PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL SCREENING AND PHARMACOLOGICAL EFFECTS OF *ARTEMISIA VESTITA* LEAF EXTRACT AND TRAGACANTH GUM BASED HYDROGEL

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Microbiology

By

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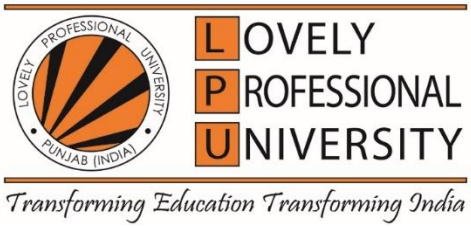

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DECLARATION

I, hereby declared that the presented work in the thesis entitled "**Phytochemical analysis, antimicrobial screening and pharmacological effects of *Artemisia vestita* leaf extract and Tragacanth gum based hydrogel**" in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of **Dr. Bhupendra Koul**, working as **Professor, in the Department of Biotechnology, School of Bioengineering and Biosciences, 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 another 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 "**Phytochemical analysis, antimicrobial screening and pharmacological effects of *Artemisia vestita* leaf extract and Tragacanth gum based hydrogel**" submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** is a research work carried out by **Shivani Dogra, 41900129**, is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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Abstract

Medicinal plants have played a crucial role in the development of therapeutic drugs and have been widely used by various ethnic communities and traditional practitioners since ancient times. Natural products remain a valuable source of bioactive compounds with significant pharmacological potential, making them a focus of extensive research. *Artemisia vestita*, a medicinal plant known for its bioactive properties, and Tragacanth gum, a natural polymer commonly used in drug delivery systems, offer promising applications. Combining these two components in a hydrogel formulation introduces an innovative strategy to improve drug stability, enhance bioavailability, and facilitate targeted delivery.

The skin serves as a crucial protective barrier, safeguarding the body against external factors such as pathogens, as well as mechanical, thermal, and chemical stressors. However, it is highly susceptible to various types of injuries, including burns, minor cuts, and chronic wounds like pressure ulcers and diabetic sores. When chronic wounds fail to heal properly, they lose their innate self-repair mechanisms, increasing the risk of severe complications if not treated effectively.

To address this critical issue, we have developed a hydrogel utilizing natural polymers to meet the growing demand for efficient wound-healing solutions. This study explores polymer-based strategies that integrate plant extracts. Many currently available skin regeneration hydrogels rely on synthetic polymers or animal-derived materials such as collagen, which may present challenges such as poor biodegradability and potential immune reactions. The underlying hypothesis of this research is that the phytochemical compounds found in *A. vestita* leaf extract possess diverse therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory effects. When incorporated into a Tragacanth gum-based hydrogel, these bioactive compounds exhibit improved stability,

controlled release, and enhanced therapeutic efficacy. This innovative hydrogel formulation is expected to exhibit strong antimicrobial activity against pathogenic microorganisms while promoting wound healing, positioning it as a promising candidate for biomedical and pharmaceutical applications.

A hydrogel is a biphasic material consisting of a porous and permeable solid network combined with a substantial amount of interstitial fluid, typically water-based, making up at least 10% of its weight or volume. The solid component of these hydrogels forms a three-dimensional, water-insoluble structure that can be derived from either natural or synthetic polymers. This structural composition allows hydrogels to absorb and retain large quantities of water or biological fluids, making them highly valuable for biomedical applications. While many hydrogels are synthetically engineered, some are sourced from natural materials.

Artemisia vestita Wall. Ex Besser, commonly known as "Russian Wormwood" or "Kubsha," is a medicinal plant with a long history in traditional healing practices. It belongs to the Asteraceae family and is recognized for its therapeutic potential. Traditionally, this plant has been used to treat wound infections, heat-related ailments, rashes, and skin irritations due to its naturally cooling properties. It is a perennial, upright shrub that can grow up to two meters tall, featuring grooved stems and fern-like leaves. The leaves are soft and hairy on the upper surface, while the underside has a white, woolly texture. The plant produces small flowers arranged in racemes, typically in clusters of 6–10, with a distinct sweet, woody fragrance and a naturally bitter taste.

Natural gums, which are plant-derived polymers, exhibit multiple functional properties such as stabilizing, emulsifying, thickening, increasing viscosity, structuring, and gelling. These gums are generally odourless, tasteless, and viscous, consisting of water-soluble polysaccharides obtained from the extracted and dried sap of plant roots. Due to their medicinal property, they are highly suitable for pharmaceutical and biomedical applications.

Given the longstanding use of *A. vestita* in traditional medicine and its frequent collection from natural forests for treating inflammation and fever, there is a need to develop an innovative hydrogel incorporating its leaf extract. Among the various extraction techniques, aqueous and alcoholic solvents have been found to yield the highest concentrations of bioactive medicinal compounds from the plant. This species has played a significant role in Tibetan and Chinese medicine, particularly in managing inflammatory conditions such as contact dermatitis, rheumatoid arthritis, and sepsis. Crushed leaves have traditionally been applied externally to control bleeding and also in relieving stomach pain.

To enhance the effectiveness of this formulation, natural gums have been incorporated into the hydrogel due to their multifunctional properties. These plant-derived polymers, obtained from dried sap, contribute to the hydrogel's stability, emulsification, thickening, and gelation, improving its overall therapeutic potential.

This research seeks to address an existing gap by introducing Tragacanth gum, a natural polymer, as a sustainable and biocompatible material for skin tissue engineering. As an eco-friendly and biodegradable alternative, Tragacanth gum breaks down naturally without producing harmful byproducts, making it a safer choice for biomedical applications. Despite its extensive traditional use, the therapeutic properties of *A. vestita* remain largely unverified through scientific studies, and its potential in tissue engineering is yet to be fully explored. Many conventional dermal gels lack a combination of antimicrobial, anti-inflammatory, and antioxidant properties, limiting their effectiveness. To overcome this challenge, this study incorporates *A. vestita* extract into a Tragacanth gum-based hydrogel, harnessing their combined bioactive properties to accelerate the wound-healing process.

Additionally, many existing dermal formulations suffer from short-lived drug release, reducing their therapeutic efficacy. The proposed hydrogel addresses this limitation by ensuring sustained release of bioactive compounds, improving treatment outcomes and promoting more efficient wound healing.

The present study focuses on *A. vestita*, commonly known as Kubsha, a traditional medicinal plant. The research aims to identify and characterize the secondary metabolites present in *A. vestita*, to assess the *in vitro* antimicrobial activity of its leaf extract, to develop and incorporate the extract into a crosslinked polymer matrix to enhance drug-loading capacity and evaluate its release profile and investigate pH-sensitive and temperature-responsive drug release mechanisms. By integrating scientific validation with traditional knowledge, this study contributes to the advancement of biomedical and pharmaceutical applications through the development of a novel, nature-based hydrogel for enhanced wound healing and skincare.

This thesis explores the phytochemical composition of *Artemisia vestita* leaf extract (ALE) and evaluates its antimicrobial activity against clinically significant pathogens, along with its antioxidant, anti-inflammatory, cytotoxic, and wound-healing properties. The phytochemical analysis was carried out by using Soxhlet extraction, followed by GC-MS profiling to identify bioactive compounds. Antimicrobial activity was assessed using the agar well-diffusion method against selected bacterial and fungal strains. The antioxidant potential of ALE was determined through DPPH, ABTS, and FRAP assays, while its anti-inflammatory effects were evaluated via a COX-II enzyme inhibition assay. The cytotoxicity of ALE on HaCaT cells was examined using the MTT assay, and its wound-healing potential was assessed using the *in vitro* scratch assay.

The antimicrobial screening demonstrated significant inhibition zones against multiple microbial strains, including *S. aureus* (14.2 mm), *E. coli* (17.6 mm), *B. subtilis* (13.1 mm), *S. pyogenes* (17.3 mm), *P. mirabilis* (9.4 mm), and *C. albicans* (17.6 mm). The GC-MS analysis identified 36 phytochemicals in ALE, with 22 major constituents. Additionally, ALE displayed strong free radical scavenging activity across different antioxidant assays and exhibited notable anti-inflammatory effects. The MTT assay confirmed ALE's cytotoxic impact on HaCaT cells, while the scratch assay demonstrated 94.6% wound

closure within 24 hours of incubation, showing results comparable to the standard positive control, Cipladine.

This study is the first to document the wound-healing potential of *A. vestita* from Himachal Pradesh, India. The findings highlight its therapeutic potential for wound care, providing valuable insights into its medicinal applications and supporting its use as a promising natural treatment in biomedical and pharmaceutical research. Furthermore, the study also investigates the invitro pharmacological effects of *A. vestita* leaf extract-loaded Tragacanth gum-based hydrogel (ALEH), with a focus on its wound-healing, anti-inflammatory, and antioxidant properties. A total of eight distinct formulations (F1 to F8) were systematically developed, incorporating *A. vestita* leaf extract, Tragacanth gum, humectants, preservatives, pH stabilizers, and deionized water. To ensure both safety and effectiveness, a preformulation study was conducted, followed by extensive testing to evaluate its functional characteristics and potential applications.

Key parameters such as drug release and swelling behaviour were analysed under different pH conditions and temperatures, with the most suitable drug release model identified based on the regression coefficient (R^2 value). The hydrogel's antimicrobial activity was assessed using the agar well-diffusion method, while its wound-healing potential was evaluated through a scratch assay on HaCaT cells. ALEH exhibited a non-Fickian diffusion mechanism, with enhanced drug release at pH 6.8 compared to pH 4.5, confirming its pH-responsive release profile. The hydrogel demonstrated notable antimicrobial activity, showing the highest antibacterial effects against *E. coli*, *S. pyogenes*, and *C. albicans*, with zones of inhibition measuring 19.6 mm, 20.3 mm, and 19.4 mm, respectively. Moderate inhibition zones of 15 mm and 15.1 mm were observed against *S. aureus* and *B. subtilis*, while *P. mirabilis* exhibited the lowest inhibition zone of 8.6 mm. Additionally, the hydrogel achieved 95% wound closure within 24 hours *in vitro*, demonstrating strong wound-healing capability.

This hydrogel formulation is well-suited for large-scale industrial production, offering a shelf-life of over one year, making it a viable candidate for pharmaceutical and medical applications. By integrating phytochemistry, microbiology, and pharmacology, the study provides the first experimental validation of a hydrogel formulation combining *A. vestita* leaf extract and Tragacanth gum, supporting its potential in skincare and wound management. The findings may contribute to the development of plant-based therapeutic agents with improved efficacy, paving the way for further translational research in drug formulation and natural product pharmacology.

PREFACE

Natural products have long been a valuable source of medicinal compounds, offering diverse bioactive molecules with therapeutic potential and their exploration has always been a cornerstone of drug discovery. Among the species of genus *Artemisia*, *Artemisia vestita* have garnered significant attention due to their pharmacological properties, including antimicrobial, antioxidant, and anti-inflammatory activities. Likewise, plant derived hydrogel system such as Tragacanth gum, a natural polysaccharide, has gained attention for its biocompatibility and potential use in drug delivery systems. The combination of these two natural resources presents an opportunity to develop innovative formulations with enhanced therapeutic benefits.

This study focuses on phytochemical composition of *A. vestita* leaf extract, investigate their antimicrobial properties, and evaluate their pharmacological effects *in vitro*. By employing advanced analytical techniques *A. vestita* is systematically examined to identify key bioactive compound which are responsible for therapeutic effects, while antimicrobial screening is conducted against various microbial strains to determine their effectiveness. Furthermore, a hydrogel formulation based on Tragacanth gum is developed as a carrier for *A. vestita* extract, and its physicochemical properties, stability, and biological activity are assessed.

The study also explores the potential of this hydrogel system as a drug delivery platform, examining its interaction with cellular models to understand its pharmacological effects. By integrating plant-based bioactive compounds with hydrogel technology, this work aims to contribute to the development of novel therapeutic strategies that combine natural medicine with modern pharmaceutical approaches.

The present thesis entitled **"Phytochemical analysis, antimicrobial screening and pharmacological effects of *Artemisia vestita* leaf extract and Tragacanth gum-based**

hydrogel" encompasses the details of the studies undertaken and analyses of results obtained are presented as eight major chapters:

Chapter 1 – Introduction: This chapter includes a brief introduction of the traditional plant *Artemisia vestita* and the natural polymer Tragacanth gum, emphasizing their potential in hydrogel formulations for skin tissue engineering. It explores their role in overcoming limitations of conventional dermal gels by offering enhanced stability, prolonged shelf-life, and sustained release of bioactive compounds to support wound healing.

Chapter 2 - Review of Literature: This chapter presents an in-depth review of previous studies on the phytochemical composition, antimicrobial properties, and pharmacological effects of *Artemisia vestita*, along with the significance of Tragacanth gum in hydrogel-based drug delivery systems. By analysing extent literature, it highlights the research gap and prepares the ground for the current study.

Chapter 3 - Hypothesis: This chapter outlines the hypothesis of the current study.

Chapter 4 - Aims and Objectives: This chapter outlines the goals and objectives of the current study.

Chapter 5 – Methodology: This chapter covers the collection, identification, and extraction of the plant, followed by phytochemical analysis and antimicrobial evaluation against specific pathogens. It also includes the formulation and characterization of the hydrogel, along with an assessment of its drug release profile**.**

Chapter 6- Results and Discussion: This chapter presents the results and discussion of the study, focusing on the identification of phytoconstituents, hydrogel formulation, and methods to enhance the stability of *Artemisia vestita* leaf extract (ALE). It examines the antimicrobial properties of both ALE and ALE-infused hydrogel (ALEH), along with their *in vitro* pharmacological effects. Incorporating ALE into a Tragacanth gum-based hydrogel

aims to improve its stability, extend shelf life, and enhance its wound-healing and antimicrobial capabilities.

Chapter 7- Summary and Conclusion: This chapter provides a summary of the study conducted in this thesis along with the findings obtained.

Chapter 8- Bibliography: This chapter includes citations of the references utilized in the current study.

Shivani Dogra

ACKNOWLEDGEMENTS

The journey of my Ph.D. has been a challenging yet transformative experience, reshaping me in ways I never anticipated. At the very beginning, I would like to express my profound gratitude and heartfelt indebtedness to the Almighty and Devta Ji, whose divine guidance has supported me throughout my research. Without this sacred blessing, my journey would not have been possible. I barely recognize the person I have become, but I have come to understand that every challenge holds meaning and purpose. As I approach the end of this journey, I embrace these changes, emerging more confident and prepared to face future obstacles. Looking back at this pivotal phase of my life, I am profoundly grateful for the support and guidance I have received from many individuals along the way.

First and foremost, I extend my sincere gratitude to my Ph.D. supervisor **Dr. Bhupendra Koul**, Professor, Department of Biotechnology, Lovely Professional University, Jalandhar, Punjab. for granting me the opportunity and an exceptional platform to be part of his research group. His unwavering commitment to excellence and passion for achieving the highest standards have been a constant source of motivation throughout my Ph.D. journey. I am profoundly thankful for his invaluable guidance and mentorship, which continuously challenged me to push my limits. His faith in me, even during moments of self-doubt, has been truly inspiring. His encouragement and unwavering support have been instrumental in shaping both my personal and professional growth. His unparalleled critical evaluations and insights have helped me evolve into a confident and independent researcher. The time spent under his mentorship has been an enriching and unforgettable experience, and I feel truly fortunate to have him as my supervisor.

I wish to convey my sincere thanks to **Dr. (Prof.) Ashish Vyas,** Head, Dept. of Microbiology; **Dr. (Prof.) Joydeep Dutta,** Head, Dept. of Zoology; **Dr. (Prof. and Dean) Neeta Raj Sharma**, Dean, School of Bioengineering and Biosciences, for her kind support. I would also like to thank **Dr. Ashok Mittal**, Chancellor, LPU; **Mrs. Rashmi Mittal,** Pro

Chancellor LPU, and **Dr. (Prof) Lovi Raj Gupta**, Pro Vice Chancellor LPU, for their vision ('Think Big') motivation and support.

I sincerely extend my deep gratitude to my Co-Supervisor, **Dr. (Prof.) Joginder Singh Panwar**, Professor, Department of Botany, Nagaland University, Nagaland, for his invaluable guidance, unwavering encouragement, constructive feedback, and steadfast moral support. His kindness, cooperation, and mentorship have been truly instrumental throughout my journey. I deeply appreciate his friendly and supportive demeanour.

