic, anti-inflammatory and abortifacient.

KEYWORDS: Apamarga kshara, Rakshoghana, kushthaghana, Kshetriya roga, Yatudhan Krimi, Paaproga .

INTRODUCTION: -

Kshara is the water-soluble ash of drug which is in the form of solutions, powders which is alkaline in nature. It is known as kshara because it has Ksharan (which is destruction of tissues) property which causes ksharan of mamsa and other dhatus. Acharya Sushruta described Kshara separately in detailed manner. He defines that Kshara have chedan (Excision), Bhedan and lekhan (Scrapping) and tridoshahar (equilibrium vata, pitta and kapha) properties. He also considers the scope of Kshara in Shalya Tantra. He has stated various drugs for the preparation of kshara and Apamarga is one of them from which Apamrga Kshara is prepared. He also mentioned that Kshara is prepared from 22 plants such as Apamarga(*Achyranthus aspera*), Snuhi(*Euphorbia nerifolia*), Amaltas(*Cassia fistula*), Kutaj (*Holarrhena antidysentrica*), Vasa(*Adhatoda vasica*), Arka(*Calotropis gigantea*), Tila(*Sesamum indicum*), etc. and kshara chikitsa has been in practice since 500 B.C.

Apamarga kshar is an alkaline preparation prepared from Apamarga *(Achyranthes aspera)* in powder form. Recently, there has been a remarkable increase in the use of herbal plants and herbal formulations in creating just as created nations which brings about raised development of natural items comprehensively. Nowadays, for medicine development and for the discovery of new drug molecules number of plants and their products and formulations are determined based on their conventional use. One of them which are being assessed for their restorative adequacy is Apamarga which is regularly known as Cheerchira (Hindi) and Prickly Chaff Flower (English). It has many therapeutic properties used as spermicidal (Shukra Stambhan), antipyretic (Jwarghan) & cardiovascular agent (Hridya).

MORPHOLOGY:-

- Apamarga is a wild perennial herb which grows up to a height of 30 – 90cm and having tap root type of branched[3].
- Stem:- It is herbaceous, hairy, above ground, erect and green in color[3].
- Leaves:- Leafs have sudden pointed apex, opposite arrangement and semiorbicularis, petiolate[3].
- Flowers:- They are bisexual, tetracyclic, small size, green in color and actinomorphic. Having spike with reflexed flower arrangement on long peduncle[3].
- Fruit:- Indehiscent fruit enclosed within persistent perianth and bracteole[3].

An overview of Sphatika (Alum)

Nitika Anand, Saurabh Singh*, Simranjeet Kaur, Sakshi Sabharwal, Dileep Singh Baghel, Vibhu Khanna, Sajisha V. S
School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar - Delhi G.T. Road, Phagwara, Punjab (India)-144411

Abstract

Sphatika is considered as very good and useful drug in Ayurveda which is known as alum in English. This is found in crystal form which resembles to salt. It is a mineral origin drug. Work done by the Greek scholars or physicians is considered as the healthy discussions on the topic of sphatika. Ancient scholar Razi from Arab gives the full details about sphatika. He characterized alum in the types of vitriol, due to the same activity like astringent qualities. Sphatika is a drug with good therapeutic efficacy. It is used as krimighan, jwaraghan, shulprashmnam. Saphatika is used as major ingredient in so many herbo-mineral formulations. This drug comes from that category of drugs which are used internally as well as externally. Shpatika is used for many kinds of antimicrobial and antiseptic purposed since ancient time. The present review highlights the important activities and detailed description of sphatika. Which will be beneficial to promote the natural potential antimicrobial drug.

Keywords: Sphatika, Alum, Natural, Antimicrobial, Ayurveda

1. Introduction

Most of the mineral origin drugs are found in crystal form. Sphatika is a mineral origin drug. Commonly it is known as fitkari in India. Alum, name given to it because of its astringent properties[1]. It is completely soluble in warm water. As per Unani medical literature it is considered as kashya[2]. It is transparent but some time slightly translucent[3]. According to historical review of alum, are firsty prepared in Asian countries. In ayurveidc literature it is reported as a best drug to treat krimighan. Its weight is lower than salt. According to review of literature, sphatika is used from the ancient era, as the best antiseptic and antimicrobial agent. It also helps to cure the various types of ulcers but it also cause dryness in mouth, ulcers as a side effect, if it is taken in regular terms or in a higher dose. It is also used as a colouring agent because it makes permanent stains of colour on the fabric when it mixed in the colour. It has seven types as per modern texts, but according to ayurvedic texts it is of two types. This is mineral origin drug in crystal form. It is used internally and externally to cure various diseases. Its dose is mentions in Vedas, this gives the evidence of presences of sphatika from vedic period. Along this range of indications, it also have various pharmacological activities.

