DEVELOPMENT AND PHARMACOLOGICAL EVALUATION OF POLYHERBAL MICROEMULSION FOR THE TREATMENT OF PSORIASIS

Thesis Submitted for the Award of the Degree of

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Ayurvedic Pharmacy

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DECLARATION

I, hereby declared that the presented work in the thesis entitled **"Development and Pharmacological Evaluation of Polyherbal Microemulsion for the treatment of Psoriasis"** 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 Associate Professor & C.O.D in the Ayurvedic Pharmacyof 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.

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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled Development and Pharmacological Evaluation of Polyherbal Microemulsion for the treatment of Psoriasis" submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Lovely School of Applied Medical Science, is a research work carried out by Arun Kumar having registration no 41800307 is bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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Abstract

The aim of present study was to develop a microemulsion formulation of *A.indica* extract for the treatment of psoriasis. There are total five drugs used in the study and on the basis of their in vitro analysis most potent extract is further incorporated in novel drug delivery system i.e microemulsion. Aqueous extract obtained from the stem bark of *A.indica* plant and further incorporated in NDDS for its better bioavalability and therapeutic efficacy as herbal drugs leads to poor solubility.

A.indica microemulsion is formulated by simple mixing method. Amount of each ternary component (on w/w basis) viz. Cardamom Oil, Smix (Tween 80/PEG 400) and aqueous phase were carefully determined from respective phase diagram. Total six batches were prepared and optimisation is done with the help of their characterisation by means of particle size, zeta potential, TEM. Transmission electron microscopy reveals round shaped droplet with average size of 212 nm having no agglomeration in their shape was observed and zeta potential is -24.66 mV. The in vitro permeation over skin of *A.indica* extract microemulsion reveals that F3 formulation is having highest flux as compare to controli.e 0.296µg/cm^2h^{-1}, which is almost ten folds increase as compare to control.

The effect of *A.indica* microemulsion was evaluated on immiquimod induced mice model for its antipsoriatic activity. The mice were treated with low and high dose of prepared microemulsion (group V & VI) shows significant ($p < 0.05$) of psoriasis. Histopathology analysis also states the reduction in Psoriasis severity index over skin, thus proved the enhanced efficacy of *A.indica* microemulsion.

PREFACE

First and above all, I praise god, the almighty for providing me this opportunity and granting me the capability to proceed successfully. This thesis appears in its current form due to assistance and guidance of several people.

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Words will fall short to express my feelings for my parents. They are the soul of my energy and motivation. Their blessings have to lead me towards achieving my goals.

Date: *Arun Kumar*

Place: Jalandhar

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List of Abbreviation

Abbreviations/symbols	Full form
%	Percentage
B	Beta
µg	Microgram
µL	Microlitre
3D	Three dimensional
ACE	Angiotensin converting enzyme
ANOVA	Analysis of variance
BMD	Bone mineral density
cAMP	Cyclic adenosine monophosphate
COX-2	Cycloxegenase
DSC	Differential scanning calorimeter
ELA	Elastase
ELISA	Enzyme linked immune sorbate assay
Eq	Equation
Fl	Flower
FTIR	Fourier transform infrared spectroscopy
HIV	Human immune deficiency virus
HPTLC	High performance thin layer chromatography
HaCaT	Human keratinocyte line
HPLC	High performace liquid chromatography
HD	High dose
IDC	Immature dendric cell
IL	Interlukin
IMQ	Immiquimod
IAEC	Indian animal ethical committee
ICH	International council for harmonisation
kV	Kilo volt

Kg	Kilogram
LDH	Lactate dehydrogenase
LD	Low dose
MTT	3 [4,5 – dimethylthiazole- 2-yl]-2,5 diphenyl tetrazolium bromide
mV	Mega volt
mRNA	Messenger ribonucleic acid
NSAID's	Non-steroiidal anti-inflammatory drugs
Nm	Nano meter
NCCS	National centre for cell science
NO	Nitric oxide
O/W	Oil in water
PASI	Psoriasis area severity index
PDI	Polydispersity index
PPM	Parts per million
PMA	Phorbol myristate acetate
PUVA	Photochemotherapy
PCL	Psoralia corylifolia
Rz	Rhizome
St.Bk	Stem bark
SOD	Superoxide dismutase
TGF-α	Transforming growth factor alpha
TCM	Traditonla chinese medicine
TEM	Trasnmission electron microscopy
V	Volt
WHO	World health organisation
W/O	Watr in oil
GBD	Global Burden of Disease

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CHAPTER-1

Introduction

1.1 PSORIASIS

At a microscopic level, psoriasis is a complex and severe inflammatory condition that lacks a definitive cause. It involves an abnormal immune response, disrupting the body's normal defense mechanisms. Psoriasis is recognized as one of the most prevalent skin disorders with strong genetic roots. It’s termed with fast multiplication of skin cells in epidermal layer, resulting in excessive cell growth. This process also hinders proper cell layering, increases blood flow to the skin, and prompts white blood cells to move out of blood vessels in response to cytokines. These changes predominantly occur within the top layer of skin, specifically epidermis & papillary dermis.(1) In 2013, following the evaluation of a comprehensive report on the impact of psoriasis on health, Executive Board of WHO proposed resolution to be presented at the 67th session of WHA (Boehncke W-H., et al 2015).(2) Resolution put forth was a request for the Director-General to initiate an awareness program addressing psoriasis as a significant global health issue. This proposal stemmed from the examination of a report that highlighted the impact of this inflammatory skin condition as a burden on health.(3)

It is an enduring autoimmune disorder signified with red, scaly patches on skin. This ancient & widespread provocative skin disorder was identified as early as 400 B.C. and was termed "psora" (meaning "to itch") by Hippocrates. Scaly patches, referred to as psoriatic plaques, signify regions of inflammation and heightened skin cell production. In the case of psoriasis, the usual 26-30 day cycle for keratinocyte cell maturation and migration to the upper skin layers is drastically shortened to just 3-4 days, leading to a phenomenon known as parakeratosis. As a result, skin accumulates rapidly at these sites, forming distinct silvery-white plaques. While these plaques commonly emerge upon knee & elbow, they can manifest upon any part of body, including scalp and genital regions.

Psoriasis is globally prevalent disorder impacting around 2% of populace.(4)It has an equal impact on both males and females(5)and can emerge at any age, although it most frequently makes its debut between 20 and 30 years of age(6)(7) While the exact cause

of psoriasis remains speculative, its development involves intricate physiological, immunological, environmental, and genetic processes. The disease is characterized by a series of interconnected changes within the skin: an overgrowth of epidermal keratinocytes, excessive blood vessel formation, dilation, infiltration of T lymphocytes, neutrophils, etc of WBC affecting skin. As an inflammatory skin disorder, the roles of NF-kB, STAT3, and TNF have been extensively studied and documented.(8)(9)

Histopathological alterations in the skin, including excessive cell growth, heightened keratin production, gathering of polymorphonuclear leukocytes into epidermis, and inflammation in dermal layer, can contribute to a more accurate diagnosis of the condition. Various factors thought to incite episodes of disease exacerbation encompass infections, stress, and shifts in climate that induce skin dryness, especially during colder weather. Skin traumas such as burns, scrapes, skin irritations, rashes, contact dermatitis, chemical reactions, infections, and sensitivity to light may trigger the formation of new plaques in roughly a quarter of individuals with the condition, a phenomenon known as the Koebner response. Medication like lithium, beta blockers, anti-malarials, and NSAIDs (such as ibuprofen) are examplescould elicit or worsen the disease. Excessive alcohol consumption might impede the expected positive response to appropriate psoriasis treatments. Psoriasis flare-ups may also be triggered by mental stress, which may cause release of peptides from cutaneous nerve cells which encourage irritation or excessive development of keratinocytes.(10)

Though psoriasis seldom results in death, it may severely diminish life standard & even need hospitalized in extreme cases. The enduring and disfiguring nature of this condition also results in notable psychological and social distress, contributing to a diminished quality of life and psychological health challenges.(11) At present, there exists no definitive cure for this ailment, which tends to alternate between periods of improvement and worsening. The available treatment options are associated with well-recognized side effects. Systemic or UV phototherapies might yield undesirable consequences; metotrexate or cyclosporine treatments can lead to liver and kidney issues, oral retinoids carry the risk of causing birth defects, and recurrent PUVA treatments could potentially lead to skin cancer.

The estimated likelihood of developing psoriasis over one's lifetime is around 24 - 28% amongst close family members. Monozygotic twins, who share identical genetic makeup, display a higher level of agreement with rates of 63 to 73%, compared to the 17 to 20% rate observed in dizygotic twins with differing genetic backgrounds. This divergence in rates further underscores the genetic underpinning of this condition. Notably, concordance rate is never 100%, indicating as external variables, such as surroundings, might have an impact upon causing signs of psoriasis. Through comprehensive genome-wide analyses, researchers have identified at least six susceptibility regions, labeled as PSORS1 to PSORS6, although the level of certainty varies among these findings.(12)(13)

1.2 Epidemiology of Psoriasis- Incidence and Prevalence

Worldwide, over 125 million individuals grapple with the persistent condition known as psoriasis. Both males and females experience this ailment in equal measure. While the symptoms of this disease can emerge at any life stage, they typically commence either between the ages of 18 and 39 or during the period of 50 to 69 years.(14)

Figure 1: Global prevalence of psoriasis

Typically, the occurrence of psoriasis is cited to be within the range of 1-3%.(15)(16)(17)(18)(19)(20)(21)(22)(23) However, investigations across diverse

populations reveal significant disparities in the prevalence of the condition. Recent geographical studies have documented psoriasis rates of 1.6% in the United Kingdom(24), 1.55% in Croatia(25), 1.4% in Norway(26), 3% in Denmark (13), 2.5% in Australia (7), 2.8% into Faroe Islands (16), & 4.8% into Norway (27). Conversely, prevalence figures range from 0.7% in Rwanda in eastern Africa(28), to a notably high 11.8% in Kazach'ye, located in the Arctic area of the former Soviet Union(29) Various studies from Hong Kong, Japan, and China, concluding that despite the distinct climatic and geographic variations across these regions, the surveys consistently demonstrated a stable and uniformly low psoriasis prevalence of around 1%. True prevalence may be greater since some people could have single lesion & fail to get it checked up.(30)

1.3 Treatment

Psoriasis ranks as the third most frequent motive for individuals to consult dermatologists, following acne and warts. Approximately 1.5 million annual visits to medical offices or hospitals are attributed to psoriasis, with 80% being to dermatologists and the remaining 20% to specialists in other fields. Many of the drugs now established as standard treatments were originally adopted based on practical experience, lacking an extensive comprehension of their alignment with T-cell pathogenesis.

The treatment strategy for controlling psoriasis is condition-specific. The primary goal of current therapy is to lessen severity of condition to manageable levels. These therapies are effective because they target distinct facets of immune system's reaction abnormal proliferation of skin cells. Topical medicines, phototherapy, & systemic remedies are 3 primary classes into which conventional therapies fall according to their mode of administration.

Table1. Marketed Formulation for the Treatment of Psoriasis(31)

Sr.No	Drug	Brand Name	Manufacturer
1.	Calcipotriene	Dovonex	Leo Pharma
2.	Tazarotene	Tazorac	Allergan Pharma
3.	Adalimumab	Humira	Abbott
4.	Etanercept	Enbrel	Pfizer

5.	Methotrexate	Rheumatrex	Dava Pharma
6.	Cyclosporine	Sandimmne Neoral	Novartis
7.	Betamethasone dipropionate	Diprolene	Schering
8.	Clobetasol propionate	Temovate	Pharmaderm
9.	Brodalumab	Siliq	Valeant Pharma
10.	Guselkumab	Tremfya	Janssen Biotech

1.4 Kustha

As per Ayurvedic point of view Psoriasis is termed as Ek-Kustha comprises of two parts 'Eka' and 'Kustha'. The term Eka signifies some special features. Ekakustha is a Vata Kapha predominant Tridosaja Vikar. View of Acharya Charak denotes that among Vata and Kapha if either dosa is predominant then the disease is not so difficult to cure. According to the view of Sushruta among 'Ksudrakustha', Ekakustha is one that is incurable. Bhavprakash had placed Ekakustha as most important among other Ksudrakustha. Clinical assessment of Ek kustha is done by observing Vedana, Varna, Akriti, Prabhav, Poorva rupa & Upadrava.(32)

The term Kustha is originated from the word 'Nikushi', which implies the meaning 'Kusita'. This word indicates worst condition of the body which cause isolation of an individual. Kustha is a disease which cause cosmetic crisis by vitiation of rakta dhatu.

In Atharva Veda 'Kustha' term is comes under herb. Acharya Hemchandra clarying Kustha is having two meaning one related to herb and other one is related to disease. 'Kustha' herb is related to S lappa which is able to cure skin disease.

1.4.1 Etiology of Ek Kustha as Per Ayurveda(33)

- **Ahara Nidana:**Taking large amount of rice, citrus fruit, heavy food, black grams, buffalo milk, curd, fish etc. Improper consumption of food in daily routine may leads to aggression of Ek Kustha.
- **Vihara Nidana:** Sexual activity, sleeping at day time, more exposures to sun rays, and excess exercise plays an important role in the etiology of psoriasis.

- **Sansargaja Nidana:** It meansEk Kusthais communicabledisease; it can be affect anyone through communicable route.
- **Poorva Janamkrata:** As per Acharya Sushruta if a person suffered from Ek Kustha in his previous life he can be suffered again with same disease**.**

1.4.2 Classification of Kustha(34)

Classification is done on the basis of Severity, symptoms &treatment. In classical book i.e Charak Samhita Nidan Sthana chapter is having only seven types of Mahakusthas, while other Kshudra Kustha is mentioned in Charak Samhita at Chikitsa sthana. Maha Kusthas are more stubborn as compare to Kshudra Kustha. In Kshudra Kustha doshas involvement is main factor for the disaease as compare to Mahakustha. Acharya Charak, Sushruta and Vaghbhatt justify kustha are as; Mahakustha is off 7 types, while Kshudra Kustha is off 11 types.

1.4.3 Doshik Involvement in Ekakustha(35)

As per Acharya Charak Kustha is a doshas disorder. There may be vitiation of all the three doshas or may be two doshas involvement for the occurance of disease. In Chikitisthasthan Kshudrakustha is mentioned due to the vitiation of doshas. Predominance of doshas is Kshudra Kustha is as follows:

Table 2: Doshik Involvement in Eka Kusth

Vatika Kustha	**Ruksha (Dryness), Sula (Pain), Parush (Rough Skin), Shosha**
Kaphaja Kustha	Shvet (White coloration), Kandu (Itching over skin), Sthairyam (Localised), Janturabhi Bakshmam (Parasite infection).
Pittaja Kustha	Dah (Burning sensation over skin), Strava (Discharge), Paka (Pustule formation), Kleda (Exces debris)

1.5 Chikisa (Treatment of Psoriasis as Per Ayurveda)(36)

Patient affected from Ek Kustha dominated with Vata roga is recommended to intake of herbal ghee internally, similarly kapha vitiated patient is recommended to take vamak dravya (emesis), while pitta vitiated patient is recommended to take virechan dravya (Purgatives). In certain cases, if patient is suffering from Kustha due to the vitiation of different doshas then shodhan (purification) therapy is recommended for that patient with proper care. Some ayurvedic therapies for the treatment of psoriasis are;

consumption extract of *B.aristata* extract along with cow urine may cure Kustha, intake of haritaki churna along with trikatu (i.e Pippali, Marich, Shunthi) with jiggery and tila taila for 30 days may cure kustha. Some ayurvedic formulation mentioned in the classical books are, **Patolamuladi kshaya, Mustadi churna, Triphaladi churna** etc.

1.6 Novel Drug Delivery to Overcome and Enhance the effect of Conventional Dosage Form

Novel drug delivery plays an important role for the treatment of various dreadful diseases. Nanomedicine plays an important role to enhance the absorption of drug through skin membrane with improved bioavailability. Herbal extract incorporated in such delivery system like; Microemulsion, Nanoemulsion, Phytosome, Ethosome, & Nanocarriers play an important role to enhance the effect of drug.

Microemulsion is the best formulation interms of topical drug delivery system. The main therapeutic potential of microemulsion is that it can act low water soluble drug into bottom of the skin layer which leads to the quick healing of inflammation/psoriatic lesion. Microemulsion possesses control release of active constituents to direct target site.

CHAPTER- 2

REVIEW OF LITERATURE

2.1 Psoriasis

As per Ayurvedic point of view Psoriasis is termed as Ek-Kustha comprises of two parts ‘Eka’ and ‘Kustha’. The term Eka signifies some special features. Ekakustha is a Vata Kapha predominant Tridosaja Vikar. The two main categories of Ayurvedic treatment principles are Shodhanakarma (biocleansing therapy) and Samshamana (palliative process). Acharya Charaka acknowledged the importance of Shodhana and said that, in contrast to Shamanachikitsa, the return of the condition is less likely when Shodhanachikitsa is used to control it. If the patient receives solely Shamanachikitsa treatment, they may eventually reoccur.

As per modern point of view epidermal hyperplasia and a significantly increased rate of epidermal turnover are hallmarks of psoriasis, a chronic skin condition. Typical lesions take the form of isolated, scaly, erythematous papules and plaques. Traditional categorization of psoriasis as a papulosquamous dermatosis is based on these morphological traits. This skin illness might manifest as a few small spots on the scalp, elbows, or knees or it can be systemic, affecting the whole body.

2.1.1 Historical aspects

In 1808, an English physician, Robert Willan, provided the first accurate description of psoriasis(37). Up to that time, this common cutaneous disease was confused with leprosy, syphilis, or other skin illnesses. According to a biblical reference in Leviticus, the differential diagnosis of leprosy leads one to surmise that many who were called "unclean," were actually suffering from psoriasis. Over the last few decades, meaningful advances have been made in studying this protean cutaneous illness. The diagnosis of psoriasis no longer offers any particular problems, and specific clinical and laboratory features of the disease have been established; however, the etiology and pathogenesis remain an enigma.

2.1.2 Clinical features of Psoriasis

Psoriasis vulgaris (Photo1) is the most common presentation. This is also the most common variant of psoriasis, seen in more than 90% of patients, also known as plaque type psoriasis.(16) Red, scaly plaques appear, usually in a symmetrical pattern. Although the scalp, elbows, knees, shins, and sacrum are particularly vulnerable, any area of skin is potentially at risk. The size of a plaque may vary greatly, from only a few millimeters to a significant portion of a body component. The skin that psoriasis affects is often very rough, and this is one of the most helpful ways to tell it apart from other dermatoses. Since the enlarged capillaries nearly reach the skin's surface, mild or pulling scales from the plaque's surface might induce bleeding (Auspitz sign). It is possible for hard, thicker, firmly adhering keratin to cover the surface of plaques instead of scaling. Not all individuals exhibit the Köbner phenomenon, in which psoriatic lesions form at areas of trauma (such as a surgical cut or a minor scratch, abrasion, or burn). Lichen planus has it, too, whereas eczematous dermatoses do not.(38) Psoriasis flare-ups may last for a few weeks to a few years and might impact a smaller or bigger percentage of the population at different times. Treatment may cause a prolonged remission, or it might happen naturally.

Guttate psoriasis (eruptive psoriasis) is the most common type of the illness in children and young adults, and it is characterized by the abrupt, widespread emergence of tiny red lesions on trunk and proximal limbs. Approximately 70% of these people will progress to the chronic plaque type, and those with chronic plaque may also have guttate flares.(39) Intertriginous erythematous lesions without scaling are characteristic of inverse psoriasis (seborrhoeic psoriasis). The afflicted area may include the middle of the chest or the sides of the nose, lips, and eyes. Chronic plaque psoriasis and this type often occur together. Localized or widespread sterile pustules on erythematous, scaling skin define pustular psoriasis.(40)

2.1.3 Etiology

Research suggests an inheritance of multifactorial mode. Numerous psychological events, environmental factors, and stressful physiologic conditions are associated with the onset as well as worsening of the diseased condition. Trauma directly to the skin can trigger/generate psoriasis (which is called as Koebner phenomenon). Throat infections caused by Streptococcal bacterial might also activate or it might even intensify the prevailing psoriatic disease. HIV infection can exacerbate the current disease and has not been shown or presented to trigger psoriasis disease. Progression of the infection often causes worsening of psoriasis. Increased risk of psoriasis and its severity or rigorousness is seen in smoking. Alcohol usage/consumption, alcohol abuse, and obesity are moreover connected with this inflammatory skin disease, such kinds of connections might not be contributing, but patients who are suffering from this skin disease may be liable to even harmful activities.(41)

2.1.3.1 Genetic disposition

A high family of psoriasis manifestation about 7% to 36% recommends that the genetic factors play a role.(15) In a German study, when one single parent is affected the risk was 14% and 41%, if both parents are affected 6% are at risk when one sibling is pretentious compared to 2% when no sibling or parent is affected. Concordance of this inflammatory skin disease is 15% in the case of dizygotic twins whereas it is 64% of psoriasis in monozygotic twins.(42) There are two pathogenically distinct forms of diseases Type 1:- is inherited and associated with strong Human Leucocyte Antigen, the initial inception of this inheritance is probably expected to be a severe one. Type 2:- is intermittent and not related to HLA, which is usually mild.(43) The average age (in years) of onset of disease in patients with a family past was an age of 23 years as compared to an age of 28 years in others.

2.1.3.2 HLA Studies

HLA-Cw6 is a single connected alternative form of genes in psoriasis. Some of the population demonstrated a prevalence of epistatic interaction between LCE3 risk variants 3 with HLA-Cw6, while some populace failed to exhibit the interaction.

LCE3A exhibited a noteworthy higher expression with homozygous risk genotype while LCE3B also exhibited a noteworthy overexpression in the patient's population with HLA-Cw6 form of genes. A combination of genetic and gene expression is permissible to recognize the real disease variants at the LCE3 cluster amongst the patients affected with psoriasis in India. (44) However, various studies revealed that these associations were secondary to an association with HLA-Cw6. HLA~Cw6 has also portrayed to influence the age of onset of psoriasis.

2.1.3.3 Environment risk factors

The current understanding favors a principally immunological mechanism in which abnormal T-Lymphocytes affecting genetically liable keratinocytes, under several environmental factors are predictable on triggering and intensifying psoriasis.

2.1.3.4 Trauma

Psoriasis lesions could look as if it is a consequence of injury in psoriasis patients to the plain skin. This type of phenomenon is termed "Kobner's phenomenon". In 1877 the German dermatologist Heinrich Kobner was the first to describe this phenomenon. An extensive array of injurious local stimuli including infective, surgical, electrical, physical, chemical, and inflammatory injuries is acknowledged to elicit psoriatic lesions. Psoriasis occurs usually 7 to 14 days after the injury. Its occurrence varies between ranges of 38% -76% of the patient populace affected with psoriasis inflammatory disease. In a patient it is all or none phenomenon i.e., psoriasis inflammatory disease might occur only in one site or it might occur at all the sites of injury. Clearance of prevailing psoriasis lesion subsequent wound is witnessed and named as contrary type of Kobner phenomenon.

2.1.3.5 Infection

Quite a lot of studies put forward that prior or current infection caused by β hemolytic streptococci which trigger guttate psoriasis, and are associated with the plaque 4 phenotype. Streptococcal M protein, which is the chief antigenic determinant of the lance field groups A, C, and G streptococci shares epitopes with keratins and as a consequence may induce immune responses in the skin.(45)

Severe exacerbation of psoriasis can be a manifestation of HIV infection and effectively treated with antiretroviral therapy. Like psoriasis, HIV-associated psoriasis has a strong association with HLA-Cw6. Interestingly, the occurrence of psoriasis in infection by HIV is no higher than in the general populace, (1% to 2% of patients), indicating that this infection is not a trigger for psoriasis but rather a modifying agent. Psoriasis is increasingly more severe with the advancement of immunodeficiency nevertheless can remit in the terminal phase. This paradoxical exacerbation of psoriasis may be in line for a loss of regulatory T cells and bigger activity of the CD8 T-cell subset. (1)

2.1.3.6 Stressful life events

Despite the lack of strong evidence, clinical observation and epidemiology studies recommend that psychological stressful life events can be involved as a potential trigger for the beginning of the diseased condition as well as worsening of psoriasis. (45)

2.1.3.7 Alcohol Consumption

Since several huge epidemiological studies appear in the direction of high intake of alcohol might trigger, exacerbate and impact the severity and the development of psoriasis.(1) A variety of mechanisms that explains the interaction between ethanol and psoriasis has been proposed by numerous studies, including increased discharge of proinflammatory cytokines, amplified lymphocytes production, leading to depression in the immune system, and increased cAMP-dependent multiplication of cells in the epidermis. (45)

2.1.3.8 Smoking

Numerous studies conducted have been associated with the startup of palmoplantar pustular and psoriasis vulgaris mainly due to smoking habits. Similarly, the habit of 5 smoking has also been associated with comorbidities like the severity of the disease condition, and a higher rate of mortality rate in patient population suffering from psoriasis.

Different mechanisms have been proposed indicating a connection between nicotine and psoriasis, including the augmentation of proinflammatory cytokines, and transformed shape, structure, particular form, and function of leukocytes.(45)

2.1.3.9 Diet and Obesity

Increased (BMI) that reflects over nutrition has been presented to trigger psoriasis, while low-calorie ingestion and a vegan intake have been demonstrated to advance severe inflammation of the skin conditions like psoriasis. High dietetic consumption of essential fatty acids, Omega (Ω)-3 and Omega (Ω) -6 has been recommended to be protective against psoriasis.(1)(45)

2.1.3.10 HIV

HIV infection has exhibited to intensify the psoriatic disease state. However the occurrence is unaffected but the severity of the disease is superior in this populace.

2.1.3.11 Climate

A number of studies endorse that psoriasis deteriorate in wintertime and recovers in the summertime. Though the radiations emitted by the sun are considered to be beneficial, in a few proportions of the patient population, severity in the diseased state may be triggered by strong emission of radiations from the sun.

2.1.3.12 Drugs

NSAIDs, Lithium, Anti-malarials, β (beta)–blockers, IFNS alpha and gamma imiquimod, ACE inhibitors, and gemfibrozil are the different categories of treatments that intensify or worsen the psoriasis disease. Withdrawal of the potent dermal or topical or systemic 6 steroids is associated with exacerbation of psoriasis. NSAIDs like oral phenylbutazones, indomethacin, diclofenac, meclofenamate, and ibuprofen are reported to precipitate psoriasis. The mechanisms are largely unknown behind the triggering progression or process of psoriasis by which kind of the medicines are involved. It has been anticipated that Lithium tends to cause exacerbation by tending to interfere or impede with the release of calcium in the keratinocytes, whereas β (beta)-blockers are assumed to impede intracellular cyclic adenosine monophosphate (cAMP) levels.(1)

2.1.3.13 Metabolic factors

During the pregnancy, psoriasis gets altered it is more likely to recover the condition of the disease than worsen. During the post-partum period, it is copious enough to worsen the diseased condition than improve the condition of the disease. In contrast, in the 3 months of post-partum period, 11 percent is improved and 54 percent has deteriorated. Hypocalcemia occurred in severe forms, especially in generalized pustular psoriasis.(45)

2.1.4 Pathogenesis

Earlier the to early 1980s a number of biochemical mediator enzymes were incriminated as being abnormal like Cyclic Adenosine mono-phosphate (cAMP), the eicosanoids, the protein kinase-C, the phospholipase–C, the polyamines, transforming the growth factor- α (TGF-α). However compelling, circumstances and experiments are evident enough that suggests a primary T- lymphocyte based Immunopathogenesis.(5)

2.1.5 Immunopathogenesis

2.1.5.1 Cellular basis of psoriasis

Characteristic fiery red plaques of flaking skin lesions called psoriasis are identified by their clinical appearance or manifestation. These types of skin lesions are elucidated by remarkable progress and dilatation of the blood vessels visible superficially and hyperplasia of the epidermal layer. In the epidermis or epidermal layer, keratinocytes 7 multiply swiftly so that the terminal cells differėntiation, which occurs in the granular type of keratinocytes, and then squamous comeocytes are unfinished. Parakeratosis is a process in which corneocytes aberrantly retain intact nuclei that result in poorly adherent stratum corneum which contributes to the characteristic scale of psoriasis /psoriatic lesion. The manner in which these mononuclear leukocytes appear in papillary dermis and neutrophils in the epidermis are either as spongiform pustules of Kogoj or in Munro micro-abscesses are the defining features of psoriasis histopathologically. The intermingling of keratinocytes and T-lymphocytes are seen into epidermis & in fairly in higher amounts in layer of the dermis.(46)

Epidermal T cells rely heavily on CD8+ T cells, with a sizable subset of these cells having developed a unique homing mechanism for epithelia. Integrin αEβ7, covalently linked to cadherin, mediates the connection between cytoskeleton and desmosomes. Dermal T lymphocytes are a heterogeneous population of CD4 (+) and CD8 (+) cells. The majority of T cells in skin lesions are memory cells that can make cutaneous lymphocyte antigen (CLA).(47)

2.1.5.2 Dendritic cells

Psoriasis skin lesions also include an increased number of antigen-presenting accessory cells, another major type of leukocytes. Langerhans cells are the kind of cell that fall under the umbrella of (iDC). In most cases, you may find these cells in the epidermis.(48) In addition to measuring MHC II or factor XIHa expression levels, dermal DCs are a broadly distributed kind of iDC. MDCs are the last stage of development for iDCs that began life as blood monocytes. T lymphocytes benefit greatly from the immunostimulatory effects of these cells. Some examples of interleukins produced by these cells include IL-12 & IL-23.(49)

A noticeable rise in the dermal DCs demonstrates or indicates lesions of Psoriasis, which are marked by an expression of XIIIa and CD11c, as amongst constant markers of myeloid DCs or mannose receptor as well. A comparable subset of dendritic cells termed an IEDC is amplified as active lesions in the 8 epidermis. Another type of DC called pDC are intensely enlarged in the skin affected with psoriasis.(50)

a) Activation of T cells: It is anticipated that the patient population develops an immune system to an anonymous skin antigen (Ag). Activation of the T cells requires three steps.
    - The first step requires binding i.e. the T cell attaches to the antigen (Ag) presenting cell (APC) through surface adhesion molecules.
    - The second step is the antigen (Ag) specific activation in which the antigen (Ag) is accessible to the T cells by the APC leading alteration of naïve T cell in antigen (Ag) specific cell.
    - The third step is co-stimulation of non-antigen specific cells.

b) T cell proliferation & differentiating: The CD4 T cells are differentiated in a Th1 phenotype under influence of IFN-γ and IL-12.

c) T cell trafficking: Activation of the T cells is the permanent process that occurs in skin where they can elicit their effects, a multi-step process that includes interaction amid the active T cells as well as the endothelium.

d) T cell reactivation: After departing the post-capillary venules, Th lymphocytes meet up with the DC into dermis layer & Cells of Langerhans into epidermis layer and consequently release proinflammatory cytokines such as TNFα and IFNγ. The ultimate result is an increase in keratinocyte proliferation, as shown in lengthening of rete ridges, damage to granular layer, parakeratosis, & proliferation of endothelium.(50)

2.1.6 Histopathology

The histopathological features of a fully developed psoriatic lesion are:

a) Uniform parakeratosis.

b) Presence of micro-abscesses (Munro) in the horny layer: they contain neutrophils with pyknotic nuclei that have been migrated from the "squirting papillae" to the spongiform pustules and then to the horny layer.

c) Absence of granular layer.

d) Rete ridges regular elongation, with a thickened portion on the lower side, sometimes resembling a shape like camel-foot, which often they tend to coalesce.

e) Regular elongation of dermal papillae that are clubbed at their upper portions.

f) Capillaries are tortuous and dilated in the papillae with perivascular mononuclear cell infiltration as well as edema.

g) The occasional existence of small pustules as a spongiform is due to the diminishing of the suprapapillary parts of the stratum malpighii.

h) The presence of spongiform pustules of Munro and Kogoj (micro abscesses) are truly indicative of psoriasis and in their absenteeism, the diagnosis can be made rarely with certainty on a histological base.

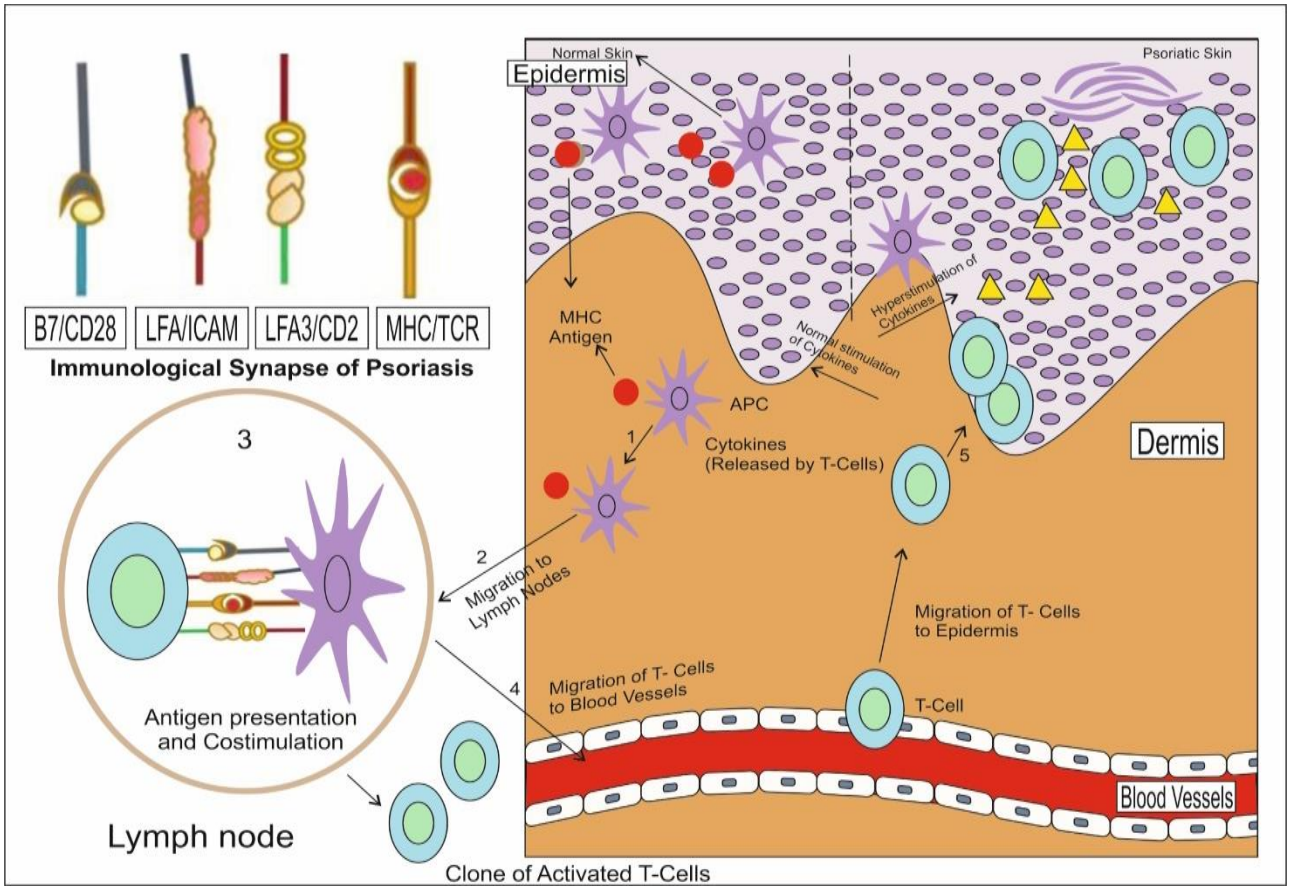


Figure 2. Inflammatory Cytokines Involved in the Pathogenesis of Psoriasis

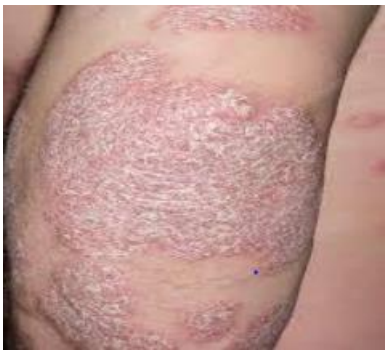
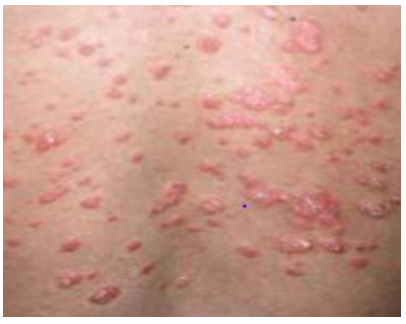
2.1.7 Clinical features

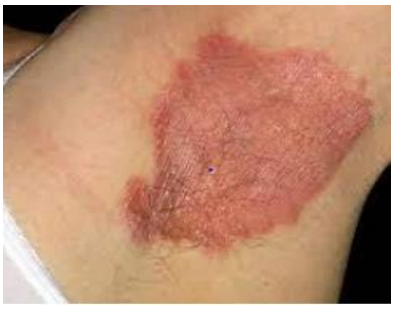
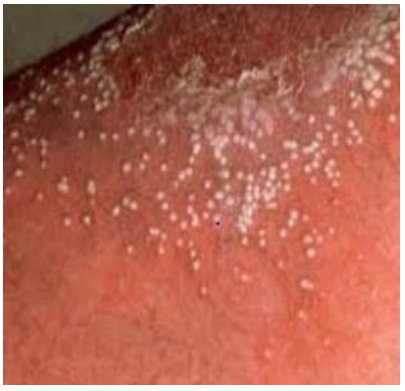
Psoriasis has various phenotypes, Psoriasis vulgaris is a type of clinical variant most commonly found. Psoriasis vulgaris is found in 85-90% of the psoriatic patient population. Erythematous plaque is characterized by well-demarcation, with a silvery scale. The most affected areas are elbows, knees, scalp, and sacral regions. Other less impacted sites are the hands and feet, nails on the fingers as well as toes. The areas where two skin areas may touch or rub together (an intertriginous area) like groin area, axilla of the arm, anogenital region, umbilicus area, retro auricular or skin folds.(51)

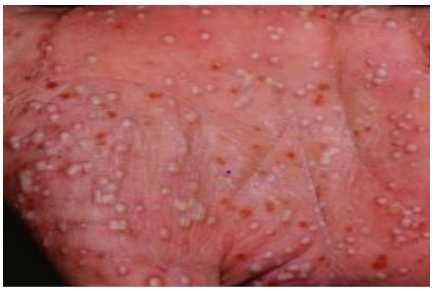
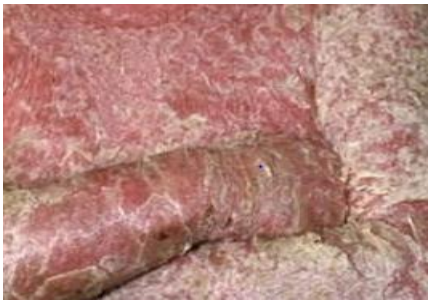
The plaques ooze and display an inflammation that is fiery red without any scaling. The actual frequency rate of the intertriginous skin psoriasis is indefinite.(52) found nearly 44% of psoriasis patients had perianal involvement.

2.1.7.1 Different kinds of Psoriasis

Table 3: The following types of psoriasis summarized in the table below can be seen in the skin:(53)

Types of psoriasis	Characteristics	Figure 3-8
Chronic plaque	This type of psoriasis is one of the much observed types of Psoriasis vulgaris. In this, the plaque is signalized by erythemato-squamous plaques either as single or multiple. These usually are distributed and seen in extensor sites of extremities like knees or elbows, sacral areas, and buttocks. The lesions can improve due to various external, internal, or endogenous factors like infections, medication, or stress which can be the main triggering factors for psoriasis. (Nast et. al., 2012, Armstrong, et al 2020)	 **Figure 3.** Chronic Plaque
Guttate Psoriasis	This is marked by the presence of drop-shaped erythematosquamous lesions. These psoriatic lesions may measure up to a diameter of 1.5 centimeters which mostly impact the entire skin surface. This type of psoriasis is commonly observed clinical appearance of psoriasis. This mainly occurs in response to an infection	 **Figure 4.** Guttate psoriasis

	(streptococcal origin) n childhood or adolescence. The guttate type of psoriasis may move to the plaque type. (Nast et. al., 2012).	
Intertriginous Psoriasis	Intertriginous psoriasis is a rare kind of psoriasis; mainly impacting the folded skin body type like submammary region, axillae, inguinal and anal region. (Nast et. al., 2012)	 **Figure 5.**Intertriginous psoriasis
Pustular psoriasis	There is a number of clinical variants of Pustular psoriasis which is categorized with presence of multiple disseminated pustules. This type even has the potential to reduce general health like fever, feeling of being in poor health, and dermopathic lymphadenopathy. Pustules are widespread in other clinical variations of psoriasis and occur with other clinical conditions (e.g. severe static plaque kind of psoriasis) which are associated with a rapid worsening of the condition. Psoriasis cum postulates its presence in such circumstances. (Nast et. al., 2012).	 **Figure 6.** Pustlar psoriasis

Palmoplantar Pustulosis	This type of Pustulosis is now included in the cluster of acropustular forms of the disease. Pustules are manifested in multiples that appear underneath the feet as well as on the concave portion of the hands. (Adriana et. al., 2019)	 **Figure7.** Palmoplantar pustulosis
Erythrodermic psoriasis	This type of psoriasis is life-threatening in which almost 90% of the total body surface is erythematous and inflamed which is considered to be an acute condition. Erythroderma type of psoriasis is such a condition that requires an emergency cure. (Adriana et. al., 2019)	 **Figure 8.**Erythrodermic psoriasis

Psoriasis can further result in decreased symptoms of health state like abnormal rise in body temperature, dehydration due to fewer intakes of fluids, high consumption of energy, and generalized fiery red skin lesion.