I would like to express my heartfelt gratitude to **Dr. HemRaj Vashist**, Pharmacy Officer, PHC Goela, Kasauli, Solan, HP, for his unwavering support and guidance. His assistance at every step was invaluable in completing my thesis. From guiding me in my early days on how to search for research papers and build a researcher profile to improving my writing skills, he has always been there to help. His support extended beyond academics, providing emotional and financial assistance while patiently addressing all my queries. Dr. Vashist is one of the most knowledgeable, dedicated, and selfless individuals I have ever met, always willing to share his expertise and support my growth. I am truly grateful for his mentorship and encouragement.

I acknowledge the efforts of **Dr. Arvind Kumar**, Centre for Aromatic Plants (CAP), Dehradun, Uttarakhand, India for his help in redrawing the structure of phytochemicals.

I would like to express my sincere gratitude to **Dr. Muhammad Fazle Rabbee**, International Research Professor, Department of Life Sciences, Yeungnam University, Gyeongsan, Republic of Korea, and **Dr. Dhananjay Yadav**, Research Scientist, Department of Neurosurgery and Brain Repair, University of South Florida Morsani College of Medicine, Tampa, USA, for their invaluable support and guidance in suggesting reputed journals.

I am also deeply thankful to **Mr. Kuldip Singh**, Deputy Superintendent, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, for his continuous assistance and encouragement in the laboratory and throughout various university-related tasks. Their contributions have been instrumental in the successful completion of my

research work.

I take this opportunity to express my gratitude to all my teachers throughout my life, whose guidance has brought me to where I am today. I also extend my heartfelt thanks to all my friends who supported my emotional well-being; without their help, I would not have come this far.

Finally, I would be indebted to my parents and other family members for their constant support. I feel honored to express my highest level of gratitude to my parents, **Mr. Kapoor Dogra** & **Mrs. Uma Dogra** and my younger brother **Mr. Shubham Dogra** for the affection, encouragement and emotional support throughout this journey, without which it would have been impossible to conceptualize the present work. I am immensely grateful for every moment we have shared, navigating challenges and celebrating triumphs together, which gave me the strength to persevere through this challenging journey.

I find myself at a loss for words when it comes to expressing my heartfelt gratitude to my special person, **Mr. Nitin Kaushal**. This journey would not have been possible without his unwavering support. I am eternally grateful for his love and encouragement during my most vulnerable moments, as he has been my pillar of strength. Through every high and low, he has always stood by my side. Whether it was accompanying me to my presentations by taking leave from work or paying attention to the smallest details that bring me happiness, his constant care and support have meant the world to me.

I thank the creator of the universe for all the blessings from the outset and for guiding my path in life.

Shivani Dogra

ABBREVIATIONS

ALE	*Artemisia vestita* Leaf Extract
µg/mL	Microgram per milliliter
µm	Micrometer
A/F	Abundance-to-frequency ratio
ABTS	2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ALEH	*Artemisia vestita* leaf extract hydrogel
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
AV	*Artemisia vestita*
B. subtilis	*Bacillus subtilis*
BC	Before Christ
BCE	Before Common Era
C. albicans	*Candida albicans*
CAOV-3	Carcinoma, Ovary 3 (Cell Line)
CFU	Colony forming units
CLSI	Clinical and Laboratory Standards Institute
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
Da	Dalton (a unit of molecular mass)
DAD	Diode-array detection
DMEM	Dulbecco's Modified Eagle's Medium
DPPH	2,2-Diphenyl-1-picrylhydrazyl
E. coli	*Escherichia coli*
FBS	Fetal Bovine Serum
FRAP	Ferric Reducing Antioxidant Power
GCMS	Gas Chromatography-Mass Spectrometry
H_0	Null Hypothesis
H_1	Alternative Hypothesis
HeLa	Henrietta Lacks (Cell Line)
HepG2	Hepatocellular carcinoma, G2 (Grade 2) cell line
HP	Himachal Pradesh
HPLC	High-performance liquid chromatography
IC50	Inhibitory Concentration 50%
IFN	Interferon
IL	Interleukin-2
IVI	Important value index
Kcal/mol	Kilocalories per mole
LD50	Lethal Dose 50%

m/z	Mass-to-charge
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MIC	Minimum Inhibitory Concentration
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaCl	Sodium chloride
NCCS	National Centre for Cell Science (India)
NIST	National Institute of Standards and Technology
OD	Optical density
P. mirabilis	*Proteus mirabilis*
PARP	Poly ADP-ribose polymerase
PC3	Prostate Cancer Cell Line 3
PCA	passive cutaneous anaphylaxis
PCl	Picryl chloride
S. aureus	*Staphylococcus aureus*
S. pyogenes	*Streptococcus pyogenes*
SD	Standard Deviation
SKOV-3	Serous Cystadenocarcinoma, Ovarian (Cell Line)
STAT1	signal transducers, activators of transcription
TCM	Traditional Chinese Medicine
TG	Tragacanth Gum
TICA	The International Cultivar Registration Authority
TNBS	tri-nitrobenzene sulfonic acid
USSR	Union of Soviet Socialist Republics
WHO	World Health Organization
ZOI	Zone of inhibition

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CHAPTER 1

INTRODUCTION

Chapter 1

“Ayu” means “Life” and “Veda” means “Knowledge”. Hence, knowledge of our life is called “Ayurveda”. - Gurudev Sri Sri Ravi Shankar.

Ayurveda, an ancient Indian system of medicine, that focuses on maintaining balance between the body, mind, and spirit (Dahiya et al. 2025). Originating from the wisdom of the Vedas, it provides deep insights into health, longevity, and overall wellness. By embracing Ayurvedic principles, individuals can cultivate a harmonious life through mindful dietary choices, balanced lifestyles, and natural healing therapies.

Herbs, play a central role in Ayurveda due to their natural therapeutic properties and ability to restore internal harmony. These medicinal plants offer gentle yet effective remedies, embodying a selfless essence as they provide healing without seeking anything in return. Their therapeutic properties reflect the core philosophy of Ayurveda—nurturing health and well-being in a way that aligns with nature’s wisdom (Sharma, 2024).

The world is rich in diverse traditional medicinal herbs, widely recognized for their role in promoting health security. According to the World Health Organization (WHO), nearly 80% of the global population depends on medicinal plants for primary healthcare. Various traditional healing systems, including Traditional Chinese Medicine (TCM), Indigenous medicinal practices, and African traditional medicine, place great emphasis on the therapeutic use of medicinal plants. Each region harbours unique plant species with distinct healing properties, showcasing both cultural diversity and ecological wealth. Despite differences in specific remedies, traditional medicine systems share a common foundation in holistic healing and the use of natural therapies.

India, known for its four mega biodiversity hotspots, is home to an impressive array of 17,500 medicinal plant species that are effectively utilized for treating a wide range of health conditions (Suresh et al. 2011; Chan, 2003; Haq, 1993). With its vast biodiversity and rich heritage of traditional medicinal practices such as Ayurveda, Siddha, and Unani, India has long relied on medicinal plants for therapeutic purposes. Many of these plants have been time-tested as traditional medicines, scientifically studied and

validated for their medicinal benefits. They are used to address both minor and chronic health concerns and play a crucial role in primary healthcare across rural and urban populations.

For centuries, these medicinal herbs have been highly valued and utilized by saints, scholars, and practitioners of various traditional medical systems, including Ayurveda, Unani, Siddha, Homeopathy, Naturopathy, Allopathy, and Traditional Chinese Medicine (Sophia et al. 2023). Traditional medicine encompasses vast knowledge, skills, and practices rooted in cultural beliefs, theories, and traditions passed down through generations, used for maintaining health and managing both physical and mental ailments, reflecting the deep wisdom and expertise of different cultures (Che et al. 2024; Pathak et al. 2024). Traditional medicine represents a valuable inheritance from nature and our cultural roots (Che et al. 2024), serving as a crucial component for maintaining wellness and preventing illness. Medicinal plants are essential components of healthcare systems worldwide, providing natural and sustainable approaches to promoting well-being and managing illnesses. Through the integration of traditional wisdom with scientific inquiry, these plants hold promise in tackling contemporary healthcare obstacles and facilitating the creation of evidence-backed medical interventions (Qiu, 2007).

Traditional Chinese Medicine (TCM): As one of the oldest and most comprehensive medical systems, Traditional Chinese Medicine incorporates a variety of healing techniques, including the use of herbal remedies, acupuncture, dietary modifications, and specialized physical exercises (Xutian & Huang, 2011).

Ayurveda: Originating in ancient India, Ayurveda seeks to restore balance and harmony in the body through herbal treatments, dietary changes, massage therapies, yoga, and lifestyle modifications (Frawley & Ranade, 2001). Rooted in the Vedic tradition, Ayurveda's principles are documented in classical texts such as the *Charaka Samhita* and *Sushruta Samhita*, which provide detailed insights into the use of medicinal plants, surgical procedures, and holistic lifestyle practices for disease prevention and overall health (Sharma & Dash, 2001).

Herbal Medicine: Also known as phytotherapy, herbal medicine involves the use of medicinal plants for promoting health and treating ailments. Recognized by the World Health Organization (WHO) as a fundamental aspect of traditional and complementary medicine, plant-based healing has been practiced for thousands of years. Historical records of herbal remedies appear in ancient texts such as the Egyptian *Ebers Papyrus* (circa 1550 BCE), the Chinese *Shennong Ben Cao Jing*, and India's *Charaka Samhita* and *Sushruta Samhita*. These classical works provide extensive documentation on the medicinal properties of various herbs and their roles in disease prevention and treatment (Sharma & Dash, 2001; Patwardhan et al., 2005).

Indigenous Healing Practices: Indigenous healing encompasses traditional therapeutic methods practiced by native communities across the globe. These approaches integrate herbal remedies, spiritual rituals, oral traditions, and communal support to foster holistic health. Various indigenous cultures, including Native American, African, Australian aboriginal, and South American tribes, have developed sophisticated healing systems that are deeply rooted in their environment, spiritual beliefs, and cultural heritage (Waldram, 2000).

Traditional African Medicine: Indigenous African communities have developed distinct healing practices that often integrate the use of medicinal plants, spiritual healing rituals, divination techniques, and ceremonial traditions (Mokgobi, 2013).

Native American Medicine: The indigenous peoples of North and South America have a diverse range of healing traditions, which include the use of herbal remedies, sweat lodge purification ceremonies, spiritual journeys, and rituals that incorporate dance and music as part of the healing process (Hult Krantz, 1992).

Unani Medicine: Originating in ancient Greece, Unani medicine later flourished in the Middle East and South Asia. This system of medicine focuses on maintaining harmony among the body's essential fluids and employs treatments such as herbal formulations, dietary modifications, and cupping therapy to promote health and well-being.

Kampo Medicine: Kampo, Japan's traditional medical practice, is deeply influenced by ancient Chinese medicine. It incorporates herbal treatments, acupuncture, and

moxibustion and is often integrated with modern healthcare approaches in Japan (Itoh & Mizuno, 2017).

One of the key advantages of herbal medicine is its relatively low risk of side effects compared to conventional pharmaceuticals, making it a safer alternative in many cases (Ved & Goraya, 2008). The use of plant-derived essential oils dates back to early civilizations, with the Egyptians being particularly renowned for their advancements in perfumery (Kotnis et al., 2004). Wild and naturally occurring plants have long contributed to human well-being by providing essential resources such as food supplements, fuel, fodder, and raw materials for various industries, offering economic support to local communities.

In many developing nations, around 80% of the population relies on medicinal plants for healthcare, with India being a major consumer. Approximately 90% of India's medicinal plant species are sourced from the Western Himalayas. Himachal Pradesh, a biodiversity-rich state in northeastern India, is home to a wide variety of medicinal flora (Rawal et al. 2013). However, as modernization progresses, traditional herbal knowledge is gradually being lost, posing a significant challenge to its preservation. Medicinal plants contain bioactive compounds that contribute to their therapeutic effects and immune-boosting properties. In the aftermath of the COVID-19 pandemic, there has been a growing global interest in herbal remedies, as people increasingly focus on natural approaches to health and wellness (Kar & Borthakur, 2008). Essential oils extracted from aromatic plants are widely used in industries such as cosmetics, perfumes, food flavoring, spices, pesticides, and pharmaceuticals (Lal et al., 2020). India, with its abundant soil diversity and favourable climate, produces approximately 15 essential oils on a pilot/experimental scale and around 20 herbal essential oils at a commercial level. These oils hold a unique economic advantage due to India's rich biodiversity.

In the context of *Artemisia*, which has various medicinal properties and has been used in traditional medicine systems across the world.

"तिक्त रसः कषायश्च, पित्तघ्नः ज्वरनाशनः।
कृमिघ्नः शोथहरश्च, हृद्यः दन्त्यः रसायनः॥

A verse from the *Charaka Samhita* (Sutra Sthana 1.3.12) describes the therapeutic attributes of certain herbs, stating that “bitter and astringent herbs help balance excess pitta, reduce fever, eliminate parasites, alleviate swelling, and serve as a heart tonic and rejuvenator” (Dwivedi, 2020). This ancient wisdom underscores the sacred nature of medicinal plants, emphasizing their role in sustaining life and highlighting Artemisia’s significance in providing relief during challenging times. In Ayurveda, various species of Artemisia are often associated with *Damanaka* (दमनक, *Artemisia pallens*) or *Nagadamani* (नागदमनी, *Artemisia indica*), owing to their distinct bitter taste, aromatic nature, and medicinal potential. These plants are valued for their healing properties and contributions to traditional herbal medicine.

The genus *Artemisia* is renowned for its rich biodiversity and is considered significant for potential exploration. Belonging to the Asteraceae family, also known as the 'Compositae family,' 'thistle family,' 'daisy family,' and 'sunflower family,'(Koul and Khatri, 2020) *Artemisia* is highly valued for its medicinal properties, botanical characteristics, and structural diversity (Bora and Sharma, 2011; Tan et al. 1998; Abad et al. 2012). This genus, particularly prevalent in the high-altitude regions of western China and Tibet, has been traditionally used in folk medicine for the treatment of inflammatory conditions (Tian et al. 2013; Yin et al. 2008). Two millennia ago, the Chinese recognized the medicinal benefits of Artemisia. In 1596, Li Shizhen recommended Qing Hao, or Artemisia tea, for alleviating malaria symptoms. The term "Artemisia" originates from the revered Greek Goddess Artemis (known as Diana in Roman mythology), as well as the Greek Queens Artemisia I & II. Notably, it was named after Queen Artemisia II of Caria, a renowned medical researcher and botanist during the 4th century BC. Artemisia, commonly known as wormwood, mugwort, and sagebrush, is distributed extensively across temperate zones in Europe, North America and India (Wang et al. 2004). Its species are utilized in folk medicine and have gained importance in the pharmaceutical sector due to their medicinal value and commercial significance (Kala et al. 2006). For centuries, people have acknowledged the healing qualities of Artemisia, with historical evidence tracing back 2000 years to China. Li Shizhen, in 1596, advocated for Artemisia tea (known as qing hao) as a remedy for malaria symptoms. With 500 species, *Artemisia* is one of the largest and most diverse

genera of the tribe Anthemideae (Martin et al. 2003; Valles and Garnatie, 2005), with a significant presence in Asia, particularly in countries like the former USSR, China, Japan, Iran, and India (Rechinger, 1986; Greger et al. 1982).

In India, several *Artemisia* species are traditionally recognized for their medicinal properties, including *Artemisia vestita*, *Artemisia dracunculus*, *Artemisia brevifolia*, *Artemisia roxburghiana*, *Artemisia dubia*, *Artemisia herba-alba*, *Artemisia japonica*, *Artemisia santolinifolia*, *Artemisia maritima*, *Artemisia scoparia*, *Artemisia absinthium*, *Artemisia verlotiorum*, *Artemisia annua* and *Artemisia vulgaris* (Mucciarelli and Maffei, 2001; Paniagua-Zambrana et al. 2024). These plants have been traditionally employed in community healthcare owing to their aromatic nature and the presence of diverse bioactive phytochemicals. While the present work concentrates on *A. vestita,* recognizing these additional species helps illustrate the wider medicinal relevance and therapeutic scope of the Artemisia genus within Indian ethnobotanical traditions. Artemisia plants exhibit a wide range of activities, including antioxidant, antimicrobial, anticoagulant, antispasmodic, antidiabetic, antihelmintic, anticancer and anti-inflammatory properties, among others (Silva et al. 2005). Despite their therapeutic benefits, differentiating between *Artemisia* species based on morphology can be challenging (Sorensen, 2005), leading to misinterpretation and misidentification of economically and commercially valuable products (Ashraf et al. 2010). Several healthcare products based on Artemisia, such as tablets, syrups, oils, and creams, have been commercialized, highlighting the growing interest and demand for Artemisia-based remedies in the healthcare industry (Trendafilova et al. 2020; Nigam et al. 2019; Orege et al. 2023).