Table 1.Synonyms and vernacular names of Sphatika:

Sanskrit	Phatikri, Surashtraj, Kmakshi, Tuvri, Siithi, Angad
Hindi	Phitkhar, Phitkar
Gujarat	Phatkari
English	Alum, Aluminous sulphate, Potash or of Aluminium, Sulphate of Alumina and Ammonium
Punjabi	Phitkari
Bengali	Phatakiri
Urdu	Phitkari
Persian	Shibb-e-Yamani, Zake safed, Zake bilore
Tamil	Patikarm, Adikhrum, Shinacruma
Canra	Phatikara
Telgu	Pattikaramu, Padikharam
Sindh	Shinaaran
Maratha	Trae phitki
Malay	Tawasa

CHAPTER XVI

FIGHTING THE COVID-19

An overview on socioeconomic impact, Diagnostics and Traditional therapies

Vibhu Khanna, Saurabh Singh, Sajisha V.S, Dileep Singh Baghel

Chapter outline

1. Introduction
2. Origin
3. Causes of covid-19
4. Epidemiological details
5. Incubation period
6. Preventive measures
7. Clinical features
8. Diagnostic procedures in covid-19
    a. laboratory diagnostic measures
    b. CT imaging changes according to stages
9. Differential diagnosis
10. Management
11. Strategic objectives of WHO
12. Treatment
13. Role of ayurveda
14. Chinese herbs in COVID-19
15. Conclusion

References

ABSTRACT

December 2019, a nasty month in Hubei province of China where a new-fangled type of pneumonia emerged, such a disease was not under the knowledge fact of W.H.O before and it named it as novel corona virus, nCoV-19 on 12th january'2020. Soon, it attracted attention of common man and scientist all over the world suggesting various diagnostic tools to combat this virus. Here, There is a review of this disease, causes, epidemiological data, diagnostic tools, first hand CT-imaging technique used in diagnosis in Zhongnan hospital of China has also been added along with the isolation & other management protocols of this disease, which decreased contaminated cases substantially with non-therapeutic control measures in China and then initiated in many countries, treatment protocols, role of Ayurveda with recent upgradations of research in Ayurveda has been also mentioned and its impact on socio-economic status of the countries. On the other hand, there is a need to evaluate, what steps should be taken to prevent such causalities in future. This paper is based on secondary information collected by various sources of published journal articles, newsletters,

13 A Synoptic Overview on Ancient Alchemy Sudha Varg (Calcium-Containing Drugs)

An applied Nanomedicine

Saurabh Singh, Simranjeet Kaur, Dileep Singh Baghel, Nitika Anand, Sakshi Sabharwal, Vibhu Khanna, Arun Kumar, and Iqbaljit Kaur

13.1 INTRODUCTION

The National Institute of Health (USA) defines nanomedicine as "molecular-scale medical intervention for the purpose of prevention, diagnosis and treatment of disease" [1]. According to the European Science Foundation, "Nanomedicine is the science and technology of diagnosing, treating and preventing disease and traumatic injury, of relieving pain and of preserving and improving human health using molecular tools and molecular knowledge of the human body" [2]. But the unique definition given by the European Technology Platform on Nanomedicine is "Nanomedicine is the application of nanotechnology to health. It exploits the improved and often novel physical, chemical, and biological properties of materials at the nanometric scale" [3].

Bhasma is an exclusive Ayurvedic herbo-mineral-metallic compound with size in nano-dimensions. This is an ancient formulation described in "*Rasa Shastra,*" which helps prevent chronic diseases and rapid absorption better as compared to herbs [4]. The benefits of nanomedicines (*Bhasma*) are multifold. Safety-related investigations already described in the classical texts of Ayurveda and the latest AYUSH guidelines for safer manufacturing practices like 'Schedule T guidelines' and toxicological evaluation as per OECD guidelines take care of ecology and environment. Calcium is now a daily requirement for people. In Ayurveda, the *sudha varga* drug is co-related with calcium compounds in the modern era and they belong to the category of minerals. The nanomedicines (*Bhasma*) prepared from these drugs are highly beneficial for the treatment of several chronic diseases and also helpful to fulfil the daily calcium supplement needs in a safer way. The drugs of *sudha varga* have been classified in ancient texts of Ayurveda, and

Serial No. 31CP2022/393

LOVELY PROFESSIONAL UNIVERSITY
Transforming Education Transforming India

Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. Vibhu Khanna has successfully participated as Delegate & Presented ~~Poster~~/ Oral Presentation on Evaluation of Physico-Chemical parameters of Aloe-vera extract & its role in wound healing in the 3rd International Conference of Pharmacy (ICP-2022) on the Theme of "Practice, Promotion & Publication of Innovation : A Way of Transforming Health" held on 09th & 10th November 2022 organized by School of Pharmaceutical Sciences in a collaboration with Indian Pharmaceutical Association (IPA) at Lovely Professional University, Punjab.