Moreover to the skin manifestations of psoriasis, it is even observed on nail affected up to 50% of psoriasis patients. (54)Nail Psoriasis signs include

- Sublingual hyperkeratosis
- Oil-drop discoloration
- Nail plate Crumbling
- Pitting

2.2. Summary of literature review of topical treatment for the management of psoriasis

Psoriasis is persistent skin ailment categorized by red, itchy, & scaly skin eruptions. Despite advancements in treatments and the introduction of innovative antipsoriatic

drugs, psoriasis remains incurable, significantly impacting patients' quality of life. To enhance the well-being of individuals with psoriasis, it's crucial to understand how specific drugs affect therapy safety, efficacy, and patient comfort during application. Therefore, this literature review explores topical treatments for managing psoriasis in-depth, aiming to develop a patient-friendly and effective local approach.

Various treatment options exist for psoriasis, dependent on the condition's severity and previous treatment responses. Topical agents are typically the initial choice, followed by light therapy, traditional systemic treatments, or systemic immunomodulatory drugs (biologics). Numerous conventional medical therapies, such as topical and systemic medications, phototherapy, or combinations thereof, are available. However, these treatments often have limited effectiveness and may lead to side effects like skin thinning, organ toxicity, cancer risk, and broad immune suppression, limiting their long-term use. Therefore, exploring herbal products as a safer alternative for psoriasis treatment is desirable.

To achieve this objective, this review utilizes electronic databases and literature references to compile current knowledge derived from animal studies and clinical trials on herbal products used topically for psoriasis treatment. The subsequent literature review delves into the realm of topical herbal treatments for psoriasis.

Table 4. Literature review of topical treatment for the management of psoriasis

S. No.	**References**	**Drug Name**	**Dosage form**	**Types of Psoriasis treated**	**Major finding reported in the study**
1.	Goldberg et. al 1991	0.05% halobetasol propionate	Ointment	Plaque psoriasis	Research findings indicate that, following a two-week timeframe, a significantly higher percentage of patients given treatment with halobetasol experienced either no illness or only mild illness

					comparing with that receiving clobetasol therapy (86% versus 70%, with a p-value of 0.023). Additionally, it became apparent that 69% of patients into halobetasol group & 56% in clobetasol group showed signs of improvement after beginning their respective treatments.(55)
2.	Van De Kerkhof et. al 1996	Tacalcitol	Ointment	Plaque psoriasis	From the results reported in this study, it has been observed that application of a 4µg/g tacalcitol ointment once daily was found to be efficient in psoriasis vulgaris in patients of the Caucasian population. Tacalcitol was also found to be safe and tolerable on the lesions on the facial place or outer appearance.(56)
3.	Lebwohl et. al	Calcipotriene and halobetasol	Ointment	Psoriasis	As per the study, the length of remission attacks of psoriasis can be increased with a regimen of pulse therapy of superpotent

					corticosteroids on the weekends as well as an additional topical application of calcipotriene ointment can increase the remission process.(57)
4.	Lebwohl et. al 2001	fluticasone propionate, 0.005%	Ointment	Psoriasis on the facial region and intertriginous areas	The author reported fluticasone propionate when applied topically over the skin in a span of 10 weeks is efficacious and lengthens the process of initiation reoccurrence of lesions with no of atrophy of skin , in the patients with acute to chronic psoriasis at threat in facial and intertriginous parts of the body over the skin surface.(58)
5.	Ulrich et. al 2003	Pime crolimus	Ointment	Psoriasis	A noteworthy therapeutic efficacy of pimecrolimus in an ointment formulation was observed when applied without occlusion to psoriatic plaques.(59)
6.	Lebwohl et. al 2004	Tacrolimus	Ointment	Facial and intertriginous psoriasis	The author has reported that as early as within 8 days, as per static sternness score, many of

					patients exhibited a promising improvement into treatment group when comparing by vehicle group. There was 24.8% betterment in the group who used tacrolimus ointment whereas in the vehicle group it was only 5.8%. On the end day of 8weeks, 31.5% of the vehicle group and 65.2% of the group with tacrolimus ointment treatment was almost clear.(60)
7.	Brune et. al 2007	Tacrolimus	Ointment	Lesional psoriasis on the facial or intertriginous areas	Children with an age of 2 years and above suffering from atopic dermatitis are treated with currently approved Tacrolimus ointment.(61)
8.	Yin-Ku Lin et. al 2007	Indigo Naturalis	Ointment	Plaque Psoriasis	The result from the study suggests that Indigo naturalis ointment was efficient for psoriasis treatment. The drug acts by modulating the multiplication as well as keratinocytes differentiation in the

					epidermis of the skin. It also inhibits the T-lymphocytes infiltration process and subsequently inflammation reactions in the psoriatic lesions.(62)
9.	Dong et. al 2012	Flumetasone	Ointment	Psoriasis vulgaris	From the clinical results obtained in the study, the author reported that a combination of 308 nm excimer laser along with flumetasone ointment was efficient and superior in psoriasis vulgaris when compared with the laser monotherapy.(63)
10.	Ilker et. al 2012	Combination of Psoralen gel or calcipotriol ointment	Gel/ Ointment	Plaque Psoriasis	The combination of targeted phototherapy and calcipotriol ointment increases the therapeutic activity of phototherapy especially in a type of plaque psoriasis.(64)
11.	Korting et. al 2012	Mometasone Furoate 0.1%	Cream	Psoriasis	A new light cream, Mometasone cream (oil-in-water type of emulsion 60/40) in combination with 0.1% of Mometasone Furoate, displays a similar therapeutic activity compared to Mometasone

					comparator. The formulations possess all the properties of light cream and as well as good tolerability. These positive attribute of the cream treatment was found to be a substitute for psoriasis with acute inflammation in skin diseases.(65)
12.	Najafizadeh et. al 2012	St Johnswort (Hypericum perforatum L.)	Ointment	Plaque Psoriasis	The major outcome in the study suggests that the PASI scores were declined in the mild type of psoriasis with plaques. Ointment of Hypericum perforatum when applied two times daily was found to give effective results.(66)
13.	Singhal et. al 2012	*Cassia tora* Linn	Cream	Psoriasis	The results were evident enough that there was a sufficient great reduction in the spleen index and relative epidermal thickness percentage. The combination of methanolic extract of Cassia Tora Linn. And topical oil in water cream possesses potent anti-

					psoriatic efficacy in the rats with UVB-induced psoriasis.(67)
14.	Ethan et. al 2014	combination with clobetasol and calcitriol	Spray/ Ointment	Plaque Psoriasis	A combination of clobetasol spray along with an application of calcitriol ointment with excimer laser therapy was found to be the best treatment therapy. This therapy was found to be effective for acute to chronic generalized psoriasis. The combination therapies possess the advantage of avoiding the threat of serious adverse events with systemic drugs.(68)
15.	Avasatthi et. al 2015	Methotrexate	Nanogel	Psoriasis	The developed methotrexate Nano lipid carrier (MTXNLC) gel formulation can be a promising substitute to existing methotrexate (MTX) Nanogels in the psoriasis treatment.(69)
16.	George et. al 2015	Calcipotriolbet amethasone dipropionate	Gel	Psoriasis vulgaris	The high level of adherence due to its advantageous physical properties and a distinct

					improvement in HRQoL makes the formulation of calcipotriol-betamethasone dipropionate gel a vital, efficient and well-tolerated topical therapy to manage psoriasis.(70)
17.	Gritt et. al 2015	Calcipotriene Plus Betamethasone Dipropionate	Gel	Psoriasis vulgaris	Calcipotriene Plus Betamethasone (Cal/BD) topical suspension Applicator was found to be an innovative applicator delivery system within the area affected with psoriasis. The Applicator containing suspension may promote prolonged adherence acceptable option of therapy.(71)
18.	Jie Wu et. al 2015	Baicalin	Cream	Psoriasis	Baicalin cream possesses an anti-inflammatory activity in CHS response, by modulating the keratinocyte differentiation in the mouse tail test activity.(72)
19.	Lambert et. al 2015	Calcipotriol and	Ointment	Psoriasis vulgaris	The author reported that the real-life study has

		betamethasone dipropionate			proved the same efficacy in both calcipotriol and betamethasone dipropionate over a therapy period of 52-weeks, the population of patients with psoriasis showed more patient satisfaction with the topical gel, proving a more convenient formulation with properties of faster and easier to apply.(73)
20.	Min Liu et. al 2015	linoleic acid-ceramide	Lotion	Psoriasis vulgaris	Topical linoleic acid-ceramide moisten alleviating of psoriasis on the skin and was found to be a suitable method for preventing and treating psoriasis.(74)
21.	Thomas et. al 2015	Kunzea oil	Ointment/ Lotion	Psoriasis	The study revealed the use of ointment lotion was found to be safer & efficient into treating of acute to chronic 41 psoriasis. The outcome reported in the study did not pose to give good results for the use of kunzea oil for psoriasis treatment.(75)

22.	Umezawa et. al 2015	Maxacalcitol	Ointment	Palmoplantar Pustulosis	OCT topical application on the pustules/vesicles was found to be demonstrating potency, which contributes to the recovery of diseases from thereby Palmoplantar Pustulosis.(76)
23.	Hiroki et. al 2016	Betamethasone	Ointment	Psoriasis	Skin inflammation after imiquimod induced psoriasis can be prevented by inhibition of gene expression of different cytokines related to Th1, Th17 cells, and keratinocytes with Betamethasone ointment.(77)
24.	John Koo et. al 2016	calcipotriene & betamethasone dipropionate	Aerosol foam/ ointment	Psoriasis vulgaris	Calcipotriene and betamethasone as an aerosol foam formulation exhibit sufficiently great therapeutic activity and the same tolerance compared with calcipotriene & betamethasone ointment in treating psoriasis.(78)
25.	Karakawa et. al 2016	Maxacalcitol	Ointment	Psoriasis	Pre-emptive usage of maxacalcitol has been found to be beneficial on

					psoriatic lesions in its maintenance phase. Adalimumab along with other systemic treatments or as single therapy was found to be more effective.(79)
26.	Kaur et. al 2017	Clobitasol propionate and calcipotriol	Gel	Psoriasis	Primary finding of research is nanoemulsion gel is superior to both free medicines & commercially available formulas in treating psoriasis in Imiquimod-induced BALB/c mice. Despite enhanced penetration in skin, resulting formulation showed no significant skin irritation..(80)
27.	Nuo Li et. al 2017	Pulian	Ointment	Psoriasis vulgaris	The results indicate that ointment formulation containing Pulian was found to be efficient and a tolerable formulation. The formulation improved the PASI score in both skin lesion areas with psoriasis

					vulgaris of the bloodheat syndrome.(81)
28.	Parmar et. al 2017	Solanum xanthocarpum	Gel	Psoriasis	The study reports scientifically warranted anti-psoriatic efficacy which is attributed to the inhibiting activity of cytokines expressions like TNF-α, IL-1β, IL-6, & IL17. One of the major contributing factors for the treatment of psoriasis is anti-microbial activity, cell multiplication activities, and antioxidant, which might endorse the combination of chlorogenic acid along with other phytoconstituents.(82)
29.	Wigger et. al 2017	Mapracorat 0.1%	Ointment	Plaque psoriasis	The author reported in the study, that considering superior challenges technically for the plaque psoriasis tests (PPTs). From the study, author recommends this occluded test standard screening test in the early development stages of the drug substance.(83)

30.	Adriana et. al, 2018	Calcipotriol/ betamethasone	Ointment	Plaque psoriasis	As a part of routine clinical practice, UVB phototherapy in combination with calcipotriol/betamethason e (Cal/BD) ointment are efficient for managing plaque type of psoriasis with a reduction in the PASI scores.(84)
31.	Gold et. al, 2018	Halobetasol and tazarotene	Lotion	Plaque psoriasis	From the results, the lotion containing Halobetasol and tazarotene was found to be excellent and proved to be superior in decreasing the signs and the symptoms of psoriasis which were observed during the study. It was also efficient on the BSA affected with psoriasis. HP/TAZ when compared with the vehicle exhibited supremacy in the statistical results obtained in the span of 2 weeks.(85)
32.	Irhan et. al, 2018	Acitretin	Gel	Psoriasis	The author reported from the results obtained in the mouse tail model, dermally applied Acitretin (Act) nano niosomal gel

					showed considerably greater orthokeratosis, the activity of the drug substance, and also decline in the thickness of the epidermis when a comparison was carried out with the control and other gel formulations.(86)
33.	Kuang et. al, 2018	Sunitinib	Ointment	Psoriasis	From the major outcomes reported in this study, it has been observed that Sunitinib eases the inflammation in lesions that has been induced by imiquimod. It acts by inhibiting the keratinocytes multiplication and apoptosis induction which works with a mechanism of phosphorylation regulation. This ointment was found to be novel and highly efficient for psoriasis as logical usage.(87)
34.	Song Yi et. al, 2018	Artemisia capillaris Extract	Cream	Psoriasis	The author reported from the results that extract obtained from Artemisia

					capillaris was formulated as cream and was found as being safer & efficient by reducing severity of symptoms due to psoriasis.
35.	Lin et. al, 2018	Citrus Peel extract	Lotion	Psoriasis	A suppressive mechanism of GL in the initiation of psoriasis strongly advocates that GL might portray probable activity to be developed in managing psoriasis.
36.	Sidgiddi et. al, 2018	Betamethasone Dipropionate	Spray	Plaque kind of psoriasis	Betamethasone Dipropionate (BD) spray 0.05% was significantly more efficient than vehicle spray, as measured by the proportion of subjects with treatment success after 14 and 28 days of two times daily treatment, and by a 50% or greater decrease in TSS at Days 4, 15, and 29.(88)
37.	Tripathi et. al, 2018	Methotrexate	Gel	Psoriasis	NLC is a potential particle carrier because it increases skin permeability &prolongs medication release.(89)

38.	Hashim et. al, 2019	Crisaborole 2%	Ointment	Intertriginous, anogenital, and facial psoriasis	In the study, 2% crisaborole was compared with the vehicle group. After a treatment therapy of 4 weeks, 66% of subjects were received crisaborole with the vehicle which was only 9% ($p = 0.0011$). by the end of week 8, 81% of the subjects in crisaborole group exhibited improvement in the psoriatic lesions from which 71% of these subjects observed clinical clearance.(90)
39.	Hemangi et. al, 2019	Plant-based butter	Moisturizing Cream	Psoriasis	Hydrating lotion shows promise as an adjunctive therapy for psoriasis.(91)
40.	Kendice et. al, 2019	Naltrexone HCl 1%	Cream	Psoriasis	From the outcomes achieved it has been reported that the formulated cream reduced the psoriasis biomarkers level suggesting that the formulated cream may alternate the inflammation and multiplication of the cells connected with in vivo psoriasis.

41.	James et. al, 2019	Ceramide/ keratolyticcontaining formulations (CeraVe® Body Cleanser and Cream)	Body Cleanser and Cream	Psoriasis	The author reported a higher acceptance level when used individually or as a combo in skincare therapy in treating of psoriasis disease. Usage of keratolytic containing formulation gives efficacy as a promising cleanser.(92)
42.	Jasmina et. al, 2019	Nicotinamide	Gel	Psoriasis	The major outcome reported in the study indicates a new emollient formulation with anti-inflammatory activity appears to be safer, helpful in adding it to the treatment therapy of armamentarium for eczema as well as psoriasis, henceforth diminishing the use of synthetic drugs like topical corticosteroids and immunomodulators.(93)
43.	Lunfei et. al, 2019	Icotinib Hydrochloride	Cream	Plaque Psoriasis	The results reported by the author indicate an excellent efficacy with a topical cream containing Icotinib in patients suffering from mild and

					moderate psoriasis. The study revealed a favourable, safe and high tolerability without any toxicity with the formulation.(94)
44.	Poonam et. al, 2019	Thymoquinone	Gel	Psoriasis	The major outcome reported in the study exhibits that Thymoquinone contains phospholipid for the treatment of psoriasis when compared with other test formulations. The topical gel of Thymoquinone showed promising efficacy.(95)
45.	Priyadarshini et. al, 2019	Dithranol	Gel	Psoriasis	Nano lipid carriers with a base as a gel containing Dithranol were seen as efficient in treating of psoriasis, but a drawback has been observed during the study a smaller amount of staining in clothes was prominent with the product prepared.(96)
46.	Sindhu et. al, 2019	Fenoldopam mesylate	Ointment	Psoriasis	Imiquimod model for inducing psoriasis was used to demonstrate the efficacy of Fenoldopam

					mesylate. The results showed greater effectiveness of the formulation in inflammatory disorders like psoriasis. This study also gives an insight into the importance of the selection of drug carriers that is going to impact the efficacy and stability status of the drug substance.(97)
47.	Sobhan et. al, 2019	Atorvastatin 5% and betamethasone 0.1%	Lotion	Scalp Psoriasis	Study results exhibited that a comparable efficacy has been observed with topical application of atorvastatin and betamethasone, indicating which can be used as a therapeutic alternate for the management of psoriasis on the scalp. (98)
48.	Starbek et. al, 2019	Silver fir (Abies alba)bark extract	Ointment	Psoriasis	The study demonstrated that silver fir bark extract in the topical formulation was safe and slightly more effective than placebo in treating psoriatic cases which are mild and moderate; however,

					statistical outcome reported that there was no performance impact with silver fir bark extract for the treatment of psoriasis.(99)
49.	Chunxia He et. al 2020	Tazarotene/Betamethasone Dipropionate	Cream	Plaque Psoriasis	Tazarotene/Betamethasone Dipropionate formulated as a cream for mild to moderate psoriasis in plaque-type was reported to be safe, effective, and well tolerable preparation in real-world conditions.(100)
50.	Lin Cai et. al 2020	1% benvitimod	Cream	Plaque Psoriasis	A therapeutically acting aryl hydrocarbon receptor (AhR) has a modulating activity that helps in protecting the skin which leads to various pathological processes under the influence of cytokines production. In this study it is evident that a 1% concentration of benvitimod formulation of cream acts as an agent to modulate and protect the skin.(101)

51.	Wang et. al 2020	Trans retinoic acid and betamethasone	Gel	Psoriasis	A liposomal flexible gel which dually comprises of Betamethasone and Trans retinoic acid, elicits an effect of synergistic activity, which indicates the gel to be a promising therapeutic option for the management of psoriatic disease when applied topically to the skin.(102)
52.	Chen et. al 2020	Tazarotene and betamethasone	Cream	Psoriasis vulgaris	As observed, it's evident as combinations of Tazarotene and betamethasone have great benefits when compared Tazarotene alone, which includes efficacy, rapid onset of activity, and decreased localized stimulation.(103)
53.	Dina et. al 2020	Anthralin	Gel	Psoriasis vulgaris	In the study, it has been reported that enhancement of performance and effectiveness of the Anthralin can be achieved by loading the drug into different ethosomal and liposomal preparations. This method of formulating the drug was

					also reported to decrease severe adverse events.(104)
54.	Eichenfield et. al, 2020	Calcipotriene (0.005%) and betamethasone (0.064% as dipropionate)	Gel	Scalp and body psoriasis	Betamethasone as dipropionate in a concentration of 0.064% and Calcipotriene concentration of 0.005% combination of gel was well tolerated and efficacious for treating psoriasis on the body and scalp in adolescents.(105)
55.	Gajanan et. al, 2020	Mometasone Furoate	Aspasomal Gel	Psoriasis	The author reported that the formulated gel preparation provided a prolonged release profile of the drug when compared with the marketed cream formulation. The permeation of the drug into the skin reveals an entrapment process in the vehicles of the dermis layer, which in turn reduces the exposures of the drug systemically, which will decrease toxicity. The supplementary uses of

					Aspasom gel include anti-oxidant, aids in skin whitening in psoriasis that will exhibit a multiple effects on the discoloration of the skin and rough skin.(106)
56.	Pinto et. al, 2020	INFLATIV(Pereskia aculeate)	Cream	Psoriasis	INFLATIV cream when compared with dexamethasone exhibited a well comparable result for efficacy in antiinflammatory action.it was observed that there was no skin atrophy triggered by the drug.(107)
57.	James et. al, 2020	Clobetasol Propionate 0.025%	Cream	Psoriasis	From the two-week study with two-time daily treatment, application of CP 0.025% concentration of cream have exhibited the same effectiveness when it was compared with the brand superiorly potent drug of CP 0.05% concentration in the cream. The data results from the clinical studies indicate that cream containing 0.025%

					concentration of CP showed a decline in the signs and symptoms in the patient population suffering from plaque kind of psoriasis.(108)
58.	Mark et. al, 2020	Roflumilast	Cream	Plaque psoriasis	From the study results reported by the author, cream of Roflumilast applied topically one time daily on the affected inflammatory regions of the psoriatic skin was found to be greater in efficacy when compared with the vehicle cream, the skin was almost clear at six weeks of a time period.(109)
59.	Shatalebi et. al, 2020	Gracilaria algae 3%	Cream	Plaque psoriasis	The topical cream comprising of 3% concentration of Gracilaria algae was reported to be efficient and nontoxic. 51 It was found to be an alternate treatment for clobestol in plaque psoriasis.(110)
60.	Xiang et. al, 2020	Oxymatrine	Ointment	Psoriasis	The Oxymatrine ointment elicits its activity by hindering the expression

					of HSP90 and HSP60 in keratinocytes through signalling the MAPK pathway. This formulation was found to be evident for relief from pruritic inflammation psoriasis condition.(111)
61.	Lebwohl et. al, 2021	Combination of halobetasol propionate 0.01%/tazarotene 0.045%	Lotion	Plaque psoriasis	The amalgamation of HP 0.01% & TAZ 0.045% lotion was found to be efficacious, nontoxic with an additional quality of its usage for a longer time of treatment period and also in managing modest and severe kinds of plaque psoriasis. (112)
62.	Linda et. al, 2021	Tapinarof	Cream	Plaque psoriasis	From the study results, it is evident that Tapinarof formulation cream performance was superficial at 2nd week with a noteworthy efficacy at 8th week which was sustained upto 16th week. No reports of major adverse events have been received. The research's adverse effects were rather minor, this represented to

					be essential progress in the 52 expansion of topically applied medications for the management of psoriasis.(113)
63.	Mahmoud et. al, 2021	Resveratrol	Gel	Psoriasis	Study results recommended that a selection of carbopol gel of resveratrol-loaded spanlastics may perhaps make the best use of resveratrol topically for its anti-psoriatic activity.(114)
64.	Fen et. al, 2021	Celastrol	Noisome Hydrogel	Psoriasis	In the study, the Niosomal hydrogel of Celastrol hinders inflammation and agitated multiplication of keratinocytes in the skin, proving to be effective in having anti-psoriatic activity by further suppression of systemic inflammation. Therefore, this formulation could be a novel drug to treat psoriasis both topically as well as for systemic effects.(115)
65.	Atheer et. al, 2021	Montelukast	Gel	Psoriasis	The study outcome indicates that topical

					application of gel consisting of Montelukast as an active drug presented substantial anti-inflammatory and anti-psoriatic actions when carried out the tests with imiquimod which induced with psoriasiform kind of inflammation in mice skin.(116)
66.	Fangyuan et al 2019	Tofacitinib		Severe chronic kind of psoriasis	The study outcome indicates tofacitinib was found to be very effectual but exhibited supplementary adverse events.(117)

2.3. Summary of literature review of different dosage forms used for the management of psoriasis

We looked for meta-analyses, RCTs, systematic reviews, & qualitative research involving psoriasis diagnosis and treatment in PubMed, & Cochrane databases etc.

The degree and location of psoriasis lesions guide treatment decisions. Phototherapy, photochemotherapy, and systemic therapy using biologicals and immunomodulators are some of the most common conventional therapies. Psoriasis treatments often consist of synthetic medications, which come with serious side effects. Factors such as patient cost, intolerance, medication resistance, and significant side effects are very important when deciding on a systemic treatment. Despite the potential life-saving effects of biologicals in extreme psoriasis cases, the hazards of infection, especially reactivation of latent TB, and the possibility of malignancy on extended therapy need safe and

effective alternatives. Natural products provide a strong foundation for treating psoriasis because of their accessibility, patient tolerability, low cost, and wide range of biochemical mechanisms of action. In contrast to conventional medications and their accompanying undesirable effects, this research chronicles the existing natural resource of diverse plant extracts and secondary metabolites described as antipsoriatic agents.

The paper also provides a summary of the many methods for treating psoriasis effectively, including:

Table 5. Literature review of different dosage forms used for the management of psoriasis

S. No	**Reference**	**Drug Name**	**Dosage form**	**Type of psoriasis treated**	**Major findings**
1.	Morimoto et. al 1986	vitamin D3	Oral	Psoriasis	The study, indicates that abnormalities due to the metabolism of vitamin D or in response of the cells of the skin with the active metabolites of vitamin D3 perhaps be intricate into pathogenesis of the illness. Outcomes of study demonstrated that vitamin D3 might react with these kinds of active metabolites.(118)
2.	Van de Kerkhof et. al 2002	Calcipotriol	Solution	Scalp Psoriasis	The combination of ointment/scalp solution of calcipotriol usage ensured no impact in the indices

					metabolism of calcium or bone turnover. It also exhibited more effective in decreasing the severity condition of the disease state and extent in patient with dithranol/tar.(119)
3.	Shanu et. al 2008	Curcuminoid C3 Complex	Oral	Psoriasis Vulgaris	Oral consumption of curcumin was found to be well accepted by patients suffering from psoriasis. The complete performance level was little which cannot be ruled whether the response was diluting the effect of placebo or remission attacks of the disease occurring naturally. However, exceptional efficacies were reported in two of the patients. Hence, huge, placebo measured randomized trials are essential to ultimately substantiate that oral consumption of curcumin is impending therapeutic agent in psoriasis treatment.(120)
4.	Papp et. al 2011	clobetasol propionate	Shampoo	Scalp Psoriasis	The author reported that clobetasol propionate was highly accepted and

					demonstrated sufficient progress clinically for the treatment of scalp psoriasis(121)
5.	Vijayalakshmi et. al 2011	Givotia rottleriformis	Oral	Psoriasis	The Givotia rottleriformis ethanolic extract of bark possesses powerful anti-psoriatic efficacy in the rats which are prior induced with UV-B.(122)
6.	Gutierrez et. al 2015	Curcuma extract	Oral	Plaque psoriasis	The outcome from the present study indicates to be safe, non-toxic, and demonstrated sufficiently great progress clinically and instrumentally for plaque psoriasis.(123)
7.	Reich et. al 2016	Apremilast, etanercept	Oral tablet	Plaque psoriasis	From the results obtained the author reported that at 16th week in biologic-naive patients having psoriasis have been given treatment with Apremilast as part of treatment therapy which exhibited great efficacy when compared with placebo. This efficacy was sustained up to a time period of 52 weeks. The study revealed, apremilast was consistently safe and non-toxic through

					the studies. Swapping of etanercept and apremilast has not ensured ant clinical significance and safety. Apremilast exhibited its efficacy up to 52 weeks.(124)
8.	Maria et. al 2017	Ichthyól, Zanthalène, Mandelic Acid, and Honey	Shampoo	Scalp Psoriasis	The outcome from the present study indicates to be safe, non-toxic, and demonstrated sufficiently great progress clinically and instrumentally for scalp psoriasis. The brand market shampoo Mellis Cap® was found to be an excellent substitute when compared with the other shampoos that are medicated into managing of psoriasis, which is minor to modest on the scalp.(125)
9.	Azevedo et. al 2017	Tofacitinib	Oral	Plaque Psoriasis	Tofacitinib administered orally was found as efficient, safe, & non-toxic in managing moderate and chronic plaque kind of psoriasis. The treatment algorithms might include Tofacitinib prior to biologic usage considering two strengths. The oral route of administration was found to

					be cost-effective and therapeutically efficient when compared to biosimilars as well as biologics.(126)
10.	Chen et. al 2017	Quercetin	Oral	Psoriasis	The study revealed that quercetin had an appreciative anti-psoriasis activity in mice which are prior induced with Imiquimod. The principle mechanism underlying may intervene in the development of antioxidant and anti-inflammatory properties by inhibiting the NF-κB signalling. Henceforth, QC, a flavone occurring naturally, quercetin poses to have therapeutic potency in anti-psoriasis activity and was found to be an excellent drug for the management of psoriasis and also in further advancement.(127)
11.	Mease et. al 2017	Tofacitinib or Adalimumab	Oral	Psoriatic Arthritis	The report in the study infers about the superiority of Tofacitinib effectiveness when compared with placebo over a time period of 3 months in patients affected with the psoriatic type of

					arthritis. These patients previously were given synthetic DMARDs a conventional method that was not effective to give a response. It has also been observed that the recurrent adverse events were more in tofacitinib than in placebo.(128)
12.	Papp et. al 2017	Adalimumab	Oral	Plaque psoriasis	A treatment dose 0·8 mg per kg of adalimumab exhibited worthy enhancement in children as well as in adolescents with chronic plaque type of psoriasis. These enhancements were observed in PASI75 scale and not a significant intensification in the patient proportion who has achieved a PGA in minimal, compared to that of methotrexate(129)
13.	Boontaveeyuwat et. al 2018	Triamcinolone	Injection	Psoriatic Nails	Temporarily efficient & safer therapy for psoriatic nails with intralesional triamcinolone injection.(130)
14.	Bilia et. al 2018	Curcumin	Oral Nanoparticle	Psoriasis	Curcumin formulated as nanoparticles administered orally characterize to be an efficacious additive

					treatment therapy in acute to chronic kinds of psoriasis in patients who are on the oral therapy of Acitretin, enhancing the profile of serum lipid.
15.	Michelle et. al 2018	vitamin D3	Oral	Plaque psoriasis	In the study author reported that direct supplementation of vitamin D3 for management of psoriasis is advantageous or not, was not able to be determined. Although, findings in the study indicate an association with an extent of severity of psoriasis and 25(OH)D, this was observed in a few of the subgroups.(131)
16.	Attwa et. al 2019	methotrexate	Oral/SC	Plaque psoriasis	The subcutaneous route of methotrexate administration has superior effectiveness with minimal side effects and a relapse rate which was reported to be lower when it was compared against administration of drug oral route with the same dose and same duration of time.(132)
17.	Marian et. al 2019	Calcipotriol Plus Betamethaso	Aerosol Foam	Scalp Psoriasis	Calcipotriol Plus Betamethasone formulated as an aerosol foam superior

		ne Dipropionate			permeation in the skin as well as greater bioavailability of the drug when compared with the traditional and conventional formulations. The author reported from the results that Cal/BD formulation of aerosol foam improves the efficacy. The cases presented in the study also confirm the advantages of this formulation for psoriasis in adult's scalp.(133)
18.	Colombina et. al, 2020	Cannabidiol	Shampoo	Scalp Psoriasis	The formulation of shampoo containing broadspectrum cannabidiol (CBD) sufficiently diminishes the extent of severity of psoriasis and also the symptoms like inflammation in a span of 14 days. The formulation poses to have a promising safety profile and tolerable with a patient satisfaction those affected from mild to moderate kind of psoriasis.(134)
19.	Ighani et. al 2020	Tofacitinib	Oral	Psoriasis and	Tofacitinib is a useful novel systemic agent in the management of the psoriatic

				psoriatic arthritis	disease. Its oral route of administration, novel JAK path- way target, short halflife, and strong recapture degrees after treatment interruption make it a unique new tool for psoriatic disease treatment.(135)

2.4. Role of Herbs for Psoriasis Treatment

Throughout the course of history, herbs have been utilized for addressing a wide array of skin conditions. Interestingly, even primates, similar to humans, have been observed employing herbal treatments. Such occurrences have been documented in numerous literary works and are publicly accessible, as noted by Huffman et al. in 2001.(136) recently, there's a notable upsurge into usage of herbs for therapeutic purposes, and this can be attributed to several factors. One key factor is the growing awareness of the adverse effects associated with synthetic drugs, which has led to a renewed interest in natural remedies for managing diseases. This shift towards natural treatment methods aligns with the ecological movement, thereby fostering the growth of organic products.

Herbal remedies have gained prominence in the management of skin disorders, and a segment of the medical community advocates for their use to enhance the management of these conditions. Particularly, countries in Asia, such as China and India, have taken the lead in the utilization of herbal treatments. These nations have a rich history of investigating and employing herbal treatments that date back centuries, as evidenced by records from earlier times.

While herbal medicines thrived in Asia, their popularity diminished in Western countries with the emergence of more predictable synthetic drugs. However, many developing nations retained their valuable knowledge and traditions related to herbal treatments. Even as Western countries embraced synthetic drugs, technologically advanced nations with a strong foundation in therapeutic herbalism continued to benefit

from this ancient wisdom. Notably, Unani remedies in the Middle East, Ayurveda and Siddha treatments in India, as well as (TCM) and Kampo Medicine in Japan, remain widely practiced.(137)(138)

Presently, a significant number of healthcare practitioners have a long-standing history of employing herbal therapies for managing psoriasis. According to WHO, nearly 80% of global population relies upon herbal medications for various aspects of basic medications.Notably, WHO reports indicate that approximately 25% of synthetic drugs used in the United States (US) are derived from plants.In modern medicine, researchers have isolated over 120 active constituents from various plants, and there is a positive correlation between the therapeutic use of about 80% of these active compounds and traditional practices.(139)

Concerning the immune-mediated model for managing psoriasis, researchers initially considered immunosuppressant medications, although the precise role of the immune system in psoriasis remained incompletely understood. Traditional approaches suggested that reducing T-cell counts could alleviate psoriasis symptoms, but paradoxically, reducing T-cell counts worsened psoriasis in HIV patients, leading to confusion among researchers. Some studies even demonstrated that reducing T-cell counts in mice could induce conditions similar to psoriasis, further complicating the picture. The histopathological aspects associated with psoriasis include angiogenesis, abnormal keratinocyte differentiation, epidermal hyperproliferation, and elevated Th-1 and Th-17 responses. Active psoriatic lesions commonly involve intraepidermal activated polymorphonuclear leukocytes, which generate reactive oxygen species causing damage to skin membranes. Reactive oxygen also triggers phospholipase (A2), releasing Arachidonic acid mediators, including PGE2, which induce dermal blood vessel dilation, leukocyte infiltration, and stimulate keratinocyte growth.(140)(141)(12)

Throughout the annals of history, humans, plants, and plant-derived products have maintained a longstanding and interconnected relationship. Plants have consistently been a preferred choice for treating diseases and managing various health conditions, spanning from addressing inflammation to combatting life-threatening illnesses like cancer. More recently, WHO has acknowledged potential of herbal drugs and plant-

derived products, endorsing them for their therapeutic benefits alongside consideration for safety.

2.5.Phytochemistry of plants used in the study

2.5.1 *Curcuma longa*

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%).(142) Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%)(143) Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated.(144) Curcumin was first isolated by Vogel and Pelletier in 1815 and its chemical structure was determined by Roughley and Whiting in 1973.(145) It melts between 176 and 177 degrees Celsius, dissolves in alcohol, alkalis, ketones, acetic acid, and chloroform, and produces a reddish-brown salt with alkali. *Curcuma longa* has a number of chemicals that are useful for medicine, cosmetics, and spices. It can be used in place of antibiotics in food products and is abundant in bioactive substances such as flavonoids, polyphenols, and antioxidants. Turmeric has both volatile and nonvolatile components, including turmerone, curlone, ar-turmerone, and zingiberene, as well as curcuminoids. Turmeric's distinctive color is caused by curcuminoids, which are the main phenolic components. Curcumin, desmethoxycurcumin, and bisdemethoxycurcumin make up the majority of them with carbohydrates, proteins, and resins serving as other vital macromolecules. The primary and beneficial active component is curcumin, which is present in 0.3-5.4% of fresh turmeric.

2.5.2. *Azadirachta indica L.*

Azadirachta indica L. (neem) is a rich source of numerous kinds of components, demonstrating a therapeutic effect in health management. The other active ingredients include nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin, although azadirachtin is by far the most significant. Leaves include compounds like nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide,

ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol. Its polyphenolic flavonoids, quercetin and ß-sitosterol, are recognized for their antibacterial & antifungal qualities, and its seeds contain important elements including gedunin & azadirachtin. The phytochemicals sodium nimbinate, gedunin, salannin, quercetin, nimbolinin, nimbin, nimbidin, nimbidol, and others that are effective against certain infections. Ascorbic acid, n-hexacosanol, amino acid, nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol are among the compounds found in leaves. (146)

2.5.3. *Psoralea corylifolia L.*

Furano coumarins and coumestrol are examples of coumarins found in PCL, whereas flavonols, dihydroflavones, isoflavonoids, and chalcones are examples of flavonoids. PCL also contains monoterpene phenols and benzofurans among its chemical components. There are also lipids like monoglyceride, diglyceride, triglyceride, and free fatty acid, glycosides like daucosterol, methylglycoside, and PCL polysaccharide, and fatty acids like palmitic acid, oleic acid, linoleic acid, and stearic acid found in volatile oils and non-volatile terpenoid oils. Bakuchicin, psoralidin, isopsoralidin, bavachin, isobavachin, bavachinin, bavachalcone, isobavachalcone, corylifolean, corylifolin, corylifolinin, Flavanoids that have been extracted from *P. corylifolia* seeds include 7-O-methyl bavachin, bavachromanol, corylin, corylidin, corylinal, 4-O-methyl bavachalcone, neobavaisoflavone, bavachromene, and neobavachalcone [3, 7].The chemical structure of flavonoids is phenylbenzopyran(147)

2.5.4. *Pongamia pinnata*

Leaves of *Pongamia pinnata* contain Fisetin tetramethyl ether, 3, 7- dimethoxyflavone, Luteolin, 7-O-ethylchrysin, 7,4′-dimethoxy-5- hydroxy flavone, Kaempferol, Quercetin; flowers contain Fisetin tetramethyl ether, Demethoxykanugin, 3, 7-dimethoxyflavone, L. Eight fatty acids—three saturated and five unsaturated—as well as six compounds—two sterols, three sterol derivatives, and one disaccharide—have been identified in *P.pinnata* seeds. The amounts of the saturated and unsaturated fatty acids—two monoenoic, one dienoic, and two trienoic—were precisely equal. The most

abundant acid was oleic (44.24%), followed by stearic (29.64%) and palmitic (18.58%) acids.(148)

2.5.5. *Woodfordia fruticosa*

Woodfordia fruticosa has mostly phenolics, hydrolyzable tannins, and flavonoids as its chemical ingredients. Desai et al. were the first to check whether *Woodfordia fruticosa* stems contained octacosanol and -sitosterol. In contrast to the presence of β-sitosterol in the leaves of Woodfordia fruticosa, the isolation of chemicals such as steroid sapogenin hecogenin and mesi-inositol from the flowers demonstrates the presence of non-phenolic elements in this plant. Dan et al. found additional chemicals, including the triterpenoids lupeol, betulin; betulinic acid, oleanolic acid, and ursolic acid, in the leaves of Woodfordia fruticosa.Not only are flavonoids and tannins extracted from this plant, but also phenolic components and gallic acids. Woodfordia fruticosa has ellagic acid in its leaves and flowers. Woodfordia fruticosa has the novel component norbergenin in its stems, the chrysophanol-8-O-glucopyranoside in its blooms, and the naphthoquinone pigment lawsone in its leaves. Six quercetin glycosides [3-rhamnoside from flowers, 3- β-L-arabinoside from flowers and leaves, and 3-O-α -D xylopyranoside] and three other flavonoid glycosides [3-O-(6"-galloyl)- β-D glucopyranoside] and one flavonoid glycopyranoside from leaves characterize the various groups. The plant yielded three different myricetin glycosides. Flowers and leaves contain 3-O-β-D-galactoside, whereas leaves contain 3-O-α-L-arabinopyranoside and 3-O-(6"-galloyl) β-D-galactopyranoside, and flowers include 3-O-glucosides of naringenin (7-O) and kaempferol (3-O) respectively. Nair et al. looked at the blooms to find the orange-red pigment, and they found pelargonidin 3,5-diglucoside. *Woodfordia fruticosa* flowers are the source of both novel and previously characterized hydrolysable tannins. Isolated tannins include 1,2,3,6-tetra-O-galloyl- β-D-glucose, 1,2,4,6-tetra-O-galloyl-β -D-glucose, 1,2,3,4,5-penta-O, galloyl- β-D-glucose, tellimagrandin, gemin D, heterophyllin A, and oenothein B.

2.6. Pharmacology of plants used in the study

2.6.1. *Curcuma longa*

The medicinal and pharmacological uses of turmeric are many. Here are turmeric's most notable medicinal and phytopharmacological qualities.