Artemisia species are valuable reservoirs in natural medicine, containing diverse bioactive compounds with potential therapeutic benefits. Ongoing exploration of their chemical makeup and pharmacological impacts shows potential for creating innovative treatments and healthcare approaches. Among these species, *Artemisia vestita* Wall. ex-Besser, though less studied, is garnering significant interest. The World Flora Online has recognized *Artemisia vestita* Wall. ex-Besser as the accepted botanical name, while *Artemisia vestita* Wall. ex-DC. and *Artemisia vestita* var. vestita are considered

synonyms of *Artemisia vestita* according to TICA records (The plant list, 2013). In recent times, there has been a significant surge in interest in natural products globally, leading to increased attention towards plants like *A. vestita* in India. This plant is commercially valuable and is obtained through various extraction methods such as distillation, hydro diffusion, expression, and solvent extraction, often facilitated by natural carriers like host organisms (Iqbal et al. 2004). Due to the high cost and limited availability of synthetic drugs (Tariq et al. 2009), local farmers and tribal communities traditionally use *A. vestita* as an anti-helminthic agent to treat parasitic infections. In the Kashmir valley, it is known as "Tethe-Ven," (Ahad et al. 2017) while in Tibet, it is referred to as "Maolianhao" or "Wannianpeng," serving as a folk medicine (Tian et al. 2013). In the Kashmir region, it is known as "RoosiTyethven" (Mochi and Riyaz, 2021). This plant has various biological activities and also recognized by various names such as "Russian Wormwood," "Gangaa Tulsi" in Ayurveda, and by several folk names like Buer, Drubsha, Seski, Kubsha, Chamariya, Kundja, and Kundiyaa. *A. vestita* belongs to the Sunflower family (Asteraceae, Compositae). Despite its widespread use, there is a lack of specific research articles providing comprehensive data on its standardization, mode of action, dose optimization and toxicity.

The skin acts as the first line of defence against external hazards and is essential for regulating temperature, sensation, and immunity (Archer, 2010). Skin problems and wounds present substantial challenges to dermatologists globally, impacting millions of people each year (Sen, 2023; Rawal et al. 2013). These issues vary from minor cuts and scrape to persistent ulcers and severe burns, affecting both physical well-being and overall quality of life. Despite its importance, skin integrity can be compromised by factors like injury, infection, chronic illnesses and environmental influences, resulting in a range of issues such as cuts, burns, ulcers, infections, and inflammation (Levine, 2024). Wound healing is a complex and tightly controlled process comprising several stages and cellular activities, including haemostasis, inflammation, proliferation and remodelling (Yang et al. 2021; Gupta, 2021; Sorg and Sorg, 2023; Pena and Martin, 2024; Mamun et al; 2024).

Hydrogels, composed of hydrophilic polymers that form three-dimensional networks capable of absorbing and retaining substantial amounts of water (Omidian and Park,

2010), have gained significant attention in biomedical research. These versatile materials offer numerous advantages in wound healing, including biocompatibility, controlled drug release, and support for tissue regeneration (Bahram et al. 2016; Zhang et al. 2021). As advancements in biomaterials and tissue engineering continue, hydrogels are emerging as essential components in clinical wound care and regenerative medicine strategies. Wound management is a critical aspect of healthcare, encompassing a wide range of injuries, from acute wounds to chronic ulcers. Traditional treatments often rely on gauze dressings and topical applications, but recent progress in biomaterials has led to more effective and innovative solutions. Among these, hydrogels are particularly noteworthy due to their unique properties and adaptability in wound treatment. Their ability to retain moisture, provide a conducive environment for cell proliferation, and support extracellular matrix (Tibbitt and Anseth, 2009) deposition enhances the healing process (Gonzalez-Díaz and Varghese, 2016; Geckil et al. 2010). Additionally, hydrogels promote angiogenesis and tissue regeneration, ultimately accelerating wound closure and improving recovery outcomes (Lai and Rogach, 2017).

Hydrogels provide a modern avenue for administering traditional remedies, amplifying their effectiveness and ease of use in therapeutic applications (Lu et al. 2024). Traditional medicine has traditionally utilized natural substances and plant extracts for healing. Hydrogels provide a contemporary means of administering these traditional remedies, improving their effectiveness and practicality by integrating herbal extracts, essential oils, and other traditional components into hydrogel compositions, the therapeutic advantages of traditional medicine can be maintained and enhanced (Mittal et al. 2024). Integrating hydrogel technology with traditional medicine presents inventive strategies for addressing diverse health issues. These fusion harnesses the insights from traditional healing methods alongside the adaptability of hydrogel technology (Omidian et al. 2024), paving the way for the creation of innovative therapeutic formulations aimed at enhancing patient outcomes and fostering better overall health, including wound healing, pain relief, skincare and oral care. Traditional medicine, deeply rooted in nature and cultural heritage, plays a vital role in promoting health and preventing disease. Medicinal plants have long been integral to healthcare

systems across the globe, offering natural and sustainable solutions for managing various health conditions (Raju and Das, 2024). By blending traditional knowledge with modern scientific research, these plant-based remedies have the potential to address current medical challenges and contribute to the development of evidence-based treatments.

Gum Tragacanth is a natural, water-soluble polysaccharide derived from the dried sap of *Astragalus* species (Emam-Djomeh et al. 2019), primarily found in the Middle East and western Asia (Taghavizadeh Yazdi et al. 2021). It has been traditionally used as a thickening, stabilizing, and emulsifying agent in pharmaceuticals, cosmetics, and food industries (Dhupal et al. 2019) due to its biocompatibility, non-toxicity, and high viscosity at low concentrations. Structurally, gum Tragacanth consists of two major fractions: Tragacanthin, which is water-soluble, and bassorin, which swells in water but does not dissolve, contributing to its unique gel-forming ability (Ahmad and Ali, 2023). Recent studies have highlighted its potential in biomedical applications such as drug delivery, wound healing, and tissue engineering (Abdi et al. 2024;). Its natural origin and excellent film-forming properties make it an ideal candidate for the development of biodegradable hydrogels and nanocomposites. Furthermore, its ability to encapsulate and sustain the release of bioactive compounds enhances its value in controlled drug delivery systems (Nazemi et al. 2023; Askari et al. 2025).

Hydrogels offer an innovative approach to delivering traditional remedies, enhancing both their efficacy and practicality in therapeutic applications. For centuries, natural substances and plant extracts have been used in healing (Sinaga et al. 2021), and hydrogels provide a modern platform for their controlled and sustained release (Yang et al. 2018; Yan et al. 2024). By incorporating herbal extracts (Xianyong et al. 2024), essential oils, and other bioactive compounds into hydrogel formulations, the beneficial properties of traditional medicine can be preserved and optimized. The integration of hydrogel technology with traditional medicinal practices paves the way for novel healthcare solutions. This combination leverages the wisdom of ancient healing methods alongside the adaptability of hydrogels, enabling the development of advanced therapeutic products. These formulations hold promise for improving patient care in

areas such as wound healing, pain management, skincare, and oral health, ultimately contributing to better overall well-being.

The combination of *Artemisia vestita* and Tragacanth gum in hydrogel formulation offers a promising approach for biomedical applications. *Artemisia* is rich in bioactive compounds known for their antimicrobial, antioxidant, and wound-healing properties, while gum Tragacanth serves as a natural polymer that enhances gel stability, water retention, and biocompatibility. This hydrogel can provide a controlled release of therapeutic agents, creating a moist environment essential for wound healing and infection prevention. Additionally, its biodegradable and eco-friendly nature makes it a suitable material for drug delivery and tissue engineering.

Research gap and justification: Although *A. vestita* has been widely used in traditional medicine, scientific studies on its wound-healing, antimicrobial, anti-inflammatory, and cytotoxic properties—particularly in relation to its leaf extract—are still scarce. Commonly referred to as Russian wormwood and locally known as Kubsha, this plant has been traditionally utilized in Himachal Pradesh to treat wounds, itching, and skin infections. The application of fresh leaf paste is well known for promoting faster wound healing. However, no scientific research has yet explored its formulation for medicinal use. To bridge this research gap, our study focuses on developing a gel formulation incorporating *A. vestita* leaf extract (ALE) with the natural polymer Tragacanth gum (TG). This hydrogel is designed to enhance the stability and shelf life of Kubsha while facilitating its commercialization for wound care and skin infection treatment. Furthermore, we assessed the drug release profile of the formulated gel to ensure its therapeutic effectiveness and affordability, aiming to make it a viable healthcare solution for the local population.

Based on the above considerations, this research was conducted with the following objectives: (1) Identification and phytochemical characterization of secondary metabolites from *Artemisia vestita*, (2) *In vitro* evaluation of antimicrobial activity of the leaf extract, (3) To develop and incorporate the drug in crosslinked customized polymer for increased drug loading capacity and evaluate its release profile, (4) To identify pH sensitivity and temperature stimulus drug release system.

Future prospects: By accomplishing these goals, our study seeks to provide scientific validation for the traditional use of Kubsha while developing a commercially viable product for skin health. *Artemisia vestita* (AV) holds significant promise as a natural skincare remedy for addressing minor cuts, redness, inflammation, wound healing, bleeding control, and microbial skin infections. If formulated into a hydrogel and commercialized by pharmaceutical industries, it could serve as a safe, effective, and affordable herbal alternative for dermatological care, with no known adverse effects.

However, further preclinical and clinical studies are essential to establish the safety, efficacy, and biocompatibility of *A. vestita* extract in combination with Tragacanth gum (TG) for wound healing and antimicrobial applications. Investigating the molecular mechanisms behind its antimicrobial, anti-inflammatory, and wound-healing properties through mechanistic studies would provide deeper insights into its therapeutic potential. Moreover, integrating advanced drug delivery techniques, such as nanotechnology or stimuli-responsive systems, could enhance targeted and controlled release for improved efficacy.

To ensure commercial feasibility, large-scale production and formulation refinement should be undertaken to improve stability and prolong shelf life. Beyond wound healing, this hydrogel formulation could have broader therapeutic applications, including the treatment of burns, diabetic ulcers, and other skin-related conditions. Additionally, sustainability-focused research should explore environmentally friendly cultivation, harvesting, and extraction techniques to support conservation efforts while promoting its global medicinal use.

CHAPTER 2

REVIEW

OF

LITERATURE

Chapter 2

2.1 Harnessing the power of traditional medicinal herbs

The world is enriched with a treasure trove of traditional medicinal herbs. They hold immense global health security. India hosts four mega-biodiversity hotspots and is home to approximately 17,500 medicinal plant species used effectively against various disorders (Ved et al. 2008; Kar and Borthakur, 2008; Suresh et al. 2011). These medicinal herbs have been time-tested and recommended by saints, maharishis, Vaidya's, and ayurvedic acharyas. They hold a significant place in traditional medicinal systems such as Ayurveda, Unani, Siddha, Homeopathy, Naturopathy, Allopathy, and Traditional Chinese Medicine, addressing ailments in both humans and animals. Herbal remedies are generally considered safer and act on underlying causes of disease with fewer side effects than allopathic drugs (Chan, 2003).

Plant-derived essential oils have played a vital role in human health and wellness since ancient times. Ancient Egyptians were skilled perfumers who taught the art of perfumery to the Hebrews around 5000 years ago (Haq, 1995). Wild and naturalized plants have supplied fuel, food, raw materials and income for communities. According to the WHO, approximately 80% of people in developing nations rely on herbal remedies. India sources 90% of its medicinal herbs from the Western Himalayas, known for its rich diversity, with 1,748 medicinal species utilized in pharmacological research, chemistry, clinical studies, and pharmacognosy. Traditional herbal knowledge is diminishing due to the growing preference for allopathic medicine. They often have side effects or adverse effects on non-target organs. Multiple phytochemicals in herbs act synergistically and may enhance immune function. Post-COVID-19, there has been a surge in interest and demand for herbal medicines as people seek improved health and peace of mind (Kotnis et al. 2004). Volatile aromatic oils derived from herbs are commercially utilized in cosmetics, soaps, perfumery, the spice industry, flavoured teas, drinks, traditional foods, pesticides, and pharmaceuticals (Lal et al. 2020). The increasing global demand for herbal essential oils has driven a decisive shift in international trade. Sharma et al (1997) reported that India produces approximately 15 essential oils on a pilot scale and 20 essential oils commercially, benefiting from its

rich soil diversity and favourable climatic conditions. The oil content and quality of these plants are influenced by the type of soil and climate.

2.2 The genus *Artemisia*

Artemisia a genus belonging to the Asteraceae (Compositae) family, daisy family, thistle family and sunflower family (Koul and Khatri, 2020). This genus is gaining attention for its remarkable medicinal properties, phytochemical diversity, and scientifically proven health benefits (Bora and Sharma, 2011; Tan et al. 1998; Abad et al. 2012; Tian et al. 2013; Yin et al. 2008). Historically, the Chinese recognized the therapeutic value of *Artemisia* over 2000 years ago. In 1596, Li Shizhen recommended tea made from *Artemisia* (*qinghao*) to treat malaria. The name *Artemisia* is derived from the Greek goddess Artemis (Roman Diana) and Queen Artemisia II (Encyclopaedia, 2004) of Caria, a 4th-century BC medical researcher and botanist.

The genus contains approximately 500 species, commonly known as wormwood, mugwort, or sagebrush. These species are distributed across temperate zones in Europe, North America, and India (Wang, 2004). The Artemisia genus consists of approximately 500 species (Martin et al. 2003; Valles and Garnatje, 2005), with the highest diversity found in Asia, particularly in the northwestern Himalayas. Among these, 150 species are found in China, 174 in the former USSR, 50 in Japan, 35 in Iran, and 35 in India (Greger et al, 1982; Rechinger, 1986).

This study emphasized *Artemisia vestita* because of its traditional relevance and notable phytochemical composition. However, several other Artemisia species occurring in India share similar ethnomedicinal significance and may exhibit comparable therapeutic effects. Species such as *A. dracunculus*, *A. roxburghiana*, *A. dubia*, and *A. brevifolia* are widely used in traditional practices, especially in the northwestern Himalayan region. Future research should investigate these species using standardized methods, including phytochemical analysis, biological activity assessment, and formulation studies. Such comparative work may identify additional candidates for herbal treatment strategies and expand the medicinal potential of the Artemisia genus. Mucciarelli and Maffei (2001) reported that species of *Artemisia* are valued for their

antioxidant, antimicrobial, anticoagulant, antispasmodic, antidiabetic, anti-helminthic, anticancer, anti-ulcer, and anti-inflammatory properties. They are used to treat coughs, colds, dyspepsia, headaches, malaria, and inflammation (da Silva et al. 2005). However, the morphological similarity (Konowalik and Kreitschitz, 2012) among *Artemisia* species often leads to misidentification, resulting in potential economic and medicinal discrepancies (Muhammad et al. 2010). Relevant research papers and official reports aligned with the theme of the current study were sourced from various search engines. To ensure a high-quality literature review, only peer-reviewed articles and authoritative publications were included. The review was limited to literature published in English, with no specific restrictions on the publication date. A comprehensive and systematic review of the available literature on *A. vestita* was conducted using multiple databases, including Web of Science, PubMed, INMEDPLAN, EMBASE, Google Scholar, and NCBI, along with information gathered from various online sources.

2.3 *Artemisia vestita*- From tradition to therapy

The World Flora Online recognizes *A. vestita Wall. ex-Besser* as the accepted botanical name, with *A. vestita Wall. ex-DC.* and *A. vestita var. vestita* listed as synonyms. In India, *A. vestita*, known by various names like "Russian Wormwood," "Kubsha," "Chamariya," and "Ganga Tulsi," is an important aromatic plant. In India, *Artemisia vestita*, a significant aromatic plant. This is commercially utilized through processes such as distillation, hydro diffusion, expression, solvent extraction, and natural carriers or host organisms (Lal et al. 2020). Traditionally, local farmers and tribal communities have used *A. vestita* as an anti-helminthic agent. This was done primarily due to the limited availability and high cost of synthetic drugs (Iqbal et al. 2004; Tariq et al. 2009). It is used in rural and tribal communities, particularly in the Kashmir Valley ('Tethe-Ven'), to treat parasitic infections (Ahad et al. 2017). In Tibet, it is widely recognized as 'Maolianhao' (also known as 'Wannianpeng'), a traditional folk medicine (Tian et al. 2013). In Kashmir, it is referred to as 'RoosiTyethven' (Mochi et al. 2021). Additionally, it is commonly known by various names, including 'Russian Wormwood,' 'Ganga Tulsi,' 'Buer,' 'Seski,' 'Drubsha,' 'Kubsha,' 'Kundja,' 'Chamariya,' and 'Kundiyaa.' Despite its wide-ranging traditional uses, scientific studies on *A. vestita* remain fragmented, with limited research on its extracts, modes of

action, standardization, dosage optimization, and toxicity. This chapter aims to bridge these gaps by providing comprehensive information on the distribution, botanical description, phytochemistry, and pharmacological activities of Russian Wormwood.