Mr. Suresh Khanna	Dr. Bimlesh Kumar	Dr. T.V. Naryana	Dr. Monica Gulati
National Hon. Gen. Secretary, IPA	Organizing Secretary	National President IPA	LOC Chairperson

Sri Herbasia Biotech

Tishk International University, Iraq

3ICP2022 PRPP5-158

Serial No. _____

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

Certificate of Merit

This is to certify that ~~Prof.~~/Dr./~~Mr.~~/Ms. VIBHU KHANNA has Presented Poster/ ~~Oral Presentation~~ on GREEN SYNTHESIS OF COPPER NANO PARTICLES FROM ALOE YERA LEAF and awarded for best poster/ oral presentation in the 3rd International Conference of Pharmacy (ICP-2022) on the Theme of "Practice, Promotion & Publication of Innovation : A Way of Transforming Health" held on 09th & 10th November 2022 organized by School of Pharmaceutical Sciences in a collaboration with Indian Pharmaceutical Association (IPA) at Lovely Professional University, Punjab.

Mr. Suresh Khanna
National Hon. Gen. Secretary, IPA

Dr. Bimlesh Kumar
Organizing Secretary

Dr. T.V. Naryana
National President IPA

Dr. Monica Gulati
LOC Chairperson

Sri Herbasia Biotech

Tishk International University, Iraq

GOVT. DEGREE COLLEGE BILLAWAR

TWO DAY INTERNATIONAL CONFERENCE

NOVEMBER 25-26, 2022

CERTIFICATE

No: GDCB/2022/1973
Date: 31.12.2022

This certificate is awarded to Prof./Dr./Mr./Ms. Vishu Khana, Assistant Prof./Research Scholar of Lovely Professional University, Phagwara for his/her ~~Invited Lecture/Chairperson/Co-Chairperson/Adjudicator~~/Participation/Presentation of Research Paper (Oral/~~Poster~~) entitled "Aloe vera: An Ethnopharmacological updated review on burn wound healing" in TWO DAY INTERNATIONAL CONFERENCE on "ETHNOBIOLOGY: PRESENT AND FUTURE PERSPECTIVES "held at Govt. Degree College Billawar on November 25-26, 2022.

PROF. YASH PAUL
Organizing Secretary

PROF. LEKH RAJ
Co-Convener

PROF. ANITA [illegible]
Principal and Convener

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

Certificate No.253328

Certificate of Presentation

This is to certify that **Dr./Mr./Ms. Vibhu Khanna** of **School Of Pharmaceutical Sciences, Lovely Professional University, Phagwara** has presented a paper on **Proportional analysis of powdered Curcuma longa and Curcuma longa Extract.** in the **"3rd International Conference on Functional Materials, Manufacturing and Performances (ICFMMP-2022)"** held on July29-30th**,** 2022, organized by **Division of Research and Development, Lovely Professional University, Punjab**.

Date of Issue: 30-08-2022

Place: Phagwara (Punjab), India

Prepared by
(Administrative Officer-Records)

Dr. Hitesh Vasudev
Convenor

Dr. Pranav Kumar Prabhakar
Organizing Secretary

Dr. Chander Prakash
Conference Secretory

DSIR RECOGNIZED

CSIR-CSIO

SERB INDIA

विज्ञान एवं प्रौद्योगिकी मंत्रालय
MINISTRY OF SCIENCE AND TECHNOLOGY
सत्यमेव जयते

Office of the Controller General of Patents, Designs & Trade Marks
Department of Industrial Policy & Promotion,
Ministry of Commerce & Industry,
Government of India

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

Application Details	
APPLICATION NUMBER	202011050143
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	18/11/2020
APPLICANT NAME	**Lovely Professional University**
TITLE OF INVENTION	NOVEL HERBOMINERAL NANO EMULSION FOR THE TREATMENT OF BURNS
FIELD OF INVENTION	BIOTECHNOLOGY
E-MAIL (As Per Record)	dip@lpu.co.in
ADDITIONAL-EMAIL (As Per Record)	
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	12/07/2021
PUBLICATION DATE (U/S 11A)	01/01/2021

Office of the Controller General of Patents, Designs & Trade Marks
Department of Industrial Policy & Promotion,
Ministry of Commerce & Industry,
Government of India

सत्यमेव जयते

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS | GEOGRAPHICAL INDICATIONS

Application Details	
APPLICATION NUMBER	202211073273
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	17/12/2022
APPLICANT NAME	**LOVELY PROFESSIONAL UNIVERSITY**
TITLE OF INVENTION	METHOD FOR EXTRACTION OF LATEX FROM CALOTROPIS PROCERA AND PRODUCT THEREOF
FIELD OF INVENTION	BIOTECHNOLOGY
E-MAIL (As Per Record)	director@bansalip.com
ADDITIONAL-EMAIL (As Per Record)	dip@lpu.co.in
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	30/12/2022

Application Status