- **Anti-inflammatory**

  The volatile oils and curcumin in *Curcuma longa* have powerful anti-inflammatory properties. Half as much curcumin, when given orally, is as effective as cortisone or phenylbutazone for treating acute inflammation. The lipoxygenase and (COX-2) inhibitory characteristics of turmeric are responsible for its fiery intensity and anti-inflammatory activity. Inflammatory changes in the joints are a common cause of rheumatic symptoms. It corrects the pathogenic alterations and underlying causes that cause inflammation. Curcuminoids contain qualities that block the production of a wide variety of inflammatory mediators, including cyclooxygenase (COX), nitric oxide (NO), (ELA), hyaluronidase (HYAL), collagenase (COL), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor (TNF), and interleukin-12 (IL-12). Curcumin prevented edema in a mouse model when given at levels between 50 and 200 mg/kg. When given at a level of 48 mg/kg of body weight, curcumin may decrease oedema by 50%. At therapeutic levels, its efficacy is comparable to that of cortisone and phenylbutazone. Applying a dosage between 20 and 80 mg/kg reduced paw inflammation and oedema in rats. A dosage of 40 mg/kg of curcumin was able to suppress formaldehyde-induced arthritis in rats, and doses up to 2 g/kg/day showed no acute toxicity. Animals with rheumatoid arthritis produced by streptococcal cell wall had their joint inflammation reduced in both the acute (75% reduction) and chronic (68% reduction) phases after receiving intraperitoneal injections of turmeric extract containing 4 mg total curcuminoids/kg/day for four days before arthritis induction.(149)

- **Antimicrobial Properties**

  Turmeric extract and *Curcuma longa* essential oil inhibit the development of several harmful microorganisms. Supplementing the food with turmeric, as Eimera maximum did, reduces small intestine lesion scores and improves weight growth. Topical use of turmeric oil suppressed dermatophytes and pathogenic fungus in guinea pigs afflicted with dermatophytes, pathogenic molds, or yeast, according to another research. After seven days of turmeric treatment, the lesions on the guinea pigs afflicted with dermatophytes and fungus had vanished. Curcumin has modest efficacy against *Plasmodium falciparum* & Leishmania major in clinical trials.(149)

- **Antidiabetic Properties**

  Experiments have shown that turmeric has a crucial role in managing diabetes. Extraction with hexane (containing ar-turmerone), ethanol (containing ar-turmerone, curcumin, demethoxycurcumin, and bisdemethoxycurcumin), and ethanol from the residue of the hexane extraction (containing curcumin, demethoxycurcumin, and bisdemethoxycurcumin) all stimulate adipocyte differentiation in a dose-dependent manner. Both curcuminoids and sesquiterpenoids are hypoglycemic, however the ethanolic extract of turmeric has a higher concentration of both, demonstrating their superiority. Turmeric has extraordinary impacts on postmeal plasma glucose and insulin levels. *C.longa* (6 grams) showed no discernible impact on glycemic response after intake. 30- and 60-minute post-OGTT insulin fluctuations are much greater when *C.longa* is present. *C.longa* administration followed by an (OGTT) results in a significant elevation in insulin AUC. Turmeric may also help reduce diabetic complications. Both turmeric and curcumin reduced blood sugar level in alloxan-induced diabetes, according to an experiment using albino rats that demonstrated the efficacy of turmeric on blood sugar and the polyol pathway.(149)

- **Antioxidant Effects**

  Curcumin, a component of turmeric, has potent antioxidant activity that is on par with that of vitamins C and E when present in water- and fat-soluble extracts. Pre-treatment with curcumin effectively reduces ischemia-induced alterations in the heart. Researchers examined the impact of curcumin on endothelial hemeoxygenase-1, an inducible stress protein, in an in vitro investigation using bovine aortic endothelial cells. The 18-hour curcumin incubation period also increased cellular resilience to oxidative damage. It may prevent oxidation in lipids and hemoglobin. Since curcumin is an antioxidant, it dramatically reduces activated macrophage production of ROS such hydrogen peroxide, superoxide anion, and nitrite radical. The antioxidant capabilities of its derivatives make them useful for preventing and treating cholelithiasis.(149)

- **Hepatoprotective Effects**

  Because of its antioxidant capabilities and its capacity to reduce the generation of pro-inflammatory cytokines, turmeric showed hepatoprotective and reno-protective

characteristics comparable to silymarin. Research on animals has shown that turmeric may protect the liver against a number of hepatotoxic insults, including *C C l* 4, galactosamine, paracetamol, and Aspergillus aflatoxin. The treatment of curcumin significantly reduced liver damage in experimental mice compared to controls that had *C C l* 4-induced acute and subacute liver injury. When tested on ducklings infected with Aspergillus parasiticus, turmeric extract was found to significantly reduce aflatoxin production by 90%. Additionally, sodium curcuminate, a salt of curcumin, prevents and treats cholelithiasis by increasing biliary excretion of bile salts, cholesterol, and bilirubin.(149)

- **Anti-Cancer Effect**

  The effects of turmeric on carcinogenesis have been the subject of a plethora of in vivo and in vitro investigations, mostly on rats and mice but also using human cell lines. Multiple in vitro studies have shown that curcumin inhibits angiogenesis, tumor promoting, and tumor development, all of which are critical steps in the carcinogenesis process. Two investigations on colon and prostate cancer found that curcumin inhibited cell proliferation and tumor development. Turmeric and curcumin also inhibit the mutagenic and carcinogenic effects of a number of other commonly used chemicals. There is evidence that the antioxidant and free-radical scavenging properties of turmeric and curcumin, as well as their ability to increase glutathione levels and, by extension, aid in the hepatic detoxification of mutagens and carcinogens, contribute to their anti-carcinogenic effects. UV rays induce mutations, whereas curcumin blocks this impact.(149)

  Turmeric, when included in the diet, has shown promise as a chemo preventive drug against stomach tumors caused by benzo (alpha) pyrene in swiss mice. Patients with malignant lesions on the skin have had positive responses to topical treatments consisting of an ethanolic extract of turmeric and a curcumin ointment. Evidence shows that turmeric's antioxidant properties may counteract the cancer-causing effects of free radicals. We observed that acetyl curcumin had no effect. In addition to acting as an antitumor agent and helping to induce apoptosis or PCD in human myeloid leukemia cells (HL—60), numerous studies have shown that turmeric can prevent the expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and Eselectin by human umbilical vein

endothelial cells. The results show that turmeric's Curcumin-I, II, and III have cytotoxic, antioxidant, and anti-inflammatory activities. Extensive studies have shown that these chemicals have potent natural activity against several cancer cell lines, including those of the leukemia, colon, CNS, melanoma, renal, and breast types.(149)

- **Cardiovascular Effects**

  By reducing cholesterol and triglyceride levels, lowering low density lipoprotein (LDL)'s sensitivity to lipid peroxidation, and blocking platelet aggregation, turmeric's antioxidant activity creates a protective impact on the cardiovascular system. Researchers found that low-dose (1.6-3.2 mg/kg body weight daily) administration of turmeric extract to 18 atherosclerotic rabbits decreased LDL's susceptibility to lipid peroxidation and lowered plasma cholesterol and triglyceride levels. The increased dosage reduces cholesterol and triglycerides but has no effect on LDL oxidation. Possible mechanisms for the cholesterol-lowering effects of turmeric extract include reduced cholesterol absorption in the intestines and enhanced hepatic conversion of cholesterol to bile acids. Further research revealed that *C.longa* anti-platelet effects arise from the plant's ability to boost prostacyclin production while simultaneously stifling thromboxane production.(149)

- **Gastrointestinal Effects**

  Sodium curcuminate and p-tolymethylcarbinol, two of *Curcuma longa* main components, have several beneficial benefits on the digestive system. Sodium curcuminate, like p-tolymethylcarbinol, enhances gastrin, secretin, bicarbonate, and pancreatic enzyme production, and it reduces intestinal spasm. The application of turmeric to rats exposed to stress, alcohol, indomethacin, pyloric ligation, and reserpine resulted in a significant increase in stomach wall mucus. In an open, phase II experiment, researchers gave 600 milligrams of powdered turmeric five times a day to 48 percent of patients with endoscopically confirmed stomach ulcer. There were no negative responses or blood abnormalities discovered. Results in mice with deliberately induced colitis showed that curcumin protected the mucosal lining of the digestive tract from damage. Curcumin reduced inflammation in rat models of experimentally induced pancreatitis. Curcumin's ability to suppress inflammatory

mediators, as assessed by histology, pancreatic trypsin, serum amylase, and neutrophil infiltration, was not limited to cerulean or ethanol-induced pancreatitis.(149)

2.6.2. *Azadirachta indica L. (Neem)*

Various neem extracts have yielded more than 135 unique chemicals. Nimbin, nimbinin, nimbidinin, nimbolide, and nimbidic are examples of physiologically active nimbin-related compounds.(150) Several studies, including one by Biswas et al. (2002), have demonstrated the anti-inflammatory, anti-pyretic, analgesic, immunostimulant, hypoglyce-mic, anti-ulcer, anti-fertility, anti-malarial, anti-bacterial, anti-fungal, anti-viral, antioxidant, hepatoprotective effects of neem tree extracts.(151) Nimbidin and sodium nimbidate have potent anti-inflammatory action against formalin-induced arthritis and carrageenin-induced acute paw oedema in rats. The anti-inflammatory effects of neem leaf extract in a rat model of cotton pallet granuloma were statistically significant.(152) In addition to our own research, Chattopadhyay et al. conducted studies on Azadirachta indica leaf extract and found that it inhibited the inflammatory edema-inducing effects of chemical mediators such 5-hydroxytryptamine (5-HT), histamine, bradykinin, and phosphodiesterase type 5 (PGE 1).

At 500 mg/kg, neem leaf aqueous extract showed considerable anti-nociceptive efficacy in rats.(153) In 1981, Okpanyi and Ezeukwa showed that neem extract had a significant anti-inflammatory effect.(154) There is a 400 mg/kg body weight dosage, which is equivalent to 50 mg/kg acetylsalicylic acid and 4 mg/kg indomithacin. At 80 mg/kg, nimbidin, a neem extract crude extract, had an anti-inflammatory effect almost identical to predinisolone at 10 mg/kg.(155)

In a study by Mosaddek and Rashid (2008),(156) By injecting rats with formalin and then giving them aqueous extract of neem leaf once daily for 7 days, researchers were able to assess the anti-inflammatory effects of the two treatments. The results of the research indicated that neem extract's anti-inflammatory effects were inferior to those of dexamethasone. Some studies have shown that nimbidin may reduce fever. In fasting rabbits, nimbidin treatment resulted in a considerable hypoglycemic impact.(155)

Nimbidin was shown to have a strong antiulcer effect in preventing both histamine- and cysteamine-induced duodenal ulcers and gastric- and serotonin-induced stomach lesions. Nimbidin may operate as an antihistamine by inhibiting H2 receptors, and it may reduce both basal and histamine- and carbachol-stimulated stomach acid flow.(155)

In 1959, researchers discovered that nimbidin and nimbin had spermicidal effects in both rats and humans.(157) Nimbidin's ability to impede Tinea rubrum's development provided more evidence of its antifungal properties. There was a 30 fold reduction in the growth of Mycobacterium tuberculosis in vitro, and it was also bactericidal. Some studies have shown that sodium nimbidinate may cause diuresis in dogs. Inhibiting *Plasmodium falciparum* growth is one way in which nimbolide displays antimalarial action.(151) Both *Staphylococcus aureus* and *Staphylococcus coagulase* are susceptible to nimbolide's antibacterial effects.

Extraction of dried neem leaves has shown promising results in the treatment of ringworm, eczema, and scabies, according to clinical trials. When applied topically, a neem leaf lotion may alleviate various skin conditions in as little as three to four days during the acute stage and two weeks during the chronic stage. Nearly 814 people100 had improvement in their scabies symptoms after using a neem and turmeric preparation. Within 3-15 days of therapy, 97% of patients had no signs of scabies returning.(151)

Since ancient times, Indian ayurvedic doctors have recommended taking a neem leaf extract orally to cure malaria. The natives of Nigeria and Haiti make a drink out of dried neem leaves to combat this illness.(151)

2.6.3. *Psoralea corylifolia L. (PCL)*

- **Anti-osteoporosis effect**

In the field of traditional Chinese medicine, PCL is now one of the most significant herbs for the clinical treatment of osteoporosis. Different concentrations of PCL aqueous extract have been shown to increase the mRNA ratio of osteoblasts osteoclastogenesis inhibitory factor and Receptor Activator of Nuclear Factor- B ligand, suggesting that PCL may have an anti-osteoporosis effect. Aqueous PCL extract has a considerable regulating influence on rat osteoblast models, especially at concentrations of 1.0 mg/mL and 10 mg/mL.6 Coryfolin stimulates the

differentiation of mesenchymal stem cells from rat bone marrow into osteoblasts by increasing the mRNA and expression of protein kinase A and cAMP response element-binding protein via the Cyclic adenosine monophosphate (cAMP) /protein kinase A /cAMP response element-binding protein signaling pathway.7 Psoralen may raise the expression of certain indicators (such as osteoblasts, glucose transporter 3, Runx2, and type I collagen [Col-I]), boost ALP activity, activate bone morphogenetic protein signaling, and promote osteoblast development.8-11 In addition to affecting osteoblasts, psoralen may also limit the activation of the activator protein-1 and protein kinase B pathways, hence reducing osteoclast differentiation.12 Psoralen has been shown to dramatically block bone resorption, decrease bone turnover rate, and alleviate osteoporosis symptoms in patients by lowering blood levels of carboxy-terminal telopeptide of type I collagen. Bone marrow mesenchymal stem cells may be induced to differentiate into osteoblasts by isopsoralen (13).In addition, psoralidin considerably increases the femoral bending strength, trabecular bone area rate, and bone mineral density (BMD) of the lumbar vertebrae and femur in ovariectomized rats, demonstrating a considerable anti-postmenopausal osteoporosis effect.15 (BMD) increases as a function of concentration of bakuchiol in a zebrafish osteoporosis model.16 Related research has also shown that the Chinese patent medicine product Xianlinggubao Capsule, which contains PCL, may improve (BMD) in the lumbar vertebrae and femur, as well as blood calcium and phosphorus levels in rats, hence producing anti-osteoporosis benefits.17 In conclusion, PCL and its active components effectively combat osteoporosis.(147)

- **Dermatosis treatment effect**

  Dermatoses including vitiligo and Sezary's disease respond well to PCL and its active components, while psoriasis responds well to PCL and its active components in combination with ultraviolet A (PUVA therapy).The active ingredient in PCL, 18 8-methoxypsoralen (methoxsalen), is effective in the treatment of vitiligo and psoriasis.19,20 A concentration of 105 mol/L corylin greatly increased the proliferation rate of ultraviolet radiation b (UVB)-induced HaCaT cells, decreased the apoptosis rate, and dramatically increased the activity of antioxidant enzymes, suggesting that corylin may be useful in preventing the death of skin cells.21

Treatment of psoriasis vulgaris with a topical PCL tincture and NB-UVB is successful.22 Another medicine that shows promise as a PUVA candidate is isopsoralen.23 Psoralen may inhibit keratinocyte activity but has only a minor effect on melanocyte survival. Ointments formulated with pharmaceutical powder containing PCL are also beneficial when used alone to treat vitiligo.24 For recalcitrant dermatoses including vitiligo and psoriasis, PCL and its active components may be helpful.(147)

- **Antidepressant effect**

  There is a significant disease burden, disability rate, and suicide rate associated with depression since it is a chronic mental disorder. Coryfolin and psoralidin have been shown to significantly increase the content of 5-HT in the hippocampus and serum of mice, as well as decrease the immobility time of mice in a behavioral limitation model (forced swimming and tail suspension) in recent years, suggesting that PCL may also have a good effect in anti-depression.25 By modulating monoamine oxidase, the hypothalamic-pituitary-adrenal axis, and oxidative processes, PCL's total furanocoumarins may have an antidepressant effect.26 Furthermore, bakuchicin may decrease the fixation time of behaviorally depressed mice and the plasma levels of epinephrine and norepinephrine in chronically stressed mice, demonstrating effective antidepressant and anti-stress effects.(147)

- **Antitumor effects**

  PCL and its active components have demonstrated promising anticancer activity in in vitro and in vivo investigations, including in the treatment of breast cancer, liver cancer, and skin cancer. Psoralen may reduce HepG2 cell proliferation or induce death of HepG2 cells by activating Caspase-3/8 and causing an increase in the expression of BCL2-associated X protein (Bax) and CCAATenhancer-binding protein homologous protein.28,29 Studies have shown that psoralen can overcome drug resistance in the human breast cancer cell line MCF-7.30 According to one study, isobavachalcone has the potential to slow the development of pancreatic cancer by boosting the body's immune system and causing cancer cells to commit suicide.31 In addition, isopsoralen may cause apoptosis or necrosis in tumor cells without causing considerable damage, and it can suppress the formation of osteosarcoma xenografts in hairless mice.32 Inhibiting HepG2 cell growth,

bakuchicin can upregulate expression of the apoptosis-inducing ligand receptors death receptor 4 and death receptor 5.33 Many types of cancer, including breast cancer, skin cancer, and stomach cancer, may be treatable with bakuchiol, according to certain studies. Using the phosphoinositide 3-kinase/protein kinase B and mitogen-activated protein kinase signaling pathways, bakuchiol was able to inhibit human gastric cancer cell lines.34 Inhibiting cell growth and causing apoptosis and cell cycle arrest are additional key mechanisms by which bakuchiol exerts its anticancer effects on SGC-7901.(147)

- **Cardiovascular relaxation effect**

  Both PCL and its active components provide some cardiovascular system protection. Human umbilical vein endothelial cells are particularly vulnerable to damage from Tumor Necrosis Factor-α, however psoralen may inhibit TNF-'s ability to produce tissue factors.36 under rat aorta tissue, bakuchicin may cause vascular relaxation under physi opathological circumstances like hypoxia.37 Psoralen and bakuchicin work by increasing the expression of the protein Endothelial Nitric Oxide Synthase in endothelial cells, which causes the arteries to dilate.38 Furthermore, PCL flavonoids may reduce high-fat diet-induced atherosclerosis. Lipoprotein receptor −/− mice.(147)

2.6.4. *Pongamia pinnata*

- **Antibacterial activity**

  This research demonstrated that numerous components of *Pongamia pinnata* were able to inhibit the growth of harmful bacteria. When tested for its antibacterial effectiveness against three different bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, the chloroform fraction came out on top. Additional processing of the study's extracts is required, although they may one day be mass-produced for commercial and medicinal uses.(158)

- **Anti-inflammatory activity**

  P.pinnata leaf hydro-alcoholic [70%] extracts shown potent anti-inflammatory action in models of acute, chronic, and subacute inflammation. The study found that the extract from the leaves had potent anti-inflammatory properties. [16].

Researchers used carrageenan-induced rear mitt edema and cotton pellet granuloma in albino rats to test the aqueous extract from P. pinnata bark for anti-inflammatory efficacy at dosages of 400 mg/kg and 800 mg/kg.(158)

- **Anti-psoriatic activity**

  Using an imiquimod-induced psoriatic mouse model, we tested the efficacy of a formulation including a hydro alcoholic extract of *P. pinnata* leaves on the disease. The results showed promising action by minimizing psoriasis-related skin scaling.(158)

- **Anti-convulsant activity**

  The anticonvulsant effects of petroleum ether extract of *P.pinnata* stem bark at varying doses were tested using picrotoxin, pentylenetetrazol, strychnine, maximum electro shock, and isoniazid generated models. The findings of the pentylenetetrazol and maximum electro shock models showed that petroleum ether extract had a significant anticonvulsant effect.(158)

- **Antidiabetic activity**

  Using an Alloxan monohydrate-induced diabetic rat model, we compared the antidiabetic potential of ethanolic and aqueous extracts of *P. pinnata* stem bark and found that while both showed potent activity, the ethanolic extracts showed a significantly lower level of serum biomarkers.(158)

- **Anti-filarial activity**

  Invitro experiments using the natural behavior of a complete *S. cervi* worm and a nerve-muscle preparation tested the efficacy of both an alcoholic and aqueous extract of Pongamia pinnata's leaves and flowers. In tests against the Cattle Filarial Parasite, both extracts showed promise as anti-filarial agents.(158)

- **Antioxidant activity**

  Chemically synthesized Ag NPs from *Pongamia pinnata* leaves shown strong antioxidant activity in DPPH, ABT+s, hydroxyl radical, superoxide anion, and nitric oxide scavenging tests. Ethyl acetate extracts from the seeds of *P. pinnata* displayed highest total phenolic content and total flavonoid content of 1.23 ± 0.04 g GAE g^{-1} and 0.95 ± 0.05 g CE g^{-1} respectively with total antioxidant displaying a IC50 value of 18.47 ± 0.33 $\mu g ml^{-1}$.(158)

- **Antinociceptive and Antipyretic Activity**

Some studies have shown that *P.pinnata* leaves may reduce fever and pain. We tested the effects of the 70% ethanolic fraction of *P.pinnata* leaves on the bodies of rats and mice using several models of pain. The effect of *P.pinnata* leaf extract on Brewer's yeast-induced fever in rats was also examined. Researchers have discovered that a *P.pinnata* leaf extract has powerful analgesic and fever-reducing properties.(158)

- **Antiviral activity**

  Testing in a Penaeus monodon animal confirmed the efficacy of an ethanolic extract of *P.pinnata* leaves in inhibiting the replication of White Spot Syndrome Virus. After testing the anti-viral properties of a coarse aqueous seed extract, researchers found that it effectively stifled the spread of herpes simplex virus types 1 and 2 at 1 and 20 milligrams per milliliter, respectively, and had no cytopathic effects on the cells. Dry leaf extract in its basic form had little effect on rotavirus.(158)

- **Nano-pesticidal effect**

  The pesticidal efficacy of zinc oxide nanoparticles derived from *P.pinnata* leaf extract was tested, and the findings showed that the nanoparticles were poisonous enough to kill the Pulse beetle, Callosobruchus macula.(158)

- **Anti-diarrheal activity**

  P. pinnata decoction of dried leaves showed promising anti-diarrheal action in a study of its antimicrobial effects against gastrointestinal-harming *Escherichia coli* labile toxin, *E. coli* stable toxin, and cholera toxin.(158)

- **Cardio protective property**

  Assessment of petroleum ether fraction from *P.pinnata* stem bark in a rat model of diabetes produced by streptozotocin and nicotinamide.The results of the investigation showed that cardiomyopathy improved in diabetic rats.(158)

- **Wound healing activity**

  The current study's findings corroborate previous research showing *P. pinnata* to be very effective in mending wounds. Reputable wound healing is a result of a number of factors, including contraction of the wound, improvement in tensile strength, elevation of hydroxyproline and hexosamine content, modification of pro-inflammatory and anti-inflammatory cytokine, moderate antibacterial activity, and In-vivo antioxidant activity.(158)

- **Anti-ulcer activity**

  Evaluation of the anti-ulcer activity of *P. pinnata* root extract in methanol revealed considerable protection against mucosal damage and the ability to minimize acetic acid generated ulcers. Inhibition of mucosal defense mechanisms such mucin production, mucosal cell glycoprotein, mucosal cell life span, cell proliferation, and protection against lipid peroxidation.(158)

- **Neuroprotective activity**

  The neuroprotective effects of an ethanol extract of *P. pinnata* stem bark were tested in rats with mono sodium glutamate-induced neurotoxicity. Oral dosing with an ethanolic fraction of *P. pinnata* stem bark resulted in 200 and 400 mg/kg. The neuroprotective effects of an ethanolic extract of *P. pinnata* plant stem bark in albino rats are comparable to those of the conventional medication Dextromethorphan, suggesting its potential use as a neuroprotective agent.(158)

- **Anticonvulsant property**

  Scientists examined the anticonvulsant potential of the petroleum ether component of *P.pinnata* branch leaves in experimental rats. Researchers have used the petroleum ether portion of *P.pinnata* branch leaves to examine the effects of picrotoxin, pentylenetetrazol, strychnine, maximum electroshock, and isoniazid on mice. The anticonvulsant effects of a petroleum ether extract of *P.pinnata* stem bark have been well-documented.(158)

2.6.5. Woodfordia fruticosa

The medicinal & pharmacological uses of this plant were subject of a number of studies. In order to verify therapeutic usefulness of this plant, more extensive scientific investigations are required, particularly in the areas of skin illnesses, immunomodulatory disorders, and diabetes.

- **Antimicrobial Activity:**

Kumaraswamy et al. found that *W.fruticosa* flower extracts have antibacterial properties when tested on bacteria and yeast. For the extraction, we employed ethanol, methanol, petroleum ether, chloroform, and water. When compared to the gold standard medication Gentamicin, only the petroleum ether extract exhibited

substantial antibacterial action. Another In-vivo investigation employing the agar well diffusion technique confirmed the antibacterial efficacy of a crude methanolic extract of *W.fruticosa* compared to the gold standard antibiotic ciprofloxacin. The most effective extract against Pseudomonas pseudoalcaligenes was methanolic. In another experiment, researchers used the disc diffusion technique to test the antibacterial activity of an extract of diethyl ether, chloroform, and petroleum ether against four different bacterial species. The extracts showed activity against all tested strains.(159)

- **Immunomodulatory Activity:**

Shah et al. found that an ethanolic extract of *W.fruticosa* flowers has immunomodulatory properties. Sulforhodamine 'B' (SRB) tests were used to assess the extract's immunomodulatory activities in vitro using murine peritoneal macrophage phagocytosis and bone marrow cell proliferation as examples. A 60% increase in bone marrow cell proliferation is indicative of bone marrow stimulation.

- **Hepatoprotective Activity:**

Aqueous extract of *W.fruticosa* flower showed hepatoprotective effect against phenytoin-induced rats and carbon tetrachloride-induced toxicity, as did petroleum ether, alcohol, chloroform, and other solvents. Baravaia et al. found that a methanolic extract of *W.fruticosa* flowers protected the livers of rats given acetaminophen and diclofenac sodium. Both investigations showed that using the *Woodfordia fruticosa* plant to treat liver diseases increased the plant's hepatoprotective effect.(159)

- **Antioxidant Activity:**

Using the ABT and DPPH free radical scavenging models, Finose et al. assessed the antioxidant activity of *W.fruticosa* flowers. The findings indicated that the plant extracts made using petroleum ether, chloroform, and methanol all had considerable antioxidant activity.

- **Antiulcer Activity:**

Mihira et al., demonstrated antiulcer efficacy of chloroform and methanol extracts of *W.fruticosa* roots against diclofenac sodium-induced female Wister albino rats. Intestinal antiulcer activity was significantly increased in female Wister albino rats.

- **Antifertility Activity:**

Kushlani et al., reported woodfordia fruticosa flower extracts were shown to have antifertility effects on female albino rats. According to the findings, the plant's alcoholic extract exhibited strong abortifacient action.

- **Anti-tumor Activity:**

Yoshida et al., the anti-cancer effects of *Woodfordia fruticosa* flower extract are well-documented. Macro-ring hydrolyzable tannin dimer Woodfordin C has been shown to have anti-tumor action.

- **Analgesic Activity:** Rose et al., analyzed the effectiveness of stem bark of Woodfordia fruticosa as an analgesic in albino rats. Each rat received 200mg/kg of body weight in a mixture of water, ethanol, chloroform, & petroleum ether extracts. The conventional treatment group received Analgin. In experiments with albino rats, both the water-based and alcohol-based extracts showed analgesic effects. The aqueous extract also had the greatest analgesic efficacy when compared to petroleum ether & alcoholic extracts.
- **Antihyperlipidemic activity:**

Khera et al., reported *W.fruticosa* methanolic floral extract showed antihyperlipidemic efficacy in mice fed a high-cholesterol diet, according to a recent study. We gave 0.5 ml of water, 30 mg of cholesterol, and 400mg/kg of a methanolic extract of *W. fruticosa* flowers to five groups of six Swiss albino mice. The extract has a lipolytic action, decreasing lipid levels in the blood. In the fight against hyperlipidemia, *W.fruticosa* has emerged as a promising new natural product.

- **Anti-inflammatory Activity:**

Baravalia et al., reported flowers of *W.fruticosa*, extracted using methanol, have been shown to have anti-inflammatory effects on rats treated with carrageenan, histamine, dextran, serotonin, and formaldehyde. The findings demonstrated potent anti-inflammatory efficacy in the animal model.

- **Antihyperglycemic Activity:**

Verma et al., reported extract of *W.fruticosa* flowers inhibits hyperglycemia & lipid peroxidation in diabetic rats caused by streptozotocin. *W.fruticosa* ethanolic extract effectively decreased fasting blood glucose and increased insulin level in

diabetic rats after 21 days of therapy. The findings indicated that ethanolic extract of flowers had a significant hypoglycemic impact.

- **Anthelmintic Activity:**

Sengupta et al., reported *W.fruticosa* anthelmintic activity against Pheritima posthuma, an Indian earthworm, was tested using methanol & petroleum ether extract of dried flowers.

Table 6. Literature review of plants used in the study

S. No	Reference	Drug Name	Dosage form	Type of psoriasis treated	Major findings reported in the study
1.	Liu et. al 2011	Curcumin	Microemulsion	Psoriasis	From the major outcome reported usage of eucalyptol as a system of microemulsion was found to promising and efficacious for the delivery of curcumin percutaneous.(160)
2.	Jun Sun et. al 2013	Curcumin	Gel	Psoriasis	In this study imiquimod was induced in a mouse model for psoriasis-like inflammation, Curcumin gel was reported to relieve inflammation which showed similar efficacy as that of clobetasol.(161)
3.	Antiga et. al 2015	Curcumin (Meriva)	Oral	Psoriasis Vulgaris	In the study, it has been reported that Curcumin when used as an adjuvant, was found to be effective in the

					treatment therapy of psoriasis vulgaris which sufficiently reduces the IL-22 levels of serum.(162)
4.	Gutierrez et. al 2015	Curcuma extract	Oral	Plaque psoriasis	In this study the author inferred a new method of treatment therapy that would be safe, non-toxic, and more effective than the existing treatments. The results also exhibited that oral Curcuma administration was found to be efficient against moderate to chronic plaque kind of psoriasis.(123)
5.	Anjali et. al 2016	Tacrolimus and Curcumin	Gel	Psoriasis	The combination of Tacrolimus and Curcumin gel liposphere has enhanced the histopathological features and also phenotypic features in the skin of psoriasis. This combo also decreases the cytokine levels which include TNF-α, IL-17, & IL-22. Study results exhibited greater and better potential with the formulation. From the study, the author also reported for an extensive way to determine the synergistic mechanism for psoriasis.(163)

6.	Bilia et. al 2018	Curcumin	Oral Nanoparticles	Psoriasis	The study results reported that oral Nanoparticles of Curcumin were found to be an efficacious adjuvant when given in the combination of Acitretin orally. It has improved the serum lipid profile in moderate to chronic psoriatic patient population.
7.	Clinton et. al 2019	CurcuminChitosan/Alginate	Nanoparticles	Psoriasis	The Curcumin-Chitosan/Alginate nanoparticles proved to pose a superior effect when compared with curcumin free alone. Along with a combination of irradiation of blue light using LED-based illumination was an efficient system of PDT in the management of psoriatic disease state.(164)
8.	Padmini et. al 2020	Curcumin and caffeine mixture	Gel	Psoriasis	The Curcumin and caffeine mixture sufficiently augmented its efficacy using numerous components. It also decreased the required onset of action. Therefore, the proposed formulation of nanogel would pose an imperative drug delivery

					system for more efficient psoriasis activity.(165)
9.	Algahtani et. al 2020	Curcumin	Nanoemulsion	Psoriasis like skin lesions	In this study imiquimod combined with curcumin as a nanoemulgel prohibited the presence of signs and symptoms psoriasis, when compared with the formulation of imiquimod gel and nanoemulgel.(166)
10.	Kesharwani et. al 2020	Curcumin	Nanogel	Psoriasis	The author reported in the study indicating Curcumin NLCs will be a promising, potential therapeutic approach in managing of diseases like psoriasis.(167)
11.	Khatoon et. al 2020	Curcumin, Thymoquinone, and resveratrol	Nanoemulsion gel	Psoriasis	The results indicate that a mice model of Balb/c exhibited improvement in anti-psoriatic activity which in turn indicates a triple natural combination of bio-actives formulation of nanoemulgel is efficient in the treatment of psoriasis therapy.(168)
12.	Nan Jin et. al 2020	Curcumin	Smart Pearls Topical	Psoriasis	The fabrication of smart Pearls Technology with the addition of GA as an adjuvant to amorphous topical curcumin in the stable form

					without using supplemental dermal matrix available. This also enhances the penetration for anti-psoriasis activity.
13.	Pandey et al	Neem leaf extract capsules	Capsules	Psoriasia	Pandey et al. Fifty people with uncomplicated plaque psorias were randomized to receive either a placebo or neem leaf extract capsules 3 times a day, in addition to a topical regimen of 5% crude coal tar & 3% salicylic acid in a Vaseline base. The treatment period lasted for 12 weeks. While at both 8 and 12 weeks post-intervention, PASI scores decreased, indicating response to the topical regimen, the difference between neem and placebo groups was statistically significant (mean PASI, 9.7 vs. 12.24, p 0.05 and 4.74 vs. 9.47, p 0.001). There were no documented side effects.(169)
14.	B.K.R. PillaP at all	Neem	Nimbidin 200mg	Psoriasis	A doubled blind clinical study conducted by B.K.R. PillaP at all on the psoriasis in this study the recruited 20 patients in each group one group was given nimbidin 200mg and

					other group given placebo. They asses the severity of the diseases and the area of body part affected and found that nimbidin have edge in the treatment of the psoriasis.(170)
15	Rajasekharan S at all	Neem extract	Nimbidin 100mg	psoriasis	A case report published by Rajasekharan S at all shows improvement in various symptoms of psoriasis by using neem extract. In this report they have taken a 72 year male with psoriatic lesion they prescribe nimbidin 100mg twice daily and his symptoms were recorded and blood investigations was done on the subsequent follow- ups it was found that patients red flat discoid disappears in 15 days, long axil papuls disappears in 66 days, white laminar scales in 45 days, erthymia in 45 days and itching in 15 days if we compaire the blood reports his ESR values also changed from 18 to 8 mm/hr. so we can say by the report that nimbics is

					effective in the treatment of psoriasis.(170)
16.	Dr. Annu Kanwar et al., 2022	*Azadirachta indica*	*Azadirachta indica* 1X	Psoriasia	This was a prospective, single blind, randomised, comparative trial with positive results and these results need further validations by conducting clinical trials. With research they concluded as *Azadirachta indica* 1X is helpfull in casese sufeering from psoriasis. No adverse effect were recorded when homoeopathic medicine *Azadirachta indica* 1X was prescribed. It is concluded from the percentage of symptomatic relief that *Azadirachta indica* 1X much useful in treating the symptoms of psoriasis. Thus, this study helps in improving clinical practice of homoeopathic physicians.(170)
17.	Sankarados s Nirmala et al., 2022	*Azadirachta indica*	Polyherbal extract ointment formulations	psoriasis	There were a variety of alkaloids, terpenoids, tannins, and other compounds found in poly herbal extract. TLC and HPTLC research revealed

			PHF I & PHF II		active components in poly herbal extract as separate spots. The formulated polyherbal extract ointment formulations PHF I & PHF II showed acceptable physicochemical properties, including a lack of irritation, antiproliferative activity in HaCaT cell lines, & significant suppression of keratinocytes in rat model of light-induced psoriasis. Antipsoriatic action in formulations was promising.(171)
18.	Shirsath N et al., 2018	*Psoralea carylifolia*	8-methoxypsoralen (methoxsalen)	Psoriasis	While ameliorating imiquimod-induced inflammation in established psoriasis, 8-methoxypsoralen plus ultraviolet A (PUVA) not only decreases the skin's reactivity to Toll like receptor activation by lowering the baseline levels of miRNA27a and 29a and interferon-γ, interleukin-17, and -9 cytokines. (172)
19.	Shirsath N et al., 2018	*Psoralea carylifolia*	8-methoxypsoralen (8- MOP)	Psoriasis	It is possible to treat psoriasis with the photosensitizer 8-methoxypsoralen (8- MOP)

					either orally (PUVA system) or topically (PUVA bath), using either warm water or ultraviolet light. (172)
20	Yongkang Zhao et al., 2022	*Psoralea carylifolia*	PCL tincture	Psoriasis vulgaris	Combining a topical PCL tincture with NB-UVB for psoriasis vulgaris is a successful therapy. (147)
21	Alalaiwe et al., 2018	*Psoralea carylifolia*	Isopsoralen	Psoriasis-like lesions	When compared to the imiquimod-treated group, the isopsoralen-treated group showed a significant decrease. The furocoumarins have the potential to alleviate the severe scaling & inflammation produced by imiquimod. In a parallel group, isopsoralen inhibited the expression of IL-1β and IL-6 produced by imiquimod.(173)
22	Kamlesh Wadher et al., 2021	*Pongamia pinnata*	Herbal gel	Imiquimod-induced psoriasis	Phytochemical screening and phytoconstituent quantification were performed initially on a hydroalcoholic leaf extract of *Pongamia pinnata*. *Pongamia pinnata* extract herbal gel was made using Carbopol 934 as the gelling agent. Different gel formulations were created

					and tested for a variety of properties, including pH, viscosity, spreadability, and in vitro diffusion. Anti-psoriatic activity of the extract was most noticeable in the imiquimod-induced psoriatic mouse model, as measured by index grading. There was a significant decrease in both the thickness and scaling of skin in the treated groups, confirming a significant improvement in psoriasis.
23	Shraddha Kothmire et al., 2023	*Pongamia pinnata*	Nanoparticle herbal gel	Psoriasis	The end of present study was to prepare tableware nanoparticle herbal gel expression containing methanolic excerpt of Karanj on psoriasis. Topical gel expression was designed by using methanolic excerpt of seeds of pongamia pinnata in varied attention. The gel was prepared by using tableware nitrate, chitosan, carbopol 934, *Pongamia pinnata* Extract (KARANJ), Methanol, propylene glycol, methyl paraben, propyl paraben,tri-ethanolamine,

					glycerin and needed quantum of distilled water. The set gels were estimated for medicine content, pH, spread capability, physical appearance, density, swelling indicator, prolixity study, unity and grit. The results prove that gel phrasings were good in appearance and unity.(174)
24	Jagruti Desai et al.,2023	*Pongamia pinnata*	Karanjin loaded emulgel	Psoriasis	Several factors were calculated when developing the karanjin-loaded emulgel. Droplet size, pH, viscosity, and phase separation were all measured and analyzed for the optimized emulgel batch. A 30-day stability test showed that Karanjin emulgel was safe to use. The mixture was subsequently tested for its ability to alleviate psoriasis in mice. The data showed that the psoriasis may be managed with Karanjin-loaded emulgel. This study came to the conclusion that Karanjin-loaded emulgel would provide a viable option for the local administration of Karanjin..(175)

25	Navdeep Raghuwanshi et al.,2019	*W. fruticosa*	Ointment gel	IMQ-Induced Psoriasis-like skin inflammation	In this work, we use structure-based drug design to isolate putative HSP70-1-targeting inhibitors from W. fruticosa flower extracts and test their efficacy against IMQ-induced psoriasis-like skin inflammation in mice. Studies validate an effective and targeted combinatorial method, which includes in-silico molecular modeling, bio-nanotechnology, and in-vivo evaluation. In comparison to the already available anti-psoriatic medicine Tretinoin, we discovered that Myricetin, Quercetin, and Ellagic acid were the top three substances extremely active against the HSP70-1 protein. The results of this research suggest that the polyphenolic chemicals found in *W. fruticosa* flowers have the potential to operate as new inhibitors of HSP70-1, which might make them effective against psoriasis. Treatment with 1% WfAuNPs-Carbopol® 934

					applied topically reduces serum cytokine levels and alleviates IMQ-induced Psoriasis-like skin irritation. Our current research paves the way for further exploration into structural & mechanistic insights that might inform the design and development of a novel medication candidate using a structure-based drug designing strategy.
26	Amit Kumar Srivastava et al 2016	*Woodfordia fruticosa*	Ointment	Psoriasis	The purpose of this work was to use an innovative in vivo screening strategy to look into the antipsoriatic activity of an ethanolic extract of *Woodfordia fruticosa* flowers (EEWF). Psoriasis was induced in Swiss albino mice by applying 0.1 ml of CFA & formaldehyde combination (1:10) to skin's dorsum for 7 days. Phenotypic & histological parameters were used in determining PSI. After inducing psoriasis, researchers analyzed the efficacy of EEWF ointments with concentrations of 0.05% and 0.1% (w/w). Psoriatic

					lesions & epidermal thickness reduced in rats treated with 0.05% & 0.1% (w/w) ointments of EEWF from day 7 to day 21 (P 0.05)..(176)

2.7. Introduction to novel drug delivery system:

An effective drug delivery system would allow for the medication to be distributed in a controlled & intended way, resulting in the intended therapeutic effect. Universal and spatiotemporal distribution of the medicine is another important goal for any drug delivery method. Consistency, logic, strength, safety, and patient compliance are all essential qualities in a dose form.

2.7.1 Advantages of novel drug delivery system:

The following are few of many benefits of the innovative medicine delivery technology.

- Modified Release
- Extended Release
- Sustained Release
- Controlled Release
- Delayed Release
- Targeted- release drug products
- Recurrence Action

2.7.2 Introduction to Microemulsion:

Hoar and Schulman initially proposed the concept of microemulsions in the 1940s. A surfactant/co-surfactant arrangement is a fluid with low consistency or an interfacial coating consisting of water, oil, and surfactant that is visually isotropic and thermodynamically stable.(19) It's a means of improving the delivery, dosing, and bioavailability of medicines.(20) Medium-chain unsaturated fatty acids are known to affect the junctions between epithelial cells, whereas long-chain unsaturated fatty acids

facilitate lipoprotein agglomeration and lymphatic absorption. Recent studies have shown promise for topical drug bioavailability enhancement using microemulsion [o/w or w/o].

It seemed like a good topical delivery mechanism for a few of active ingredients with medical and therapeutic uses. Because of the inner stage's large surface area, topical microemulsion facilitates the rapid entry of dynamic particles, and the segments reduce the stratum corneum's obstructive feature. Therefore, microemulsions are a promising vehicle for transdermal medicine administration since they increase skin absorption in comparison to conventional schemes. In this article, we will go over the main differences between emulsions and microemulsions.

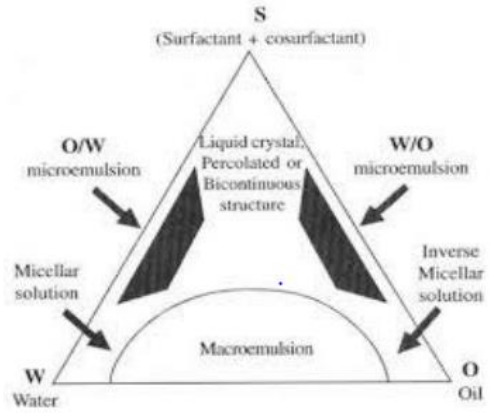


Figure 9. Regions of Macroemulsion and Microemulsion

2.7.2.1 Winsor Classification of microemulsions:

Winsor discovered that when oil, water, and surfactant are mixed, four various types of situations might occur.