2.4 Diversity and distribution

Artemisia vestita is widely distributed across East Asia, including the Himalayan regions of Nepal, Pakistan, India, and Tibet, extending to southern and central China (Figure 1). It can be found in a variety of habitats such as hills, rocky slopes, grasslands, shrublands. The outer forest margins in regions including Gansu, northwest Guangxi, north Hubei, Guizhou, Liaoning, west Sichuan, Qinghai, Xizang, Xinjiang, Yunnan, and parts of north India, Nepal, and northern Pakistan. The chemical composition of its essential oil varies significantly due to geographical and seasonal factors.

In India, *A. vestita*, commonly known as Drubsha, is found in Himachal Pradesh, Kashmir, and Uttarakhand at altitudes ranging from 2,100 to 3,000 meters. In the Pooh region of Kinnaur district, the plant's distribution across different elevations revealed a density of 1.27 plants per hectare, a frequency of 10%, an abundance of 12.67, an abundance-to-frequency ratio (A/F) of 1.27, and an important value index (IVI) of 5.12 at elevations of 2,700–3,200 meters. At elevations of 3,200–3,700 meters, the density increased to 2.25/ha, frequency to 15%, abundance to 15, A/F to 1, and IVI to 7.28. At higher elevations of 3,700–4,200 meters, the density decreased to 1.17/ha, frequency to 11.67%, abundance to 10, A/F to 0.86, and IVI to 53.53 (Verma et al. 2010). The important value index is the sum of frequency, density, and dominance of the species in the area.

In Himachal Pradesh, it is found in Kotgarh. Kotgarh lies in the mid-hill zone of Himachal Pradesh, with altitudes ranging between 1,800 and 2,700 meters above sea level. It experiences a temperate climate marked by clear seasonal distinctions. Summers are generally cool and pleasant, while winters are cold and often bring snowfall. The region receives moderate to high annual rainfall, supporting rich biodiversity and dense vegetation. The climatic features of Kotgarh, including its mild temperatures and consistent rainfall, provide optimal conditions for the growth of medicinal herbs such as *A. vestita*. These plants naturally flourish in well-aerated soils

and cooler environments. Such favourable surroundings not only support healthy plant development but also encourage the production and accumulation of biologically active compounds, which are responsible for their medicinal benefits. The region's varied landscape and supportive climate make Kotgarh a suitable site for both the cultivation and scientific exploration of therapeutic species like *A. vestita*.

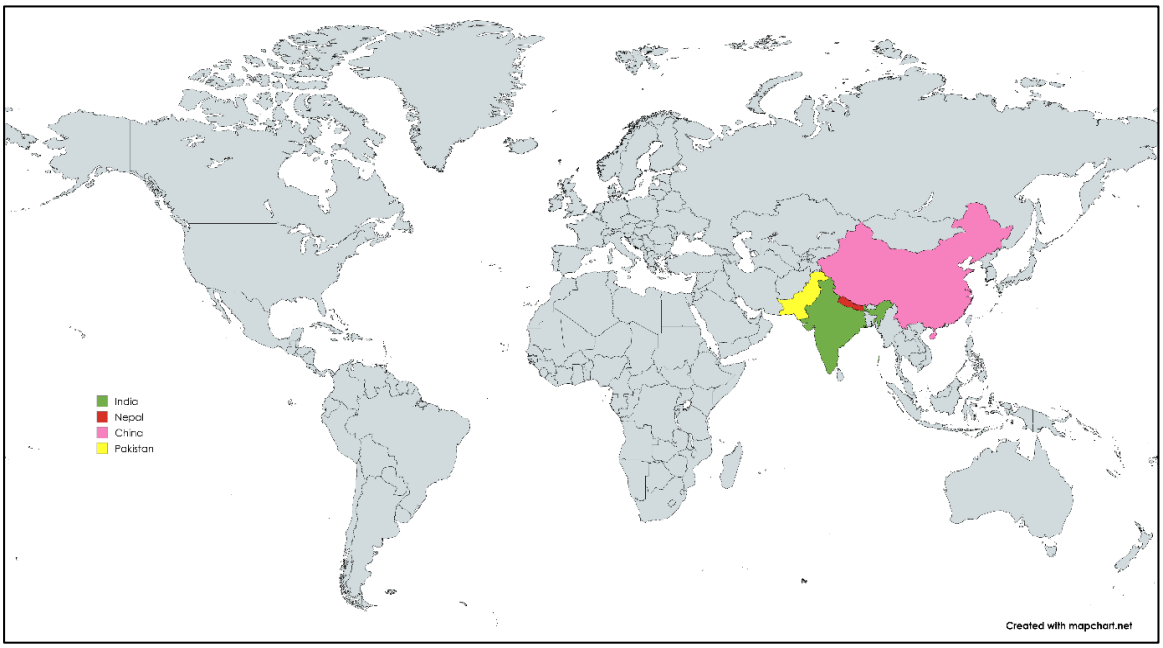


Figure 1. World map depicting the distribution of *Artemisia vestita* (coloured region).

2.5 Botanical description

Morphology: *Artemisia vestita* Wall. Ex Besser is an aromatic, erect, perennial shrub that can grow up to 2 meters (5–120 cm) (Putievsky, 1991). The leaves are fern-like, soft, and covered with fine hairs on the upper surface, while the underside is white and similarly hairy. The leaves are pinnately cut. The small flowers are creamy yellow, arranged in racemes (6–10), and the flower heads are long, hairy, and compound, hanging gracefully from slender, nodding stalks. The fruits are shiny and smooth, and the bracts are oblong and membranous (Figure 2).

The leaves have distinct abaxial and adaxial surfaces. On the adaxial surface, elongated epidermal cells are partitioned and grooved, with ridged margins. The tertiary sculpture is aggregated, and the stomata are depressed, surrounded by thick, flat peristomal rims. The inner ledges are thick, and the guard cells have a gradually concave surface with transverse polar folds. The lobule tips are cap-like and swollen, and stomata are also present in this region, with cells displaying significant undulations in a V-shape and being elongated. On the abaxial surface, the cell outline is similar but deeply undulated with loose V-shaped undulations. The surface is tertiary sculptured and coarsely granular, with the guard cells featuring oblique folds (Mehrotra et al. 1990; Jackson, 1990; WFO, 2022). The image illustrates the adaxial (left) and abaxial (right) surfaces of *A. vestita* leaves. The adaxial surface is the upper side, typically exposed to sunlight, while the abaxial surface is the lower side, often lighter in colour and containing stomata for gas exchange.

Figure 2. Leaves of *Artemisia vestita* adaxial (left) and abaxial (right) surfaces.

Vegetation details: *Artemisia vestita* can dominate grassland ecosystems (Zeng et al. 2020). The plants are hermaphroditic and pollinated by insects, with seeds ripening between August and October. Its habitat includes woodland edges, gardens, sunny positions, cultivated beds, hills, rocky slopes, grasslands, shrublands, and the outer margins of forests, typically at altitudes ranging from 2,000 to 4,300 meters above sea level (Huxley and Griffiths, 1992).

Cytology: Cytologically, *A. vestita* has a meiotic chromosome count of 2n = 2x = 36 (ploidy level 4x), with a pollen fertility rate of 78–82% and pollen grain size of 22–24 µm, as reported by Gupta et al. (2014) from plants collected at Haripur Dhar and Chur Dhar (Himachal Pradesh) at altitudes of 2,400 and 3,650 meters, respectively. Additionally, hexaploidy (2n = 54) (Marhold et al. 2011) and diploid (2n = 18) (Kaul and Bakshi, 1984) cytotypes have also been reported.

Chlorophyll: Chlorophyll content varies with altitude. Variations in chlorophyll and anthocyanin levels, as well as the chlorophyll-to-carotenoid ratio, have been observed in the temperate species in the temperate species of *A. vestita* found at altitudes of 550 and 3,600 meters (Nautiyal, 1986) above sea level in the Garhwal Himalayas, the total chlorophyll content in the lower leaves was 1.716 and 1.470, in the middle leaves was 0.902 and 1.650, and in the top leaves was 0.863 and 1.205, respectively. The molar chlorophyll-to-carotenoid ratio was lower in temperate species (1) compared to tropical (1.7) and subtropical species (1.3) (Todaria, 1986). At higher altitudes, *A. vestita* shows greater adaptability compared to lowland species, owing to a higher osmotic concentration, increased lignification, and osmoregulation in tissues, resulting in the conversion of starch to sugar (Nautiyal, 1983).

A palynological study of *A. vestita* found the plant's pollen characteristics to include a polar axis of 19.38 ± 1.52 µm, an equatorial axis of 18.09 ± 1.51 µm, a P/E (sphericity) of 1.07, exine thickness of 2.13 ± 0.67 µm, and a colpus length of 11.81 ± 1.69 µm. The spinules on the pollen grains were also found to be prominent (Hayat et al. 2010).

2.6 Medicinal uses

A. vestita has long been used in folklore medicine and was traditionally harvested from wild forests for its anti-inflammatory and antifebrile properties. Both aqueous and alcoholic solvents extract the maximum medicinal compounds from the plant compared to other solvents. It is widely used in Tibet and China to treat various inflammatory conditions, such as contact dermatitis, rheumatoid arthritis, and sepsis (Nazemizadeh and Masoudi, 2017; Qiangba et al. 2002). The leaves are crushed and applied externally

to the skin as a haemostatic agent (Gupta and Tandon, 2004). Additionally, the plant is used for treating stomach-aches (Pathak and Karnick, 1980).

2.7 Phytochemistry

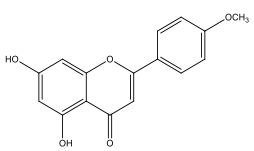
For the phytochemical analysis Gas Chromatography-Mass Spectrometry (GC-MS) is a highly versatile and widely utilized analytical method designed for the detailed qualitative and quantitative assessment of complex chemical mixtures. This technique integrates the efficient separation ability of Gas Chromatography (GC) with the precise molecular identification capabilities of Mass Spectrometry (MS), allowing comprehensive profiling of volatile and semi-volatile compounds, particularly in natural product studies. During GC-MS analysis, the sample is initially vaporized and introduced into the gas chromatograph. Within the GC system, individual components are separated based on their volatility and their interaction with the stationary phase of the chromatographic column. Each compound elutes at a distinct retention time that reflects its chemical properties. Following separation, the eluted compounds are transferred into the mass spectrometer, where they undergo ionization, typically via electron impact. This process results in the formation of charged molecular fragments. These ions are then detected based on their specific mass-to-charge (m/z) ratios, generating a unique mass spectrum for each substance. The obtained spectra are compared to established spectral libraries, such as NIST or Wiley databases, for accurate compound identification. GC-MS is widely recognized for its high sensitivity, specificity, and reproducibility. Its application provides critical structural and compositional insights into various analytes, making it particularly useful for the identification of phytochemicals, secondary metabolites, essential oils, and other biologically active compounds in plant extracts. In the present study, GC-MS was employed to analyze the chemical constituents of ALE. The analysis enabled the identification of key bioactive compounds through interpretation of chromatographic retention times and corresponding mass spectra, offering valuable information regarding the antimicrobial and pharmacological potential of the extract (Sparkman et al., 2011).

The essential oils of Kubsha are volatile, complex mixtures of sesquiterpenes, which give the plant its distinct, strong aroma. These oils are typically extracted using steam distillation or hydro-distillation methods. Various plant parts, including leaves, stems, bark, aerial parts, inflorescences, whole plants, fruits, seeds, flowers, and roots, are utilized for extraction, with the resulting oils being widely used to address human ailments. However, the composition of the essential oils varies depending on altitude.

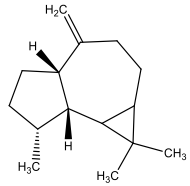
2.8 Essential Oil

A total of 202 biochemical compounds have been identified from various parts of *A. vestita* (stem, leaves, roots). These include flavonoids, terpenoids, oxygenated monoterpenes, sesquiterpene lactones, sesquiterpenoids, hydroxyl cinnamic acids, monoterpene hydrocarbons, azulenes, sesquiterpene hydrocarbons, sterols, phenylpropanoids, monoterpenoids, hydroxycoumarins, coumarins, flavonoid glycosides, organosulfonic acids, oxygenated triterpenes, and aromatic aldehydes. The bioactive compounds present in Russian wormwood have distinct chemical structures (Figure 3), contributing to the plant's medicinal properties (Figure 4). The characteristic odour of the plant's essential oil is determined by compounds like thujone and eucalyptol (1,8-cineole), which provide a fresh, woody, and herbaceous fragrance with a slight sweetness reminiscent of sage and balsamic notes. These compounds are reported to be effective against dermatophytes (Vecino et al. 2017). The essential oil is safe for use in perfumery, scented soaps, and cosmetics, with camphor, borneol, and eucalyptol playing a key role in its pleasant aroma, as well as contributing to antifungal and antibacterial properties.

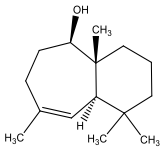
When the flowering tops of *A. vestita* are subjected to steam distillation, they yield a yellow, orange, or brown-coloured oil with a sweet-woody scent. Essential oil samples from regions like Nainital, ShimLa, and Kashmir have been analysed for their physiochemical properties (Sarin et al. 1978; Joshi et al. 2016). Key characteristics of the oil include a refractive index of 1.4915, ester value of 55.45, acid value of 1.3, carbonyl percentage of 20.80, ester value of 124.4 after acetylation, solubility in 95% alcohol (1:1), and specific gravity of 0.910.