- Type – I System - O/W microemulsions are a part of it, and they work well with the surplus oil phase. The surfactant is easily soluble in water and forms stable oil in water (O/W) microemulsion (Winsor I). In addition to the surfactant-rich water phase, the oil phase, in which surfactant is only present as monomers at low concentration, also occurs.
- Type – II - The W/O microemulsions are in sync with the surplus water level. Most surfactants exist as water-in-oil (W/O) or oil-in-water (O/W)

microemulsions. The oily stage, which is rich in surfactants, coexists with the watery stage, which is lacking in surfactants (Winsor II).

- Type – III - It combines an excess of water with an excess of oil in a microemulsion stage. Winsor III or center stage microemulsion is a three-stage structure consisting of a surfactant-rich central stage, an abundance of water surfactant-poor outer stages, and an abundance of oil surfactant-rich outside stages.
- Type – IV - Because of the micellar arrangement's single stage (isotropic), an inexhaustible supply of liquid and surfactant is available at all times.

2.7.2.2 Shortcomings of microemulsions:

- Low capability of dissolving high-melting compounds utilized in system.
- Environmental factors, like temperature and pH, influence microemulsion stability. Microemulsion delivery to patients causes a shift in these variables.
- Microemulsion has the same phase separation problems.

2.7.2.3 Methods of preparation:

A. Phase titration method: (Spontaneous emulsification method)

It is possible to depict the process of creating a microemulsion using a stage graph, since this method employs a rather unrestricted emulsification approach (the stage titration technique). The creation of a stage graph is an efficient method of dealing with the tangled web of interdependencies that might result from the blending of disparate elements. Understanding their equilibrium states and the boundaries between states is a crucial aspect of the research process. The microemulsion zone and other discrete regions may be located using a pseudo ternary stage graph, in which each side of the outline describes a hundred percent of the corresponding segment. Simply taking into account the district's organizational characteristics—whether it is oil rich or water rich—allows for its separation into w/o or o/w microemulsion. It's important to form opinions in a way that rules out the possibility of a metastable framework.

B. Phase inversion method

Expansion of the microemulsion's overabundant stage causes problems for the process of reversing the stage. When one process gives way to another, it triggers profound physiological changes, such as a shift in molecular estimation that modulates drug release. While cooling, this framework deviates from its intended state of zero unrestrained bend and low surface strain, leading to the formation of an orderly pattern of finely dispersed oil beads. Controlled expansion of lower alkanols (butanol, pentane, and hexane) in smooth emulsions yields simple configurations including o/w or w/o or colloidal scatterings, and hence, microemulsions.Co-surfactants are often lower alkanols. They reduce oil-water interfacial tension to a level where arrangement may occur with few constraints.

2.7.2.4 Applications of microemulsion:

- Parenteral delivery
- Oral drug delivery
- Topical drug delivery
- Ocular drug delivery
- Pulmonary drug delivery
- Microemulsion in biotechnology

2.7.2.5 Mechanism of action of drug release from microemulsion:

Two models are useful for explaining the release of medication from a microemulsion. Both models regard the interfacial border between the drop and the surrounding environment as the rate-deciding progress of medicine discharge; however one model explains sedate dispersion throughout the bead as the limiting factor. The most effective model for drug release from a microemulsion depicted a combination of mass adjustments and directs dependency on mass transitions on application.

Determining the mass exchange constants of the pharmaceuticals via the organic layer that separates the microemulsion from the recipient stage is the primary consideration in planning for drug release from the microemulsion. In mixtures of oil and surfactant, the mass exchange constant is proportional to the segment coefficient of the medicine.

The oil-water ratio, bead size, and drug distribution during microemulsion phases are the primary determinants of medicine arrival. The pace at which drugs move from the scatter stage to the constant stage and then from the constant stage via the organic film is another indicator of the discharge pattern.

Convergence of medicine in the watery stage of ME is thought to be crucial for the penetration of hydrophilic medication through the organic layer and into the microemulsion, and the opposite is true for lipophilic medication.

2.8. Need for Topical Nanotechnological based Drug Delivery System

Formulation creation for the therapy of psoriasis is difficult because the disorder causes epidermal changes (such as thicker, scalier horny barriers and hard, inflammatory keratodermas) that are difficult to account for. Currently on the market, standard formulations fall short of expectations due to drawbacks including a lack of aesthetic and cosmetic characteristics, potential toxicity, and low patient compliance when used over the long term. Novel medication delivery technologies that increase the dermatological advantages might be more worthwhile than the current obstacles. In addition, they have a number of additional benefits that make them ideal for treating psoriasis, including being simple to use, requiring fewer doses each day, allowing for gradual drug release over time, and increasing therapeutic efficacy at the appropriate dermal location.

There is great promise for improving medication bioavailability and efficacy via the use of nanotechnology-based drug delivery systems, particularly for lipophilic medicines. Psoriasis, like many skin conditions, is best treated with topical medications that work just at the point of application, rather than being absorbed into the body systemically. Therefore, the focus of topical medication delivery methods is on the point of application. Topical drug administration has certain benefits over systemic delivery for some medications and diseases, but a key difficulty to successful drug delivery through this route is the resistance to drug transport over the skin barrier. One or more of the ingredients in the topical formulation may cause skin irritation or contact dermatitis. Some medications have low pharmacological response because they don't pass through the skin well.

Possible issues include allergic responses and the breakdown of medicines by enzymes in the epidermis. Psoriatic skin becomes rigid due to an increase in cholesterol and a decrease in ceramides. The skin of a person with psoriasis is dry and flaky because NMFs like water are nearly nonexistent. Because of these obstacles, the topical administration of drugs for psoriasis presents significant difficulty. In order to counteract the lipid imbalance and restore the normal moisturizing factors, a lipid-based carrier system is required.

Table 7. List of topical novel drug delivery systems for psoriasis

Novel Drug Delivery Systems	**Anti-Psoriatic Drug**	**Reference**
Liposome-gel	Hydrocortisone	(Kim et al., 1998)
Transfersome in gel	Tacrolimus	(Lei et al., 2013)
Drug β-Cyclodextrine complexes into SLNs	Hydrocortisone	(Cavalli et al., 1999)
Cyclodextrin inclusion complex incorporated in multivesicular liposomes	Fluocinolone acetonide	(Vafaei et al., 2015)
Chitosan coated microemulsion	Methoxsalen	(Behera et al., 2010)
PLGA/PLA nanoparticles(plus zinc)	Betamethasone	(Ishihara et al., 2005)
PLGA (poly(D,L-lactic-coglycolic acid)) nanoparticles	Betamethasone	(Özcan et al., 2013)
PLGA nanoparticles	Ciclosporine Dexamethasone	(Jain, Mittal and K. Jain, 2011) (Gómez-Gaete et al., 2007)
PLGA microspheres	Clobetasol-17-propionate	(Badıllı, Şen and Tarımcı, 2011)
Poly(ε-caprolactone) (PCL) nanocapsule	Dexamethasone	(Friedrich et al., 2008)

PCL nanoparticles	Hydrocortisone	(Rosado, Silva and Reis, 2013)
Polymeric micelle (PEGdihexPLA diblock copolymer)	Ciclosporine	(Lapteva, Santer, et al., 2014)
Poly(NIPAM-co-BA) nanogel	Methotrexate	(Singka et al., 2010)
Methoxy-poly(ethylene glycol)-dihexyl substituted polylactide (MPEG- dihexPLA) diblock copolymer micelle	Tacrolimus	(Lapteva, Mondon, et al., 2014)
Niosome	Anthocyanin complex Dithranol Methotrexate	(Priprem et al., 2015) (Agarwal, Katare and Vyas, 2001) (Abdelbary and Aboughaly, 2015)
Lecithin/chitosan nanoparticles	Betamethasone Clobetasol-17-propionate	(Özcan et al., 2013) (Şenyiğit et al., 2010)
Liposome	Tacalcitol Clobetasol-17-propionate Dithranol Tacrolimus Tretinoin Triamcinolone	(Körbel et al., 2001) (Rao and Murthy, 2000) (Abdelbary and Aboughaly, 2015) , (Kumar, Deep and Agarwal, 2015) (Özcan et al., 2013) (Raza, Singh, Singla, et al., 2013) (Yu and Liao, 1996)
Pegylated liposomes	Calcipotriol	(Knudsen et al., 2012)

Transferosome	Dexamethasone Hydrocortisone Triamcinolone	(Cevc and Blume, 2004) (Fesq et al., 2003)
Flexible vesicles and conventional vesicles	Cyclosporine	(Guo et al., 2000)
Deformable liposomes	Methotrexate	(Srisuk et al., 2012) (Trotta et al., 2004)
Ethosome	Ciclosporine Methotrexate Psoralen Tacrolimus Tretinoin Tretinoin	(Verma and Fahr, 2004) (Dubey et al., 2007) (Zhang et al., 2014) (Li et al., 2012) (Raza, Singh, Lohan, et al., 2013)
SLNs (Solid lipid nano-particles)	Betamethasone Ciclosporine Clobetasol-17-propionate Dithranol Methoxsalen. Mometasone furoate Prednicarbate Psoralen Tretinoin Triamcinolone	(Zhang and Smith, 2011) (Kim et al., 2009) (Kalariya et al., 2005) (Gambhire, Bhalekar and Gambhire, 2011) (Battaglia et al., 2012) (Madan, Dua and Khude, 2014) (Raza, Singh, Lohan, et al., 2013) (Schlupp et al., 2011) (Fang et al., 2008) (Pradhan, Singh and Singh, 2016) (Sonawane et al., 2014)

SLN-hydrogel	Betamethasone dipropionate and calcipotriol Halobetasol Tacrolimus	(Bikkad et al., 2014) (Ruihua Wang, 2012) (Lin et al., 2010)
Nano-structured Acitretin (Antônio Dantas Silva et lipid carriers (NLCs)-hydrogel	Acitretin	(Antônio Dantas Silva et al., 2012)
NLCs	Calcipotriol and methotrexate Clobetasol-17-propionate Fluocinolone acetonide Methotrexate Methoxsalen Psoralen Tacrolimus Tretinoin	(Pradhan, Singh and Singh, 2015) (Pinto et al., 2014) (Nam, Ji and Park, 2011) (Baboota et al., 2011) (Marepally et al., 2014) (Sonawane et al., 2014) (Raza et al., 2011) (Raza, Singh, Singla, et al., 2013)
Microemulsions in hydrogel	Betamethasone dipropionate and salicylic acid Methoxsalen	(Baroli et al., 2000) (Lei et al., 2013)
Microemulsion	Dithranol Methoxsalen Tacrolimus	(Sah, Jain and Pandey, 2011) (Shinde et al., 2013) (Wohlrab et al., 2012)
Nanoemulsion	Methoxsalen	(Vicentini et al., 2013)
Liquid crystalline nanoparticles	Cyclosporine A Tacrolimus	(Vicentini et al., 2013) (Thapa and Yoo, 2014) (Petrilli et al., 2016)

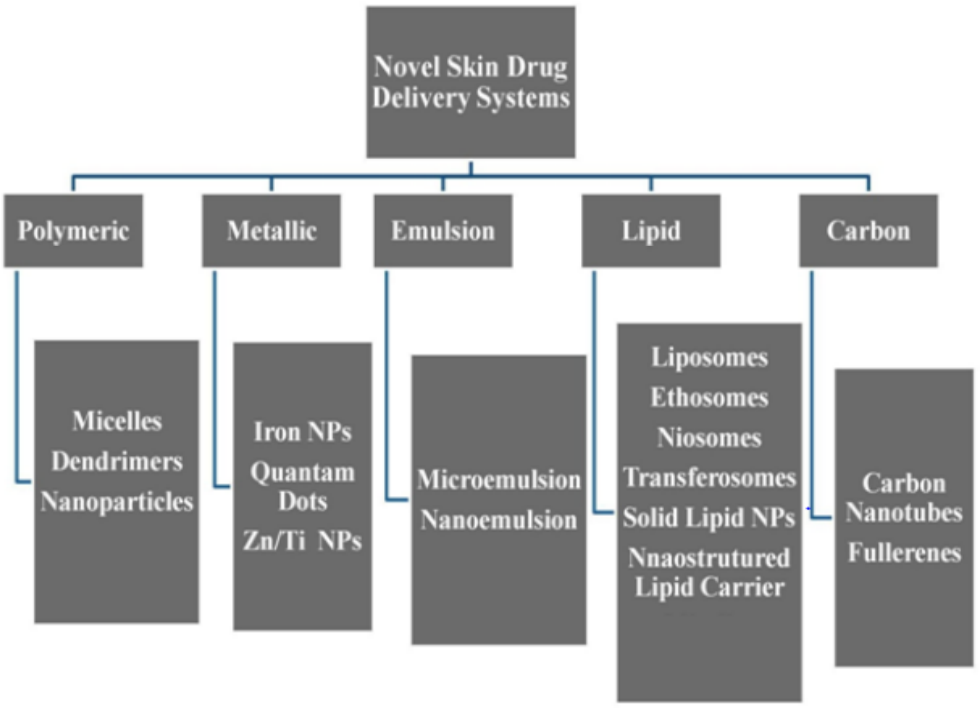


Figure 10. Categorical representation of different kinds of nanocarriers for topical skin delivery

Micellar drug delivery systems and nanoemulsion are two examples of effective nanosystems for dermatological applications because of their superior skin penetration capabilities, skin compatibility, and high stability.

2.9. **Patents available on Psoriasis**

Table 8. Patents available on Psoriasis

Patent No.	**References**	**Descriptions**
WO2013074569	Joel Wolf et al., 2014	According to patent WO2013074569, an antibody targeting IL-36R is being considered as a treatment for psoriasis.(177)
US 10,668,132 B2	Sveinsson., 2020	Compositions & compounds which act as CGRP antagonists or inhibit its activity are the focus

		of this invention because of their potential use in the treatment and prevention of psoriasis.(178)
US 9,090,689 B1	Hoffman et al. 2015	USE OF TNFa. INHIBITOR FORTREATMENT OF PSORASIS(179)
US 2018 / 0346583 A1	Liu et al ., 2018	PSORIASIS TREATMENT BY MEANS OF AN IL-17 RECEPTOR ANTIBODY (180)
US 9,072,725 B2	Ehrhardt et al., 2015	METHOD FOR TREATING PSORASIS(181)
US 8,753,883 B2	Edinger et al., 2014	TREATMENT OF PSORIASIS USING PLACENTAL STEM CELLS(182)
US 11,534,490 B2	Guettner et al ., 2022	TECHNIQUES OF TREATING PSORIASIS USING IL - 17 ANTAGONISTS(183)
US 2016/0200809 A1	Willian et al., 2016	USES AND COMPOSITIONS FOR TREATMENT OF PSORASIS(184)
US 11,517,552, B2	Silver 2022	TECHNIQUE FOR TREATING PSORIASIS(185)
US 7,223,393 B2	Landolfi et al., 2007	AMPHIREGULIN ANTIBODIES AND THEIR USE TO TREAT CANCER AND PSORASIS(186)
US 11,648,256 B2	Dow et al., 2023	TOPICAL COMPOSITIONS AND METHODS FOR TREATING PSORIASIS(187)

CHAPTER-3

Rationale of the Study

3.1 Hypothesis

- According to a WHO estimate, psoriasis affects about 100 million individuals globally. According to the worldwide report, there is no known cause or treatment for this type of illness.
- According to GBD 2019, 95% of psoriasis cases occurred in people having age group 60 to 69 years.
- Conventional dosage form has many limitations which include poor drug solubility, insufficient drug concentration, poor absorption, low permeability, rapid metabolism and elimination, drug distribution to other tissues combined with high drug toxicity and short half-life.
- Novel Drug delivery is a promising strategy to overcome these side effects and offer many advantages which include increased safety & efficacy, drug targeting specificity and lowering of system drug toxicity. Small and relatively narrow sized distribution with novel carrier permit the specific delivery to the skin with improved drug solubilization of hydrophobic drugs and better bioavailability. The objective of present research work is to develop Micro emulsion for psoriasis containing chief constituent of herbal drug with potent lipid complex.

3.2 Aim of Study

Development and Pharmacological evaluation of Poly-herbal Micro emulsion for the treatment of Psoriasis.

3.3 Objective

- Authentication of herbal drugs
- To study the Pharmacognostic & Phytochemical profile of herbal drugs
- Preparation of Phytoextract of Each drug
- *In Vitro* Study of each extract
    - ✓ MTT Assay,
    - ✓ ELISA technique
    - ✓ LDH Assay & Confocal Microscopy
- One most potent extract selected for Micro-emulsion preparation
- Development of Micro-emulsion

- Characterization of Micro-emulsion
- Stability study as per ICH guidelines
- In Vivo study for Anti-psoriatic activity in Swiss Albino Mice.

Chapter 4

Methods and Materials

4.1 Materials and Instruments

4.1.1 Materials

The list of materials used throughout the study was listed in table along with respective sources

Table 9. Various materials employed in the research work

Material(s)	Source(s)
Tween 20	Himedia Lab Pvt. Ltd., India
Tween 40	Himedia Lab Pvt. Ltd., India
Tween 60	Loba ChemiePvt.Ltd., India
Tween 80	Loba ChemiePvt.Ltd., India
Tween 81	Himedia Lab Pvt. Ltd., India
Cremophor EL	Loba ChemiePvt.Ltd., India
Span 80	S.D. Fine Chem.Ltd., India
Span 20	S.D. Fine Chem.Ltd., India
Ethanol	Himedia Lab Pvt. Ltd., India
Isopropyl Alcohol	Loba ChemiePvt.Ltd., India
Polyethylene glycol 400	Loba ChemiePvt.Ltd., India
Polyethylene glycol 600	Himedia Lab Pvt. Ltd., India
Propylene Glycol	Himedia Lab Pvt. Ltd., India
Glycerol	Himedia Lab Pvt. Ltd., India
Cardamomum Oil	Central Drug House Pvt Ltd India
Arachis oil	Central Drug House Pvt Ltd India
Castor oil	Central Drug House Pvt Ltd India
Ceedarwood oil	Central Drug House Pvt Ltd India
Eucalyptus oil	Central Drug House Pvt Ltd India
Olive oil	Central Drug House Pvt Ltd India

Orange peel oil	Central Drug House Pvt Ltd India
Peppermint oil	Central Drug House Pvt Ltd India
Anise oil	Central Drug House Pvt Ltd India

4.1.2 Instruments and equipment

The list of instruments and equipment used in various stages of research work were enlisted in **Table** along model/manufacturer details

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

Instruments/equipment	Model/Manufacturer details
Analytical balance	Shimadzu Analytical Pvt Ltd,
Hot air Oven	Lab fit India Pvt Ltd
Muffle furnace	Lab fit India Pvt Ltd
Mechanical Shaker	REMI India Pvt Ltd
Water bath	Lab fit India Pvt Ltd
Magnetic stirrer	REMI India Pvt Ltd
Stability Chamber	Thermo Lab
Transmission electron microscopy	Thermo Fischer Scientific
UV Spectrophotometer	UV-1800, Shimadzu, Japan
Tissue homogenizer	REMI India Pvt Ltd
Heating mantle	Labtronics

4.2 Methods

4.2.1. Collection of *C.longa, P.Corylifolia, P.Pinnata, W.fruticosa&A.indica*

The crude raw herb is obtained from Shree Dhanwantri Herbals Amritsar Punjab India as a gift sample. The sample specimen was authenticated from Department of Environmental and botanical sciences Guru Nanak Dev University Amritsar Punjab India. A voucher specimen 490 of Rhizome, Seed, Flower & Stem bark were deposited into the herbarium of Guru Nanak Dev University Amritsar Punjab India

4.2.2. Physicochemical standardization of powdered Rhizome, Seed, Bark, Flower(188)

4.2.2.1. Determination of Foreign Matter

Weigh 100-500gm of the drug sample to be examined or minimum quantity prescribed in the monograph and spread it out in a fine layer. Check the foreign matter with naked eye. Separate out and weighed again it and calculate the %age.

Foreign matter= (Weight of sample−Weight loss)/ (Weight of sample) ×100

Eq. (4.1)

4.2.2.2. Loss on Drying

Place about 10g of drug (without preliminary drying) after accurately weighing it in a tarred evaporating dish. After placing the above-said amount of the drug in the tarred evaporating dish dry at 105ºC for 5 hours, and weigh. Continue the drying and weighing at one-hour interval until the difference between two successive weighing corresponds to not more than 0.25 percent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01g difference.

4.2.2.3. Determination of total ash

2-3gm accurately weighed ground drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon, kept in desiccators for cooling and weighed. Percentage of ash with reference to air dried drug was calculated

% Total Ash = (Weight of Ash)/ (Weight of sample) × 100 **Eq. (4.2)**

4.2.2.4. Determination of Acid-insoluble ash

Boil the ash obtained for 5 minutes with 25ml of dilute hydrochloride acid 25ml of dilute hydrochloric acid; collect the insoluble matter in Gooch crucible or on ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid- insoluble ash

% Acid insol. Ash = (Wt of final)- (Wt of crucible)/(Wt of sample)×100

Eq.(4.3)

4.2.2.5. Extractive Value: This was carried out to determine the nature of chemical constituent present in the drug

4.2.2.5.1. Determination of ASEV

Macerate 5g of air-dried drug; coarsely powdered, with 100ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporator 25ml of the filtrate to dryness in the tarred flat-bottomed shallow dish, and dry at 105℃, to constant weight. Calculate the percentage of alcohol soluble extractive with reference to the air-dried sample.

% Alcohol Soluble Extractive Matter = (Weight of residue)/ (Weight of Sample) ×100 **Eq. (4.4)**

4.2.2.5.2. Determination of WSEV

Macerate 5g of air-dried drug; coarsely powdered, with 100ml of distilled in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporator 25ml of the filtrate to dryness in the tarred flat-bottomed shallow dish, and dry at 105℃, to constant weight. Calculate the percentage of alcohol soluble extractive with reference to the air-dried sample.

%Water Soluble Extractive Matter = (Weight of residue)/ (Weight of Sample) × 100 **Eq. (4.5)**

4.2.3 Preparation of the Extract(189)(190)(191)(192)

4.2.3.1. Method of Preparation of *C.longa* Extract by Soxhlet extraction

Take 4 gm of coarsely powdered drug and placed in filter paper (Whatman no1) thimble in soxhlet apparatus fitted with 250 ml of round bottom flask containing 150 ml of solvent. Extraction was performed at boiling temperature of respective solvent for 5 hr with completion of up o seven cycles through siphon mechanism in case of extraction with acetone and ethanol. In case of extraction with water, time required for completion of one cycle was significantly more hence, with water extraction was carried out for longer time with the completion

of up to seven to eight cycle through siphon mechanism. After the completion of first extraction step, residue in the thimble was again extracted twice with suitable amount of respective solvents. Filtrate of each solvent from three extraction steps were taken out and their volumes were noted.

4.2.3.2. Method of Preparation of *A.indica* Extract by Soxhlet extraction

Take 4 gm of coarsely powdered drug and placed in filter paper (Whatman no1) thimble in soxhlet apparatus fitted with 250 ml of round bottom flask containing 150 ml of solvent. Extraction was performed at boiling temperature of respective solvent for 5 hr with completion of up to seven to eight cycle through siphon mechanism in case of extraction with ethanol. In case of extraction with water, time required for completion of one cycle was significantly more hence, with water extraction was carried out for longer time with the completion of up to seven to eight cycle through siphon mechanism. After the completion of first extraction step, residue in the thimble was again extracted twice with suitable amount of respective solvents. Filtrate of each solvent from three extraction steps were taken out and their volumes were noted.

4.2.3.3. Method of Preparation of *P.corylifolia* extract by soxhlet extraction

Take 4 gm of coarsely powdered drug and placed in filter paper (Whatman no1) thimble in soxhlet apparatus fitted with 250 ml of round bottom flask containing 150 ml of solvent. Extraction was performed at boiling temperature of respective solvent for 5 hr with completion of up to seven to eight cycle through siphon mechanism in case of extraction with petroleum ether, chloroform &ethanol. In case of extraction with water, time required for completion of one cycle was significantly more hence, with water extraction was carried out for longer time with the completion of up to seven to eight cycle through siphon mechanism. After the completion of first extraction step, residue in the thimble was again extracted twice with suitable amount of respective solvents. Filtrate of each solvent from three extraction steps were taken out and their volumes were noted

4.2.3.4. Method of Preparation of *P.pinnata* extract by soxhlet extraction

Take 4 gm of coarsely powdered drug and placed in filter paper (Whatman no1) thimble in soxhlet apparatus fitted with 250 ml of round bottom flask containing 150 ml of solvent. Extraction was performed at boiling temperature of respective solvent for 5 hr with completion of up to seven to eight cycle through siphon mechanism in case of extraction with ethanol, methanol & chloroform respectively. In case of extraction with water, time required for completion of one cycle was significantly more hence, with water extraction was carried out for longer time with the completion of up to seven to eight cycle through siphon mechanism. After the completion of first extraction step, residue in the thimble was again extracted twice with suitable amount of respective solvents. Filtrate of each solvent from three extraction steps were taken out and their volumes were noted.

4.2.3.5. Method of Preparation of *W.fruticosa* extract by soxhlet extraction

Take 4 gm of coarsely powdered drug and placed in filter paper (Whatman no1) thimble in soxhlet apparatus fitted with 250 ml of round bottom flask containing 150 ml of solvent. Extraction was performed at boiling temperature of respective solvent for 5 hr with completion of up to seven to eight cycle through siphon mechanism in case of extraction with n-hexane, acetone & chloroform respectively. In case of extraction with water, time required for completion of one cycle was significantly more hence, with water extraction was carried out for longer time with the completion of up to seven to eight cycle through siphon mechanism. After the completion of first extraction step, residue in the thimble was again extracted twice with suitable amount of respective solvents. Filtrate of each solvent from three extraction steps were taken out and their volumes were noted.

4.2.4. Phytochemical Screening of Extracts(193)(194)

The extract of different solvents was subjected to preliminary screening for analyzing the presence of metabolite present in it

The following test was carried out:-

1. **Test for Alkaloids**

- **Dragendroff's test:** Dragondroff reagent was sprayed on Whatmann no 1 filter paper and the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with dragondroff reagent with the help of capillary tube. Development of an orange red color on the paper indicated the presence of alkaloids.
- **Mayers Test:** to a little of the test filtrate taken in a watch glass a few drops of the above reagent were added. Formation of cream colored precipitate showed the presence of alkaloids.
- **Wagners Test:** When few drops of this reagent were added to the test filtrate, a brown flocculent precipitate was formed indicating the presence of alkaloids.
- **Hagers Test:** When the test filtrate was treated with this reagent an orange yellow precipitate was obtained indicating the presence of alkaloids.

2. **Test for Tannins**

- **Ferric Chloride Test:** 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. If dark green or deep blue color is obtained, tannins are present.
- **Lead Acetate Test:** A 10 % w/v solution of basic lead acetate in distilled water was added to the test filtrate. If precipitate is obtained tannins are present
- **Iodine test:** Extract was treated with diluted iodine solution separately. Appearance of transient red colour indicated the presence of tannins and phenolic compounds

3. **Test for Triterpenoids**

- **Noller test:** Extract was warmed with tin and thionyl chloride. Pink coloration indicates the presence of triterpenoids

- **Salkowaski Test:** Extract were treated in chloroform separately with few drops of concentrated sulphuric acid, shaken well and allowed to stand for some time, yellow coloured lower layer indicated the presence of triterpenoids.

4. **Test For Glycoside:**

- Legal test: To the drug, add few ml of pyridine and 2drops of nitroprusside and a drop of 20% sodium hydroxide solution. A deep red colour is produced.
- Baljet Test: Extract is treated with sodium picrate reagent yellow to orange color indicate the presence of glycoside.

5. **Test For Flavanoids:**

- **Shinoda Test:** Extract, 5ml. 95% ethanol was added separately. Each mixture was treated with 0.5g magnesium turnings and few drops of conc. HCL. Pink colour, if produced, may confirm the presence of flavonoids.

4.2.5. Preliminary screening of Parameters – factorial experiments

4.2.5.1. Solvent Selection for extraction of *C.longa* (Rz)

Selection of best suitable solvent plays s significant role in isolating a compound with maximum yield. The main criterion of solvent selection is based on the nature of phyto-constituents that give more yields when treated with respective extract. Some previously reported extraction solvent of *C.longa* rhizome extraction are acetone, ethanol & water. Thus these three solvent select for extraction purpose to get increased yield. All the extract obtained after extraction procedure were subjected to water bath to obtain its concentrate.

4.2.5.2. Solvent Selection for extraction of *A.indica (*St bk)

Selection of best suitable solvent plays s significant role in isolating a compound with maximum yield. The main criterion of solvent selection is based on the nature of phyto-constituents that give more yields when treated with respective extract. Some previously reported extraction solvent of *A.indica* St bk extraction are ethanol & water. Thus these three solvent select for extraction purpose to get increased yield. All the extract obtained after extraction procedure were subjected to water bath to obtain its concentrate.

4.2.5.3. Solvent Selection for extraction of *P.corylifolia* (Sd)

Selection of best suitable solvent plays s significant role in isolating a compound with maximum yield. The main criterion of solvent selection is based on the nature of phyto-constituents that give more yields when treated with respective extract. Some previously reported extraction solvent of *P.corylifolia* **(Sd)** extraction is petroleum ether, chloroform ethanol & water. Thus these three solvent select for extraction purpose to get increased yield. All the extract obtained after extraction procedure were subjected to water bath to obtain its concentrate.

4.2.5.4. Solvent Selection for extraction of *P.pinnata* (Sd)

Selection of best suitable solvent plays s significant role in isolating a compound with maximum yield. The main criterion of solvent selection is based on the nature of phyto-constituents that give more yields when treated with respective extract. Some previously reported extraction solvent of *P.pinnata* (Sd) extraction are ethanol, methanol Chloroform& water. Thus these three solvent select for extraction purpose to get increased yield. All the extract obtained after extraction procedure were subjected to water bath to obtain its concentrate.

4.2.5.5. Solvent Selection for extraction of *W.fruticosa* (Fl)

Selection of best suitable solvent plays s significant role in isolating a compound with maximum yield. The main criterion of solvent selection is based on the nature of phyto-constituents that give more yields when treated with respective extract. Some previously reported extraction solvent of *W.fruticosa* (Fl) extraction is n-hexane, acetone, Chloroform & water. Thus these three solvent select for extraction purpose to get increased yield. All the extract obtained after extraction procedure were subjected to water bath to obtain its concentrate.

4.3. Preformulation Study of Prepared Extract's via HPTLC Analysis(195)

HPTLC (High Performance Thin Layer Chromatography) plays an important role for the identification and characterization of drug genuinecity whether the drug is original or not. Chief active phyto-constituents plays and important role for the treatment of various dredful disease, but with the help of HPTLC analysis we cn easily detect

whether that particular actie constituents present in that drug or not. Both qualitative as well as quantitative detection of active constituents is possible through HPTLC analysis.

4.4. In Vitro Antipsoriatic activity(196)(197)(198)(199)(200)

4.4.1. Cell Culture:

The keratinocyte cell line HaCaT was obtained from NCCS, Pune Bangalore. Chemically defined Serum-free keratinocyte culture Growth medium (KGM) were provided by Gibco Co. (North Andover, MA, USA). The HaCat cells were maintained in KGM medium at 37 degree Celsius, 5 % Co2 until 70 % confluency.

4.4.2 MTT assay

The cells were treated with positive control (PMA), compound A (*A.indica* extract), B (*C.longa* extract), C (*P.pinnata* extract),D (*W.fruticosa* extract) & E (*P.corylifolia* extract) for 24 hours. The Compound A, B,C,D& E were evaluated for cytotoxic activity against HaCatkeratinocyte cell line. The cells were plated separately in 96 well plates at a cell density of 5000 cells/well and incubated at 37°C, 5 % Co2 until 70 % confluency. After 48h, cells were washed twice with 100 µL of Phosphate Buffer saline. After washing, cells were treated with the test material (Compound A, B,C, D& E) at concentration of 10ug/ml for 24 h. At the end of the 24-hour after treatment period, the medium was aspirated. The MTT was prepared in PBS (pH = 7.2) at concentration of 5mg/ml. The serum free medium KGM containing 20 ul of MTT (5 mg/mL) was added and incubated for 3 h at 37ºC in a CO_2 incubator. The MTT containing medium was then discarded and the cells were washed with PBS (200 µL). The cells were then incubated with 100 µL of DMSO for dissolving crystals. This was mixed properly by pipetting up and down. Spectrophotometric absorbance of the purple blue formazan dye was measured in a micro plate reader at 570 nm (Biorad 680). The experiment was done three times in triplicates and the average of the viability was calculated.

4.4.3 ELISA technique

The cells were treated with positive control (PMA), compound A, B, C,D& E for 24 hours and after incubation the ELISA experiment was conducted as per manufacturer kit instructions. This sandwich kit is for the accurate quantitative detection of human IL-10,IL-12 and IL-13 cytokines. These kits are solid- phase sandwich ELISA. The 96 well plates have been pre-coated with human antibody of IL-10,IL-12 and IL-13 cytokines. All the reagents, standard solutions and samples were prepared as per instruction manual. All reagents were brought to room temperature before use. The assay was performed at room temperature. 50µl of standard were added to standard well. 40µl of cells homogenate sample were added to sample wells and then add 10µl of antibody IL-10,IL-12 and IL-13 cytokines in their respective wells were added to sample wells and after that 50µl streptavidin-HRP were added to sample wells and standard wells (Not blank control well).All the reaction mixture were mixed well. The plate was covered with a sealer. The plate was incubated for 60 minutes at 37°C.Ater incubation, plate was washed for 5 times with the wash buffer, which was provided with the kit. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. After washing, 50µl of substrate solution A was added to each well and then 50µl substrate solution B were added to each well. The plate was incubated for 10 minutes at 37°C in the dark. After incubation, 50µl of stop Solution were added to each well and the blue color would change into yellow immediately. The optical density (OD value) of each well was determined immediately using a micro plate reader at 450 nm wavelength within 10 minutes after adding the stop solution.

4.4.4 LDH Assay

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalysis the inter-conversion of pyruvate and lactate. Cells release LDH into the bloodstream after tissue damage or red blood cell hemolysis. Since LDH is a fairly stable enzyme, it has been widely used to evaluate the presence of damage and toxicity of tissue and cells. Quantification of LDH has a broad range of applications.

Lactate Dehydrogenase Activity Assay Kit has been used for measuring the activity of lactate dehydrogenase (LDH) in the cell culture medium. We have used Lactate Dehydrogenase Activity Assay Kit (MAK066, Sigma Aldrich) according to manufactures instructions. In this kit, LDH reduces NAD to NADH, which was specifically detected by colorimetric (450 nm) assay. The LDH Activity Assay kit has quantifies LDH activity in cells of treated and control group. All the vials were briefly centrifuged before opening. The ultrapure water was used for the preparation of reagents. LDH Assay Buffer were allowed to come to room temperature before use. LDH Substrate Mix was reconstituted in 1 mL of water. All the reagents were mixed well by pipetting and kept at cold while in use. Substrate Mix was stable for one week at 4 °C and 1 month at –20 °C. 1.25 mM NADH Standard were reconstituted in 400 μL of water to generate 1.25 mM standard solution. All the reagents were mixed well by pipetting and kept at cold while in use. The NADH standard solution was stable for one week at 4 °C and 1 month at –20 °C. LDH Positive Control were reconstituted in 200 μL of LDH Assay Buffer. We used 5 μL of the prepared LDH Control as positive control. All samples and standards were run in duplicate. NADH Standards were added with volume of 0, 2, 4, 6, 8, and 10 μL in duplicate into a 96 well plate, generating 0 (blank), 2.5, 5, 7.5, 10, and 12.5 nmole/well standards. After that,LDH Assay Buffer were added to a final volume of 50 μL. Treated, control and Positive control cells (1×106) wererapidly homogenized on ice in 500 μL of cold LDH Assay buffer. Centrifugation at 10,000 × g for 15 minutes at 4 °C was done in order to remove insoluble material. The soluble fraction of homogenate was used for assay. 20 μL of samples were added into duplicate in 96 well plates. Then, the final volume of 50 μL was done by adding LDH Assay Buffer. After that, the reaction mixture was mixed well using a horizontal shaker or by gentle pipetting. The absorbance at 450 nm at the initial time $(A450)_{initial}$was measured. The plate was further incubated the plate at 37 °C and measurements (A450) were taken every 5 minutes until the value of the most active sample is greater than the value of the highest standard (12.5nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve. The final

measurement [$(A450)_{final}$] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve. The time of the penultimate reading is T_{final}.

4.4.5 Confocal Microscopy

The HaCat cells were maintained in KGM medium at 37 degree Celsius, 5 % Co2 until 70 % confluency and were seeded in chamber slides (Nunc,USA)at a density of 2×10^5 cells/well. The cells were treated with positive control (PMA), compound A, B, C, D& E for 24 hours. After incubation of 24 hour with treatment, the cells were fixed with 4% PFA for 15 min at room temperature, permeabilized in 0.1% Triton X100 for 5 min and blocked for 30 min in 2.5% BSA. The proteins of interest were then detected using, the caspase-3 rabbit polyclonal antibody (1:200 dilution) (apoptosis marker), followed by Alexa Fluor® 488-labelled goat anti-rabbit IgG (H + L) (1:1000 dilution, Invitrogen). The nucleus of HaCat cells were stained with 1ml of 4,6-diamidino- 2-phenylindole (DAPI) (10mg/ml) and were visualized in cells treated under blue laser. Cell images were obtained using a confocal microscope (Olympus Cell Observer with an Infinity multibeam confocal scanning head) and mean florescence intensity were analyzed and quantified using Image.

4.4.6 Statistical Analysis

Each measurement was repeated in triplicates. Results are expressed as the mean + SD. Weused one-way ANOVA, to compare the effects of various treatments with the untreated control cells. Different p-value of less than 0.05 was considered statistically significant.

4.4.7. Pre-formulation Studies

Pre-formulation studies are carried out to understand the physicochemical characteristic features of active substance and excipient before moving into various phase of formulation development.

4.4.8.Physical characterization of the Neem Extract (NE)

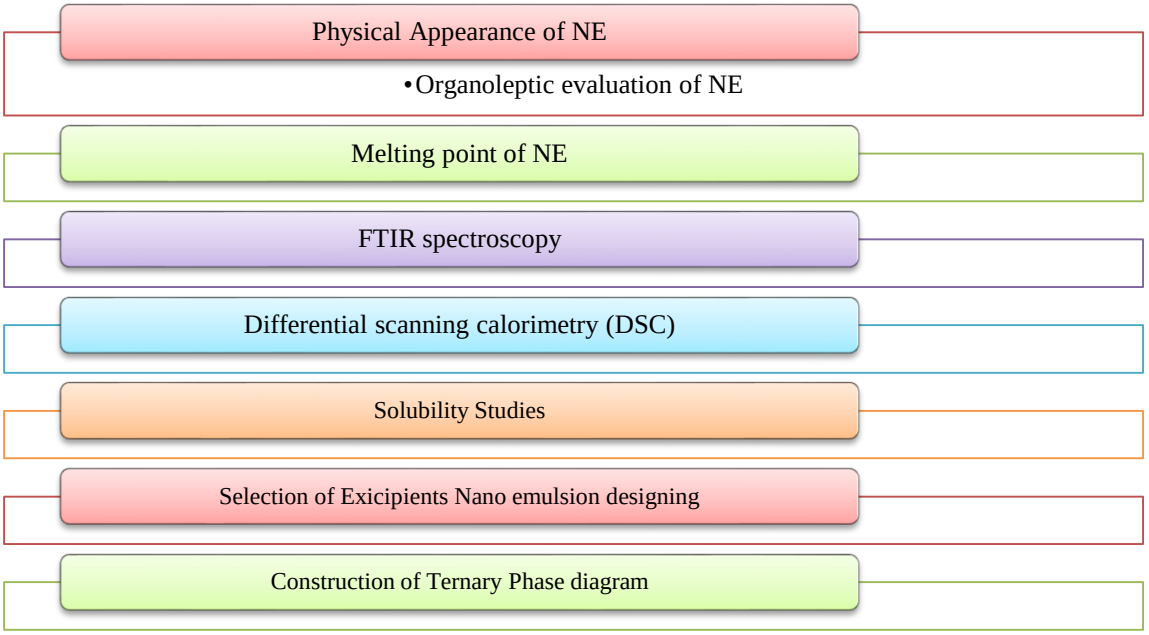


Figure 11. Physical Characterization of *A.indica* Extract

4.4.9. Physical Appearance

Neem extract (NE) was procured/generously gifted from GR Herbals, India. Physically, it was existed as free flowing powder. Sample coloration was buff-yellowish green. Powder was coherent, has tendency of take up moisture and/or frees flowing. Sample (NE) was non-viscous, non-sticky and possessed characteristic order. These features resembles the physical appearance of sample was almost identical to the certification of analysis provided by the vendor/manufacturer. Sample kept in open environment tend to absorb moisture at room temperature conditions. Sample has following details(201)

Physical state of sample: Powder form

Nature of sample: coherent, non hygroscopic

4.4.10. Organoleptic nature of sample

Sample was subjected to organoleptic characterization where its color, odor and taste were assessed. Sample coloration was greenish with slight pungency order and bitter taste.

Colour: yellowish green

Odour: characteristic aroma of pungency, bitter

Taste: bitter

4.4.11Melting point Determination

A clean capillary tube was taken and its end was sealed. Through its open end little quantity of extract was filled, so that a half of the inner space of capillary tube was filled with sample. The tube was tapped, so that the collected material was settled down. Capillary tube filled with the sample was then placed in the melting point apparatus over its base. The temperature at which sample was gradually increased and melting event of sample was noted down through thermometer and compared with literature value

4.4.12FTIR spectroscopy

FTIR spectra of sample powder were analysed using Pottasium chloride (KBr) pellet method. Dried powder sample of neem extract (approx. 10mg) and KBr (100mg) was placed in in Silica mortar, triturated uniformly and mixed. Powder sample was then uniformly placed in KBr die. By applying hydraulic pressure on the sample preparation die, a KBr pellet of sample was prepared. The pellet was then placed in a sample holding port of FTIR instrumentation (Model no.: 8400S- SHIMADZU, Japan) and allowed to run the program through software operated system. Samples spectra were scanned several times in the range of 400-4000 cm^{-1}, FTIR data of sample was recorded.(203)

4.4.13. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (Perkin Elmer Instruments, USA) was used to take thermo grams of sample and excipients. To determine the DSC of sample, the procedure was as followed. Sample (5-10mg) were placed in a DSC pan and it was crippled down so that sample placed in it. Sample filled DSC pan was placed into the sample holder of instrumentation and allowed to heat at a rate of 10°C per minute from 50°C to 350°C and data was recorded using computer program.(204)

4.4.14. Solubility of Drug/ sample

To develop a Micro-emulsion based drug delivery system, drug solubility criterion is very important since concentration gradient was developed across the system through which drug entered into the body. Generally solubility of drug was determined using equilibrium solubility method in individual surfactants, oil phases and co-solventscomponent. NE should be capable to have high solubility in each micro emulsion component for further development.

Drug solubility in different oils e.g. Arachis oil, castor oil, ceedarwood oil, eucalyptus oil, olive oil, orange peel oil, peppermint oil and palm oil, Anise oil and cardamom oil were examined Amongst various oils, drug showed highest solubility in cardamom oil (CO) as phase components. (Figure-7).The major fractions of CO were oxygenated monoterpenes (40.7–66.7%), monoterpene hydrocarbons (23.1–58.6%), and sesquiterpenes (0.1–2.0%). Among these, monoterpenoids are α-terpinyl acetate (29.9–61.3%) followed by 1,8-cineole (15.2–49.4%), α-terpineol (0.83–13.2%), β-linalool (0.44–11.0%), and sabinene (1.9–4.9%). Two sesquiterpene constituents, cardinen and nerolidol and p-cresol (a phenol derivative) were also reported. (R1)Because CO constituents can easily interact with the surfactants and penetrate the oil-water interface, it has been a good emulsification potential. Tween 20 and PEG 400 were having highest solubility for drug. Because the molecular interaction between the oil and water, or sheath of oil/surfactant provides drug embedding has been utilized in the fabrication of nanoemulsion.(205)

4.4.15. Selection of Excipients Micro emulsion designing

To determine the exact composition of Micro-emulsion different components namely oil, surfactant and co surfactant, were screened for the self- emulsification i.e. their ability to form micro-emulsion in aqueous media. The region of self-nano emulsification was assessed by visual examination with gradual addition of water to pre-concentrate. Dilution of preconcentrate mix with water resulted in formation of clear system or produce turbidity. Transparent system was examined visually until it further diluted with water and final examined for clarity. After

visual inspection, the quantity of water required to make the preconcentrate remained in transparent form was determined.

Miscibility of lipophilic components (Oil and Surfactant/co solvent mix) with aqueous phase is another aspect to be considered in the designing of micro emulsion system. The selection of surfactants/Cosurfactant in above study was mixed with selected oil. Several combinations were investigated taken dilution of different Smix and Oil. The miscibility study reveals that selected lipophilic component has tendency to produce micro emulsion region. In present work, the choice of co-surfactant and surfactant (together forming Smix) combined with oil phase will be examined to give clear dispersion or not.

4.4.16 Ternary Phase diagram

Phase diagram provides information on various types of dispersions formed when a specific combination of binary or ternary components are mixed together. It may either be existed usually in two phase or single phase region system and provide details of dispersions represented through several distinct phase boundaries. It will provide information whether a possible combination of ternary system consisted of surfactant/co-surfactant mixture, oil and water phase behaved like micelle, reverse micelle, coarse dispersion, microemulsion and liquid crystalline states. Furthermore micro-emulsion region could be existed in three separate entities like *o/w*, *w/o* or bi-continuous structures regions. To identify where exactly micro-emulsion region existed, we need to draw phase diagram between the ternary components.

Procedure: Accurately weighed quantity of surfactant and co-surfactant was taken in a beakerin the weight ratio (1:1) and blended together (S_{mix}). Similarly, several combinations containing of Smix and Oil phase were blended. To each blend, fixed weight of S_{mix} (say 400mg) and variable oil phase (weight) were taken in quantities 10, 20, 25, 30, 40, 45 and 50 mg) taken in separate 10mL beakers. Each blend was sonicated for five minute at 25°C. Each blend was titrated against distilled water (taken in the burette). Initially, little quantity of aqueous phase (20mg~20µL) was added and mixed to S_{mix}/Oil mixture and carefully

examined for the turbidity/ cloudiness formation in the beaker which may be lost within 2-3 minutes upon mixing of the components. Disappearance of clarity/ transparency of the ternary admixture or the system in the beaker or it may become slightly turbid /blushed indicate completion of titration. Weight of the aqueous phase consumed in the titration was noted down. To recheck further completion of titration, a small volume of (10mg≈ 10µL or less), accomplished using micropipette) S_{mix} to beaker which may result in loss of transparency/ clarity in the system. Specific gravity of S_{mix} and oil phase was determined using specific gravity bottle method at 25°C. Volume of aqueous phase consumed was converted into the weight basis using specific gravity of water. The amount of aqueous phase consumed, S_{mix} and oil taken in the beaker was calculated (gm). Weight of each component of ternary component viz. S_{mix}, oil phase and aqueous phase was then calculated into percentage basis by dividing the individual component weight to total weight of system followed by multiplication by 100. Pseudo ternary phase diagram was drawn between the oil phase, S_{mix} and aqueous phases on w/w basis.(206)

4.5 Formulation of *A.indica* Micro-emulsion

Neem-oil based microemulsion formulations were prepared by simple mixing method. Amount of each ternary component (on w/w basis) viz. Cardamom Oil, Smix (Tween 80/PEG 400) and aqueous phase were carefully determined from respective phase diagram previously drawn at preformulation step at specified Smix ratio. On Individual basis, each ternary component was accurately weighed, mixed to specified amount of Neem oil (20mg) using sonication and resulting mixture was transferred to screw capped container; Precaution should be taken into account and bubble formations must be avoided in the container during the transfer of Smix and the entire admixture of ternary components were subjected to 2min sonication cycle at 25C. Previously 1:2 Smix (Surfactant/ cosolvent) was prepared by taking weight ratio of surfactant and co-solvent in the ratio of 1:2 mixed using shake flask method followed by 2-min sonication cycle. Resulting mixture of ternary components was stored in the tightly closed amber coloured container and stored in the refrigerated conditions till further usage

4.5.1Experimental design for optimization of Neem Micro-emulsion

NE formulations were designed on the following basis of high & Low cardamom content (CO) content, low high smix& intermediate smix.

Table 11. Optimization of *A.indica* Micro-emulsion

Formulation Code	Cardamom Oil (mg)	Tween 80 (mg)	PEG 400 (mg)	Smix ratio	Neem extract (mg)	Aqueous phase (mg)	Criterion of selection
F1	50	170	170	11	20	20	High % of oil
F2	30	255	255	11	20	20	High Smix
F3	30	105	105	11	20	20	Low Smix
F4	30	187.5	62.5	31	20	20	High Smix
F5	30	340	170	21	20	20	High Smix
F6	30	170	170	11	20	20	Low oil
Control	30	10	…	..	20	Variable	Coarse emulsion

Rationale behind the selection of six microemulsion formulations of neem oil was quite obvious as to investigate the effect of different Smix ratio, high or low proportion of cardamom oil could affect the physical characteristics of developed microemulsion formation viz droplet size, in vitro drug release, permeation across the stratum coarneum . Since surfactant has excellent solubilisation tendency for poorly water soluble drugs, or it offers excellent solubiliation of oily liquid like neem oil or CO. Higher proportion of surfactant produces very fine

microemulsion with low droplet size. Surfactants employed in microemulsion designing for topical or transdermal systems were shown to have solubilzation of stratum corneaum and therefore it may produce skin irritation tendancy when topical microemulsion was applied. Hence lower weight fractions of smix were taken into account. Higher Smix ratio had dubious effect in the formulation designing; as it may retard the release of poorly soluble drug from microemulson base from in vitro dissolution studies data. Interestingly several reports concludes that higher smix ratio could affect the drug permeation across skin by increasing the drug flux.

4.5.1.1. Physical characterization of formulations

The characterization of the developed Neem Micro-emulsion was carried out to evaluate physicochemical properties of the formulations.

4.5.1.2. Zeta Potential

The zeta potential of Neem micro emulsion was also evaluated using zeta sizer using different cuvette. The samples were filled into omega cuvettes that were maintained at a target temperature of 25 ℃. The sample cell was diluted with water and adjusted with 200 V. Zeta potential of each sample were recorded in triplicate.

4.5.1.3. Percentage transmittance

A UV spectrophotometer was used to determine the percent transmittance (UV 1601 Shimadzu, Japan). The formulation (2.0 ml) was put in a sample quvette and the percent transmittance was measured at 250 nm.(207)

4.5.1.4. Refractive index

Refractive index of the micro-emulsion formation is a measure ofangle of refraction of white light passed into and out of the microemulsion system under study. It provides information on the nature of the microemulsion system; particularly in phase behavioural phenomena of microemulsion system involving aqueous phase. Different ternary components were mixed together to yield six microemulsion formulations had vary fraction of each component. The refractive

index of the final optimized *A.india* microemulsion was evaluated with the help of Abbe's refractometer at 25±0.5°C. A drop of *A.indica* micro-emulsion was placed on the prism and refractive index of *A.indica* micro-emulsion noted from the refractive scale.(208)

4.5.1.5. Electrical Conductivity

Electrical conductivity of Prepared *A.indica* micro-emulsion was measured with the help of conductivity meter by using conductivity cell by using with a cell constant of 1.0 consisting of two platinum plates separated by a variable distance contains liquid between them act as conductor. Clean and swipe the conductivity electrodes before calibration (using cell constant value). Place the formulation (3ml) into separate 10 ml beaker and dip the electrode into container(209)

4.5.1.6. Transmission Electron Microscopy

Morphological analysis of the dispersed oils globules was carried out using TEM. It was operated at an accelerating voltage of 200 kV with a resolution of 0.194 nm (point) and magnification of 1500000. Optimized Neem micro-emulsion (100µL) was diluted with 100 mL of millipore water and a drop of the diluted sample was placed on the carbon coated copper grid and allows standing for one minute. The, the excess amount of sample was removed to perform negative staining with 2% w/v of phosphotungstic acid. The grid was air dried and thin sample specimen was analyzed and images were recorded.

4.6. Evaluation of Formulations

4.6.1.*In vitro* drug release studies

Drug release characterization is very critical as Smix and oil play very critical region since sheath around oil where drug is embedded will release very slowly and hence produced poor release behavior. *In Vitro* diffusion studies of *A.indica* - microemulsion were performed by using a vertical Franz diffusion cell having (area 3.4618 cm^2) with a cellulose membrane and a thermostatic water bath system attaché with it. The dialysis membrane -110 having molecular weight cutoff value of 12,000- 14,000 Dalton with pore size of 2.4 nm was used. The membrane was washed thoroughly in running water. Then the membrane was soaked in diffusion medium which comprise of 1:1 ratio of mixed phosphate buffer (pH 7.0) absolute ethanol for 30 h. The receptor compartment was filled with diffusion medium

which was maintained at a temperature of 32℃ ± 0.5℃ with a stirring speed of 550 rpm using a magnetic stirrer. The dialysis membrane was placed carefully in between the receptor compartment and donor compartment. The samples were placed onto the donor compartment and aliquots were periodically withdrawn at suitable time interval of 40, 80, 120, 160, 200, 240. During each withdrawal, a fresh volume of diffusion medium was replaced into the receptor compartment to maintain sink condition. Then each aliquot was subjected to HPLC analysis.

4.6.2. Collection and Preparation of Skin Specimen

Porcine ear skin was collected from slaughterhouse and cleaned thoroughly under running water. The hair on the skin was removed using Veet hair removing cream. The dorsal ear skin was carefully separated from cartilage using sterile surgical blade. The isolated skin samples were cleaned with cotton pads that are soaked with 0.9% w/v of sodium chloride solution. Then samples were dried using cloth, packed in an aluminum foil and stored at -20℃ until use but not longer than a month.

4.6.3. Ex vivo skin permeation studies

The *ex vivo* permeation studies were carried out in the porcine ear skin having a surface area of

2.54cm^2, prior to the experimentation, the skin was soaked in the mixed phosphate buffer (pH7.2): ethanol (1:1) for 1 h. Then, the skin was carefully placed in between the cells of the Franz diffusion cell.The outer skin surface faces the donor compartment and inner skin surface rest onthe receptor compartment, which is in contact with the diffusion medium. The medium was gently stirred at 550 rpm and the temperature was maintained at 32±1°C using a thermo statically controlled magnetic stirrer. The samples were placed in the donor compartment.An aliquot of 1 mL was withdrawn from the receptor compartment at regular time intervals of 30, 60, 120, 180, 240, 360, 480, 720, 1200, 1440 h and during each withdrawal, an equal volume of thefresh medium was replaced. The amount of neem micro-emulsion permeated at each selected time was quantifiedusing the developed HPLC method. The cumulative amount of drug permeated through the skin per unit area was calculated.

4.6.4. Stability Studies

Stability study was carried out in order to assess the effect of formulation components on the stability of neem extract in the optimized micro-emulsion formulation i.e. (*F3)* Effect of ternary components might affect the stability of Neem extract (nimolidine). Besides the physiochemical stability of drug, micro-emulsion parameters were also evaluated under accelerated conditions of temperature and humidity. These studies were designed in order to follow the guidelines furnished by ICH (Q) which involve the stability testing and accelerated stability testing of drug for the determination of shelf life of optimized formulations.

4.6.5. Pharmacodynamic Studies(210)

After characterization of the optimized AGE NANE, pharmacodynamics studies were performed to evaluate the functional response and dose-response effect on suitable experimental model. In this research, anti-angiogenic activity was carried out in Hen's egg test-chorioallantoic membrane (HET-CAM) model, acute dermal toxicity was carried out in SD rats and anti-psoriasis activity was carried out in IMQ induced mouse model.

4.6.5.1. Animals

Experimental animals were used after the approval of experimental protocol (IAEC/KSOP/2022-23/05) from the Institutional Animal Ethics Committee (IAEC) of KIET School of Pharmacy, Ghaziabad (UP), India (Reg. No.: CPCSEA Reg No.1099/PO/RE/S/07/CPCSEA). Forty-two albino mice (25-30 g) were used in the study. Animals were housed under standard room temperature and relative humidity (23 ± 2°C, 60 ± 5%) with a 12 h light and dark cycle.

4.6.5.2. Drug Treatment Schedule

Mice were randomly divided into seven groups of 06 mice per group: (1) Normal control; (2) IMQ-induced psoriasis; (3) Psoriasis + micro-emulsion of neem (low dose [LD]) (0.05%); (4) Psoriasis + micro-emulsion of neem (high dose [HD]) (0.1%); (5) Psoriasis + neem extract (1%); (6) Psoriasis +standard drug i.e., Tazorac Gel (Tazarotene 0.05%); (7) Psoriasis + Placebo (i.e. cardamom oil, 1%); Doses were selected on the basis of our earlier laboratory studies and other

published reports. Animals were treated for once daily for 14 days after induction of psoriasis. The entire study design has been shown below (Table 1).

4.6.5.3. Psoriasis induction

Swiss albino mice were divided into 7 groups, six mice in each group. The hairs of the dorsal back surface of the mice were removed using hair removing cream. Initial body weight of all the mice was noted. Now lesion was developed by applying a daily dose of 62.5 mg of 5 %w/w IMQ cream. The dosing was done in the morning, except group I (i.e Normal control) and remaining all the group were exposed for the development of psoriatic lesion. The selected dosing was applied to the shaved dorsal skin (50mg of IMQ cream) for seven consecutive days.(211) The detailed of the dosing is mentioned below in the table below

Table 12. Grouping of Animal and Dosing Schedule

Sr.No	Group	Dose Schedule	No of animals
1.	Normal Control	No treatment applied	6
2.	Experimental Control	5% imiquimod topical application induced psoriasis	6
3.	Extract Treated	Psoriasis + Extract	6
4.	Vehicle Treated	Psoriasis + Vehicle	6
5.	*A.indica* micro-emulsion LD	Psoriasis + Micro-emulsion (LD)	6
6.	*A.indica* micro-emulsion HD	Psoriasis + Micro-emulsion (HD)	6
7.	Standard Group	Psoriasis + Standard drug	6

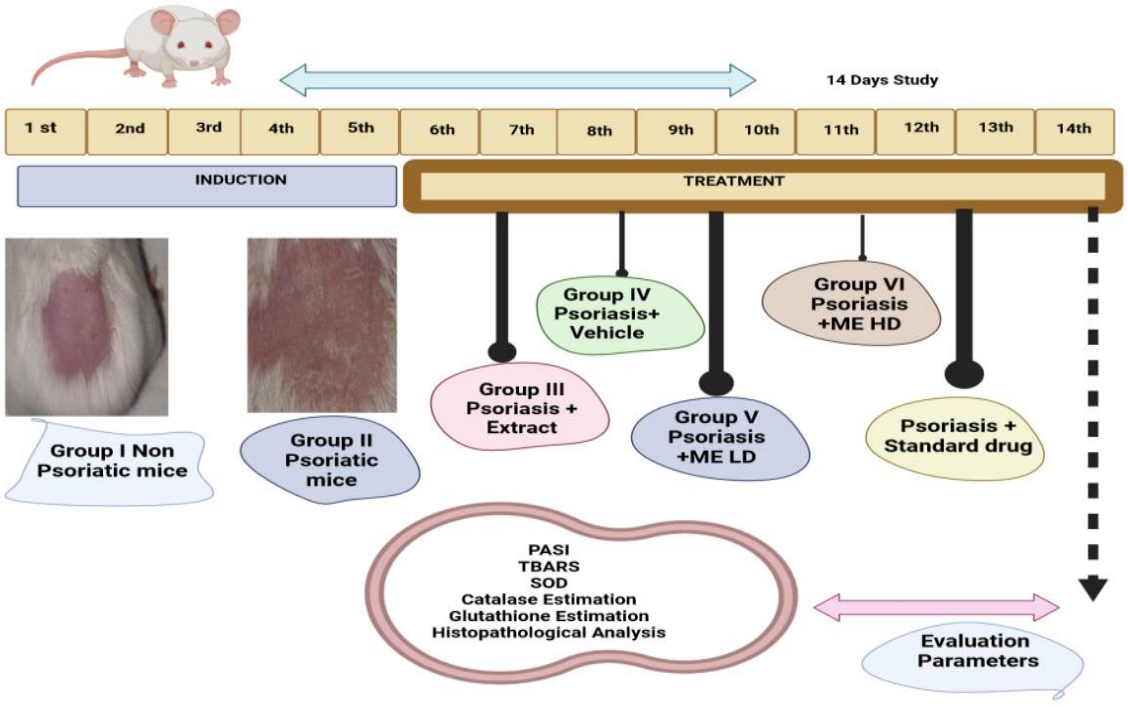


Figure 12. Graphically representation of Study

4.6.5.4.Dissection and homogenization(212)

After completion of treatment, blood samples were collected through retro-orbital route. Serum was separated and frozen at -20°C and used for the estimationsof inflammatory markers (IL-17, IL-23) and estimation of oxidative stress and antioxidant enzymes.The animals were sacrificed for the histo pathological examination and Spleen weight/body weight estimation.

4.6.5.5. Spleen to Body Weight Index

After treatment period was over, the boy weight of mice were noted. After euthanasia the spleen was collected, cleaned thoroughly with PBS (pH7.4) and weighed accurately. After that spleen to body weight is calculated.

4.6.5.6. PASI assessment (220)

The severity of skin inflammation was evaluated by the PASI score. The scoring tool included parameters like erythema, and roughness. The scale used in the study is written below:

0, none; 1, slight; 2, moderate; 3, marked; and 4, Severe

The PASI scoring was recorded in the first day before induction of Psoriasis and on third day, sixth day (the induction of psoriasis, followed by ninth day, twelfth day and fourteenth day treatment. The percentage in PASI scoring reduction after treatment was compared with scoring before treatment begun.

4.6.5.7. Tissue homogenate

The animals were anaesthetized with light ether. The skin tissues were removed quickly, rinsed in ice cold saline and dried on filter paper for biochemical estimations and for histopathology, tissues were stored in 10 % formalin solution. Homogenate (10 % w/v) was prepared for biochemical estimations using a Teflon tissue homogenizer.(213)

4.6.5.8. Estimation of inflammatory markers

Inflammatory markers viz. IL-17, IL-23, IFN-α and TNF-α levels were measured in the serum by using commercially available ELISA kits (G-Bioscience, St. Louis, Mo, USA). These levels were estimated through ELISA reader using manufacturer's protocol. A 100 µl aliquot of each serially diluted standard sample or supernatant sample was added into the microplate and incubated at 37 °C for 1 h. The plate was supplemented with 100 µl 1x antibody solution against IFN-α, TNF-α, IL-17 or IL-23 in each well and incubated at 37 °C for another 1 h. The plate was then treated with 100 µl horseradish peroxidase-linked secondary antibody for incubation at 37 °C for 30 min. The plate was washed with 100 µlPBS with Tween 20 (PBST) three times before the last incubation. Finally, the plate was incubated with 100 µl substrate in the dark for 15 min; the specific binding optical density was assessed immediately at 450 nm with a spectrophotometer.(214)

4.6.5.9. Measurement of lipid peroxidation (TBARS)

The extent of lipid peroxidation in the tissue homogenate was determined by measuring MDA content which represents the level of thiobarbituric acid reactive substance (TBARS) and concentration is expressed in as nmol MDA eq./g of the tissue at 37°C. For the estimation of MDA a reaction mixture containing 1mL of tissue homogenate, 1mL trichloroacetic acid (10%) and 1mL of thiobariburic acid (0.67%) were mixed together and incubated in boing water for 45 min. After completion of time the reaction mixture was cooled and centrifuged at 10000 g for 10 min. The supernatant obtained was extracted with 4mL of n-butanol. The pink colored n-butanol layer was separated and absorbance was measured at 532 nm. The final concentration was determined from the standard calibration curve. The measurement of TBARS indicated the extended of lipid peroxidation.

4.6.5.10. Catalase estimation

A measured volume of tissue homogenate supernatant (20µL) was added to 1 mL of hydrogen peroxide solution (10mM) into the cuvette. The reduction in the optical density of this reaction mixture was measured at 240 nm. The rate of decrease in the optical density after addition of tissue homogenate was noted from the time time of addition till the completion of reaction. Approximately 3 min was considered as an indicator of CAT activity present in the tissue samples. The final concentration of catalase was determined by using a standard calibration curve and concentration was expressed in moL/min/mL.

4.6.5.11. Glutathione estimation

The estimation of GSH content in the tissue homogenate was determined using Ellman's reagent which is chemically 5,5-dithio-bis (2-nitrobenzoic acid). The tissue homogenate (20µL) was treated with 1 mM DTNB solution (180µL) at room temperature which resulted in yellow coloration. The optical density of the reaction mixture was measured at 412 nm and concentration GSH was expressed as µg/mg of protein. The final concentration was determined by using standard calibration curve.

4.6.5.12. Superoxide dismutase (SOD)

The supernatant obtained after homogenization was added into the mixture containing 500 mM of sodium carbonate solution (20µL), 0.3% Triton X-100 (2 mL), 1.0 mM of ethylenediamine tetra acetic acid (20µL), 10 mM of nitro blue tetrazolium (240µM) was added and the optical density of the mixture was measured at 560 nm for up to 3 min with a time interval of 1 minute. The rate of increase in the measured optical density was considered as the SOD activity. The final concentration of SOD determined using a standard calibration curve was expressed in U/mL.

4.6.5.13. Histopathological analysis

After completion of the treatment period, mice were taken and anaesthetized in a glass jar containing cotton previously soaked with diethyl ether, and euthanized by cervical dislocation. After that dorsal back skin were removed immediately and rinse with normal saline for evaluation of pathological changes in skin. Place this skin specimen in 10% formalin. The samples were now stained with hematoxylin and eosin followed by mounted on the glass slides. Now observed these glass slides under microscope at a total magnification of 100 x and 400 x.

Photomicrograph of the dorsal skin were captured by using 100 x microscopic magnification and measured at 100 µm scale.

4.6.5.14. Statistical analysis of data

Graph Pad Prism (Graph Pad Software, San Diego, CA) was used for all statistical analysis. One specific group of twelve (n = 6) animals was assigned to a specific drug treatment. All the values were expressed as mean ± SEM. The data was analyzed using one way analysis of variance (ANOVA) and two way ANOVA followed by Tukey's test. In all the tests, criterion for statistical significance was $P<0.05$.

Chapter- 5

Results and Discussion

5.1 Procurement of Herbal Drugs

All the herbal drugs were obtained as a gift sample from Shree Dhanwantri Herbals AmritsarPunjab

5.2 Authentication of Herbal Drugs:

Authentication of herbal drugs was done from Shree Guru Nanak Dev University Amritsar Punjab having vouchered no 490.

Figure 13. (A) Rhizome of *C.longa*, (B) Seed of *P.pinnata*(3) Seed of *P.corylifolia*(4) Flower of *W.fruticosa* (5) St.bark of *A.indica*.

5.3 Physicochemical Evauation Herbal drugs

The physicochemical parameters were evaluated to ensure the quality of raw herb, which has to be used further for extraction of active constituents.

Table 13. Physicochemical evaluation of coarsely powdered *C.longa* rhizome(215). Degradation of plant material directly related to amount of moisture present in that material. Excess content of water leads to fungus growth in that plant material. The loss on drying of c.longa rhizome is 1.78 %. Total ash parameter clarifies the inorganic material present in the plant part. Analytical testing shows total ash value of *C.longa* was 6.18 %. The parameter of acid insoluble ash determines the siliceous matter present in plant material i.e 0.55%. The extractive value shows the extraction that particular drug in different solvent medium i.e water and alcohol. The water soluble extractive value is 54.86 % while alcohol soluble extractive value is 14.92 %. The presence of heavy metal in *C.longa* is not detected in the rhizome of *C.longa* sample.

Sr.No	**Parameters**	**Observation (%w/w) Avg Mean of triplicate analysis**	**API Limits**
1.	Foreign matter	Nil	NMT 2%
2.	Loss on drying	1.78	----
3.	Total ash	6.81	NMT 9 %
4.	Acid insoluble ash	0.55	NMT 1%
5.	Water soluble extractive value	54.86	NLT 12 %
6.	Alcohol soluble extractive value	14.92	NLT 8%
7.	Heavy Metal Analysis		
8.	Lead (Pb)	Not detected	NMT 10.0 ppm
9.	Cadmium (Cd)	Not detected	NMT 3.0 ppm
10.	Mercury (Hg)	Not detected	NMT 1.0 ppm
11.	Arsenic (As)	Not detected	NMT 3.0 ppm

Table 14. Physicochemical evaluation of *P.pinnata* (Sd)**(215).** Degradation of plant material directly related to amount of moisture present in that material. Excess content of water leads to fungus growth in that plant material. The loss on drying of *P.pinnata* seed is 1.95 %. Total ash parameter clarifies the inorganic material present in the plant part. Analytical testing shows total ash value of *P.pinnata* was 7.15 %. The parameter of acid insoluble ash determines the siliceous matter present in plant material i.e 0.69%. The extractive value shows the extraction that particular drug in different solvent medium i.e water and alcohol. The water soluble extractive value is 53.34 % while alcohol soluble extractive value is 10.64 %. The presence of heavy metal in *P.pinnata* is not detected in the seed of *P.pinnata* sample.

Sr.No	**Parameters**	**Observation (%w/w) Avg Mean of triplicate analysis**	**API Limits**
1.	Foreign matter	Nil	NMT 3%
2.	Loss on drying	1.95	-----
3.	Total ash	7.153	NMT 11%
4.	Acid insoluble ash	0.693	NMT 2%
5.	Water soluble extractive value	53.34	NLT 17%
6.	Alcohol soluble extractive value	10.643	NLT 3.5 %
7.	Heavy Metal Analysis		
8.	Lead (Pb)	Not detected	NMT 10.0 ppm
9.	Cadmium (Cd)	Not detected	NMT 3.0 ppm
10.	Mercury (Hg)	Not detected	NMT 1.0 ppm
11.	Arsenic (As)	Not detected	NMT 3.0 ppm

Table 15. Physicochemical evaluation of *P.corylifolia* (Sd)**(215).** Degradation of plant material directly related to amount of moisture present in that material. Excess content of water leads to fungus growth in that plant material. The loss on drying of *P.corylifolia* seed is 1.54 %. Total ash parameter clarifies the inorganic material present in the plant part. Analytical testing shows total ash value of *P.corylifolia* was 5.88 %. The parameter

of acid insoluble ash determines the siliceous matter present in plant material i.e 0.57%. The extractive value shows the extraction that particular drug in different solvent medium i.e water and alcohol. The water soluble extractive value is 65.943 % while alcohol soluble extractive value is 59.18 %. The presence of heavy metal in *P.corylifolia* is not detected in the seed of *P.corylifolia* sample.

Sr.No	**Parameters**	**Observation (%w/w) Avg Mean of triplicate analysis**	**API Limits**
1.	Foreign matter	Nil	NMT 1 %
2.	Loss on drying	1.54	---
3.	Total ash	5.88	NMT 8 %
4.	Acid insoluble ash	0.576	NLT 13 %
5.	Water soluble extractive value	65.943	NLT 11 %
6.	Alcohol soluble extractive value	59.18	NLT 13 %
7.	Heavy Metal Analysis		
8.	Lead (Pb)	Not detected	NMT 10.0 ppm
9.	Cadmium (Cd)	Not detected	NMT 3.0 ppm
10.	Mercury (Hg)	Not detected	NMT 1.0 ppm
11.	Arsenic (As)	Not detected	NMT 3.0 ppm

Table 16. Physicochemical evaluation of *W.fruticosa* (Fl)**(215).**Degradation of plant material directly related to amount of moisture present in that material. Excess content of water leads to fungus growth in that plant material. The loss on drying of *W.fruticosa* Fl is 1.85 %. Total ash parameter clarifies the inorganic material present in the plant part. Analytical testing shows total ash value of *W.fruticosa* was 8.64 %. The parameter of acid insoluble ash determines the siliceous matter present in plant material i.e 0.80 %. The extractive value shows the extraction that particular drug in different solvent medium i.e water and alcohol. The water soluble extractive value is 53.27 % while

alcohol soluble extractive value is 54.79 %. The presence of heavy metal in *W.fruticosa* is not detected in the Fl of *W.fruticosa* sample.

Sr.No	Parameters	Observation (%w/w) Avg Mean of triplicate analysis	API Limits
1.	Foreign matter	Nil	NMT 1 %
2.	Loss on drying	1.85	---
3.	Total ash	8.64	NMT 10 %
4.	Acid insoluble ash	0.80	NMT 1 %
5.	Water soluble extractive value	53.27	NLT 28 %
6.	Alcohol soluble extractive value	54.79	NLT 7 %
7.	Heavy Metal Analysis		
8.	Lead (Pb)	Not detected	NMT 10.0 ppm
9.	Cadmium (Cd)	Not detected	NMT 3.0 ppm
10.	Mercury (Hg)	Not detected	NMT 1.0 ppm
11.	Arsenic (As)	Not detected	NMT 3.0 ppm

Table 17. Physicochemical evaluation of *A.indica* (St.bk)**(215).** Degradation of plant material directly related to amount of moisture present in that material. Excess content of water leads to fungus growth in that plant material. The loss on drying of *A.indica* St.bk is 1.91 %. Total ash parameter clarifies the inorganic material present in the plant part. Analytical testing shows total ash value of *A.indica* was 6.33 %. The parameter of acid insoluble ash determines the siliceous matter present in plant material i.e 0.46 %. The extractive value shows the extraction that particular drug in different solvent medium i.e water and alcohol. The water soluble extractive value is 55.71 % while alcohol soluble extractive value is 37.01 %. The presence of heavy metal in *A.indica* is not detected in the St.Bk of *A.indica* sample

Sr.No	Parameters	Observation (%w/w) Avg Mean of triplicate analysis	API Limits
1.	Foreign matter	Nil	NMT 2%
2.	Loss on drying	1.91	---
3.	Total ash	6.33	NMT 7%
4.	Acid insoluble ash	0.46	NMT 1.5%
5.	Water soluble extractive value	55.71	NLT 5%
6.	Alcohol soluble extractive value	37.01	NLT 6%
7.	Heavy Metal Analysis		
8.	Lead (Pb)	Not detected	NMT 10.0 ppm
9.	Cadmium (Cd)	Not detected	NMT 3.0 ppm
10.	Mercury (Hg)	Not detected	NMT 1.0 ppm
11.	Arsenic (As)	Not detected	NMT 3.0 ppm

The physicochemical parameter is mainly evaluated to check the quality of drug by comparing it with monograph mentioned in API. All the parameters are within the standard mentioned in API.

5.4 Preparation of Extract

Extraction of the all fivedrugs is done via using soxhlet extraction technique with different different solvent to maximize the percentage yield of extract as well as active phyto-components present in them.

5.4.1 Preparation of *C.longa*(Rz) extract

Extraction of *C.longa* was done by following the method given by Singh I, et al, 2019

Soxhlet Extraction of *C. longa* Rhizome

Figure 14. Extraction of *C.longa*

5.4.1.1Extraction yield of *C.longa* extract by using different solvents

Table 18. Extraction yield with different solvents

Name of Drug	**Solvent**			
	Acetone	**Ethanol**	**Water**	**Mean**
Curcuma longa	18.41±0.2	22.27±0.1	24.17±0.1	21.61±0.3

5.4.2. Preparation of *P.pinnata* (Sd) extract

Extraction of *P.pinnata* was done by following the method given by D.M.Madhuri, et al, 2018

Soxhlet Extraction of *P.pinnata* Seed

Figure 15 . Extraction of *P.pinnata (Sd)*

5.4.2.1. Extraction yield of *P.pinnata* extract by using different solvents

Table 19. Extraction yield with different solvents

Name of Drug	Solvent				
	Ethanol	**Methanol**	**Chlorofom**	**Water**	**Mean**
Pongamia pinnata	26.41±0.12	29.10±0.3	24.21±0.2	46.20±0.25	31.48±0.5

5.4.3. Preparation of *P.corylifolia* (Sd) extract

Extraction of *P.corylifolia* was done by following the method given by Abey Sekara , et al, 2012

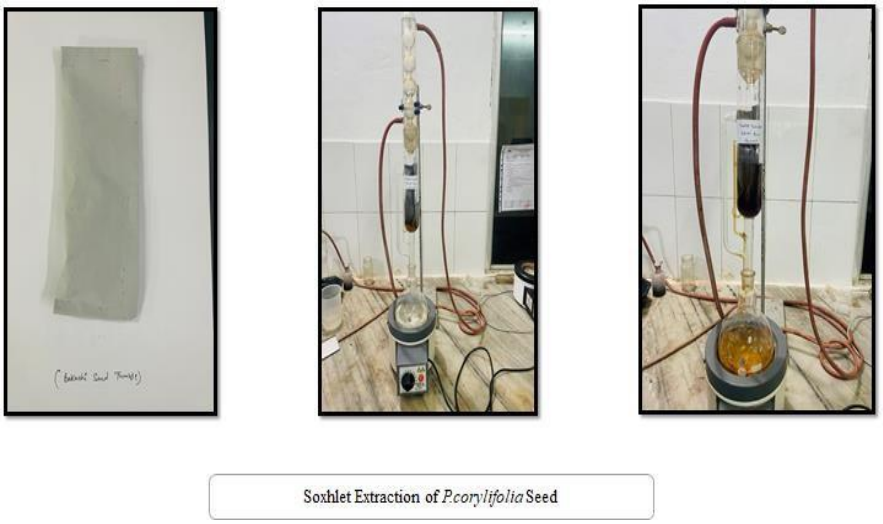


Figure 16. Extraction of *P.corylifolia (Sd)*

5.4.3.1. Extraction yield of *P.corylifolia* extract by using different solvents

Table 20. Extraction yield with different solvents

Name of Drug	Solvent			
	Petroleum Ether	**Chloroform**	**Water**	**Mean**
Psoraliya corylifolia	22.13±0.23	30.96±0.1	42.10 ±0.31	31.73±0.17

5.4.4. Preparation of *W.fruticosa* (Fl) extract

Extraction of *W.fruticosa* was done by following the method given by A.Finose, et al, 2011

Soxhlet Extraction of *W.fruticosa* Flower

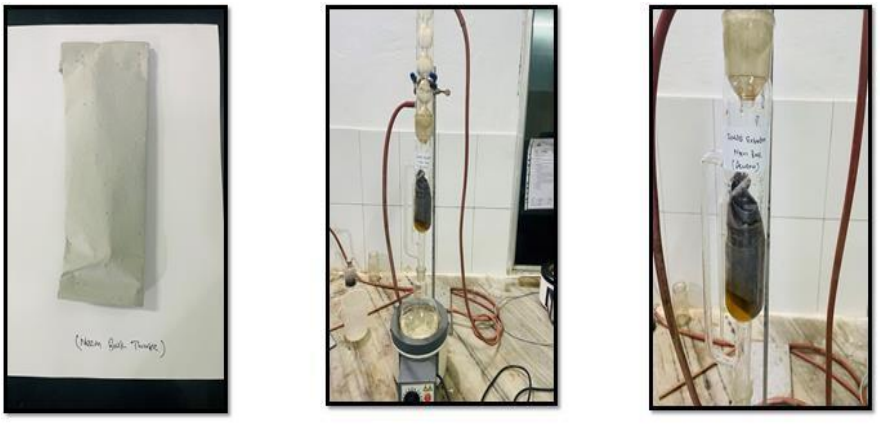
Figure 17. Extraction of *W.fruticosa (Fl)*

5.4.4.1 Extraction of W.fruticosa extract by using different Solvents

Table21. Extraction yield with different solvents

Name of Drug	Solvent				
	n-hexane	**Acetone**	**Chloroform**	**Water**	**Mean**
W.fruticosa	23.21±0.15	24.92±0.12	22.41±0.2	42.10±0.29	28.16±0.2

5.4.5. Preparation of *A.indica* (St.bk) extract



Soxhlet Extraction of *A.indica* Bark

Figure 18. Extraction of *A.indica (St bk)*

5.4.5.1 Extraction yield of *A.indica* extract by using different solvents

Table 22. Extraction yield with different solvents

Name of Drug	Solvent		
	Ethanol	**Water**	**Mean**
Azadirachta indica	18.56±0.31	29.10±0.27	28.95±0.2

5.5. Phytochemcial analysis of herbal extract

The obtained extract was further subjected to phytochemical screening for the detection of secondary metabolites present in them. The result of phytochemical screening are mentioned below in table

5.5.1. Phytochemcial analysis of herbal extract

The obtained extract was further subjected to phytochemical screening for the detection of secondary metabolites present in them. The result of phytochemical screening are mentioned below in table

5.5.1.1. Phytochemical analysis of *C.longa* extract

Table 23: Phytochemical Analysis of *C.longa* extract

Drug Name	Chemical Constituents	Type of Extract	Inference
C.longa	Alkaloids		
	Mayer	Acetone	+
	Dragondroff	Ethanol	+
	Hagers	Water	+
	Wagner		
	Tannins		
	5% Fecl3 Soln	Acetone	_
	Lead acetate	Ethanol	+
	Dilute iodine	Water	+
	Triterpenoids	Acetone	-

	Nollers Test Salkowaski Test	Ethanol Water	+ +
	Glycosides Legal Test Baljet Test	Acetone Ethanol Water	+ + +
	Flavanoids Shinoda Test	Acetone Ethanol Water	+ + +
	Carbohydrates Molish Test Fehling Test	Acetone Ethanol Water	+ + +
	Test For Proteins Biuret's Test	Acetone Ethanol Water	- + +

5.5.1.2 Phytochemical analysisof *P.pinnata* extract

Table 24. Phytochemical Analysis of *P.pinnata* extract

Drug Name	Chemical Constituents	Type of Extract	Inference
P.pinnata	Alkaloids Mayer Dragondroff Hagers Wagner	 Acetone Ethanol Water	 + + +
	Tannins 5% Fecl3 Soln Lead acetate Dilute iodine	 Acetone Ethanol Water	 + + +
	Triterpenoids Nollers Test	Acetone Ethanol	- -

	Salkowaski Test	Water	+
	Glycosides Legal Test Baljet Test	Acetone Ethanol Water	+ + +
	Flavanoids Shinoda Test	Acetone Ethanol Water	+ + +
	Carbohydrates Molish Test Fehling Test	Acetone Ethanol Water	+ + +
	Test For Proteins Biuret's Test	Acetone Ethanol Water	- + +

5.5.1.3. Phytochemical analysisof *P. corylifolia* extract

Table 25. Phytochemical Analysis of *P. corylifolia* extract

Drug Name	Chemical Constituents	Type of Extract	Inference
P.corylifolia	Alkaloids Mayer Dragondroff Hagers Wagner	 Acetone Ethanol Water	 + + +
	Tannins 5% Fecl3 Soln Lead acetate Dilute iodine	 Acetone Ethanol Water	 - + +
	Triterpenoids Nollers Test Salkowaski Test	Acetone Ethanol Water	- + +
	Glycosides Legal Test	Acetone Ethanol	+ +

	Baljet Test	Water	+
	Flavanoids Shinoda Test	Acetone Ethanol Water	+ + +
	Carbohydrates Molish Test Fehling Test	Acetone Ethanol Water	+ + +
	Test For Proteins Biuret's Test	Acetone Ethanol Water	- + -

5.5.1.4. Phytochemical analysisof *W.fruticosa* extract

Table 26. Phytochemical Analysis of *W.fruticosa* extract

Drug Name	Chemical Constituents	Type of Extract	Inference
W.fruticosa	Alkaloids Mayer Dragondroff Hagers Wagner	 Acetone Ethanol Water	 + + +
	Tannins 5% Fecl3 Soln Lead acetate Dilute iodine	 Acetone Ethanol Water	 + + +
	Triterpenoids Nollers Test Salkowaski Test	Acetone Ethanol Water	- + +
	Glycosides Legal Test Baljet Test	Acetone Ethanol Water	- + +

	Flavanoids Shinoda Test	Acetone Ethanol Water	+ + +
	Carbohydrates Molish Test Fehling Test	Acetone Ethanol Water	+ + +
	Test For Proteins Biuret's Test	Acetone Ethanol Water	+ + +

5.5.1.5. Phytochemical analysisof *A.indica* extract

Table 27. Phytochemical Analysis of *A.indica* extract

Drug Name	Chemical Constituents	Type of Extract	Inference
A.indica	Alkaloids Mayer Dragondroff Hagers Wagner	 Acetone Ethanol Water	 + + +
	Tannins 5% Fecl3 Soln Lead acetate Dilute iodine	 Acetone Ethanol Water	 + + +
	Triterpenoids Nollers Test Salkowaski Test	Acetone Ethanol Water	- + -
	Glycosides Legal Test Baljet Test	Acetone Ethanol Water	+ + +
	Flavanoids	Acetone	-

	Shinoda Test	Ethanol Water	+ +
	Carbohydrates Molish Test Fehling Test	Acetone Ethanol Water	- + +
	Test For Proteins Biuret's Test	Acetone Ethanol Water	- + +

Note: (+) indicates the presence of compound

(-) indicates the absence of compound

The phytochemical screening of extracts in different solvents has confirmed the presence of Alkaloids, tannins, triterpenoids, glycosides, flavonoids, carbohydrates & protein s respectively.