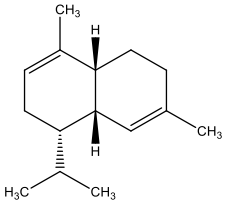
Acacetin



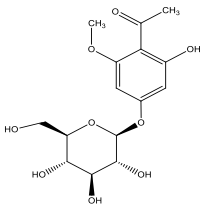
allo-Aromadendrene



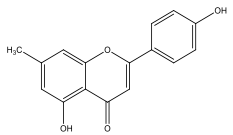
allo-Himachalol



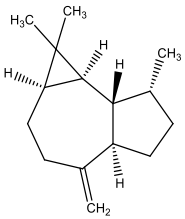
Amorphene



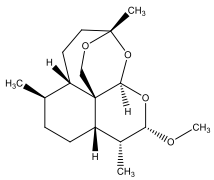
Annphenone



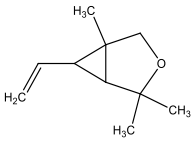
Apigenin



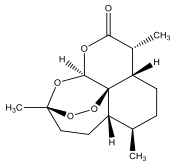
Artemether



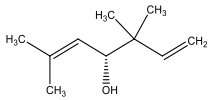
Artemisinin



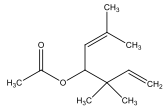
Aromadendrene



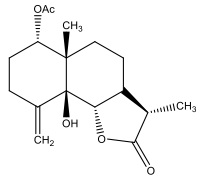
Artemiseole



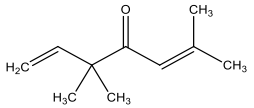
Artemisia alcohol



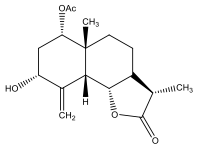
Artemisyl acetate



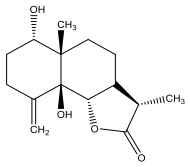
Artemivestinolide-A



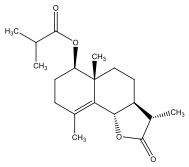
Artemisia ketone



Artemivestinolide B



Artemivestinolide C



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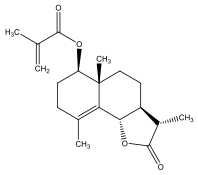
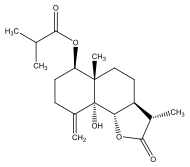


Artemivestinolide E



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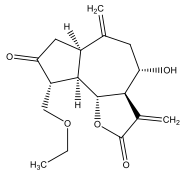
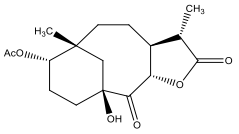


Artemivestinolide G



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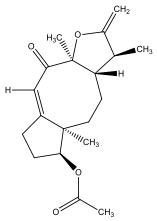

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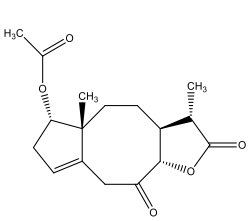
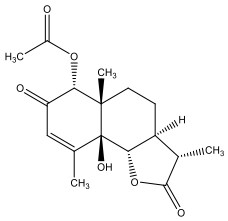
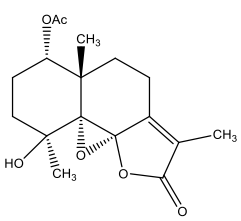


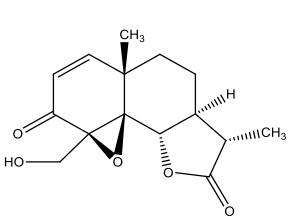
Arvestolide C



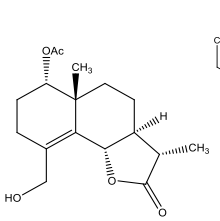
Arvestolide D



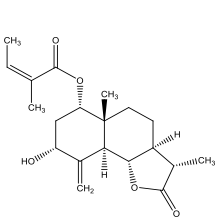
Arvestolide E



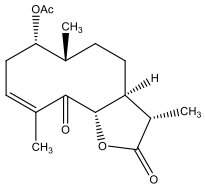
Arvestolide F



Arvestolide G



Arvestolide H



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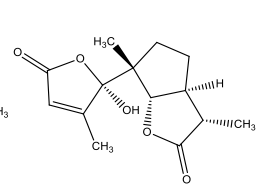

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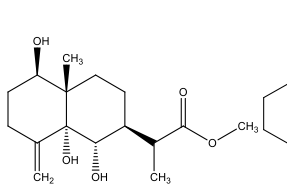
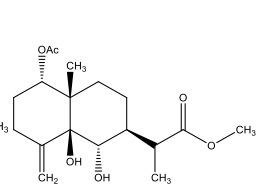


Arvestonate A



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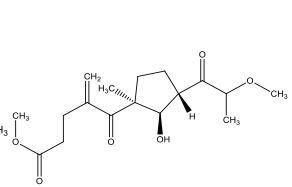
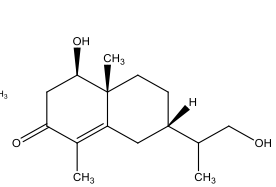
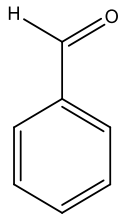


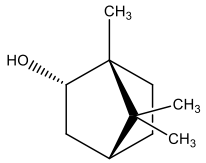
Arvestonate C



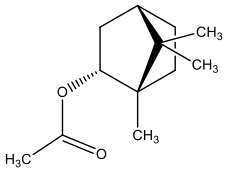
Arvestonol



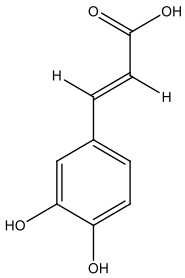
Benzaldehyde



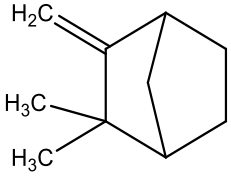
Borneol



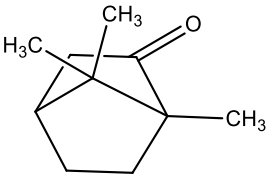
Bornyl acetate



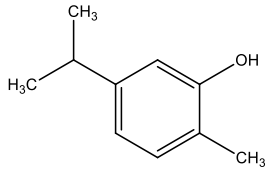
Caffeic acid



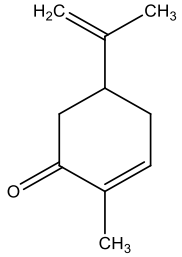
Camphene



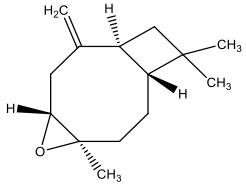
Camphor



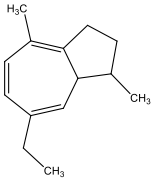
Carvacrol



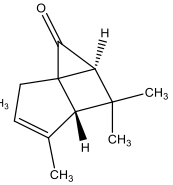
Carvone



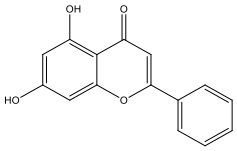
Caryophyllene oxide



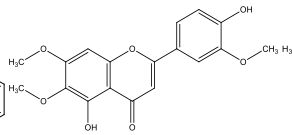
Chamazulene



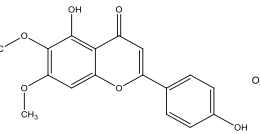
Chrysanthenone



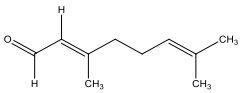
Chrysin



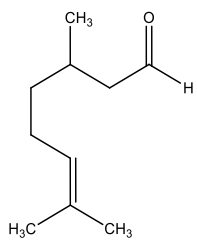
Cirsilineol



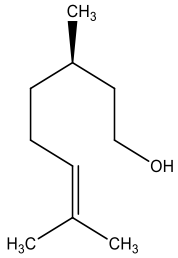
Cirsimaritin



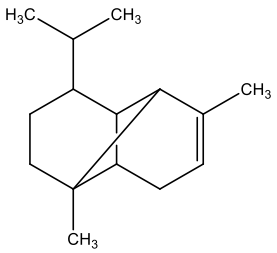
Citral



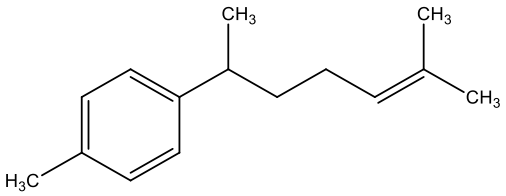
Citronellal



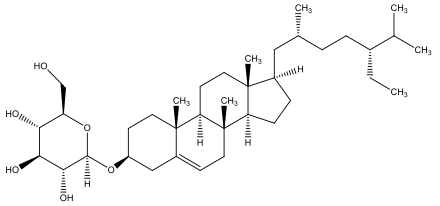
Citronellol



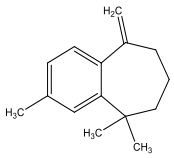
Copaene



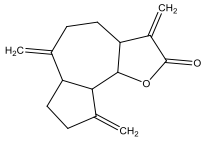
Curcumene



Daucosterol



Dehydro-ar-himachalene



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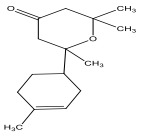

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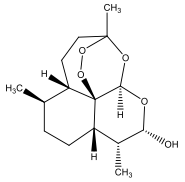

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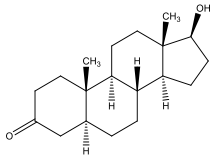

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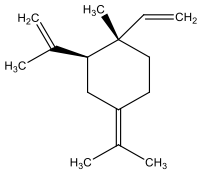

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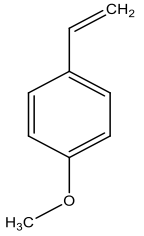

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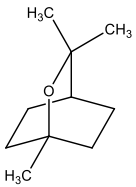

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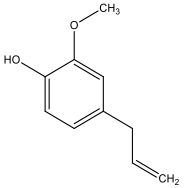

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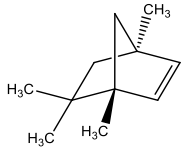
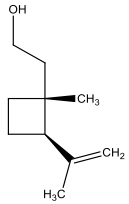


Fenchene



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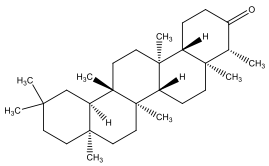

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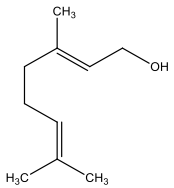

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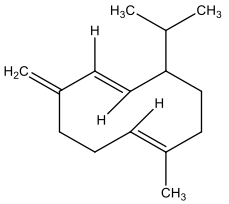

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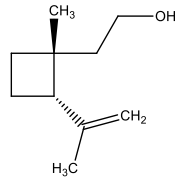

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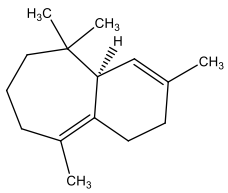
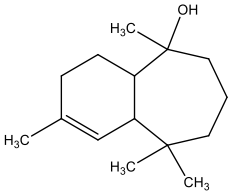
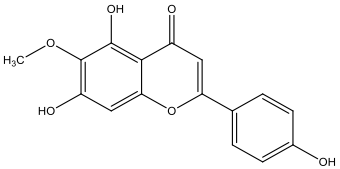


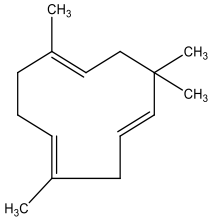
Himachalene



Himachalol



Hispidulin



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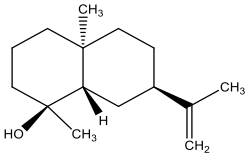
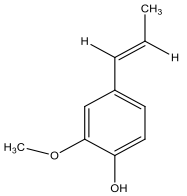
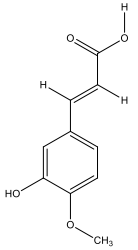


Intermedeol



Isoeugenol



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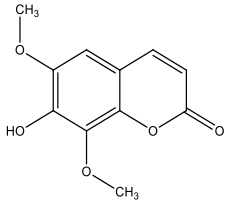

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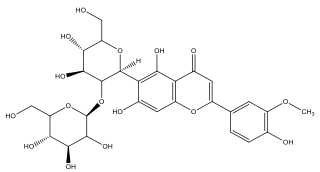
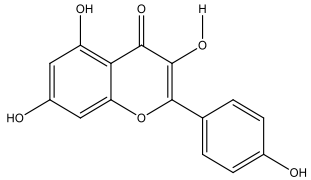
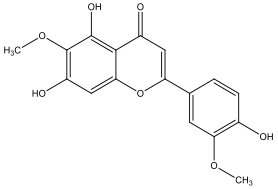


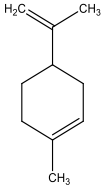
Isoscopoletin-*O*-glucoside



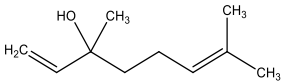
Jaceosidin



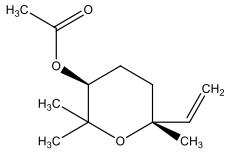
Kaempferol



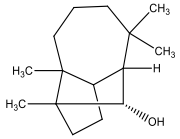
Limonene



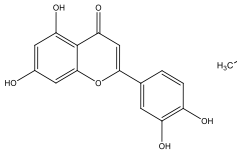
Linalool



Linalool oxide acetate



Longiborneol



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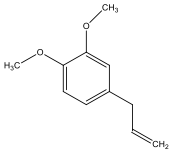
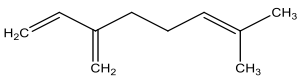
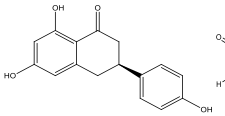


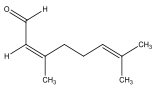
Methyl eugenol



Myrcene



Naringenin



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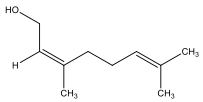
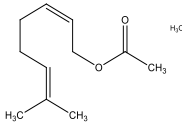
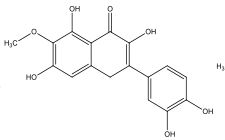


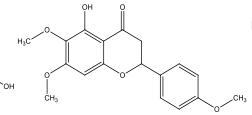
Nerol



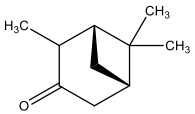
Neryl acetate



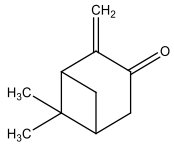
Patuletin



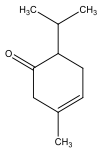
Pectolinaringenin



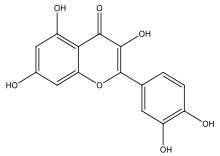
Pinocamphone



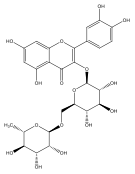
Pinocarvone



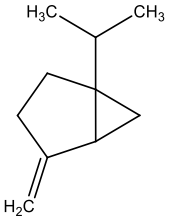
Piperitone



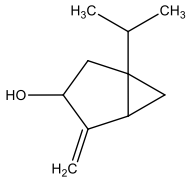
Quercetin



Rutin



Sabinene



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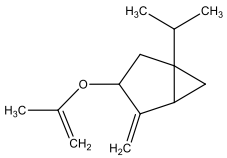
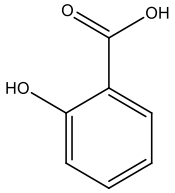
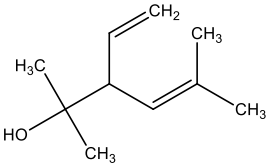


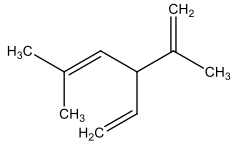
Sabinylacetate



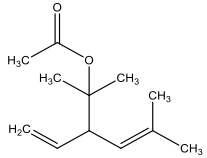
Salicylic acid



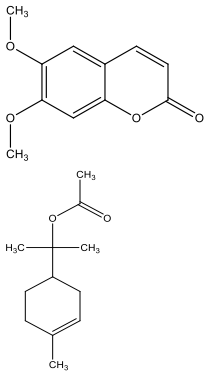
Santolina alcohols



Santolina triene



Santolinyl acetate



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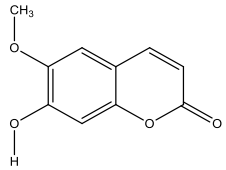
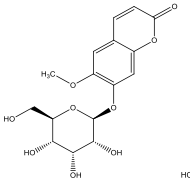
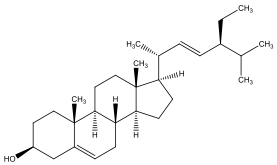


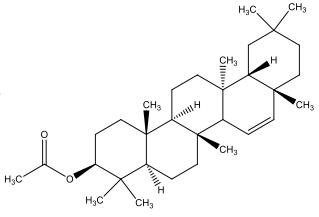
Scopoletin



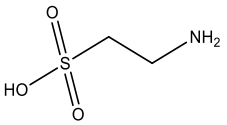
Scopolin



Stigmasterol

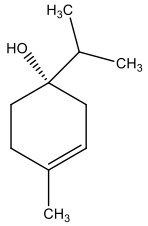


Taraxerolactetate

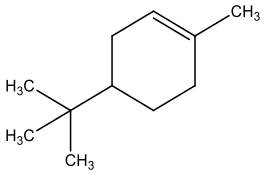


Taurin

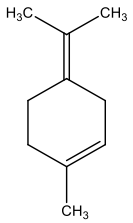
Terpenyl acetate



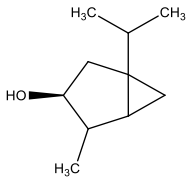
Terpinen-4-ol



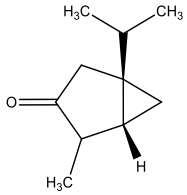
Terpineol



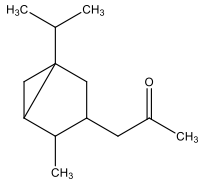
Terpinolene



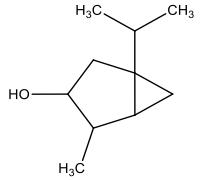
Thujanols



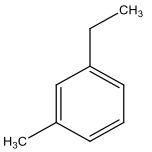
Thujone



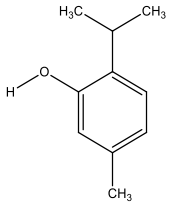
Thujyl acetate



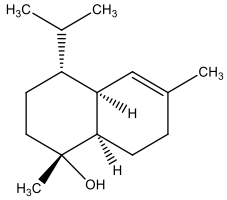
Thujyl alcohol



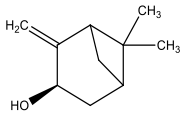
3-Farnesene



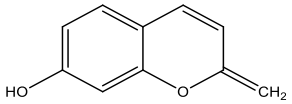
Thymol



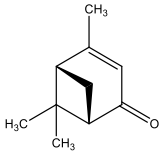
Torreyol



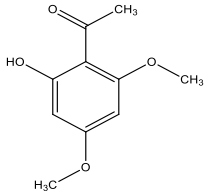
***trans*-Pinocarveol**



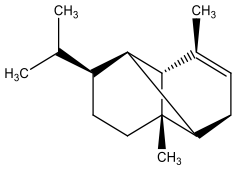
Umbelliferone



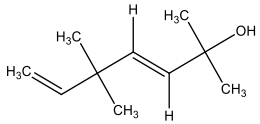
Verbenone



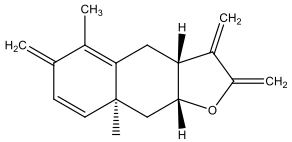
Xanthoxylin



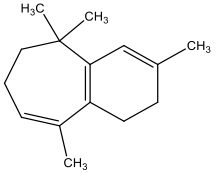
Ylangene



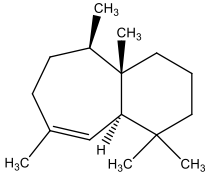
Yomogi alcohol



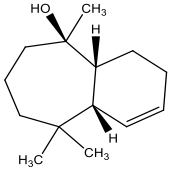
Yomogin



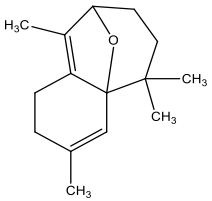
(+)-3-Himachalene



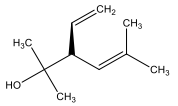
(+)-Allohimachalol



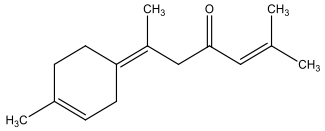
(+)-Himachalol



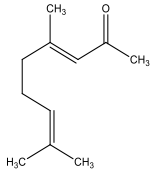
(+)- Oxidohimachalene



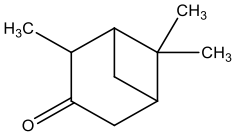
(+)-Santolina alcohol



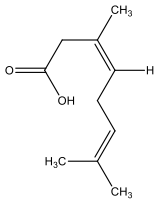
(E)- or (Z)-γ-Atlantone



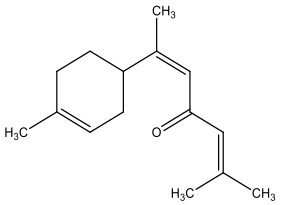
(E)-Citral



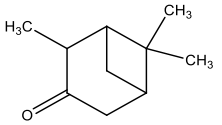
(E)-Pinocamphone



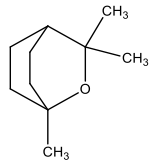
(Z)-Isogeranic acid



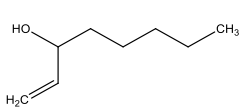
(Z)-α-Atlantone



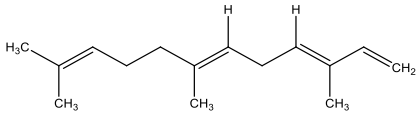
(Z)-α-Santalol



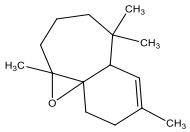
1,8-Cineole



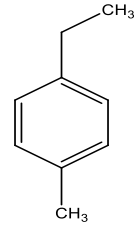
1-Octen-3-ol



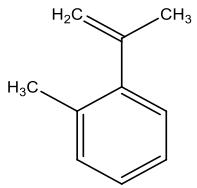
3-Ethyltoluene



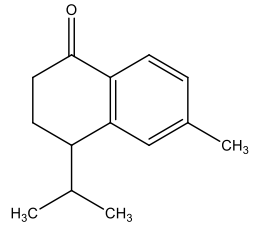
3-Himachalene oxide



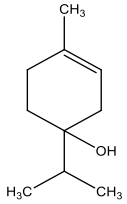
4-Ethyltoluene



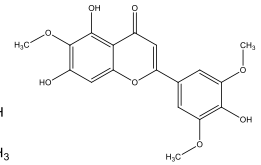
4-Isopropenyltoluene



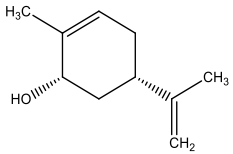
4-Isopropyl-6-methyl-1-tetralone



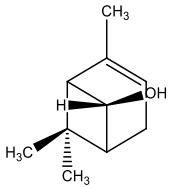
4-Terpineol



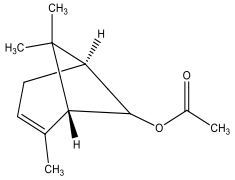
6-Methoxytricin



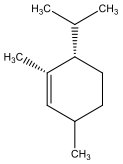
***cis*-Carveol**



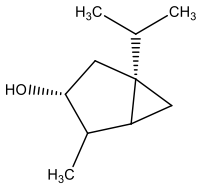
***cis*-Chrysanthenol**



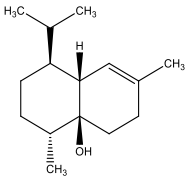
***cis*-Chrysanthenyl acetate**



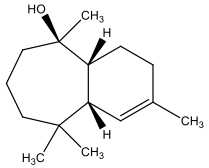
***cis*-Piperitol**



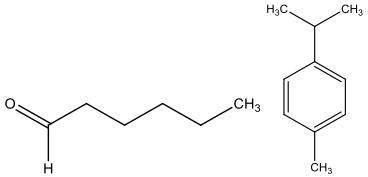
***cis*-Thujanol**



epi-Cubenol

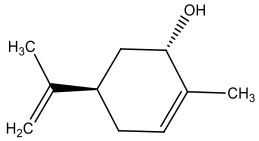


epi-Himachalol

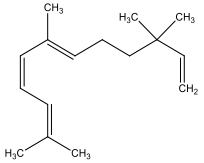


***n*-Caprylaldehyde**

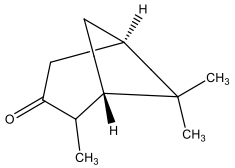
***p*-Cymene**



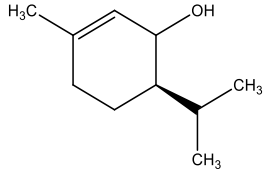
***trans*-Carveol**



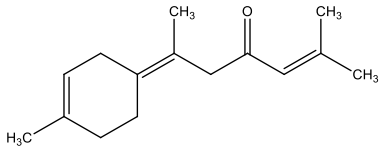
***trans*-Nerolidol**



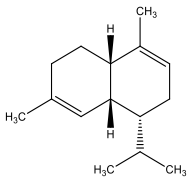
***trans*-Pinocamphone**



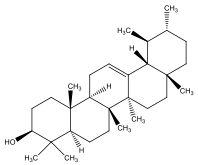
***trans*-Piperitol**



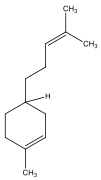
***trans*-γ-Atlantone**



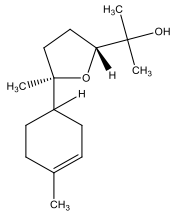
α-Amorphene



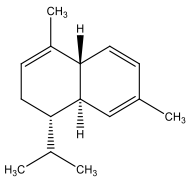
α-Amyrin



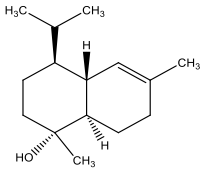
α-Bisabolol



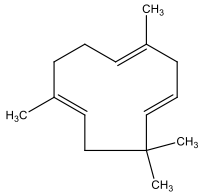
α-Bisabolol oxide B



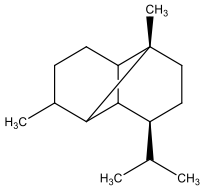
α-Cadinene



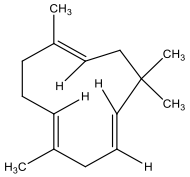
α-Cadinol



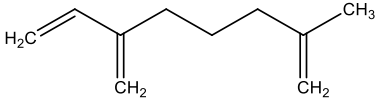
α-Caryophyllene



α-Copaene



α-Humelene



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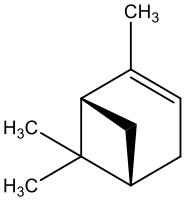

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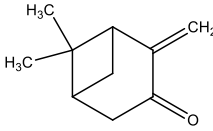
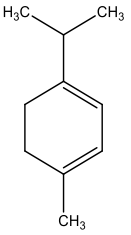
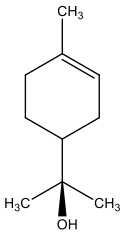


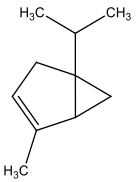
α- Pinocarvone



α-Terpinene



α-Terpineol



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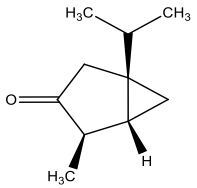

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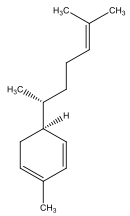
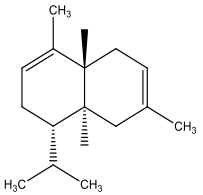
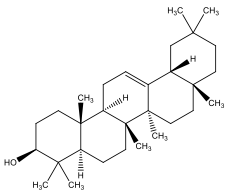


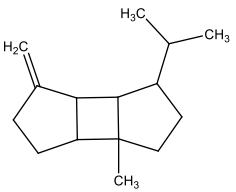
α-Zingiberene



β- Cadinene



β-Amyrin



β-Bourbonene

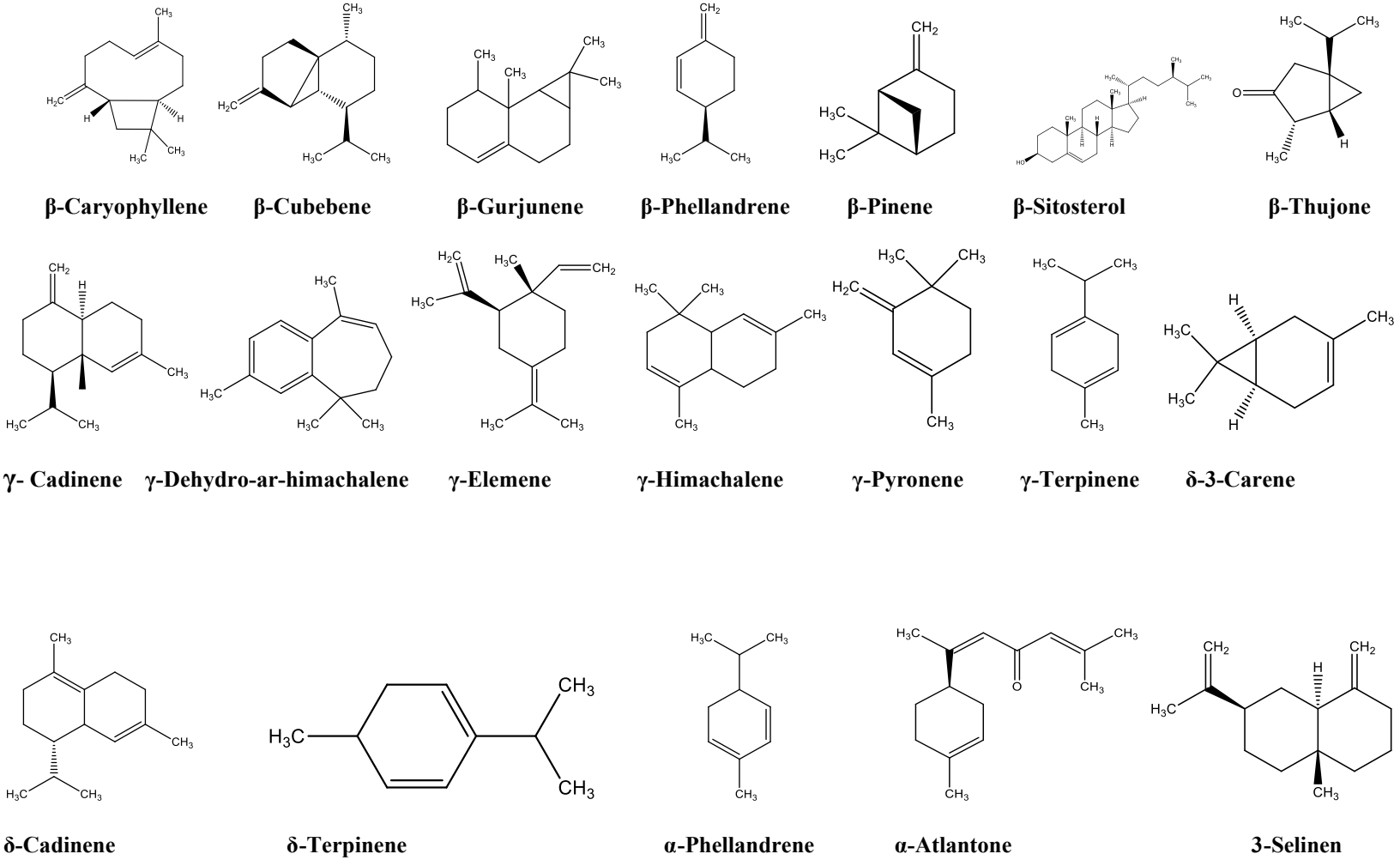


Figure 3. The chemical structures of bioactive compounds present in *Artemisia vestita*.

Figure 4. Biological properties of *Artemisia vestita*.

2.9 Pharmacological activity

Artemisia vestita (Kubsha) is rich in various phytochemicals that contribute to its extensive pharmacological properties (Table 1). These include, anti-inflammatory (antiphlogistic), antipyretic (antifebrile), antifeedant, anthelmintic, antibacterial, antifungal, antiviral, antitumor, antiproliferative, antidote, immunosuppressive, diuretic, hypoglycaemics,

antiepileptic, antioxidative, healing, relieving itching, treating ringworms, skin infections, and respiratory tract infections.

In ethnic medicine, *A. vestita* is used for colds, sinus drainage, improving ventilation, reducing inflammation, and managing asthma. Further studies are needed to explore its anti-adipogenic activity (Prakash et al. 2021).

Table 1. The pharmacological activity of phytochemicals reported in *Artemisia vestita*.

Isolated compound/Extract/Methodology	Pharmacological Activity	Reference(s)
Eucalyptol and grandisol	Antimicrobial activity	Yang et al. (2015)
Sesquiterpenoid Allohimachalol	Spasmolytic activity	Rather et al. (2017)
Ethanolic extract	Antibacterial, antiprotozoal, antifungal, antiviral, anthelmintic, diuretic, hypoglycemic, and anticancer	Qiangba et al. (2002)
Essential oil	Antibacterial and antifungal in dilution	Khare (2008)
Essential oil	Immune-suppressive agents	Sahoo and Banik (2018)
Alcoholic extract	Antifeedant and antifungal activity	Ding et al. (2019)
Alcoholic extract	Anti-helminthic activity	Irum et al. (2015)
Essential oil	Insecticidal activity	Chu et al. (2010)
Jaceosidin compound on human ovary cancer cells CAOV-3, SKOV-3, PC3, and HeLa cells	Antitumor	Sheng et al. (2008)
Annphenone compound on HepG2 cells (liver hepatocellular carcinoma)	Antiproliferative activity	Long et al. (2013)
Rutin on 3T3-L1 cells	Anti- adipogenic activity	Singh et al. (2021)
Cirsilineol	Human inflammatory bowel diseases	Sun et al. (2010)
Plant aerial part extract	Anti-inflammatory and immune suppressive effect	Sun et al. (2006); Yin et al. (2008)

- **Wound healing**: Fresh leaf paste of *Artemisia vestita* is applied to wounds or cuts to stop bleeding and reduce inflammation (Jung et al. 2018). In traditional Chinese medicine, *A. vestita* is used as an alternative and complementary treatment for skin diseases. Known for its cold nature, it is effective in treating skin eruptions, heat, and itching (Uniyal and Shiva, 2005). The paste of the leaves is used for skin infections, inflammation, ringworm, wounds, respiratory tract infections, as well as in ethnic therapies for colds, sinus drainage, and asthma (Frohlich, 1968; Rantzsch et al. 2009; Rana et al. 2021; Nin et al. 1995).
- **Antidote:** *A. vestita* has been used in the treatment of snake bites due to its high content of monoterpenes, flavones, and sesquiterpenoids found in the leaf extracts.
- **Antimicrobial:** The essential oils of *A. vestita* are primarily composed of a fragrant mixture of sesquiterpenes, monoterpenes, and aromatic compounds, well known for their antimicrobial properties in naturopathy. Two major compounds, grandisol and 1,8-cineol, have demonstrated both in vivo and *in vitro* antibacterial activity against bacteria responsible for respiratory infections. The oil exhibited minimum inhibitory concentration (MIC) values between 20 and 80 µg/mL, while the individual compounds showed MIC values between 130 and 200 µg/mL. In vivo studies indicated significant results, with no toxic effects observed in mice (Yang et al., 2015). Eight components with antibacterial activity, including α- and β-thujone, terpinen-4-ol, linalool, nerol, geraniol, α-pinene, and 1,8-cineole, were identified in the oil (Sahoo and Banik, 2018). The plant extract and its formulated gel also showed notable results, with the plant extract exhibiting MIC values between 100 and 240 µg/mL, and the formulated gel (extract + natural polymer) exhibiting MIC values between 30 and 85 µg/mL against bacterial and fungal species causing skin infections.
- **Immunosuppressive activity:** Plant constituents of *Artemisia vestita* are valuable in treating immunological disorders, such as autoimmune diseases and organ transplantation, due to their immune-suppressive properties (Oyedeji et al. 2009). It has been noted that the essential oils of plants containing higher levels of α- and β-thujone typically have minimal or trace amounts of eucalyptol and camphor (He et al. 2011).