5.6. HPTLC Analysis of *C.longa, A.indica, P.pinnata, P.corylifolia, W.fruticosa*

5.6.1. HPTLC Analysis of *C.longa*

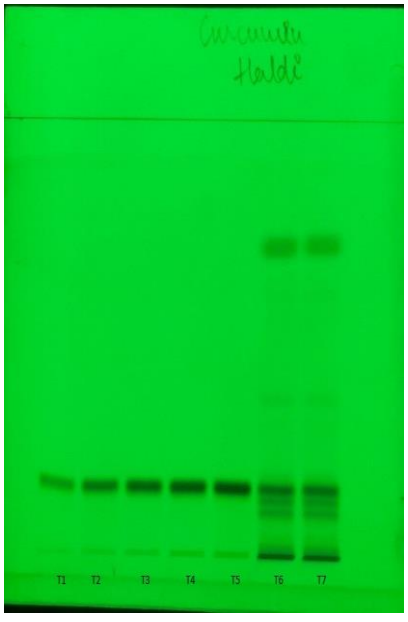


Figure 19.TLC Plate at 254 nm

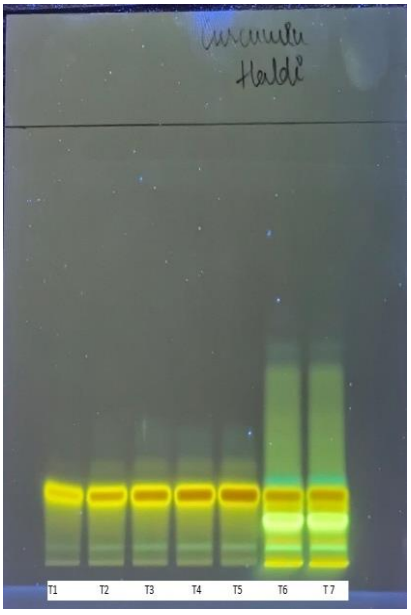


Figure 20 TLC Plate at 366 nm

5.6.1.1. Chromatogram of Standard i.e Curcumin

Track 1 Std Curcumin

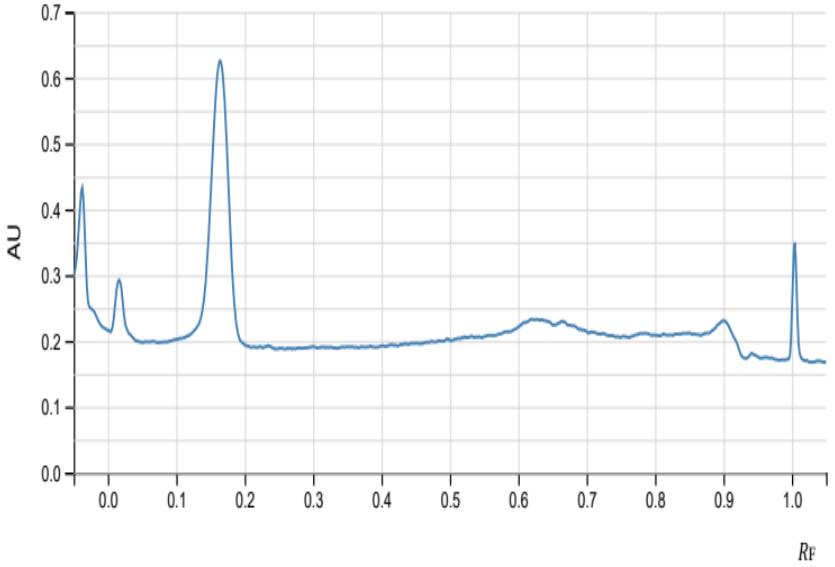


Figure 21 . Chromatogram of curcumin

5.6.1.2. Chromatogram of *C.longa* extract

Track 6 *C.longa* Extract

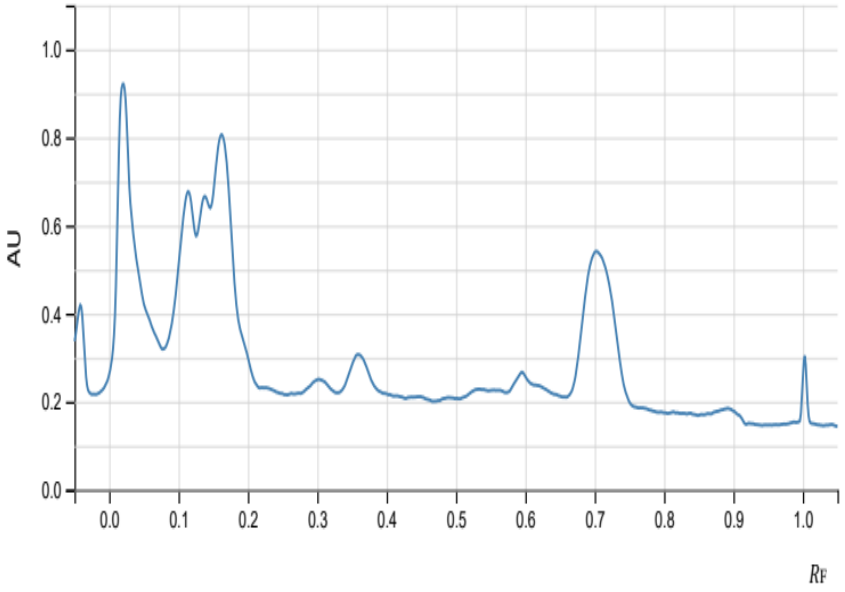


Figure 22. Chromatogram of *C.longa* extract

Table 28.HPTLC Peak table of Standard i.e Curcumin

Track 1	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.122	0.00	0.164	0.424	0.208	0.00	0.012	100.0	No	Curcumin
	0.123	0.00	0.165	0.064	0.210	0.081	0.014	100.0	No	
	0.122	0.00	0.160	0.562	0.199	0.003	0.0169	100.0	No	
	0.122	0.00	0.166	0.064	0.209	0.284	0.013	100.0	No	
	0.124	0.00	0.167	0.429	0.211	0.109	0.012	100.0	No	

Table 29.HPTLC Peak table of Sample i.e *C.longa* extract

Track 6	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.136	0.129	0.164	0.377	0.208	0.00	0.011	100.0	No	*C.Longa*
	0.139	0.136	0.167	0.378	0.211	0.00	0.011	100.0	No	
	0.124	0.00	0.164	0.409	0.190	0.00	0.012	100	No	
	0.126	0.00	0.167	0.406	0.193	0.00	0.012	100	No	
	0.164	0.00	0.185	0.213	0.226	0.00	0.006	100	No	

5.6.2. HPTLC Analysis of *A.indica*

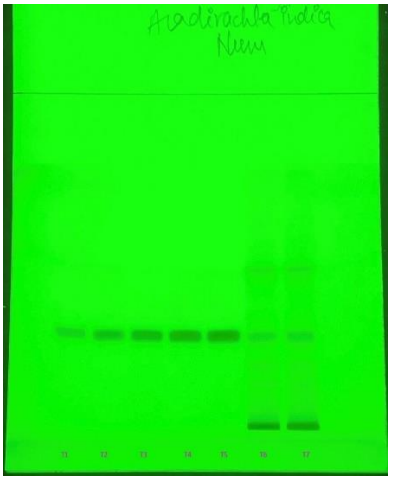


Figure 23 TLC Plate at 254 nm

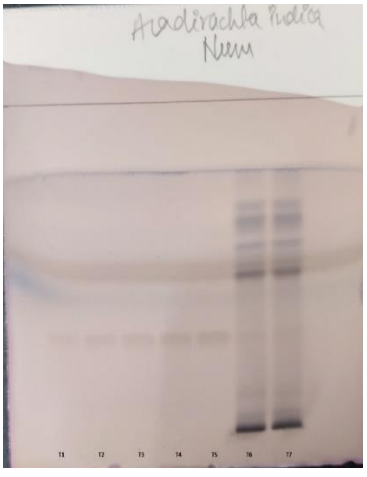


Figure 24 TLC Plate at 366 nm

5.6.2.1. HPTLC Chromatogram of *A.indica* i.e Nimbolide

Track 1 Std Nimbolide

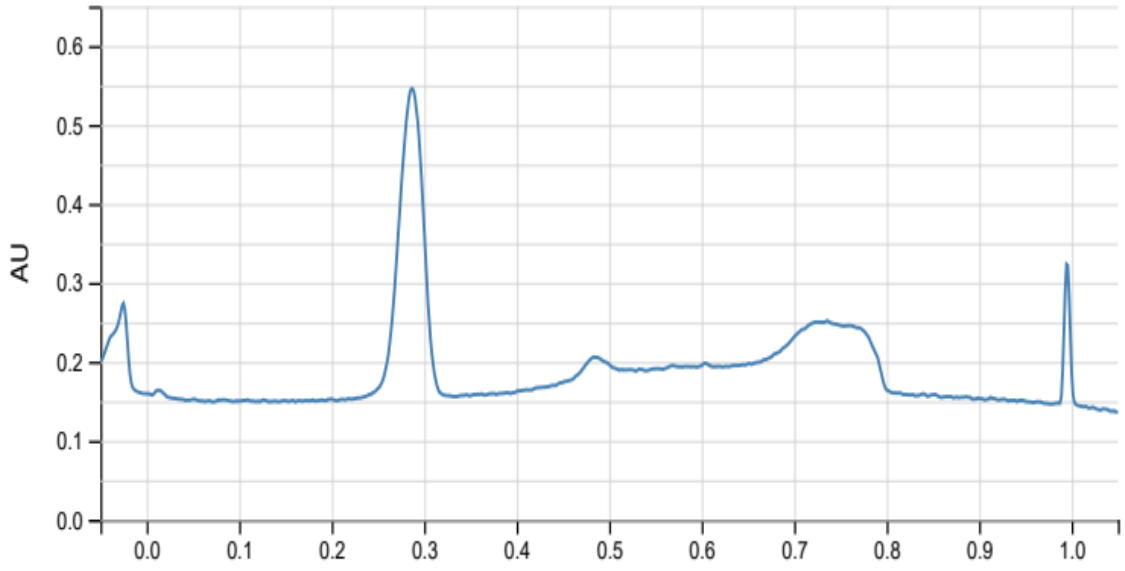
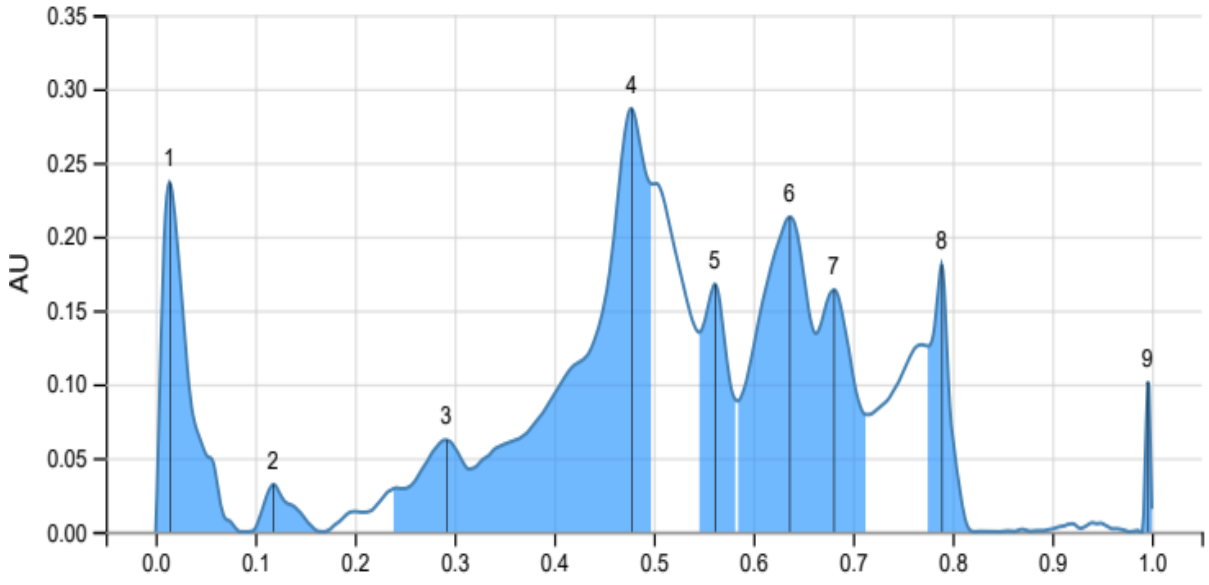


Figure 25. Chromatogram of *A.indica* standard

5.6.2.2. Chromatogram of *A.indica* extract



Track 6 *A.indica* extract

Figure 26. Chromatogram of *A.indica* extract

Table 30.HPTLC Peak table of Standard i.e Nimbolide

Track 1	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.222	0.00	0.287	0.391	0.332	0.00	0.012	100.0	No	Nimbolide
	0.122	0.00	0.281	0.535	0.324	0.002	0.017	100.0	No	
	0.222	0.00	0.279	0.607	0.319	0.00	0.021	100.0	No	
	0.222	0.00	0.279	0.679	0.315	0.007	0.025	100.0	No	
	0.021	0.0041	0.229	0.041	0.278	0.00	0.005	100.0	No	

Table 31.HPTLC Peak table of Sample i.e *A.indica* extract

Track 6	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.096	0.070	0.122	0.082	0.142	0.051	0.003	5.84	No	*A.indica*
	0.142	0.0518	0.164	0.0850	0.192	0.276	0.0031	5.61	No	
	0.193	0.0033	0.224	0.0696	0.276	0.0169	0.004	7.06	No	
	0.225	0.0652	0.442	0.540	0.454	0.224	0.018	32.0	No	
	0.456	0.224	0.464	0.290	0.485	0.133	0.006	11.27	No	

5.6.3 . HPTLC Analysis of *P.pinnata* extract

Figure 27 TLC Plate at 254 nm

Figure 28 TLC Plate at 366 nm

5.6.3.1. HPTLC Chromatogram of *P.pinnata* i.e Gallic acid

Track 1 Std Gallic Acid

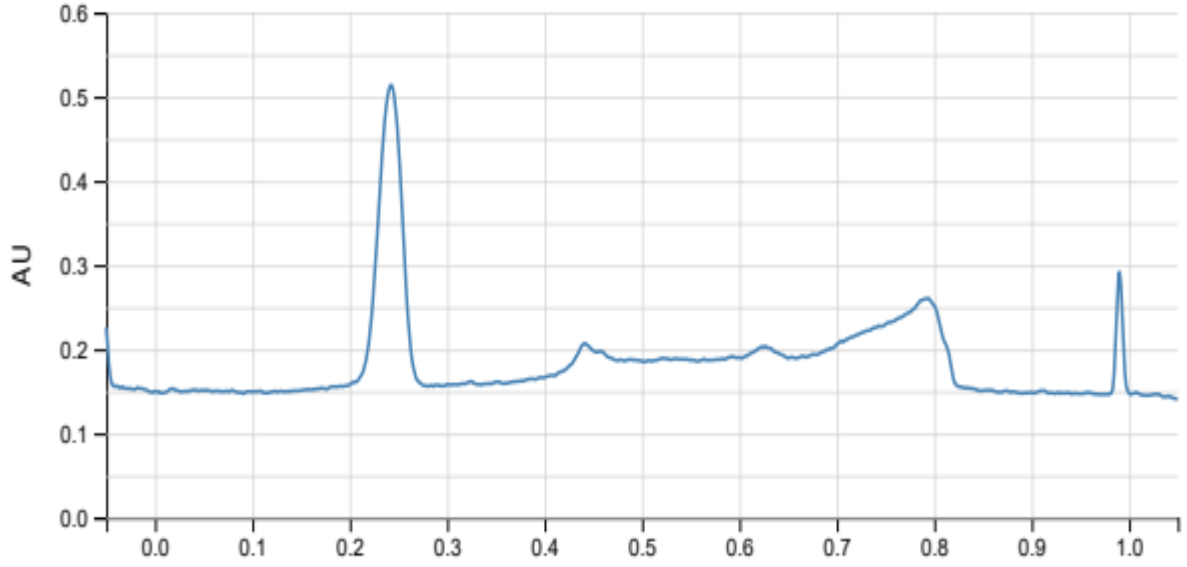


Figure 29. Chromatogram of *P.pinnata* standard

5.6.3.2. Chromatogram of *P.pinnata* extract

Track 6 *P.pinnata* extract

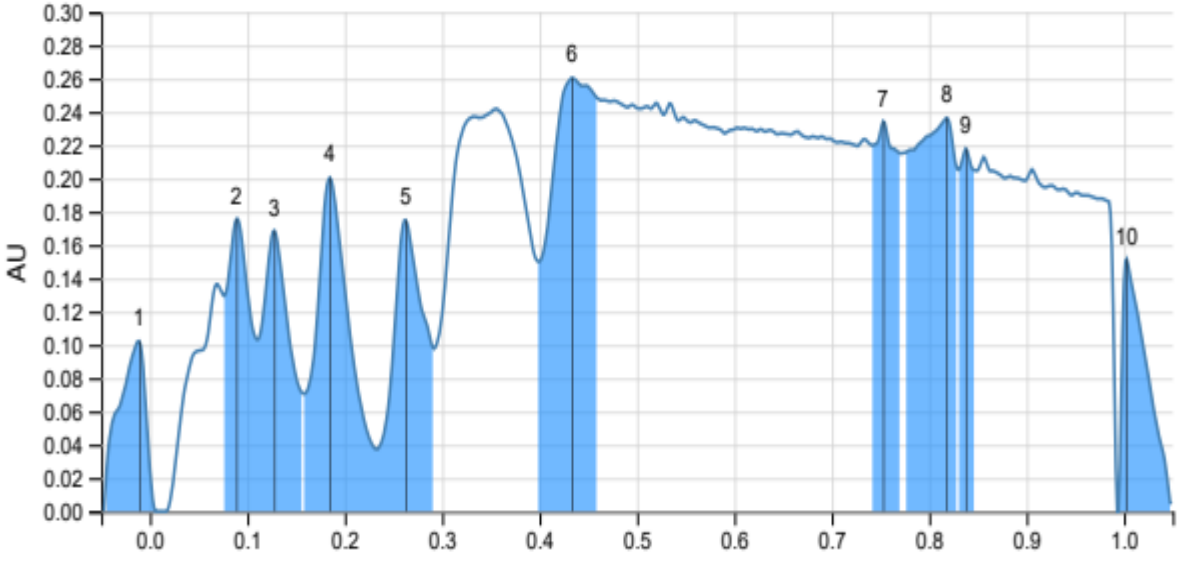


Figure 30. Chromatogram of *P.pinnata* extract

Table 32. HPTLC Peak table of Standard i.e Gallic Acid

Track 1	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.186	0.00	0.242	0.357	0.279	0.00	0.010	100.0	No	Gallic Acid
	0.185	0.00	0.236	0.478	0.271	0.006	0.0144	100.0	No	
	0.151	0.098	0.171	0.140	0.178	0.115	0.003	10.39	No	
	0.378	0.152	0.386	0.182	0.394	0.153	0.002	9.54	No	
	0.499	0.163	0.507	0.246	0.522	0.164	0.004	15.40	No	
	0.767	0.166	0.785	0.253	0.793	0.183	0.005	17.80	No	

Table 33. HPTLC Peak table of Sample i.e *P.pinnata* extract

Track 6	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.185	0.00	0.232	0.533	0.261	0.00	0.021	100.0	No	*P.pinnata*
	0.186	0.00	0.236	0.495	0.267	0.00	0.021	100.0	No	
	0.233	0.037	0.263	0.174	0.292	0.097	0.006	9.49	No	
	0.399	0.150	0.433	0.260	0.463	0.247	0.014	21.03	No	
	0.742	0.219	0.753	0.234	0.771	0.215	0.006	9.28	No	
	0.776	0.215	0.818	0.236	0.829	0.205	0.003	16.93	No	

5.6.4. HPTLC Analysis of *P.corylifolia* extract

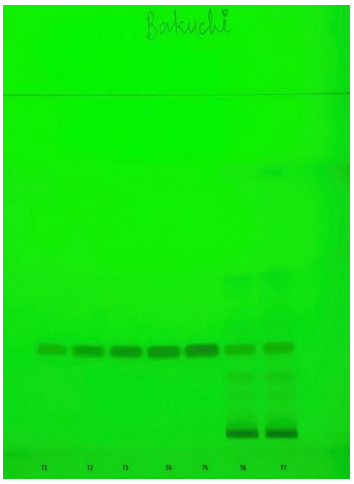


Figure 31.TLC Plate at 254 nm

Figure 32.TLC Plate at 366 nm

5.6.4.1. HPTLC Chromatogram of *P.coryfolia* with reference i.e Gallic acid

Track 1 : Standard Gallic Acid

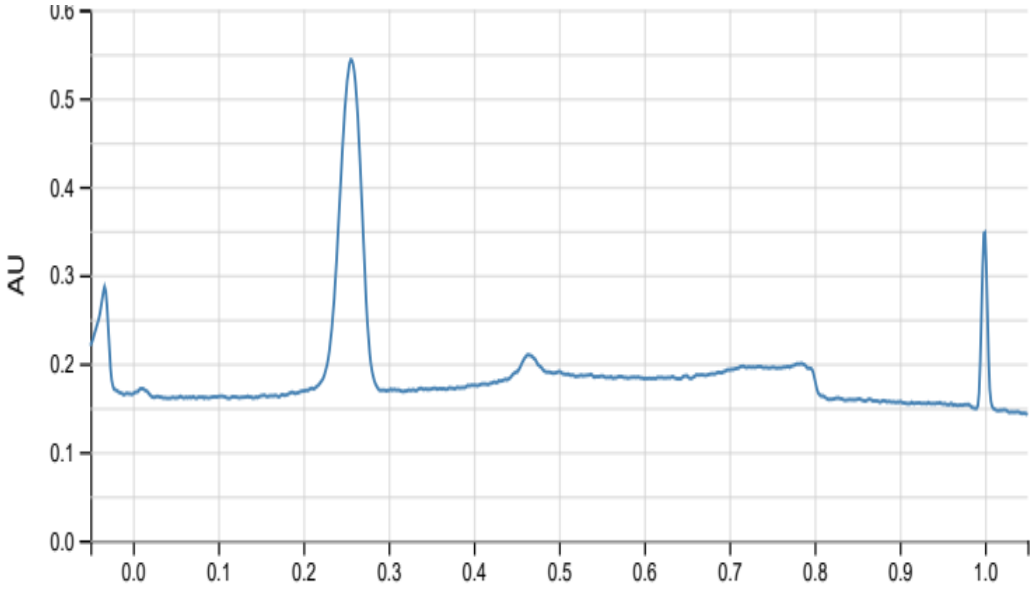


Figure 33. Chromatogram of *P.corylifolia*

5.6.4.2. Chromatogram of *P.corylifolia* extract

Track 6 *P.corylifolia* extract

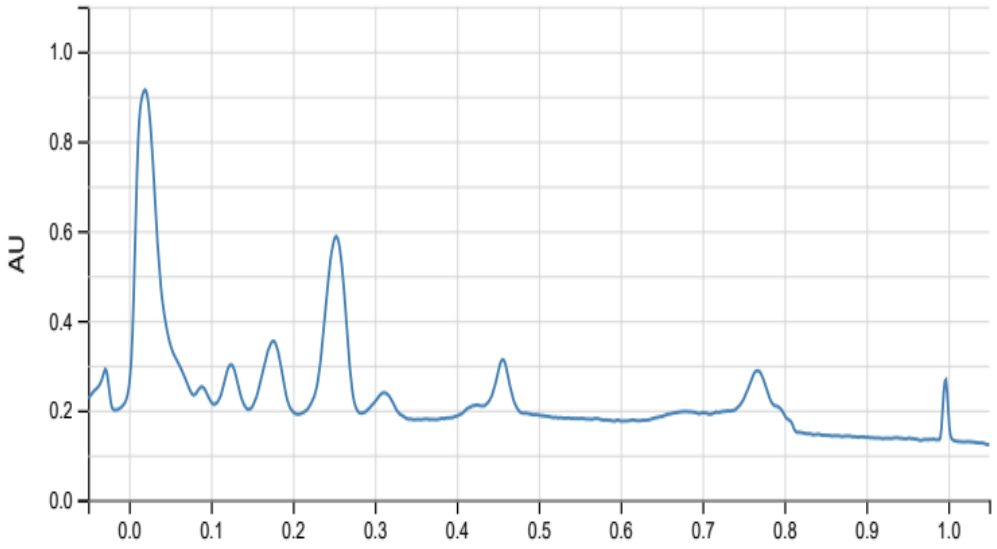


Figure 34. Chromatogram of *P.corylifolia* extract

Table 34. HPTLC Peak table of Standard i.e Gallic Acid

Track 1	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.211	0.00	0.256	0.372	0.292	0.00	0.011	100.0	No	Gallic Acid
	0.211	0.00	0.251	0.531	0.289	0.00	0.016	100.0	No	
	0.211	0.00	0.253	0.394	0.283	0.00	0.011	100.0	No	
	0.178	0.00	0.211	0.139	0.250	0.00	0.005	100.0	No	
	0.178	0.00	0.211	0.141	0.256	0.00	0.006	100.0	No	

Table 35. HPTLC Peak table of Sample i.e *P.corylifolia* extract

Track 6	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.211	0.00	0.253	0.394	0.283	0.00	0.011	100.0	No	*P.corylifolia*
	0.211	0.00	0.258	0.394	0.289	0.00	0.011	100.0	No	
	0.211	0.00	0.249	0.611	0.287	0.00	0.019	100.0	No	
	0.211	0.00	0.247	0.650	0.283	0.00	0.021	100.0	No	
	0.211	0.00	0.251	0.681	0.292	0.00	0.024	100.0	No	

5.6.5. HPTLC Analysis of *W.fruticosa* extract

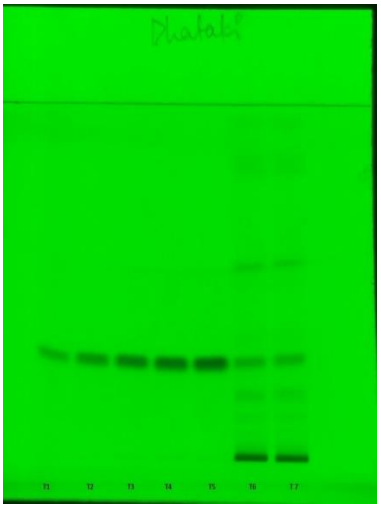


Figure 35.TLC Plate at 254 nm

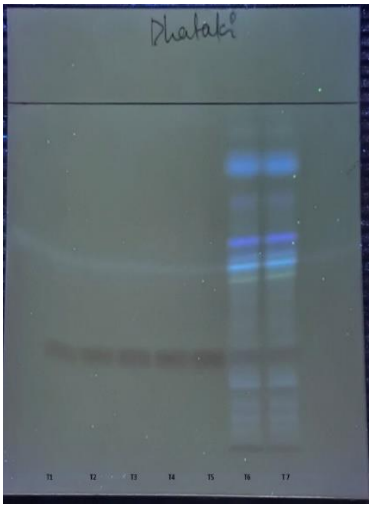


Figure 36.TLC Plate at 366 nm

5.6.5.1. HPTLC Chromatogram of *W.fruticosa* i.e Gallic acid

Track 1 Std Gallic Acid

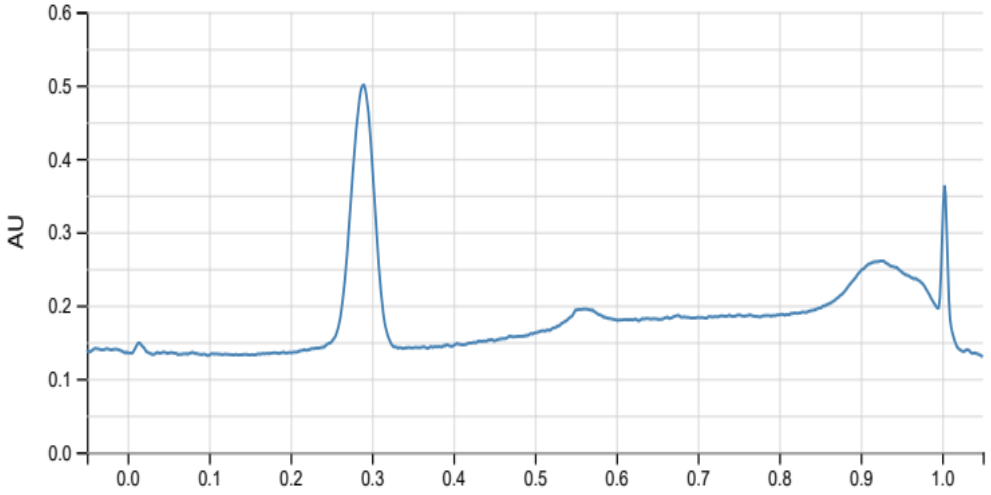


Figure 37. Chromatogram of *W.fruticosa*

5.6.5.2. Chromatogram of *W.fruticosa* extract

Track 6 *W.fruticosa* extract

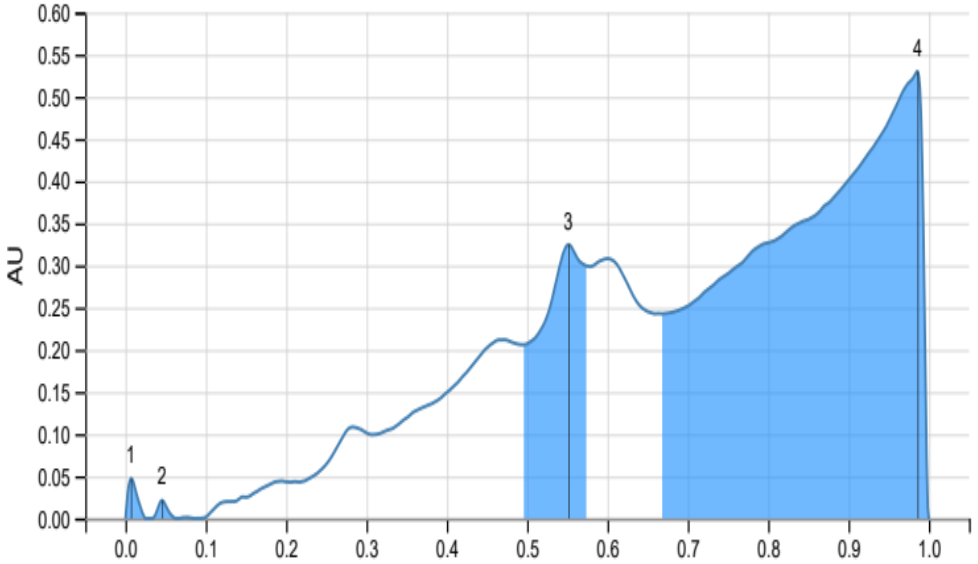


Figure 38. Chromatogram of *W.fruticosa* extract

Table 36. HPTLC Peak table of Standard i.e Gallic Acid

Track 1	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.221	0.00	0.289	0.359	0.336	0.00	0.012	100.0	No	Gallic Acid
	0.217	0.00	0.278	0.517	0.318	0.0015	0.0179	100.0	No	
	0.360	0.006	0.992	0.064	0.490	0.0376	0.006	100.0	No	
	0.321	0.021	0.447	0.073	0.487	0.044	0.007	100.0	No	
	0.678	0.00	0.753	0.011	0.800	0.000	0.006	100.0	No	

Table 37.HPTLC Peak table of Sample i.e *W.fruticosa* extract

Track 6	Start		Max		End		Area		Manual Peak	Sub.Name
	Rf	H	Rf	H	Rf	H	A	%		
	0.224	0.00	0.276	0.338	0.310	0.00	0.011	100.0	No	*W.fruticosa*
	0.229	0.00	0.286	0.325	0.321	0.000	0.010	100.0	No	
	0.126	0.112	0.149	0.156	0.175	0.087	0.006	6.63	No	
	0.183	0.072	0.207	0.152	0.276	0.0178	0.009	11.40	No	
	0.276	0.017	0.313	0.122	0.343	0.090	0.005	6.84	No	

5.7. In Vitro Antipsoriatic activity of *C.longa, A.indica, P.corylifolia, P.pinnata, W.fruticosa,*

Table 38. Coding detail of sample used in the Study

Sr.No	Code Name	Drug Name
	Compound A	*A.indica* extract
	Compound B	*C.longa* extract
	Compound C	*P.pinnata* extract
	Compound D	*W.fruticosa* extract
	Compound E	*P.corylifolia* extract

5.7.1. MTT Assay analysis

The HaCat cells were treated for 24 hours with compound A, B, C,D& E at concentration of 10ug/ml. After the 24 hours of treatment with these compounds, the viability of cells was significantly affected as shown in figure 33. In cells, treated with compound A were shown to have significantly decreased in mean viability of 45 ± 3.9 %(p value = 0.0001). Similarly, the cells that were treated with compound Balso were shown to have significantly decreased in mean viability of 55 ± 3.4 % (p value = 0.0001) as compared to control cells. Also, the cells, which were treated with compound C, were also found to have significantly decreased in mean cell viability by 80.2 ± 10.1%. However, cells treated with compound D & E have also decreased in cells viability by 86 % but they were not found to be significant as compared to control cells.

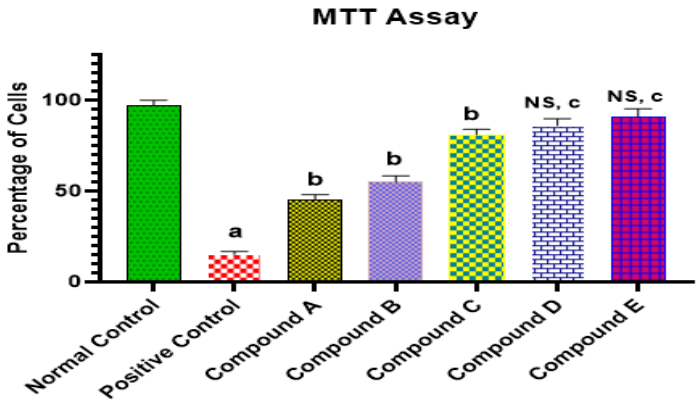


Figure 39. MTT assay was done to assess cell viability. Cells were treated with 10µg/ml of compound A, B, C, D and E for 24 hours. Values for the data shown are representative of 3 experiments and are given as mean ± SD. Statistical analysis was performed by one-way analysis of variance with all pair wise multiple comparison procedures done by Tukey test. Different p-value of less than 0.05 was considered statistically significant.

5.7.2. LDH Assay

In this experiment, we assessed the LDH activity of cells treated with PMA, compound A, B, C, D and E after 24 hour.We have used Phorbol 12-myristate 13-acetate (PMA) which was also known as 12-O-tetradecanoylphorbol 13-acetate (TPA). This is a

specific activator of Protein Kinase C (PKC) and hence activates nuclear factor-kappa B (NF-κB). NF-κB is a transcription factor that regulates numerous physiological functions and is involved in the pathogenesis of inflammations. PMA is the most common and potent phorbol ester. It is active at nano molar concentrations and activates NF-κB in a dose-dependent manner. PMA is also possessing high lactate dehydrogenase activity and hence used as positive control. For calculating the LDH activity, we have measured thethe change in measurement from $T_{initial}$ to T_{final} for the samples. ΔA450 = (A450)final – (A450)initial. The LDH activity of a sample may be determined by the following equation:

LDH Activity = B × Sample Dilution Factor (Reaction Time) × V B = Amount (nmole) of NADH generated between $T_{initial}$ and T_{final}.

Reaction Time = Tfinal – T initial (minutes)

V = sample volume (mL) added to well LDH activity is reported as nmole/min/mL = milliunit/mL.So, one unit of LDH activity is defined as the amount of enzyme that catalyzes the conversion of lactate into pyruvate to generate 1.0 μmole of NADH per minute at 37 °C. Hence, positive control(PMA) was shown to have highest LDH activity with mean ± SD of 0.38± 0.12 as shown in figure 34. However, we observed significant reduction of LDH acitivity in cells treated with compound A by mean of 0.08 ± 0.005 (p value = 0.0001) as compared to positive control. Similarly, significant reduction of LDH activity in cells treated with compound B by mean of 0.11 ± 0.05 (p value = 0.0001) as compared to positive control. Also, the cells, which were treated with compound C and D were also found to have significantly decreased in mean LDH activity by (0.25± 0.07) and (0.2 ± 0.03) (p value = 0.05)as compared to positive control. However, cells treated with compound E have also decreased in cellular LDH activity but they were not found to be significant as compared to control cells.

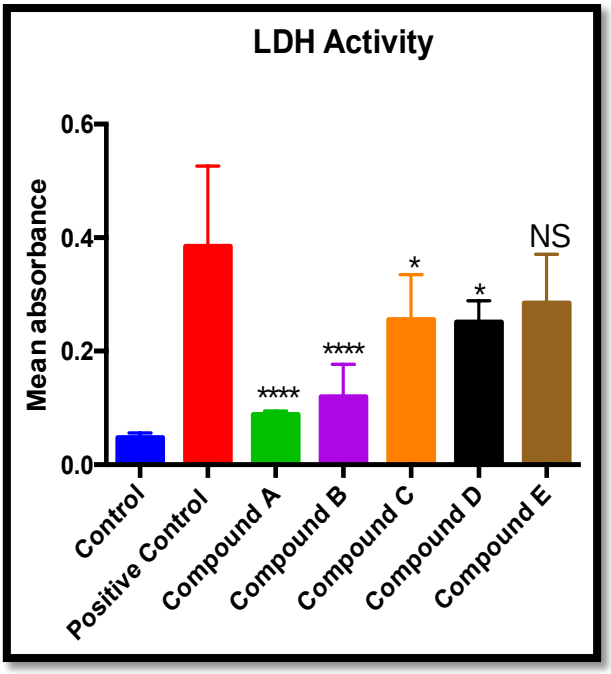


Figure 40. LDH assay was done to assess cellular inflammatory response. Cells were treated with 10µg/ml of compound A, B, C, D and E for 24 hours. Phorbol 12-myristate 13-acetate (PMA) used as positive control at 10nM concentration. Values for the data shown are representative of 3 experiments and are given as mean ± SD. Statistical analysis was performed by one-way analysis of variance with all pairwise multiple comparison procedures done by Tukey test. Multiple comparisons were done against positive control (PMA). Different p-value of less than 0.05 was considered statistically significant.

5.7.3. ELISA Assay

In this experiment, we have also measure the protein expression of IL-12, IL-13 and IL-10 in cells treated with PMA and different compounds (A to E).

IL-12

Interleukin-12(IL-12) is pro-inflammatory cytokines that appears to regulate the inflammation. Here, in our study, we found significant reduction of IL-12 levels with mean value of 0.09 ± 0.014 in cells treated with compound A as compared to cells treated with PMA(p value = 0.0001). Similarly, significant reduction of IL-12 levels in cells treated with compound B by mean of 0.12 ± 0.05 (p value = 0.0001) as compared

to positive control. Also, the cells, which were treated with compound C and D were also found to have significantly decreased in IL-12 levels mean by (0.35± 0.11) and (0.28± 0.05) (p value = 0.0001) as compared to positive control. The cells treated with compound E have also significantly decreased in IL-12 levels mean by (0.28 ± 0.07) (p value = 0.0001) as compared to positive controlAll the respective data is shown in **This shows that compound A & B have reduced IL-12 levels and hence are less pro-inflammatory.**

IL-13

Interleukin-13 (IL-13) cytokines is majorly responsible for inhibiting the pro-inflammatory cytokine and chemokine production in vitro and has known to possess the potent anti-inflammatory activities. IL-13 is an anti-inflammatory cytokine that plays a unique role in the induction and maintenance of IgE production and IgE-mediated allergic responses. Here, in our study, we found significant increase in IL-13 levels with mean value of 0.5± 0.06 in cells treated with compound A as compared to cells treated with PMA (p value = 0.0001). Similarly, significant up regulation ofIL-13 levels in cells treated with compound B by mean of 0.42 ± 0.12(p value = 0.0001) as compared to positive control. Also, the cells, which were treated with compound C and D were also found to have significantly slight increase in IL-13levelsmean by (0.28± 0.07) and (0.21± 0.06) (p value = 0.01) as compared to positive control. The cells treated with compound E have also slight increase in IL-10levelsmean by (0.24± 0.07) (p value = 0.05) as compared to positive control but they are not significant. All the respective data is shown in figure 35 shows that compound A & B have high IL-13 levels and hence exhibit anti-inflammatory response.