Jaceosidin, a flavonoid from *A. vestita*, exerts immunosuppressive effects both *in vitro* and in vivo by modulating T-cell proliferation. This activity is linked to the down-regulation of interferon (IFN)-gamma, signal transducers, activators of transcription (STAT1), and the transcription factor T-box (TBX-21) signaling pathway (Yin et al. 2011). Additionally, flavones such as apigenin, cirsilineol, and 6-methoxytricin from *A. vestita* have demonstrated both immunosuppressive and anti-inflammatory effects (Yin et al. 2008). These flavones specifically inhibit passive cutaneous anaphylaxis (PCA), which induces contact hypersensitivity, while also promoting lymphocyte proliferation and enhancing CD-25 expression in T-cells, further indicating their immunosuppressive activity.

- **Anti-inflammatory:** *A. vestita* extracts demonstrate notable anti-inflammatory activity, including degranulation inhibition in mast cells and the modulation of inflammatory cytokine production (Sun et al. 2006). The extracts have been shown to inhibit the proliferation of mouse splenocytes and mixed lymphocytes, while reducing interleukin-2 (IL-2) levels and the level of metalloproteinase-9, both *in vitro* and in vivo (Yin et al. 2008). The plant's LD50 is reported to be less than 1000 mg/kg, indicating no significant antiprotozoal, antibacterial, antifungal, antiviral, anthelmintic, diuretic, hypoglycaemic, or anticancer effects. However, the extract's effects on guinea pig respiration, ileum, nictitating membrane, cardiovascular system, and central nervous system were observed (Gupta and Tandon, 2004). Additionally, when *A. vestita* extract was cross-linked with Tragacanth gum (a polyacrylic acid-based hydrogel), it exhibited anti-inflammatory activity. The inhibition of cyclooxygenase-1 (COX-1) by the plant extract and the formulated gel was found to be 97.96 ± 0.89% and 69.81 ± 0.91%, respectively, at a 500 µg/mL concentration. Moreover, significant inhibition of cyclooxygenase-2 (COX-2) was also observed, with the plant extract and the formulated gel showing inhibition percentages of 89.47 ± 1.40% and 52.76 ± 1.11%, respectively (unpublished work).
- **Anti-epileptic activity:** Hispidulin, a flavonoid naturally found in *A. vestita*, exhibits strong anti-epileptic activity. In silico docking studies revealed that hispidulin has a binding score of 21.1893 Kcal/mol when targeting the human enzyme glycogen phosphorylase-b/chrysin. This suggests that hispidulin has significant potential as an

inhibitor molecule, which may make it a promising candidate for development into an effective antidiabetic drug to manage hyperglycaemia in type-2 diabetes. However, further in vivo and *in vitro* studies are necessary to fully evaluate its efficacy as an anti-hyperglycaemic agent (Ding et al. 2019; Choudhury et al. 2014).

- **Anti-feedant activity:** The compound artemivestinolide, isolated from *Artemisia vestita*, demonstrates significant antifeedant activity against third-instar larvae of *Plutella xylostella*, with EC50 values ranging from 25.3 to 42 µg/ML. It also exhibits antifungal activity, showing minimum inhibitory concentrations (MIC) of 256 mg/L against *Fusarium oxysporum*, 128 mg/L against *Pyricularia oryzae*, and 256 mg/L against *Botrytis cinerea* (Irum et al. 2015). Additionally, the ethanolic extract of *A. vestita* has shown anti-inflammatory, anti-helminthic, and insecticidal properties, particularly against *Haemonchus contortus* and *Sitophilus zeamais* (Husain, 1992; Wu et al. 2016; Irum et al. 2015).

Whole-plant extracts, particularly those containing vegetative shoots, led to an 87.2% reduction in faecal egg counts in *Haemonchus contortus* at a dosage of 100 mg/kg, demonstrating significant activity against both adult worms and larvae over 28 days (Irum et al. 2015). In fumigant bioassays, the essential oil of *A. vestita* exhibited promising insecticidal activity, with an LC50 value of 13.42 mg/L and an LD50 value of 50.62 mg against adult *Sitophilus zeamais*.

Monoterpenes, sesquiterpenoids, and flavones isolated from *A. vestita* contribute to its diverse bioactivities (Yin et al. 2011). Specifically, 1,8-cineol is known for its cold-relieving, mucolytic, and expectorant properties. Both 1,8-cineol and camphor present in the essential oil act as fumigants, demonstrating broad insecticidal activity (Rozman et al. 2007; Obeng- ofori et al. 1998; Obeng- ofori et al. 1997). These compounds show great potential as natural fumigants for insect control in stored products (Lee et al. 2004; Abdelgaleil et al. 2009), offering advantages over conventional fumigants due to their non-persistence, biodegradability, low toxicity to mammals, and easy availability (Liu et al. 2012).

- **Known Hazards:** Although no specific toxicity reports have been recorded for *A. vestita* extracts, it is important to note that the *Artemisia* genus contains allergenic sesquiterpenoid lactones, which have the potential to cause skin reactions or dermatitis in sensitive individuals (Foster and Duke, 1990).
- **Cytotoxicity:** The essential oil of *A. vestita* contains volatile terpenoids and monoterpenes, such as pinene, eugenol, 1,8-cineole, limonene, citronellol, terpinolene, citronellal, thymol, and camphor, which contribute to its repellent or toxic activity (Mann and Kaufman, 2012). The compounds Arvestolides H and I showed inhibitory effects on nitric oxide production in BV-2 cells, with IC50 values of 43.2 μM and 39.9 μM, respectively (Tian et al. 2018). Additionally, flavonoids like cirsilineol, apigenin, and 6-methoxytricin inhibit T-cell proliferation, suggesting their potential in treating T-cell-mediated inflammation (Yin et al. 2008). Sesquiterpenes, coumarins, and flavones were reported in wormwood (Zhu et al. 2013). The *A. vestita* leaf extract also alleviates picryl chloride (PCl)-induced contact hypersensitivity by blocking T-lymphocyte activation (Wang et al. 2006). In cytotoxicity studies, both S2 (extract) and S4 (extract + polymer) showed high cell viability, with S2 exhibiting 18.2 ± 0.35% cytotoxicity and S4 showing 19.7 ± 0.29% at 1000 μg/mL concentration against HaCat cells. These findings indicate that *A. vestita* has significant anti-inflammatory and anti-cancer effects, supporting its use in wound healing and anti-cancer therapies in traditional medicine.

2.9.1 Biological activity of compound

Annphenone: Annphenone demonstrates specific and potent antiproliferative activity against HepG2 cells, with an IC50 value of 2.0 ± 0.4 μg/mL. Cell cycle analysis showed that annphenone arrests HepG2 cells in the G0/G1 phase, as observed through immunocytochemistry. It is believed that annphenone inhibits catenin expression by blocking its localization transfer, leading to a reduction in cyclin D1 protein expression. Molecular docking simulations suggest that annphenone acts as a ligand for the ASGP-R (asialo glycoprotein receptor), highlighting its selectivity for hepatocellular carcinoma cells and potential for antiproliferative activity (Long et al. 2013). In addition to its anti-

tumor effects, annphenone, present in the aqueous extract, reduced contact sensitivity in mice by down-regulating the adhesion, activation, and metalloproteinase production in T-lymphocytes (Wang et al. 2005). The ethanolic extract of *A. vestita* also exhibited anti-sepsis activity by down-regulating the NF-kB and MAPK pathways, further supporting its therapeutic potential (Sun et al. 2006).

Cirsilineol: Cirsilineol (4′,5-dihydroxy-3′,6,7-trimethoxyflavone), found in *A. vestita* extracts, exhibits potent anti-tumor and immune-suppressive properties (Yin et al. 2008; Sheng et al. 2008). It significantly inhibits the proliferation of various cancer cells, including Skov-3, PC3, Caov-3, and Hela cells, in a concentration-dependent manner. Cirsilineol induces apoptosis in Caov-3 cells through a dose-dependent process, as demonstrated by annexin V/propidium iodide double staining. The apoptosis mechanism involves the activation of caspase-9, caspase-3, and poly ADP-ribose polymerase (PARP), alongside the loss of mitochondrial membrane potential (MMP), which leads to the release of cytochrome c into the cytosol. This mitochondrial pathway of apoptosis is crucial to its anti-proliferative effects on cancer cells. Additionally, cirsilineol is effective in ameliorating TNBS (tri-nitrobenzene sulfonic acid)-induced colitis in mice, likely due to its immunoregulatory activities, particularly the selective inhibition of the IFN-γ/STAT1/T-bet signalling pathway in colonic lamina propria CD4+ T-cells, making it a potential therapeutic candidate for Crohn's disease (Sun et al. 2010).

Jaceosidin: The regulation of transcription activator and signal transducer (STAT1) is being explored as a therapeutic strategy for bowel diseases, with limited reports of chemicals targeting STAT1/IFN-γ signaling for Crohn's disease treatment. Cirsilineol, a natural compound isolated from *A. vestita*, has shown significant potential in ameliorating TNBS (trinitro-benzene sulfonic acid)-induced T-cell-mediated colitis in mice (Sun et al. 2010). This effect is linked to reduced activation and proliferation of auto-reactive T-cells. Cirsilineol therapy also modulates the balance of anti-inflammatory and pro-inflammatory cytokines by increasing regulatory T-cell activity (through up-regulation of TGF-β and IL-10) and decreasing effector Th-1 cell activity (via down-regulation of IFN-γ). Notably, the

inhibition of STAT1/IFN-γ signalling by cirsilineol appears reversible in the presence of higher levels of IFN-γ, suggesting its potential as a treatment for T-cell-mediated bowel diseases like Crohn's.

Similarly, jaceosidin, a flavone isolated from *A. vestita*, exhibits antiproliferative effects on several human cancer cell lines, including SKOV-3, PC3, HeLa, and CAOV-3. It significantly reduces cell proliferation in a concentration-dependent manner, with CAOV-3 cells showing time-dependent inhibition. Jaceosidin induces apoptosis in these cells, as evidenced by PARP cleavage, caspase-3 activation, and increased levels of cleaved caspase-9 and cytochrome c in the cytosol. These findings highlight jaceosidin's potential as an anti- tumour agent (Sheng et al. 2008).

Herbal flora is gaining significant attention from scientists for developing strategies and understanding the therapeutic potential of novel herbal constituents to treat various health disorders. Information regarding the use of *A. vestita* as a folkloric medicine has largely been confined to native inhabitants. The phytochemicals reported in *A. vestita* belong to diverse chemical classes, including flavonoids, terpenoids, oxygenated monoterpenes, triterpenes, sesquiterpenes, hydroxycinnamic acids, mono- and diterpene hydrocarbons, aromatic aldehydes, azulenes, sesquiterpene hydrocarbons, sterols, phenylpropanoids, monoterpenoids, coumarins, and organosulfonic acids. Phenolic compounds in the plant's oil contribute to its antioxidant activity, while flavones and sesquiterpenes exhibit anti-tumour and anti-inflammatory properties, respectively. The anti-inflammatory potential of *A. vestita* leaf extracts have been validated through traditional use and preliminary research. Additionally, *A. vestita* shows promise as a source of anti-COVID-19 remedies. Studies suggest an inverse correlation between the antiviral activity of artemisinin content and total flavonoid content. Artemisinin, either alone or in combination with other compounds, acts synergistically to block post-entry viral infections. Furthermore, essential oils and extracts from the pre- and post-flowering stages of *A. vestita* have demonstrated antifungal activity against phytopathogenic fungi, indicating potential as an effective treatment for dermatophytes.

Despite its traditional use in treating various ailments, further standardization and extensive clinical studies are needed to optimize dosage and validate its efficacy. The existing scientific data on *A. vestita* remain fragmented, with inconsistent results limiting broader acceptance and application.

Future research should focus on developing value-added *A. vestita*-based products and enhancing the efficacy of its extracts through synergistic blending with other natural extracts. As a reservoir of phytochemicals, the conservation of this species is essential to ensure its continued availability for medicinal research and its potential contributions to health security.

CHAPTER 3

HYPOTHESIS

Chapter 3

3.1. Hypothesis

The *Artemisia vestita* leaf extract and Tragacanth gum-based hydrogel contain bioactive phytochemicals with significant antimicrobial and pharmacological properties, making them potential candidates for therapeutic and biomedical applications.

Objective 1: Identification and phytochemical characterization of secondary metabolites from *Artemisia vestita*.

Alternate hypothesis (H_1): *Artemisia vestita* leaf extract contains bioactive secondary metabolites with distinct phytochemical properties that can be identified and characterized using analytical techniques. They exhibit significant antimicrobial, antioxidant, anti-inflammatory, and wound-healing activities, making them potential candidates for therapeutic applications.

Null hypothesis (H_0): *Artemisia vestita* leaf extract does not contain significant secondary metabolites, or the identified compounds do not exhibit notable antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties. We reject the null hypothesis and accept the alternate hypothesis.

Objective 2: *In vitro* evaluation of antimicrobial activity of the leaf extract.

Alternate hypothesis (H_1): The *Artemisia vestita* leaf extract exhibits significant antimicrobial activity against the tested skin microbial strains *in vitro*.

Null hypothesis (H_0): The *Artemisia vestita* leaf extract does not exhibit significant antimicrobial activity against the tested skin microbial strains *in vitro*. We reject the null hypothesis and accept the alternate hypothesis.

Objective 3: To develop and incorporate the drug in crosslinked customized polymer for increased drug loading capacity and evaluate its release profile.

Alternate hypothesis (H_1): The incorporation of the drug into the crosslinked customized polymer significantly improves drug loading capacity and alters the release profile,

resulting in a controlled and sustained release compared to conventional formulations. Furthermore, the drug release from the crosslinked polymer adheres to a specific kinetic model (e.g., Zero-order, First-order, Higuchi, or Korsmeyer-Peppas), ensuring a predictable and prolonged release pattern in contrast to conventional systems.

Null hypothesis (H_0): The incorporation of the drug into the crosslinked customized polymer does not significantly enhance drug loading capacity or modify the drug release profile. We reject the null hypothesis and accept the alternate hypothesis.

Objective 4: To identify pH sensitivity and temperature stimulus drug release system.

Alternate hypothesis (H_1): The drug release rate from the system varies significantly with changes in pH and temperature, demonstrating pH-sensitive and thermoresponsive behaviour that enables controlled and targeted drug release.

Null hypothesis (H_0): The rate of drug release from the system doesn't significantly vary despite variations in pH or temperature, suggesting a lack of pH-sensitive or thermoresponsive characteristics. The drug release system does not demonstrate notable sensitivity to pH or temperature, with drug release remaining consistent across different conditions. We reject the null hypothesis and accept the alternative hypothesis.

CHAPTER 4

AIMS

&

OBJECTIVES

Chapter 4

Aims and objectives

The aim of the present study was to conduct a comprehensive phytochemical analysis of *Artemisia vestita* leaf extract, evaluate its antimicrobial activity, and assess its pharmacological effects. Additionally, the study aims to develop a Tragacanth gum-based hydrogel incorporating the leaf extract, investigate its antimicrobial potential, and analyse its biomedical applications, particularly in drug delivery and wound healing.