IL-10

Interleukin 10 (IL-10)cytokine were considered to be potent anti-inflammatory cytokinethat plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis. Here, in our study, we found significant increase in IL-10 levels with mean value of 0.5± 0.05 in cells treated with compound A as compared to cells treated with PMA (p value = 0.0001). Similarly, significant up regulation of IL-10 levels in cells treated with

compound B by mean of 0.47 ± 0.08(p value = 0.0001) as compared to positive control. Also, the cells, which were treated with compound C and D were also found to have slight increase in IL-10levels mean by (0.28 ± 0.06)(p value = 0.01) and (0.18± 0.09) as compared to positive control. The cells treated with compound D have also significantly slight increase in IL-10levels mean by (0.16± 0.06) (p value = 0.05) as compared to positive control All the respective data is shown in figure 35. This shows that compound A & B have high IL-10 levels and hence exhibit anti-inflammatory response.

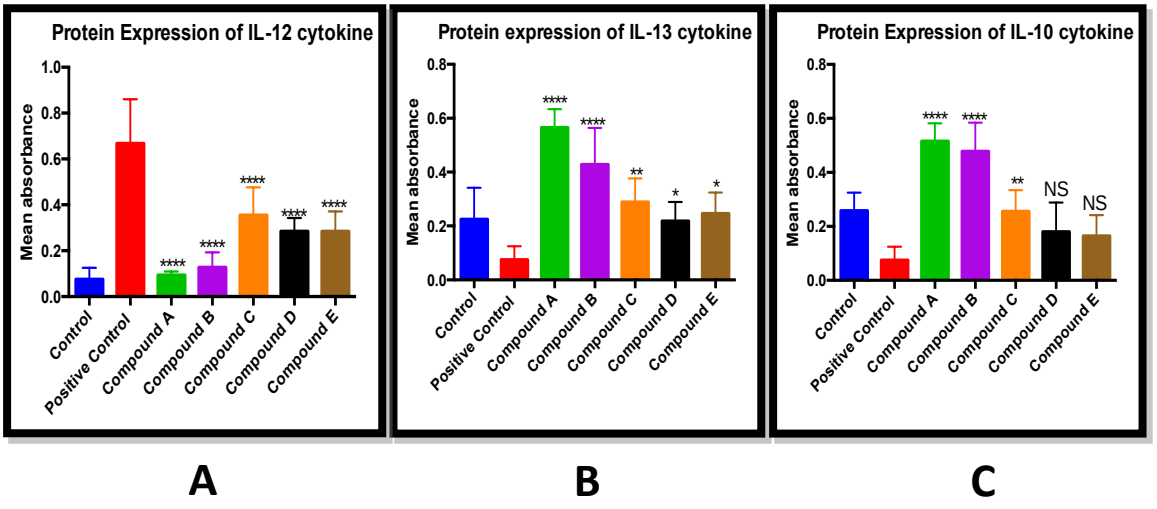


Figure 41. (A-C): ELISA of different cytokines was done to quantitate the protein expression of IL-12, IL-13 and IL-10. Cells were treated with 10µg/ml of compound A, B, C, D and E for 24 hours. Phorbol 12-myristate 13-acetate (PMA) used as positive control at 10nM concentration. Values for the data shown are representative of 3 experiments and are given as mean ± SD. Statistical analysis was performed by one-way analysis of variance with all pairwise multiple comparison procedures done by Tukey test. Multiple comparisons were done against positive control (PMA). Different p-value of less than 0.05 was considered statistically significant.

5.7.4. Confocal Microscopy of Caspase 7

We have also examined the effect of 10µg/ml of compound A, B, C, D and E for 24 hourson Caspase 7 localization and expression through immunofluorescence. The immunofluorescence of treated cells was done, target protein localization and

expression was analyzed using confocal microscopy. We have observed weak cytoplasmic expression of Caspase 7protein in case of compound A&B, but intense cytoplasmic expression in positive control as compared to control cells . Similarly, the cytoplasmic expression of Caspase 7 was decreased in cells treated with C, D & E but they seem similar to control.

Further, we have also calculated the mean fluorescence intensity of confocal images of cells treated with PMA, compound A, B, C, D and E. The expression of Caspase 7 were shown to be significantly down regulated in cells treated with compound A & B with mean value of (39.5 ± 5.2)% and (52.3 ± 6.0)% as compared to control cells. Also, the cells, which were treated with compound C, were also found to have decreased in Caspase 7 expression by 74± 6.6 %. However, cells treated with compound D & E have also decreased in Caspase 7 expression by 76 % but they were not found to be significant as compared to positive control cells.

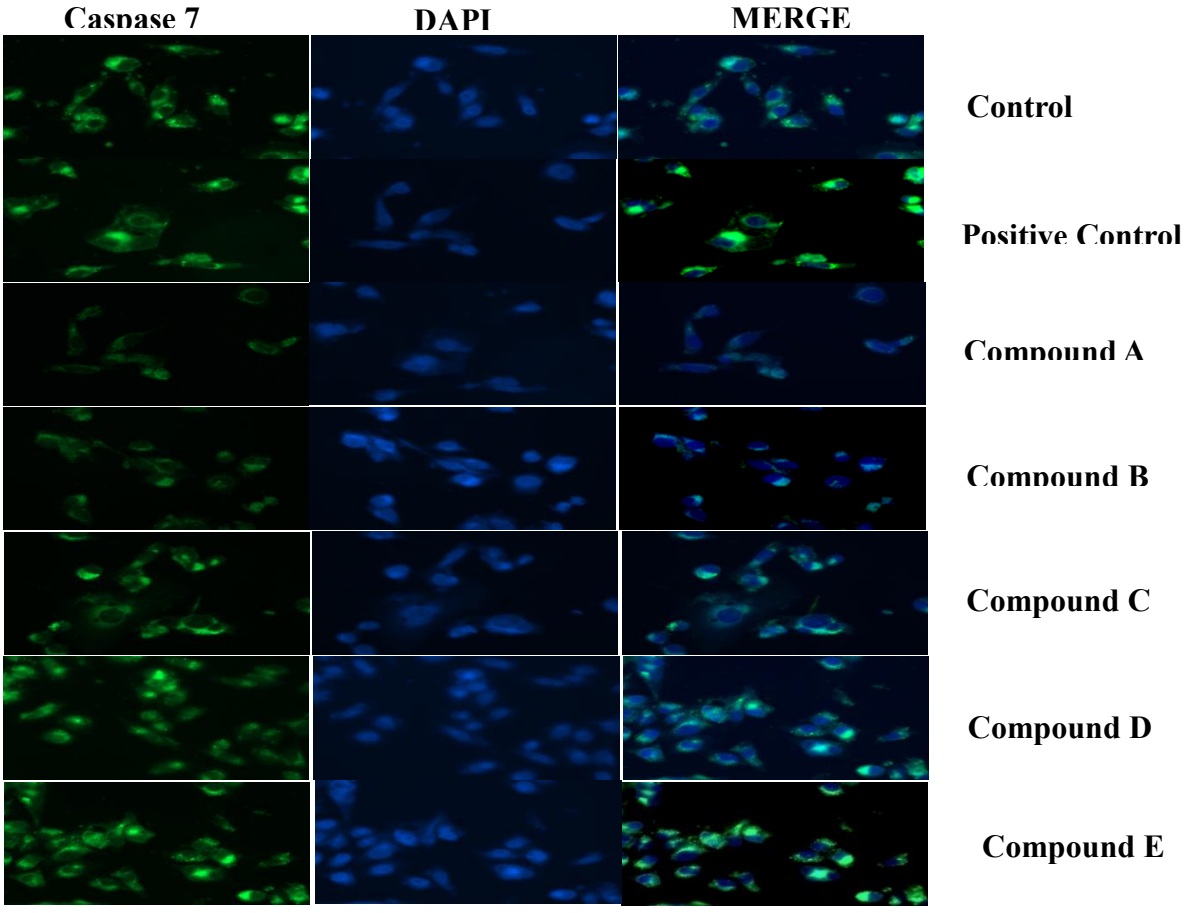


Figure 42. We have observed weak cytoplasmic expression of Caspase 7protein in case of compound A & B, but intense cytoplasmic expression in positive control as compared to control cells. Similarly, the cytoplasmic expression of Caspase 7 was decreased in cells treated with C, D & E but they seems similar to control

5.8. Pre-formulation Studies

Pre-formulation studies are carried out to understand the physicochemical characteristic features of active substance and excipient before moving into various phase of formulation development

5.8.1. FTIR spectroscopy

FTIR spectra of sample, excipients, were measured using KBr pellet method. Samples were scanned in the range of 4000 to 400 cm^{-1}using Fourier Transformed infrared (FT-IR) spectrophotometer (Shimadzu). Instrumentation was previously calibrated at with specification of 4 cm^{-1}

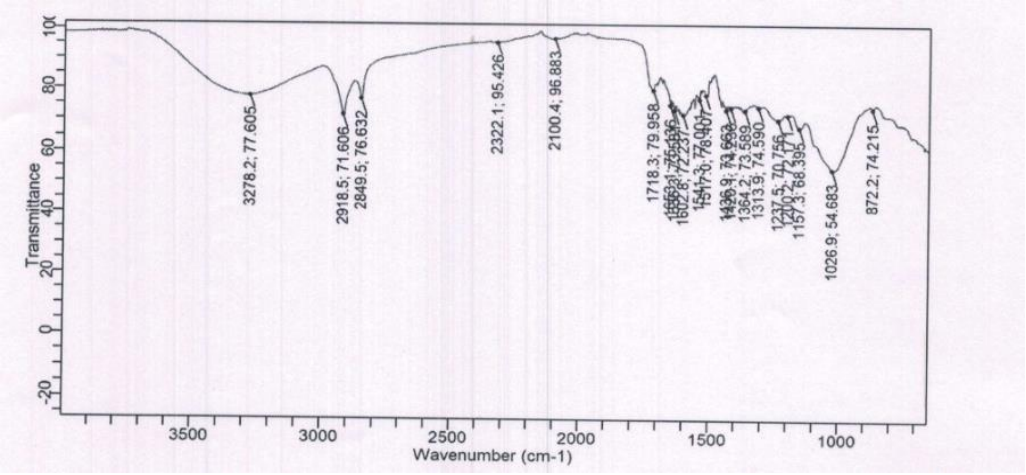


Figure 43. FTIR Spectra of *A.indica*

5.8.2 Differential scanning calorimetry (DSC)

A differential scanning calorimeter (Perkin Elmer Instruments, USA) was used to take thermo grams of sample and excipients. The mpt of Neem extract is 170 ℃.

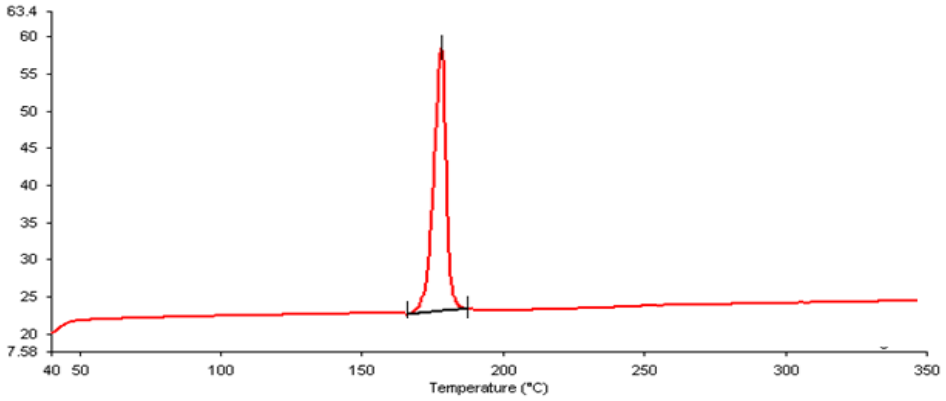


Figure 44. DSC thermogram of *A.indica*

5.8.3 Solubility of Drug/ sample

The solubility of Neem extract in selected components of Neem micro emulsion such as oil, surfactant, and co-surfactant was carried out to choose the best suitable components. Selection of components was based on amount of Neem extract solubilized in each component which was analyzed by developed method. Visual examination of various Neem extract mixture was also carried out to find out the chances of sedimentation of extract.

Natural oils such as Arachis oil, castor oil, cedar wood oil, eucalyptus oil, olive oil, orange peel oil, peppermint oil, cardamom oil, Anise oil & palm oil respectively was included in the study to analyze the solubility profile of neem extract. Synthetic oil such as Tween 20, tween 40, tween 60, tween 80, tween 81, cremophor EL, Spam 80, span 20

The observation for solubility of neem extract in various oils and surfactants are shown in figure 5.16 respectively.

The solubility of neem extract in each selected oil/surfactant/co-solvent is mentioned below in decreasing order:

Cardamomum oil (40.8± 0.1%) > Anise oil (38.5±0.2)> Peppermint oil (37.5±0.2)> Orange peel oil (33.1±0.4) olive oil (22.6±0.2) > Eucalyptus oil (21.8±0.4) > Tween 80 (21.8±0.6) > Tween 20(21.4±0.2) >Arachis oil (20.4±0.1) >Tween 81(20.4±0.1)

>Spam 80 (19.5±0.7) >Ceedarwood oil (18.7±0.3) > tween 60 (18.7±0.4) > Span 20 (18.7±0.5) >Castor oil (17.8±0.2) > tween 40 (15.8±0.5) >Palm oil (12.4±0.2) >Cremophore El(12.1±0.4) >PEG 600 (9.8±0.5) >PEG 400(9.5±0.2) >Glycerol(7.4±0.5)>Ethanol(7.4±0.4) >IPA(4.8±0.1).

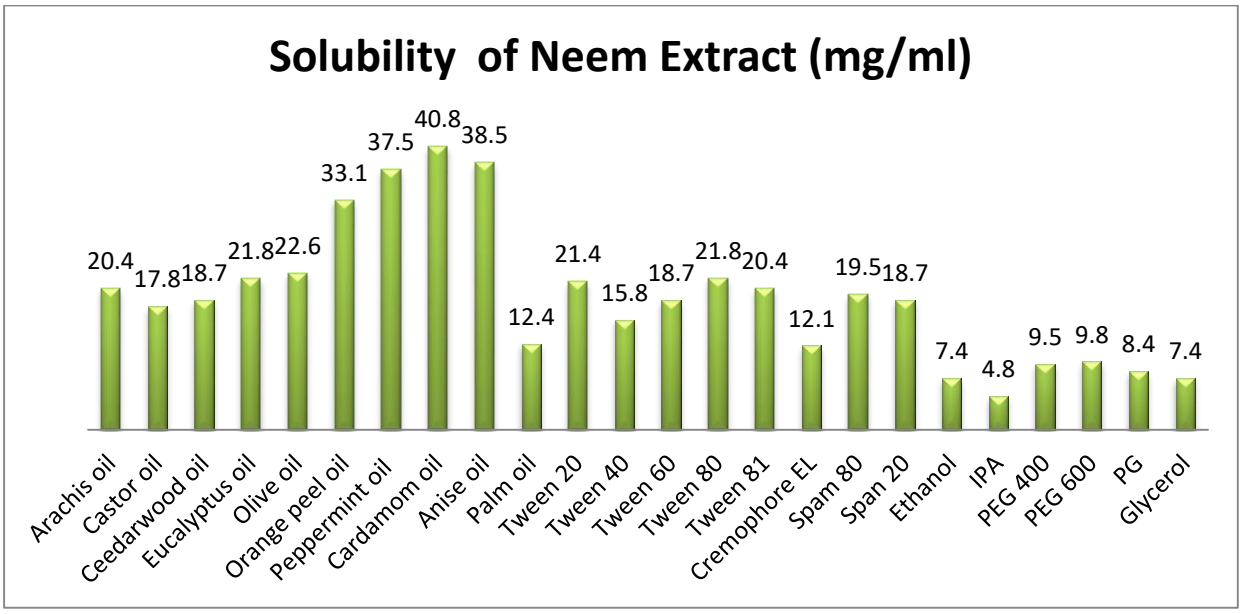
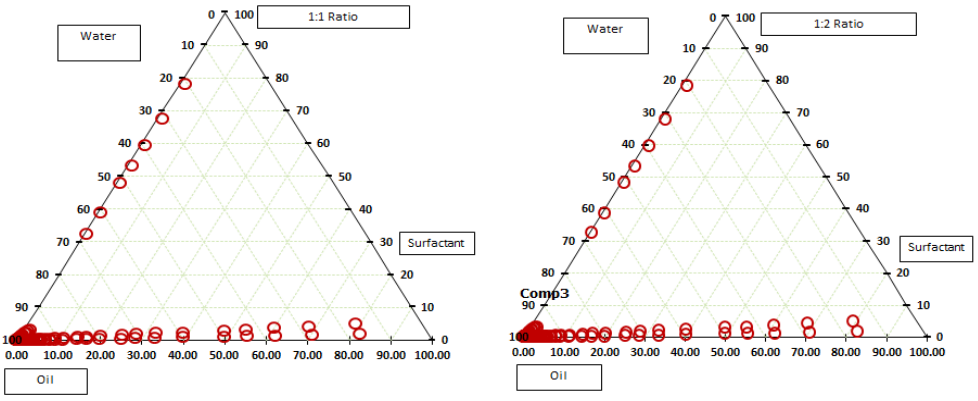


Figure 45. Solubility analysis of Surfactant, Co surfactant and Solvent system

5.8.4. Choosing of oil phase and non-aqueous polar phase

The phase separation was used as a tool for selection of oil phase and non-aqueous phase. The oils (cardamom oil) and non-aqueous polar phase (tween 80 & PEG 400) were mixed in ratio of 1:1 and observed for phase separation

5.8.5. Pseudo Phase ternary diagram of Oil : Surfactant and water system



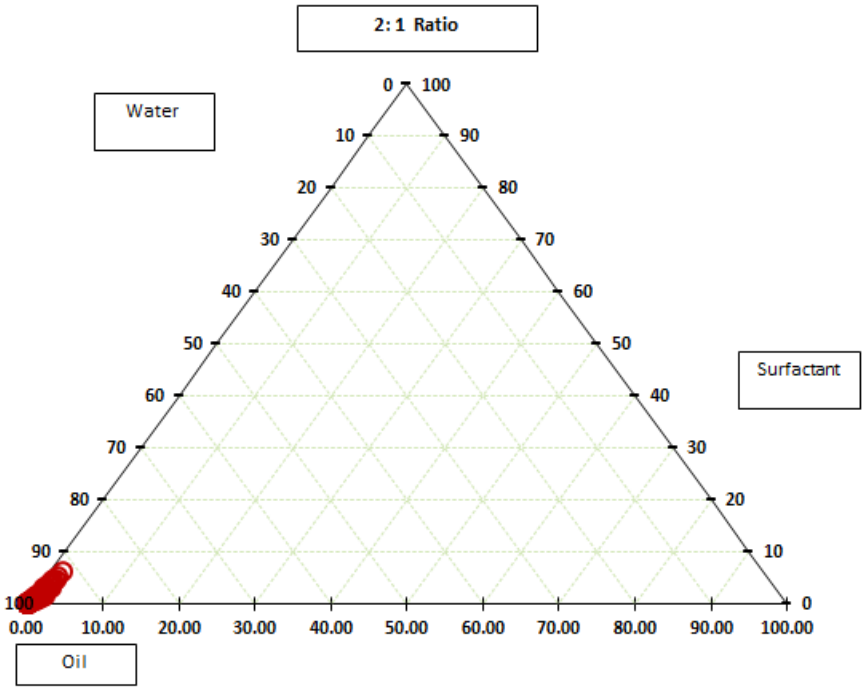


Figure 46. Pseudo ternary phase diagram of the oil/surfactant/ water system at 1:1, 1:2 & 2:1

5.8.6. Formulation of *A.indica* Microemulsion

A.indica extract based microemulsion formulations were prepared by simple mixing method. Amount of each ternary component (on w/w basis) viz. Cardamom Oil, Smix (Tween 80/PEG 400) and aqueous phase were carefully determined from respective phase diagram drawn at specified Smix ratio. On Individual basis, each ternary component was accurately weighed, mixed to specified amount of neem extract (20mg) using sonication and resulting mixture was transferred to screw capped container; Precaution should be taken to avoid bubble formations in the container during the transfer of Smix and the entire admixture of ternary components were subjected to 2min sonication cycle at 25C. Previously 1:2 Smix (Surfactant/ co-solvent) was prepared by taking weight ratio of surfactant and co-solvent in the ratio of 1:2 mixed using shake flask method followed by 2-min sonication cycle. Resulting mixture of ternary components was stored in the tightly closed amber coloured container and stored in the refrigerated conditions till further usage.

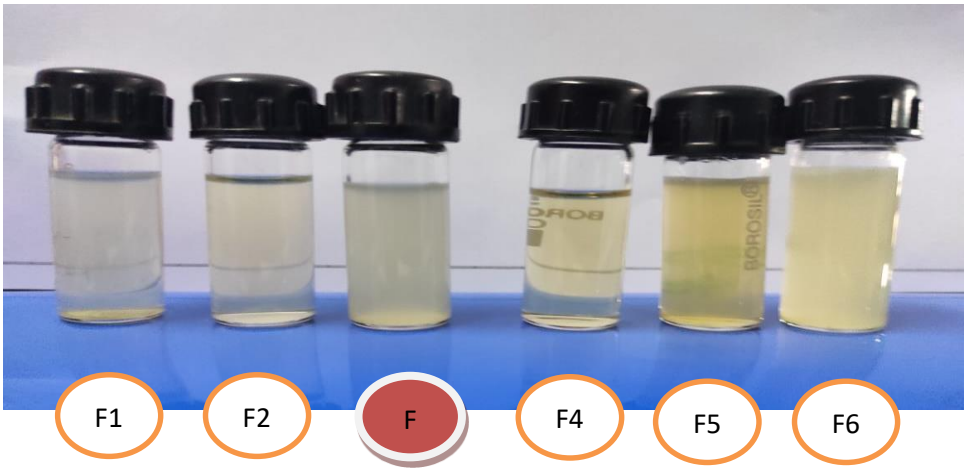


Figure 47. Different batches prepared of A.indica extract Micro-emulsion

5.9. Characterization of Neem Micro-emulsion

5.9.1 Globules size analysis and PDI

Prepared *A.indica* micro-emulsion was found to be transparent having a droplet size of 762.3 nm ± 28.62 nm and a uniform PDI of 0.773. The uniformity in the droplet size and narrow size distribution of globules were observed which shows the ability of ability of surfactant to form a closely packed film at the interface of two immiscible phases. Smaller the size of droplet may further leads to stability of the formulation.

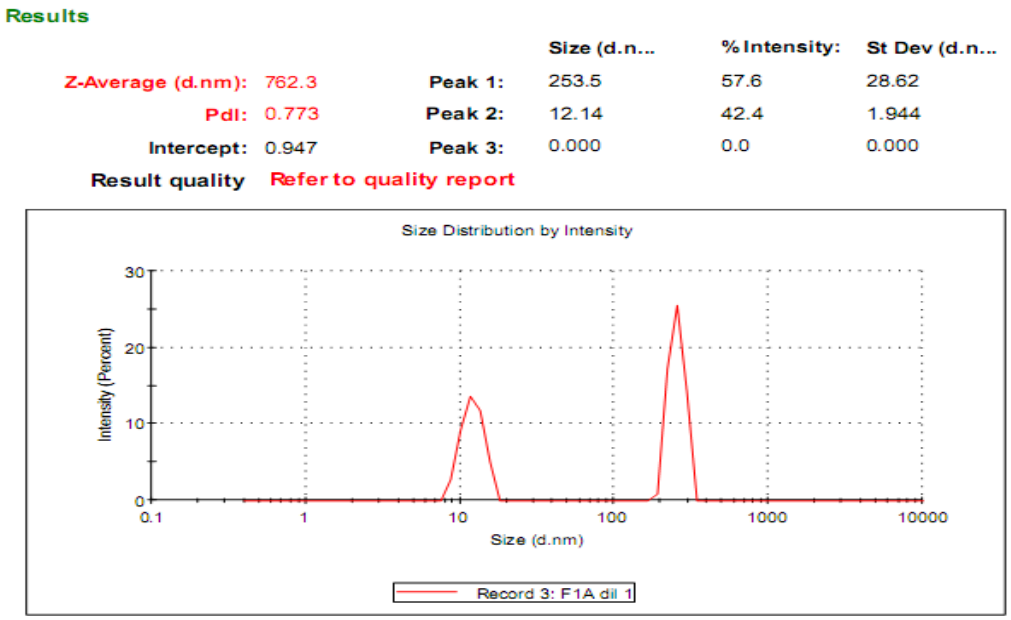


Figure 48. Droplet size analysis and PDI of final optimized Neem micro-emulsion

5.9.2 Zeta Potential

The surface charge of the droplet and the electrostatic force between these charged globules decreases the possibilities of coalescence. Zeta potential plays an important role in terms of stability of the formulation. The zeta potential of Neem micro emulsion is – 24.69 mV.

Table 39. Statistics Analysis of Zeta Potential

Name	**Mean**	**Standard Deviation**	**RSD**	**Minimum**	**Maximum**
Zeta Potetial (mV)	-24.69	--	--	-24.69	-24.69
Zeta Peak 1 Mean (mV)	-24.69	--	--	-24.69	-24.69
Conductivity (mS/cm)	0.1464	--	--	0.1464	0.1464
Wall Zeta Potential (mV)	-21.73	--	--	-21.73	-21.73
Zeta Deviation (mV)	6.856	--	--	6.856	6.856
Derived Mean Count Rate (kcps)	3.246 E +05	--	--	3.246 E +05	3.246 E +05
Reference Beam Count Rate (kcps)	1715	--	--	1715	1715
Quality Factor	2.619	--	--	2.619	2.619

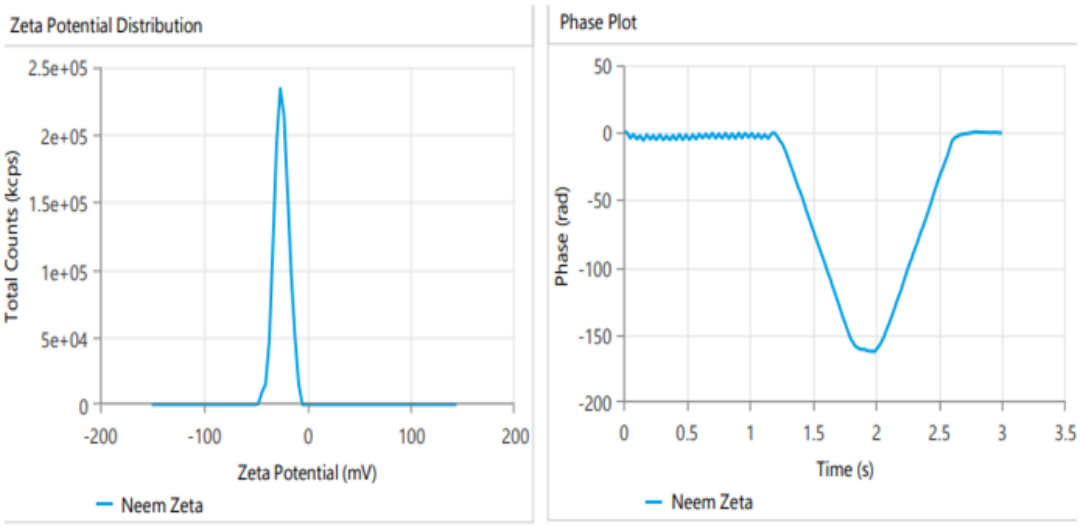


Figure 49. Zeta Potetial of final optimized *A.indica* micro-emulsion

5.9.3 Percentage transmittance

For formulation batches, the percentage transmittance ranged from 96.00 to 99.84% indicating that nanoemulsions were transparent and clear. Results obtained from % transmittance data was quite obvious since misicibilty and phase diagram studies confirmed that addition of aqueous phase in the ternary dispersion system produced clear dispersion.

Table 40. Percentage transmittance of various batches prepared

Formulation Code	% Transmittance
F1	98.89±0.3
F2	97.43±0.1
F3	96.27±0.2
F4	99.12±0.1
F5	97.38±0.1
F6	98.37±0.2

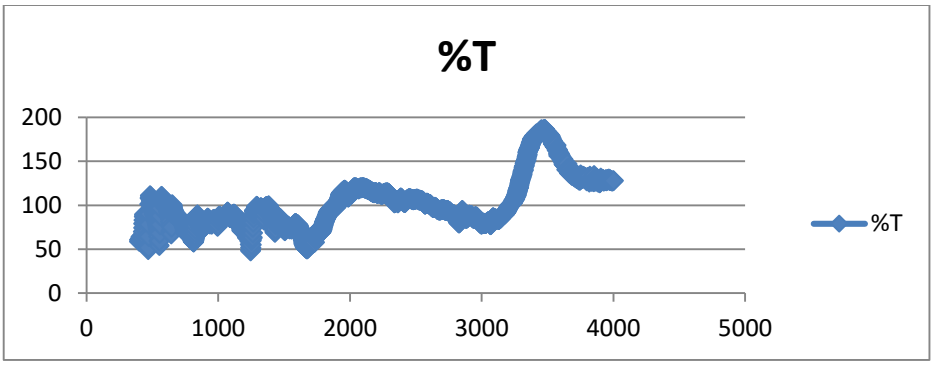


Figure 50. Percentage transmittance of optimized *A.indica* micro-emulsion

5.9.4 Refractive index

Formulations F4 & F5 were possessed comparatively larger RI values than rest of others systems. It could be linked with possession of higher Smix ratios in the microemulsion formulation. It could be interpreted as formation of thick surfactant sheath due to high proportion of Smix around the microemulsion droplets. Interaction of light could be taken place with this thick sheath around the droplet and resulted higher RI values.

Table 41. Refractive index of different prepared batches

Formulation Code	Ref. Index (units)
F1	1.341±0.004
F2	1.347±0.003
F3	1.331±0.005
F4	1.412±0.004
F5	1.415±0.003
F6	1.333±0.002

5.9.5. Electrical Conductivity

Conductometric data of microemulsion formulations provide information on the conductive species formed in each system and their relative positioning in the microemulsion droplets. Electrical conductivity of formulation was resulted due to incorporation of aqueous phase into the system. Conductivity data of each formulation was given in the table (blue) formulations represented very low conductivity value could suggest that aqueous domain forming the inner core of droplet while higher conductivities, could be possibly due to aqueous domain was present outside.This

interpretation further explains that lower values of microemulsion formulation could be existed as w/o microemulsion system due to orientation of aqueous phase domain present in the microemulsion system. Conversely higher values indicates that microemulsion existed in o/w type and the same justification be given. Data obtained from EC suggested that formulation designed were water continuous systems.

5.9.6. Transmission Electron Microscopy

The morphological characterization of the final optimized Neem micro-emulsion formulation was carried out using TEM shown in Figure … The images justify the round spherical shape of droplets and the average measured size of droplet is 212 nm.

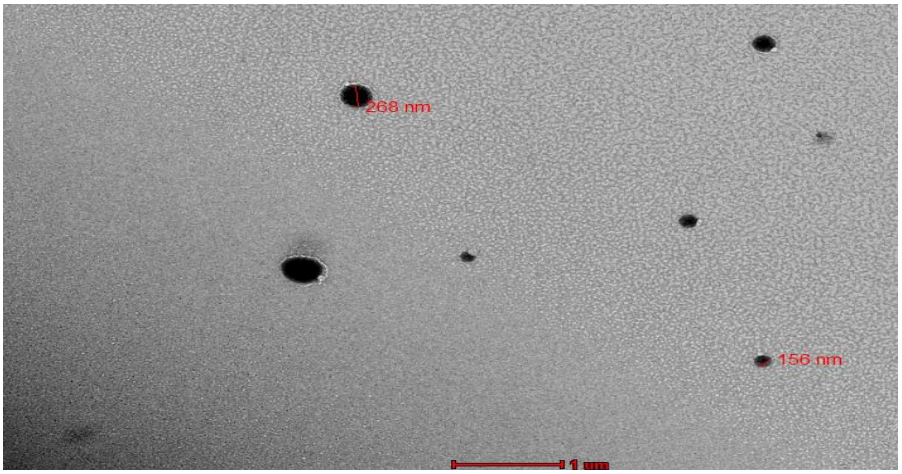


Figure 51. TEM image of optimized F3 (out of zoom)

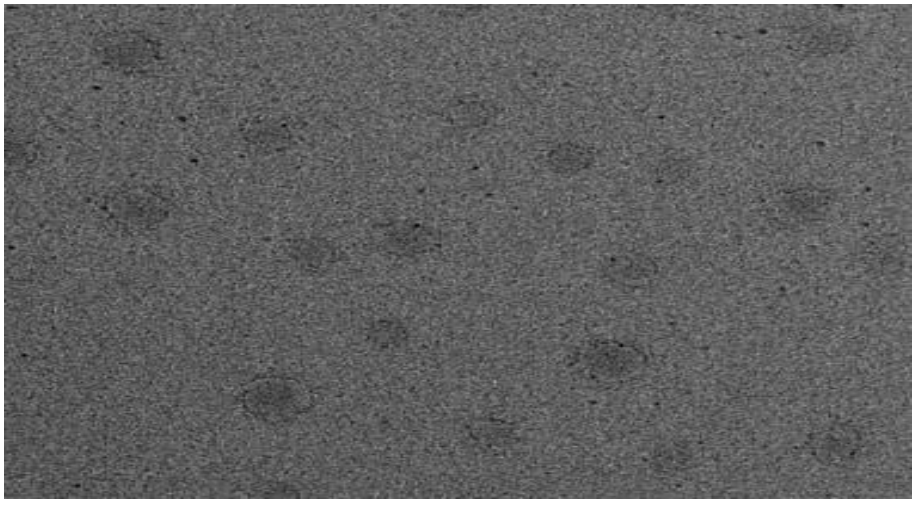


Figure 52. TEM image of F3 (zoomed)

5.9.7. In vitro drug release study

The in vitro drug release diffusion study of *A.indica* extract was performed using dialysis membrane.

Table 42. Drug release profile of six batches prepared

Time (hr)	F1	F2	F3	F4	F5	F6	Control
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.5	0.295	0.161	0.361	0.028	0.361	0.361	0.028
1	0.435	0.335	0.518	0.302	0.585	0.585	0.035
2	0.659	0.475	1.092	0.442	0.775	1.175	0.309
3	0.982	0.832	1.449	0.599	1.049	1.682	0.382
4	1.356	1.123	1.773	0.773	1.423	2.123	0.523
6	1.913	1.446	2.313	1.096	1.980	2.730	0.680
8	2.553	1.737	3.053	1.470	2.620	3.403	0.853
12	3.410	2.044	4.094	1.827	3.477	4.877	1.044
20	4.251	2.967	6.017	2.284	4.451	7.001	1.317
24	4.974	3.724	9.401	2.658	5.274	8.274	1.491

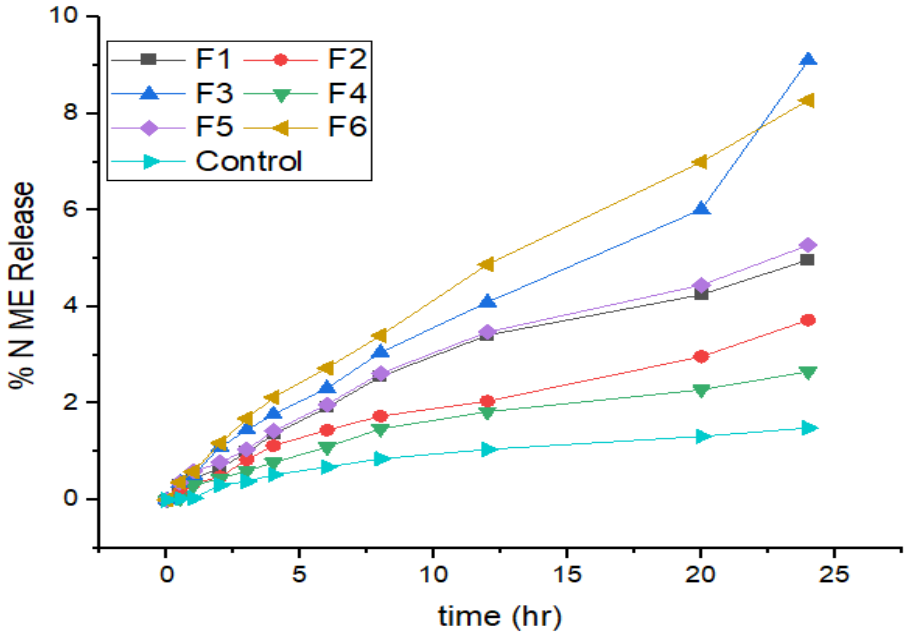


Figure 53. Drug release profile of optimized *A.indica* micro-emulsion

5.9.8 Ex Vivo permeability across Skin

Skin permeation studies performed on microemulsion formulations assessed the potential of drug to cross the skin membrane. Permeation characteristic of Neem oil based microemulsion formulations were examined across stratum corneum. Its data is summerized in the table below. Drug flux represents the amount of drug permeated per unit time and in per unit area of membrane. Higher flux of microemulsion formulation signifies better formulation in terms of drug permeation characteristics[2] since formulation design allows relatively large amount of drug cross the membrane compared to formulations having lower flux. Graphically, flux of microemulsion formulations were represented as.

Table 43. Ex Vivo skin permeability of prepared batches

Time (hr)	F1	F2	F3	F4	F5	F6	Control
Flux	0.133	0.060	0.241	0.054	0.105	0.296	0.030
% Improved	4.420	1.997	8.017	1.791	3.516	9.879	na

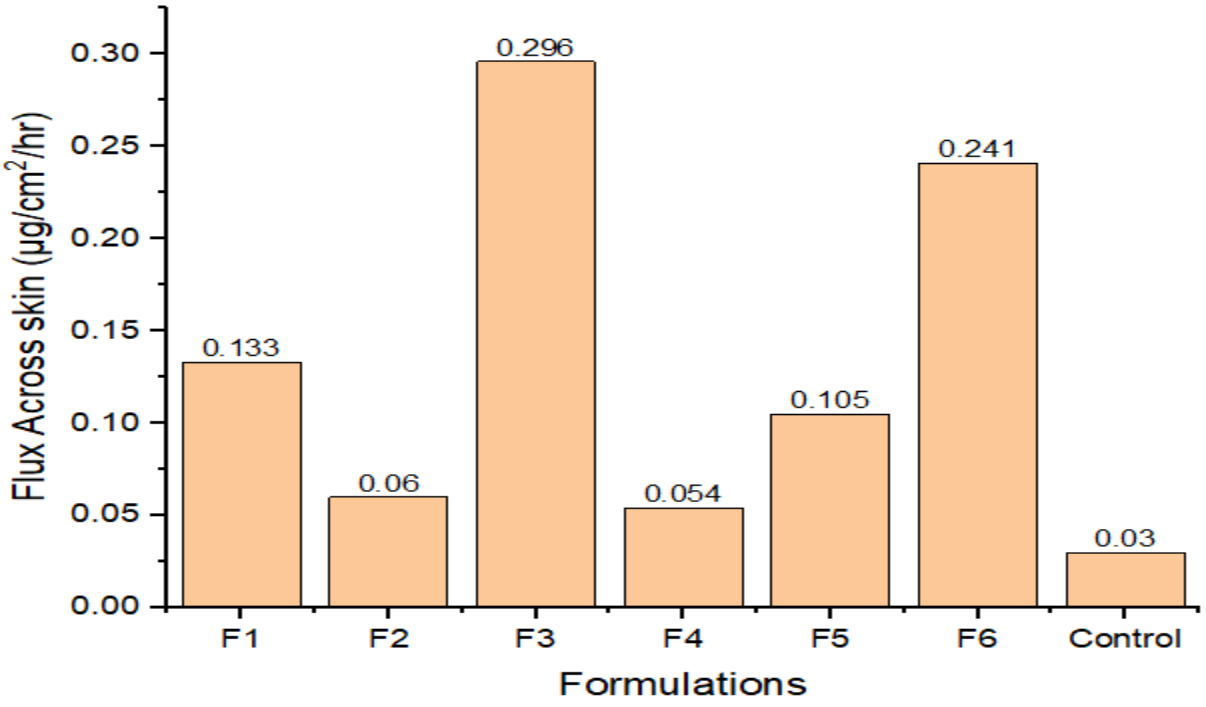


Figure 54. F3 formulation produced highest flux and permeating over control

5.10. Stability studies on optimized formulations as per ICH guidelines

The stability studies were conducted as per the ICH (Q) guidelines. Optimized system i.e. F3 (containing 20mg Neem extract) were weighed and filled in the amber-coloured glass vials and hermetically sealed to prepare a sample meant for stability evaluation. Sufficient numbers of such samples were placed at 30ºC and 75% RH in a humidity chamber for the period of six months. The sample was withdrawn carefully at the interval of 0, 1, 2, 3 and 6 months and evaluated for physical examination i.e. weight change/loss, release time of $t_{90\%}$ or $t_{50\%}$ droplet size, dilution pattern. The sample was analyzed for drug assay. A validated, stability indicating, high performance liquid chromatography (HPLC) method (Suttiarporn P, 2020) with slight modification was used for estimation of nimbolidine present in the sample. Briefly, the HPLC system (Waters,) with UV-VIS PAD-detection was used. Mobile phase and other details specified in the reference. (Methanol: water 70:30) at 217nm.

Table 44. Standard plot showing area under curve against different concentrations of *A.indica* micro emulsion stability indicating HPLC method**.**

Concentration(µg/ml)	Area under curve (AUC)
1.0	11074±978
2.0	440578 ±39857
5.0	8745128 ±97857
10.0	220401227 ±1845789

Results are the mean of six readings; Equation of line: y=2350310x –1990006; r^2 = 0.996

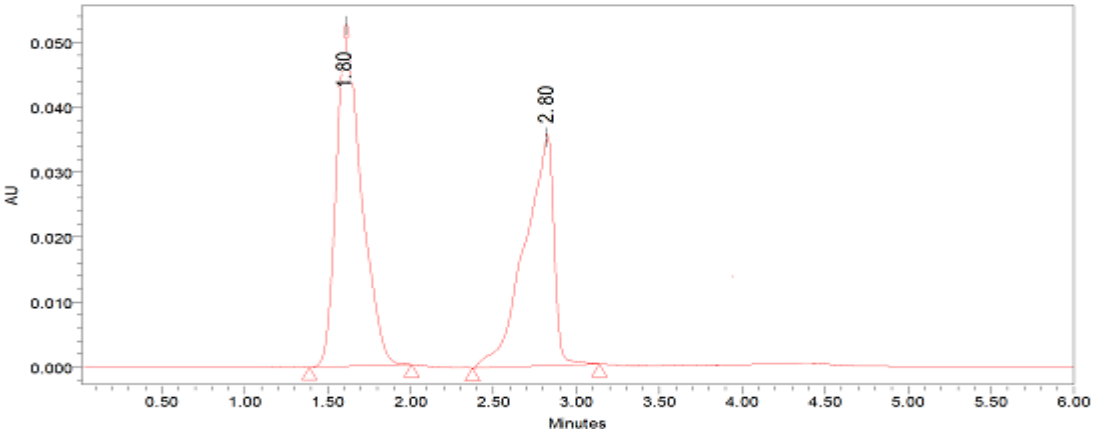


Figure 55. HPLC Chromatogram of Nimbolidine (as marker compound)

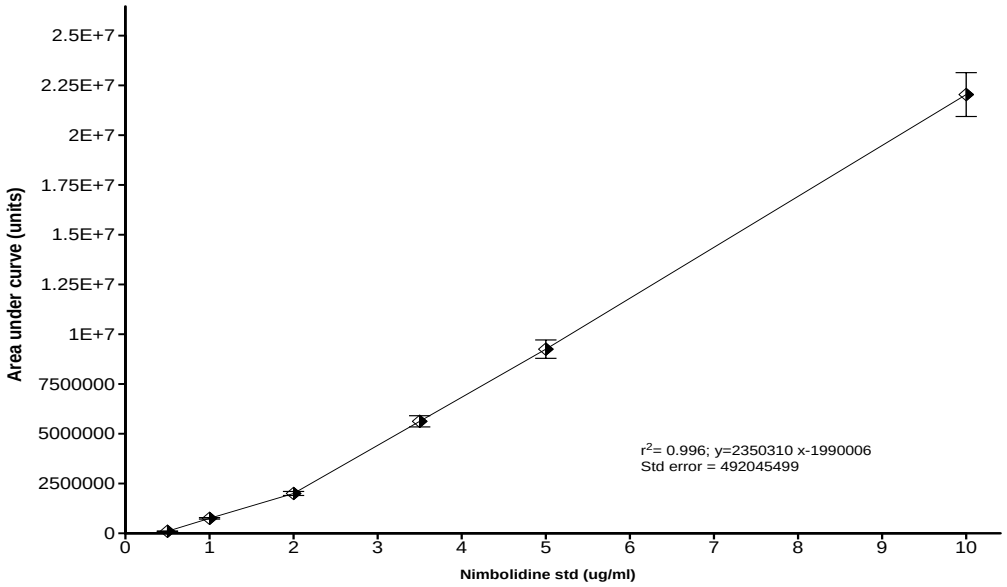


Figure 56. Standard plot of NM

Table 45. Mean *A.indica* micro emulsion content from optimized formulation during stability assessment

	Formulations F3 (Storage conditions 40±0.5ºC, 75%RH)					
Time (days)	**Neem extract content (mg)**	**Label claim amount of NE (ug) 0.05% of Neem extract**	**(%) NE**	**Log PDR**	**Slope**	**Degradation rate constant, k (Day^{-1})**
0	20.00	10	100	2	-3.37×10^{-5}	6.5974×10^{-5}
30	20.00	9.97	99.8	1.999131		
60	20.00	9.91	99.5	1.997823		
90	20.00	9.88	99.125	1.996183		
180	20.00	9.74	98.65	1.994097		

Results are the mean of three readings; MDC=mean NM content; PDR= percent NM remaining Weight of nanoemulsion taken 20mg of neem extract (20 mg of 0.05% of nimolidine=10ug)

Table 46. Post stability assessment of evaluation parameters of NM

Evaluation parameters of *F-3* optimized formulation						
Time (days)	**Weight (mg)**	**Neem extract Loading (%)**	**Assay Nimbo-lidine basis (%)**	**Droplet size nm F3**	**Dilution % Transmitance**	**Variation in drug release (Neem extract time of F3) compared at t=0**
0	260	100	99.99	255±30	99.9±0.1	±3.8
30	260	99.70	99.98	272±25	98.9±0.2	±4.7
60	260	99.10	99.50	288±15	98.9±0.1	±8.7
90	260	98.88	99.10	214±18	97.9±0.2	±6.2
180	258.45	97.40	98.80	236±17	97.9±0.3	±7.9

5.11Pharmacodynamic Studies

5.11.1 Induction of psoriasis

For pre-clinical research in monkeys, mice, and rats, more practical and quick psoriasis models have recently been created. Albino mice were employed in the study. In particular, imiquimod (IMQ) generated psoriasis-like skin inflammation in mice is thought to share many characteristics with human psoriasis and is consequently utilised to study the pathophysiology of inflammatory psoriasis as well as the development of novel treatment agents .

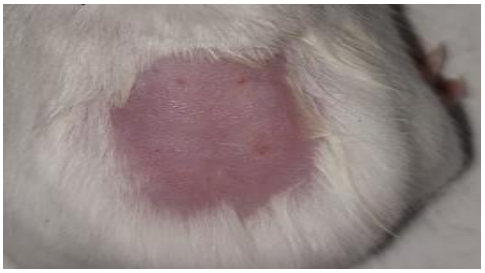
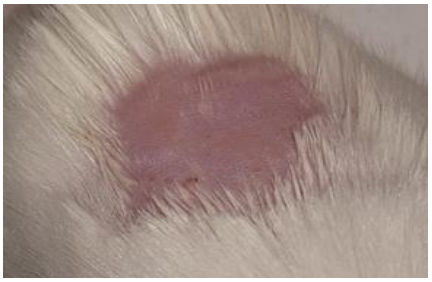
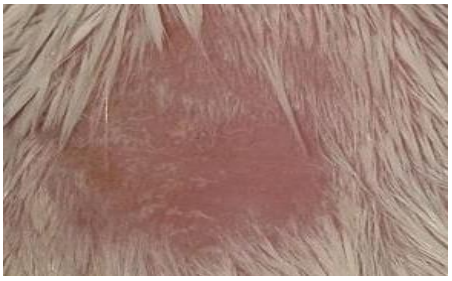
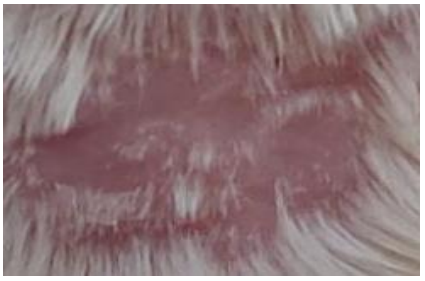
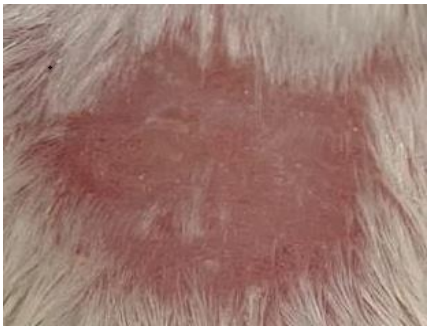
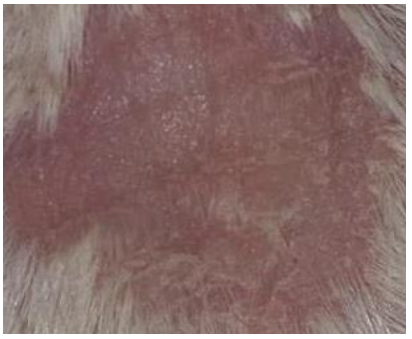
Day 0	Day 1
	
Day 2	**Day 3**
	
Day 4	**Day 5**
	

Figure 57. Represent Day first to day 5th Psoraitic skin development after induction

5.11.2 Psoriasis Area and Severity Index (PASI) score: Effect of extract, micro-emulsion low dose and high dose on psoriatic mice

Five days treatment with IMQ caused significant changes in skin (e.g. psoriasis like dermatitis, skin inflammation, keratitis erythema) as compared to normal control group and IMQ + placebo. Fourteen days of treatment with psoriasis + micro-emulsion of neem (LD) (0.05%), psoriasis + micro-emulsion of neem (HD) (0.1%); Psoriasis + neem extract (1%); Psoriasis + standard drug (Tazarotene 0.05%) significantly

attenuated changes in skin (e.g. psoriasis like dermatitis, skin inflammation, keratitis erythema) compared to IMQ group. However treatment with Psoriasis + neem extract (1%) and psoriasis + micro-emulsion of neem (LD) (0.05%) less significantly attenuated changes in skin compared to Psoriasis + standard drug (Tazarotene 0.05%). Moreover treatment with Psoriasis + neem extract (1%) and psoriasis + micro-emulsion of neem (LD) (0.05%) less significantly attenuated changes in skin compared to psoriasis + micro-emulsion of neem (HD) (0.1%) (Figure: 2).

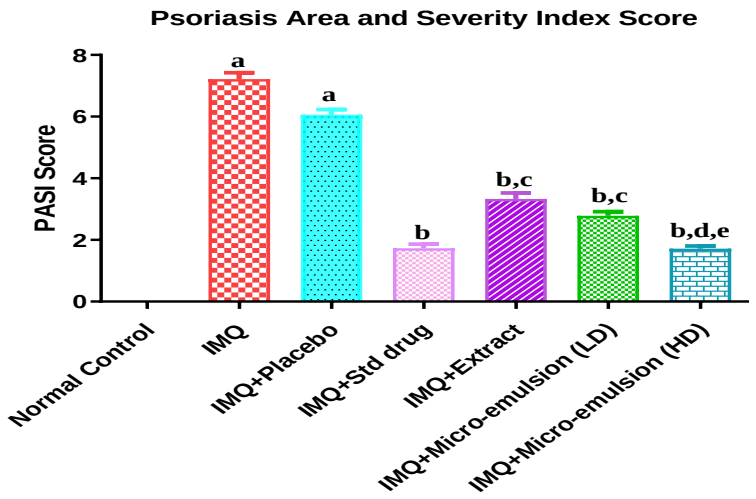


Figure 58. Effect of treatment on PASI score.

Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+Std, [d]P<0.05 as compared to IMQ+Extract, [e]P<0.05 as compared to IMQ + Micro-emulson (LD).

5.11.3 Effect of micro-emulsion of neem on Spleen wt/Body wt ratio on psoriatic mice

Five days treatment with IMQ caused significant changes in the spleen wt/body wt ratio as compared to normal control group and IMQ + pacebo. Fourteen days of treatment with psoriasis + micro-emulsion of neem (LD) (0.05%), psoriasis + micro-emulsion of neem (HD) (0.1%); psoriasis + neem extract (1%); psoriasis + standard drug (Tazarotene 0.05%) significantly attenuated changes in the spleen wt/body wt ratio compared to IMQ group. However treatment with psoriasis + neem extract (1%) and

psoriasis + placebo less significantly attenuated the changes in the spleen wt/body wt ratio compared to psoriasis + standard drug (Tazarotene 0.05%) groupMoreover treatment with Psoriasis + neem extract (1%) less significantly attenuated changes in the spleen wt/body wt ratio compared to psoriasis + micro-emulsion of neem (HD) (0.1%) (Figure: 3).

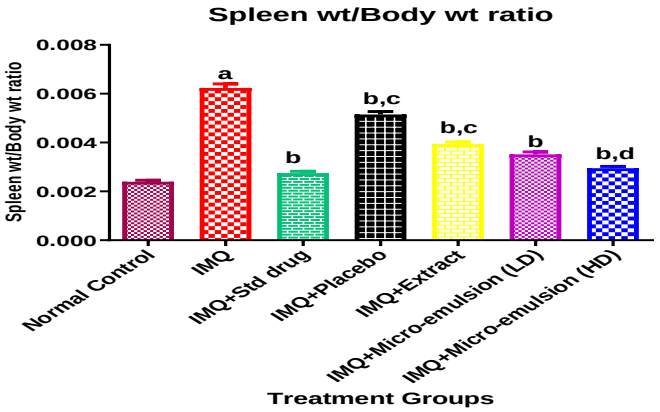


Figure 59 . Effect of micro-emulsion of neem on Spleen wt/Body wt ratio**.**

Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+Std, [d]P<0.05 as compared to IMQ+Extract.

5.11.4Effect of micro-emulsion of neem on SOD

Five days treatment with IMQ caused significant attenuated the SOD level as compared to normal control group. Fourteen days of treatment with psoriasis + standard drug (Tazarotene 0.05%) significantly attenuated the SOD level compared to IMQ group. However treatment with psoriasis + micro-emulsion of neem (HD) (0.1%) significantly attenuated the SOD level compared to Psoriasis + neem extract (1%) group

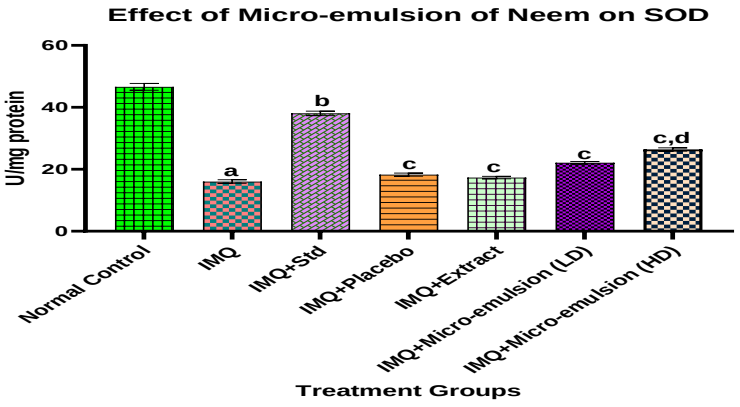


Figure 60. Effect of micro-emulsion of neem on SOD.Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+Std, [d]P<0.05 as compared to IMQ+Extract.

5.11.5Effect of micro-emulsion of neem on Catalase

Five days treatment with IMQ caused significant attenuated the catalase level as compared to normal control group. Fourteen days of treatment with psoriasis + standard drug (Tazarotene 0.05%) significantly attenuated the catalase level compared to IMQ group. However treatment with psoriasis + micro-emulsion of neem (LD) (0.05%) significantly attenuated the catalase level compared to Psoriasis + Placebo group. Moreover treatment with psoriasis + micro-emulsion of neem (HD) (0.1%) significantly attenuated the catalase level compared to Psoriasis + neem extract (1%) group.

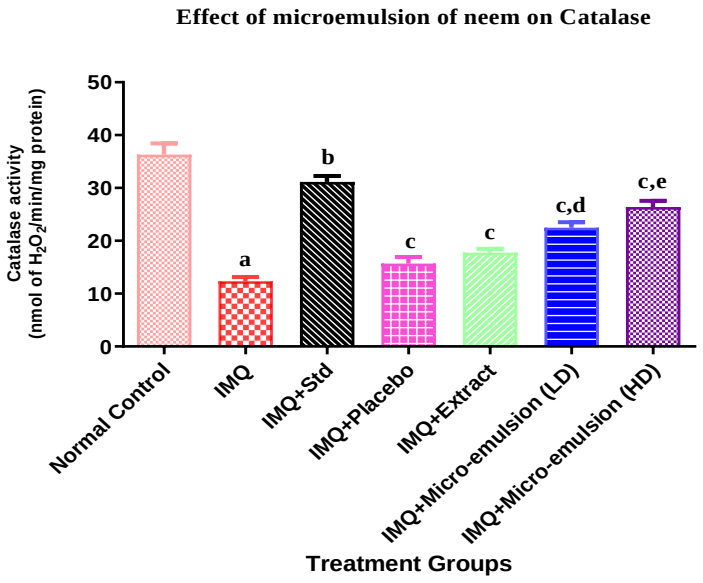


Figure 61. Effect of microemulsion of neem on Catalase

Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+Std, [d]P<0.05 as compared to IMQ+Placebo, [e]P<0.05 as compared to IMQ+extract.

5.11.6 Effect of Micro-emulsion of Neem on Glutathione

Five days treatment with IMQ caused significant attenuated the glutathione level as compared to normal control group. Fourteen days of treatment with psoriasis + standard drug (Tazarotene 0.05%) significantly attenuated the glutathione level compared to IMQ group. However treatment with psoriasis + micro-emulsion of neem (LD) (0.05%) and psoriasis + micro-emulsion of neem (HD) (0.1%) significantly attenuated the glutathione level compared to Psoriasis + Placebo group.

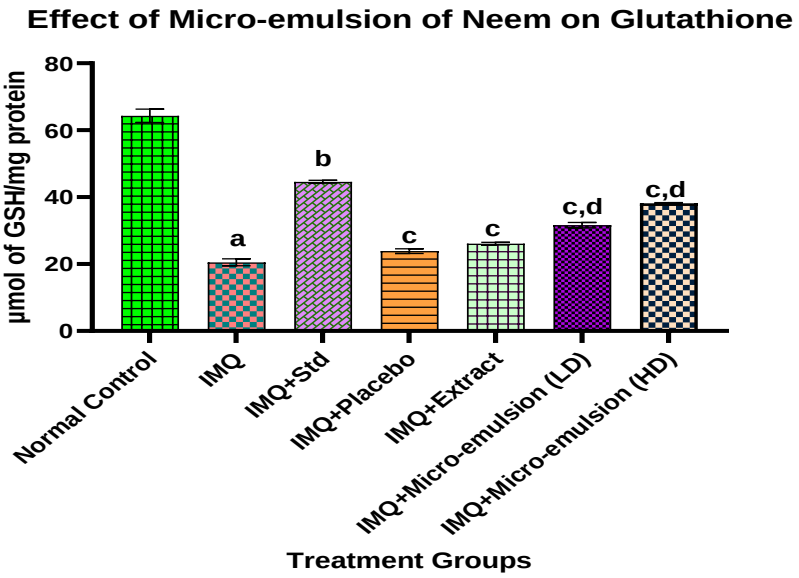


Figure 62. Effect of Micro-emulsion of Neem on Glutathione

Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+Std, [d]P<0.05 as compared to IMQ+Extract, [e]P<0.05 as compared to IMQ, [f]P<0.05 as compared to IMQ and IMQ+Extract.

5.11.7 Effect of micro-emulsion of neem on TBARS

Five days treatment with IMQ caused significant increases the TBARS level as compared to normal control group. Fourteen days of treatment with psoriasis + standard drug (Tazarotene 0.05%), psoriasis + micro-emulsion of neem (LD) (0.05%) and psoriasis + micro-emulsion of neem (HD) (0.1%) and psoriasis + extract significantly lower the TBARS level compared to IMQ group. However treatment with psoriasis + extract less significantly lower the TBARS level compared to Psoriasis + std

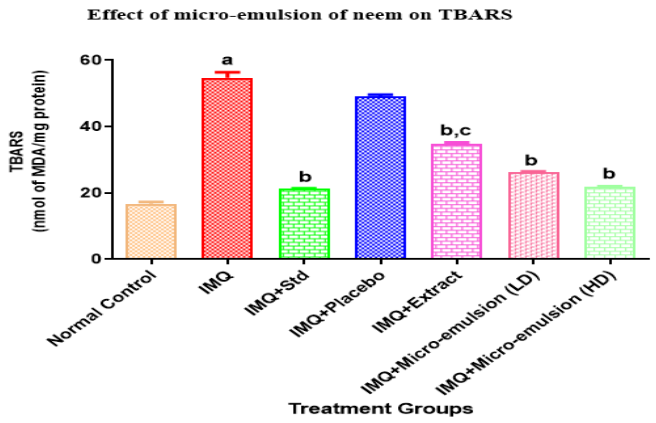


Figure 63. Effect of microemulsion of neem on TBARS**.** Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+Std.

5.11.8 Effect of micro-emulsion of neem on IFN-α.

Five days treatment with IMQ caused significant increases the IFN-α level as compared to normal control group. Fourteen days of treatment with psoriasis + standard drug (Tazarotene 0.05%), psoriasis + micro-emulsion of neem (LD) (0.05%) and psoriasis + micro-emulsion of neem (HD) (0.1%) and psoriasis + extract significantly lower the IFN-α level compared to IMQ group. However treatment with and psoriasis + micro-emulsion of neem (HD) (0.1%) less significantly lower theIFN-α level compared to Psoriasis + extract

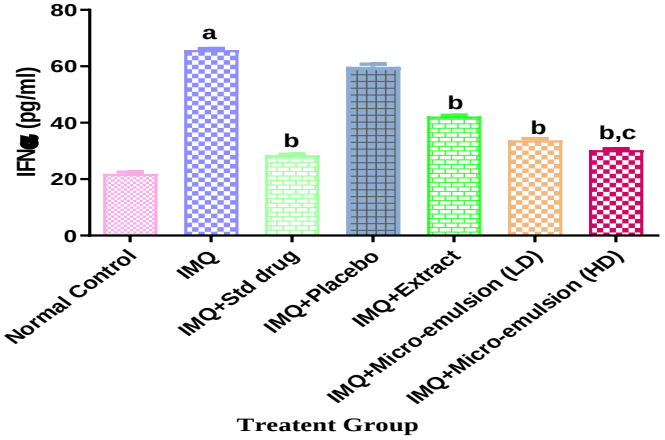


Figure 64. Effect of microemulsion of neem on IFN-α.Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+extract.

5.11.9 Effect of micro-emulsion of neem on IL-17.

Five days treatment with IMQ caused significant increases the IL-17 level as compared to normal control group. Fourteen days of treatment with psoriasis + standard drug (Tazarotene 0.05%), psoriasis + micro-emulsion of neem (LD) (0.05%) and psoriasis + micro-emulsion of neem (HD) (0.1%) and psoriasis + extract significantly lower the IL-17 level compared to IMQ group. However treatment with and psoriasis + micro-emulsion of neem (HD) (0.1%) less significantly lower theIL-17 level compared to Psoriasis + extract (Figure: 9).

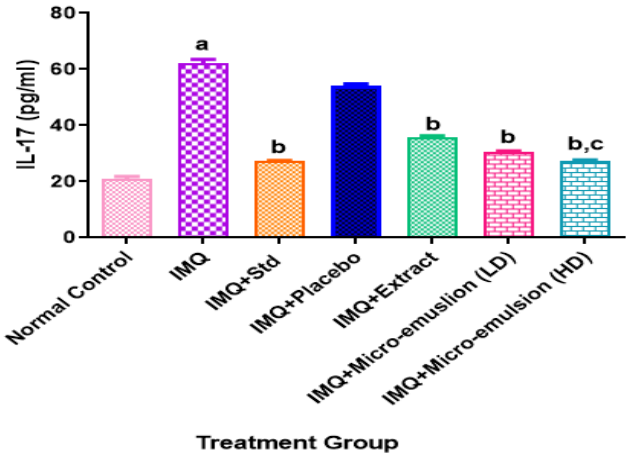


Figure 65. Effect of micro-emulsion of neem on IL-17Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+extract.

5.11.10. Effect of micro-emulsion of neem on IL-23.

Five days treatment with IMQ caused significant increases the IL-23 level as compared to normal control group. Fourteen days of treatment with psoriasis + standard drug (Tazarotene 0.05%), psoriasis + micro-emulsion of neem (LD) (0.05%) and psoriasis + micro-emulsion of neem (HD) (0.1%) and psoriasis + extract significantly lower the IL-23 level compared to IMQ group. However treatment with and psoriasis + micro-

emulsion of neem (HD) (0.1%) less significantly lower theIL-23 level compared to Psoriasis + extract (Figure: 9).

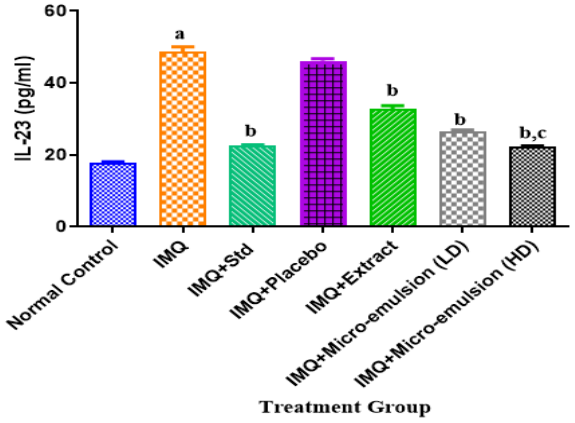


Figure 66. Effect of micro-emulsion of neem on IL-23

Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+extract.

5.11.11. Effect of micro-emulsion of neem on TNFα.

Five days treatment with IMQ caused significant increases the TNFα level as compared to normal control group. Fourteen days of treatment with psoriasis + standard drug (Tazarotene 0.05%), psoriasis + micro-emulsion of neem (LD) (0.05%) and psoriasis + micro-emulsion of neem (HD) (0.1%) and psoriasis + extract significantly lower the TNFα level compared to IMQ group. However treatment with and psoriasis + micro-emulsion of neem (HD) (0.1%) less significantly lower theTNFα level compared to Psoriasis + extract

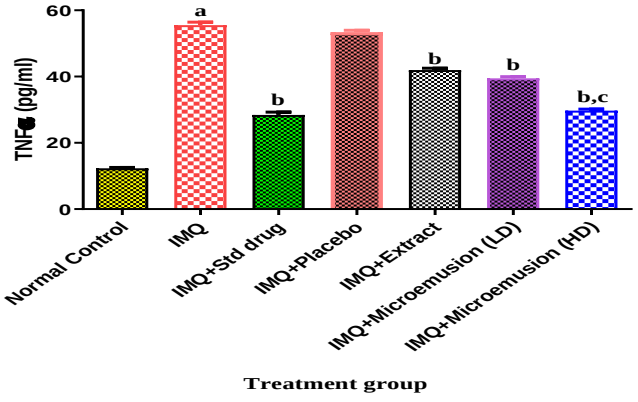


Figure 67. Effect of micro-emulsion of neem on TNFα Values are expressed as Mean ± SEM (One-way ANOVA followed by Tukey's Test, n=6, [a]P<0.05 as compared to Normal control, [b]P<0.05 as compared to IMQ, [c]P<0.05 as compared to IMQ+extract.

5.11.12Histopathological examination

The results of the histology analysis are shown in below figure. The normal control group did not exhibit any signs of keratosis suggesting the intact and normal skin. The skin was thick (coated with white desquamation), keratosis and hyperkeratosis with scales and erythema in the IMQ group. The changes were reversed after treatment with various formulations.

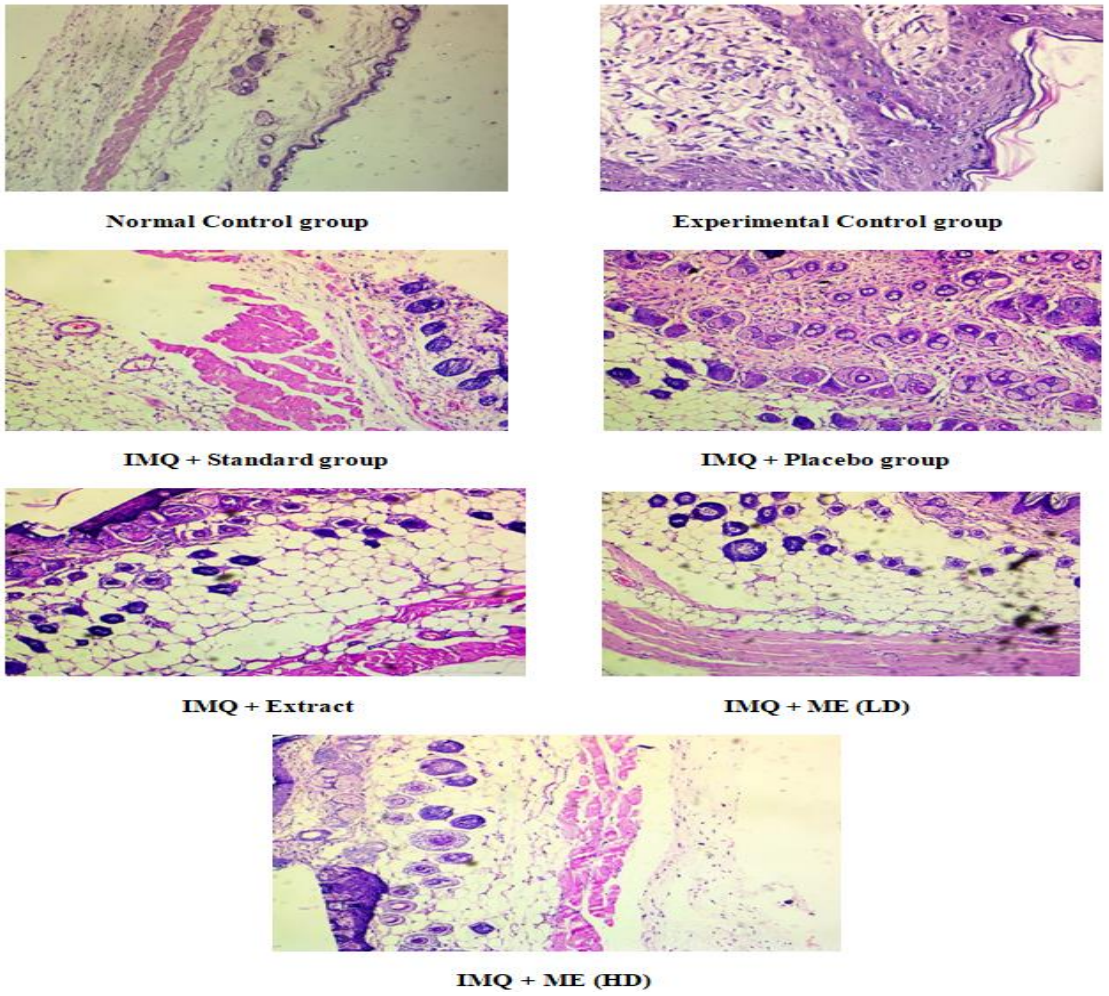


Figure 68. Above figure shows histopathological analysis A (Normal Control group), B (Experimental control group), C (Immiquimod with standard drug), D (Immiquimod with Placebo), E (Immiquimod with extract), F (Immiquimod with Micro-emulsion LD), G (Immiquimod with Micro-emulsion H

CHAPTER -6

Conclusion and Future Perspective

Conventional dosage form has many limitations which include poor drug solubility, insufficient drug concentration, poor absorption, low permeability, rapid metabolism and elimination, drug distribution to other tissues combined with high drug toxicity and short half-life. The present study emphasizes the role of herbal phytoactive for the treatment of psoriasis. Herbal phytochemicals plays an important role for the treatement of various dreadful diseases. Five herbal drugs i.e *A.indica*, *Psoralia corylifolia*, *P.pinnata*, *W.fruticosa* & *C.longa* has been selected for the study. On the basis of *In vitro* anti-psoriatic activity by performing MTT, ELISA, LDH and confocal microscopy one most potent extract is selected further for the micro-emulsion preparation. Extract of *A.indica* shows great decrease in mean viability as compare to extract treated group or compound B,C,D and E respectively. Compound A i.e *A.indica* extract having great cellular inflammatory response ass compare to other group. Extract of *A.indica* also shows weak cytoplasmic expression of caspase 7 proteins of compound A &B but positive control group is having increased cytoplasmic expression. *In vivo* pharmacodynamics studies are performed for 14 days and show great effect over psoriatic lesion over swiss albino mice skin. Fourteen days of treatment with psoriasis + micro-emulsion of neem (LD) (0.05%), psoriasis + micro-emulsion of neem (HD) (0.1%); Psoriasis + neem extract (1%); Psoriasis + standard drug (Tazarotene 0.05%) significantly attenuated changes in skin (e.g. psoriasis like dermatitis, skin inflammation, keratitis erythema) compared to IMQ group. PASI score, SOD activity, spleen to body weight index and histopathological examination of skin surface shows recovery in *A.indica* micro-emulsion (HD).

Moreover, overall study depecits the successful development of *A.indica* micro-emulsion for the treatment of psoriasis. The prepared formulation is having great botanical potential when incorporated in NDDS. From pilot batch to scale up of the formulation should also be possible in NDDS leads to new platform for herbal potentials and researcher also.

CHAPTER -7

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Annexure- 1

LOVELY PROFESSIONAL UNIVERSITY

Centre for
Research Degree Programmes

LPU/CRDP/PHD/EC/20200108/000577

Dated: 23 Sep 2019

Arun Kumar
VID: 41800307
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 23 Sep 2019 by accepting your research proposal entitled: "Development and Pharmacological evaluation of Polyherbal microemulsion for the treatment of psoriasis"

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.

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Annexure- 2

ਬੋਟੈਨੀਕਲ ਐਂਡ ਐਨਵਾਇਰਨਮੈਂਟਲ ਸਾਇੰਸਿਜ਼ ਵਿਭਾਗ
ਗੁਰੂ ਨਾਨਕ ਦੇਵ ਯੂਨੀਵਰਸਿਟੀ, ਅੰਮ੍ਰਿਤਸਰ-143 005

Department of Botanical & Environmental Sciences
Guru Nanak Dev University, Amritsar-143 005, India
(Established by the State Legislature Act No. 21 of 1969)
Accredited at "A" grade level by NAAC and awarded "University with Potential for Excellence" status by UGC

Ref. No. 490 Bot. & Env. Sc.
Dated 12/11/2020

To Whom It May Concern

The plant specimen(s) brought by Arun Kumar Student of Ph-D student of Lovely Professional University, belongs to the following species.

1. Azadirachta indica
2. Woodfordia fruticosa.
3. Psoralea corylfolia
4. Pongamia pinnata
5. Curcuma longa

Signature of Student

Herbarium Assistant Avtar Singh

Teachers Incharge

12/11/2020
Head

Head
Department of Botanical
& Environmental Sciences,
Guru Nanak Dev University, Amritsar

Phone: +91-0183-2451048; PABX: 0183-2258802-09, Extn.: 3193; Fax: 0183-2258819-20
Website: http:/www.gndu.ac.in e-mail: gndu_botanical@hotmail.com

Annexure- 3

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Certificate

This is to certify that the project proposal no. **IAEC/KSOP/2022-23/05** entitled **"Evaluation of Herbal Formulation (Microemulsion) in the Treatment of Psoriasis"** submitted by **Mr. Pawan Prajapati** has been approved/recommended by the IAEC of **KIET School of Pharmacy, Ghaziabad** in its meeting held on 08/10/2022 and **42 Albino mice** have been sanctioned under this proposal for a duration of next **06** months.

Authorized by	Name	Signature	Date
Chairman	Dr. K. Nagarajan		19/10/22
Member Secretary	Dr. Vinay Kumar		19/10/22
Main Nominee of CPCSEA	Dr. Babita Kumar		19/10/2022

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Annexure- 4

Pharmacogn. Res., 2023; 15(4):831-840.
https://www.phcogres.com

Original Article

Physicochemical and *in vitro* Analysis of Herbal Drugs *A. indica, C. longa, P. pinnata, P. corylifolia, W. fruticosa* for Potential Effect in Psoriasis

Arun Kumar, Saurabh Singh*, Bimlesh Kumar, Sheetu Wadhwa, Dileep Singh Baghel, Narendra Kumar Panday, Sachin Kumar Singh, Shivani, Vikas Kumar Pal

School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, INDIA.

ABSTRACT

Background: *Curcuma longa* (Zingiberaceae), *Azadirachta indica* (Meliaceae), *Psoralea corylifolia* (Fabaceae), *Pongamia pinnata* (Fabaceae), and *Woodfordia fruticosa* (Lythraceae) stand as renowned medicinal plants in India. Traditionally, different parts of these plants have been employed for healing, particularly in the context of treating psoriasis and various other ailments. This catalyzed our investigation into the potential antipsoriatic properties of these botanicals. **Objectives:** We set out to evaluate the preliminary phytochemical analysis and *in vitro* antipsoriatic capabilities of acetone, ethanol, and water extracts derived from *Curcuma longa, Azadirachta indica, Psoralea corylifolia, Pongamia pinnata,* and *Woodfordia fruticosa*. **Materials and Methods:** The antipsoriatic potential of these extracts was assessed through the MTT assay, using HaCaT cells. Additionally, we conducted LDH assays and utilized confocal microscopy to gain further insights. **Results:** Our investigations unveiled the promising antiproliferative activity of these plants on skin keratinocytes. Notably, compounds A, B, and C demonstrated a significant decrease in cell viability compared to the control group. In contrast, compounds D and E did not exhibit significant differences compared to the control. **Discussion and Conclusion:** These findings substantiate the traditional uses of these plants in the treatment of psoriasis and underscore their potential as valuable resources in the quest for effective antipsoriatic treatments.

Keywords: *Curcuma longa, Azadirachta indica, Psoralea corylifolia, Pongamia pinnata, Woodfordia fruticosa*, haCaT cells.

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INTRODUCTION

Psoriasis, an ailment affecting the skin, manifests when the body's immune system sends erroneous signals, leading to an accelerated skin cell growth cycle. This condition is a non communicable, multifaceted autoimmune skin disorder, recognizable by the presence of reddish, silvery patches on the skin, excessive keratinocyte proliferation, heightened dermal vascularity, and persistent inflammation.[1,2] This malady has been known for centuries, and presently, approximately 2-3% of the global population grapples with it. Psoriasis carries significant social, psychological, and economic implications.[3] Its impact on quality of life closely resembles that experienced by individuals coping with other chronic health conditions, such as diabetes and depression.[4,5]

Numerous scientific studies have provided evidence those certain medicinal plants, such as Radix pae, *Rubia cordifolia* Linn. (Rubiaceae), *Coptis chinensis* Franch. (Ranunculaceae), *Alpinia galangal* Linn. (Zingiberaceae), *Annona squamosa* Linn. (Annonaceae), *Curcuma longa* Linn. (Zingiberaceae), and others, exhibit effectiveness in the treatment of psoriasis.[6-12]

In this research, we focused on five Indian medicinal plants, namely *Curcuma longa* (Zingiberaceae) *Azadirachta indica* (Meliaceae) *Psoralea corylifolia* (Fabaceae) *Pongamia pinnata* (Fabaceae) and *Woodfordia fruticosa* (Lythraceae) to assess their impact on skin keratinocyte proliferation. These plants have a well-established history of use by traditional healers for the treatment of chronic skin inflammation and conditions resembling psoriasis. Despite the historical usage of these plants, there is a scarcity of scientific research examining their anti-psoriatic potential. The primary objective of this study was to uncover the therapeutic benefits these medicinal plants may offer in the context of psoriasis treatment. To achieve this, we employed a cultured HaCaT cell line, which is a well-established *in vitro* model for assessing the antipsoriatic properties of novel treatments, owing to its highly preserved differentiation capacity.

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Cont.

An overview on: Anti Psoriasis Phytocomponents Used in Novel Drug Delivery Systems

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Abstract

Psoriasis is proliferative autoimmune inflammatory skin disease which is mainly affecting 2 % of world population. The initial sign and symptoms is mainly characterized by itching, redness and silver scaly plaques over the skin. The pathogenesis of disease includes activation of T cells which may cause release of cytokines leads to inflammation over skin. Various subtypes of psoriasis is reported i.e Plaque Psoriasis, Flexural Psoriasis, Pustular psoriasis, Generalized Pustular Psoriasis, Palmo plantar psoriasis, Guttate psoriasis, Psoriatic nail disease diagnosed by skin biopsy. Many herbal remedies alone or in with combination is being used for the treatment of various disease. Herbal chief constituents incorporated in novel drug delivery system plays an important role for the treatment of psoriasis. The aim of this review is to understand the importance of herbal active constituents which are used in the treatment of psoriasis by inhibiting cell proliferation and lipid peroxidation over skin. The review also discussed the potential approach of novel drug delivery over conventional drug delivery for treatment of psoratic lesions. *In vitro* studies also demonstrate the therapeutic potential of herbal phytoactive for the treatment of psoriasis. Plants selected in this review article is having great therapeutic potential in terms of treating psoriasis.

KEYWORDS

Psoriasis, Types, Pathophysiology, Herbal Phytoactive, Novel Drug Delivery In-*Vitro* Antipsoriatic activity

INTRODUCTION

Psoriasis is autoimmune inflammatory skin disease which may leads to increase in cell proliferation. The disease is mainly characterized by silvery scaly plaques over the skin which may leads to itching, redness and inflammation over affected part and cause papules over skin so this type of disease is also called papulo squamous disease [1]. This skin disease approximately affect about 4-5 % of the world population [2]. In this type of skin disease the upper most layer of skin i.e epidermis move towards the surface and then continuously shed form the skin. The name psoriasis is derived from greek word 'psora' which means itching and 'sis' means action [3]. The white colour plaques frequently appear on elbows, knee, genital area and may affect any part of the body, fingernail and toenails are the main affected area in psoriasis [4].

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to me, Vikas, Saurabh, Shivani, Bimlesh, Dileep, Kalvatala, Narendra, Kalpana, Sheetu, Aswin, Amrik, Avijit, Gurmeet

Arun Kumar, Vikas Kumar Pal, Saurabh Singh Saurabh, Shivani, Bimlesh Kumar, Dileep Singh Baghel, Kalvatala Sudhakar, Narendra Kumar Pandey, Kalpana Thakur, Sheetu Wadhwa, Aswin viswanath, Amrik Singh, Avijit Mazumder, Gurmeet Singh (Author):

We have reached a decision regarding your submission to Journal of Natural Remedies, 36242-"Physicochemical and Phytochemical Evaluation of P. corylifolia (seed) and its Extract: Evaluation of P. corylifolia (seed) and its Extract".

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