The main objectives of the proposed work were:

1. Identification and phytochemical characterization of secondary metabolites from *Artemisia vestita*.
2. *In vitro* evaluation of antimicrobial activity of the leaf extract.
3. To develop and incorporate the drug in crosslinked customized polymer for increased drug loading capacity and evaluate its release profile.
4. To identify pH sensitivity and temperature stimulus drug release system.

CHAPTER 5

MATERIALS

&

METHODS

Chapter-5

5.1 Plant material collection and preparation

5.1.1 Collection and authentication of *Artemisia vestita*

The *Artemisia* species used in this study were harvested from the natural forested regions of Kotgarh (approximately 31.31° N latitude and 77.47° E longitude), located in the Shimla district of Himachal Pradesh (HP), India (Figure 5). This region forms part of the Western Himalayan range and features rugged mountainous landscapes with dense forest cover. Most of the area drains into the Sutlej River, although specific areas, such as the Shilaroo Block, are drained by tributaries of the Giri River (Chauhan and Jishtu. 2022).

The collection of *A. vestita* leaves was carried out during its peak flowering season in the months of April and May (2021) to ensure optimal phytochemical yield. Following collection, the leaves were subjected to shade drying for a period of 15 days to retain their chemical constituents before proceeding to extraction and further analysis (Figure 6). Plant specimens were authenticated (Figure 7) by botanists at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh (Herbarium Accession No. 13916).

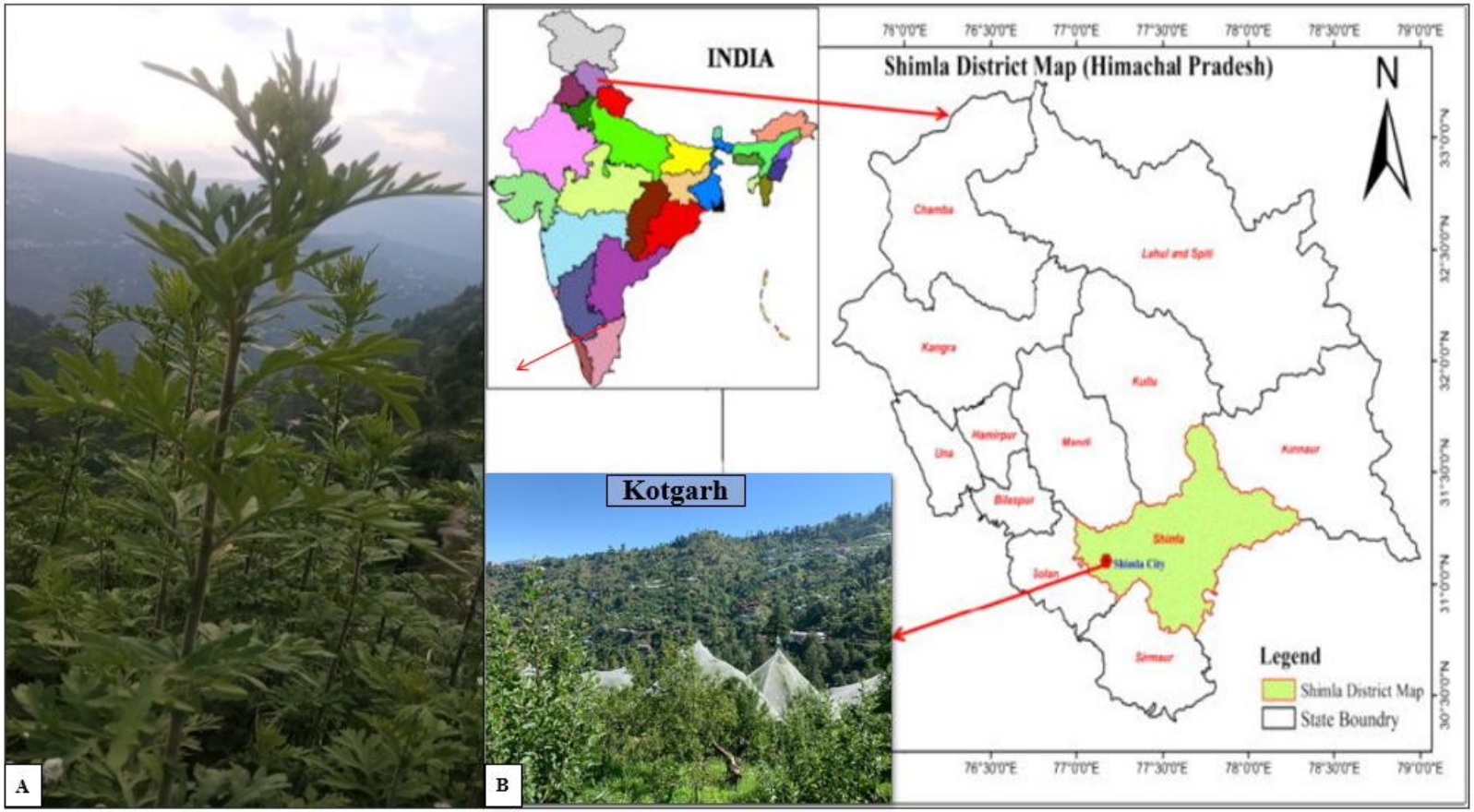


Figure 5: *Artemisia vestita* **(A)** Plant habit and **(B)** Study site (Kotgarh, Shimla, HP).

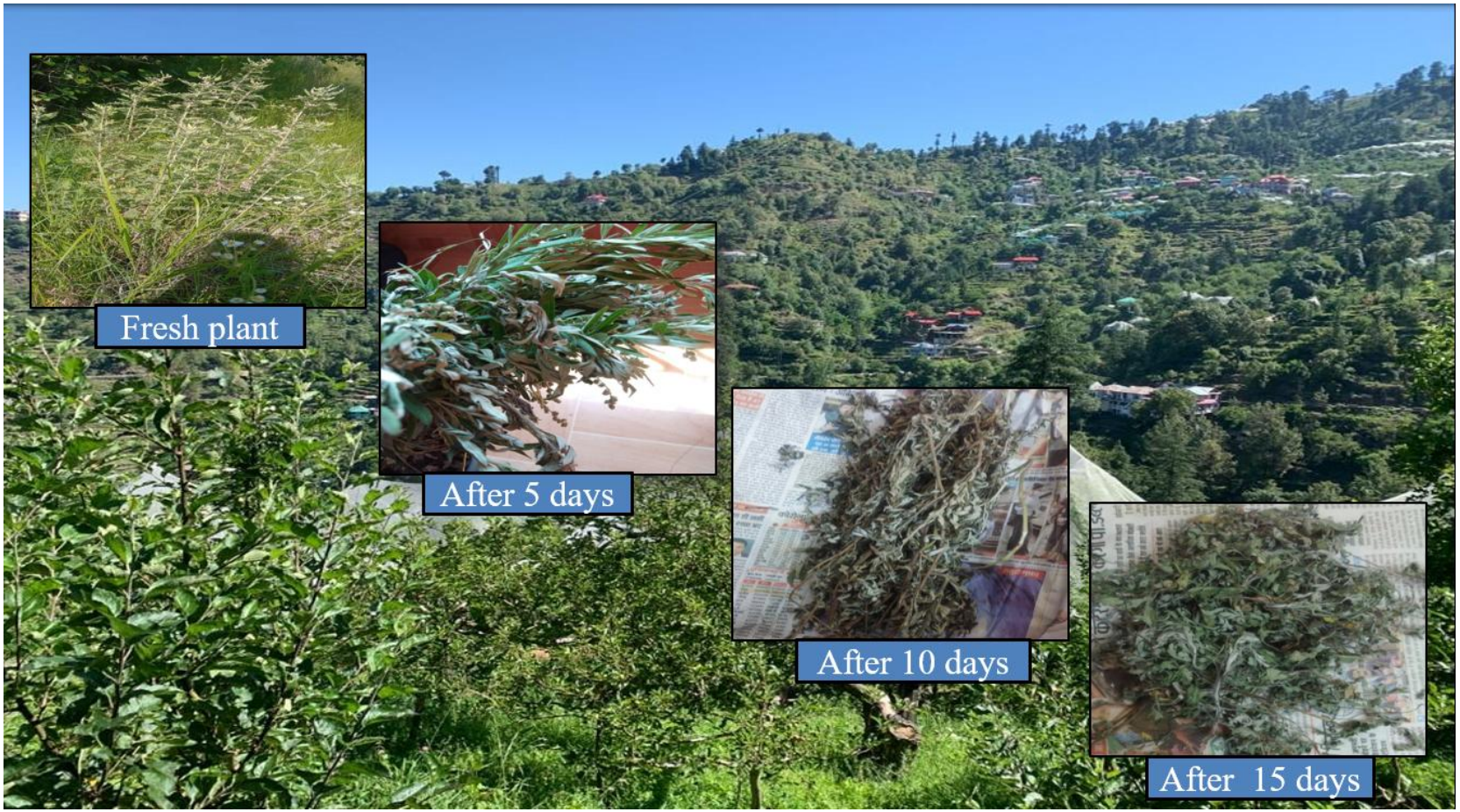
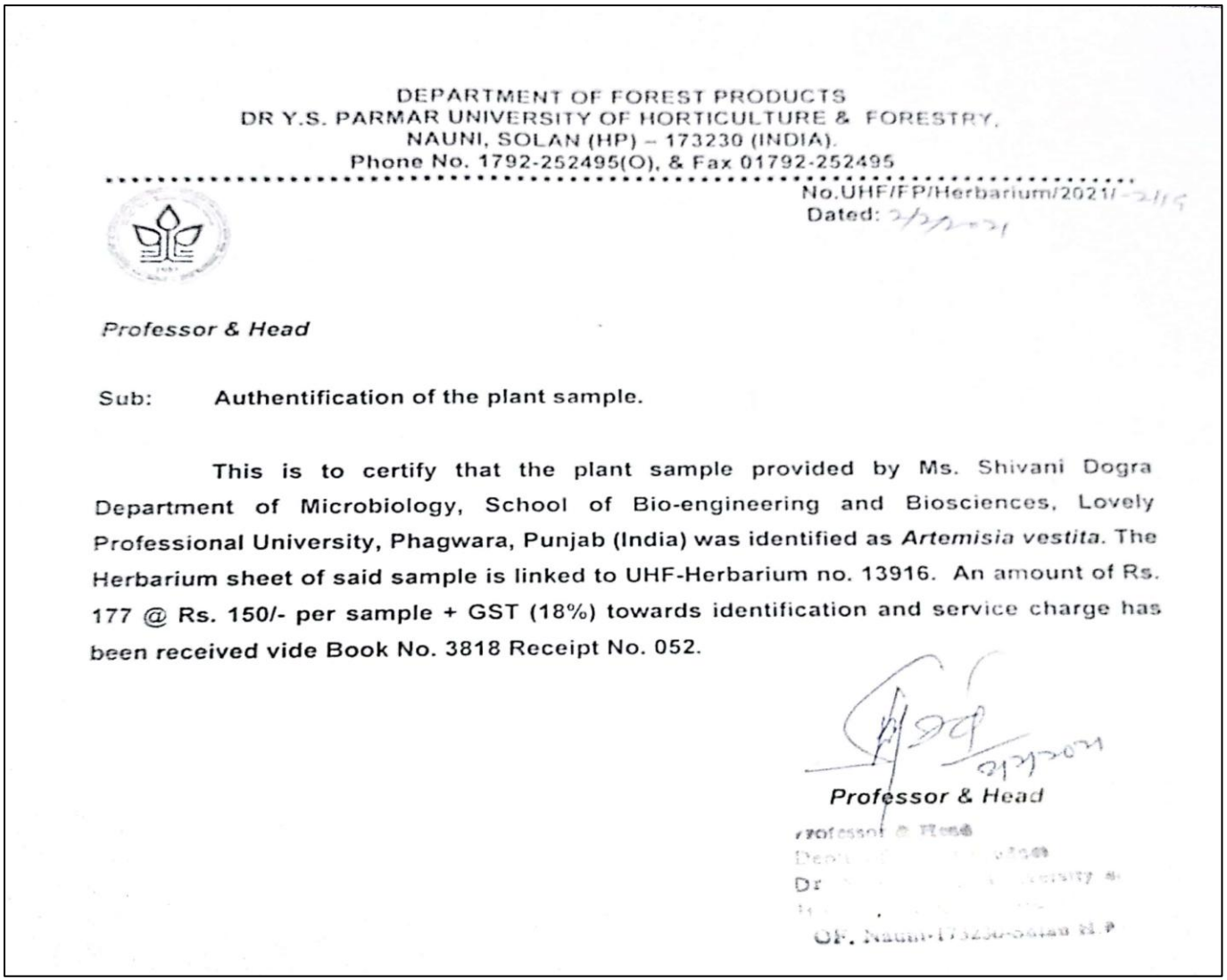


Figure 6: *Artemisia vestita* collection site and drying period.



DEPARTMENT OF FOREST PRODUCTS
DR Y.S. PARMAR UNIVERSITY OF HORTICULTURE & FORESTRY,
NAUNI, SOLAN (HP) – 173230 (INDIA).
Phone No. 1792-252495(O), & Fax 01792-252495

No.UHF/FP/Herbarium/2021/-2116
Dated: 2/12/2021

Professor & Head

Sub: **Authentification of the plant sample.**

This is to certify that the plant sample provided by Ms. Shivani Dogra Department of Microbiology, School of Bio-engineering and Biosciences, Lovely Professional University, Phagwara, Punjab (India) was identified as *Artemisia vestita*. The Herbarium sheet of said sample is linked to UHF-Herbarium no. 13916. An amount of Rs. 177 @ Rs. 150/- per sample + GST (18%) towards identification and service charge has been received vide Book No. 3818 Receipt No. 052.

2/12/2021

Professor & Head

Figure 7: Authentication certificate of plant specimen *Artemisia vestita*.

5.1.2 Preparation of *Artemisia vestita* leaf extract (ALE)

Fresh leaves of *A. vestita* were collected and thoroughly rinsed with distilled water to eliminate surface impurities such as dust and soil particles. The cleaned leaves were shade-dried at room temperature (~25°C) for approximately 15 days to avoid degradation of heat-sensitive bioactive compounds. Once completely dried, the leaves were pulverized into a fine powder using a mechanical grinder and stored in airtight containers until for later use. A hydroalcoholic solvent was prepared by combining ethanol and distilled water in a 70:30 (v/v) ratio. This solvent system was selected to efficiently extract a broad spectrum of phytochemicals, targeting both polar compounds (such as flavonoids and phenolics) and semi-polar constituents (like terpenoids).

For the extraction, 25 grams of the powdered plant material was placed in a thimble and loaded into the main chamber of a Soxhlet apparatus. A total of 250 mL of hydroalcoholic solvent was used in the round-bottom flask. The extraction was carried out continuously for 6–8 hours at a controlled temperature, allowing repeated cycles of solvent percolation as per standard Soxhlet extraction protocol (Belokurov et al. 2019; Rodrigues et al. 2017; Hidayat and Wulandari, 2021).

After the extraction period, the mixture was first filtered through a muslin cloth, followed by filtration with Whatman No. 1 filter paper to remove plant residues. The remaining plant marc was gently pressed to recover any residual liquid. The collected filtrate was then concentrated under reduced pressure using a rotary evaporator at 40–45°C for approximately 15 minutes to evaporate the ethanol content.

Subsequently, the remaining aqueous portion of the extract was further concentrated using a water bath until a semi-solid, crude extract was obtained. This step was carefully performed to preserve thermolabile constituents. The final crude extract was weighed to determine extraction yield and stored in amber-coloured, airtight containers at 4°C to prevent exposure to light and microbial contamination. The extract was retained under these conditions until further application in phytochemical analysis, antimicrobial studies, and hydrogel formulation.

$$\text{Percentage Yield} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of dried plant powder used (g)}} \times 100$$

5.2 Preliminary screening of phytochemicals

5.2.1 Qualitative phytochemical analysis

The *A. vestita* leaves extract (ALE) was analysed using standard qualitative phytochemical methods to detect the presence of various secondary metabolites. This screening aimed to confirm the occurrence of key bioactive constituents in the extract. Specific tests were employed for each class of compound: Dragendorff's reagent was used to test for alkaloids; the presence of flavonoids was verified through the Shinoda reaction; terpenoids were identified using the Salkowski test; saponins were detected by the froth formation method; and the presence of tannins and phenolic compounds was confirmed by the reaction with 5% ferric chloride solution (Herborne, 1973; Trease and Evans, 1987).

1. **Alkaloid Detection (Dragendorff's Test)**: To test for alkaloids, a few drops of Dragendorff's reagent were added to a test tube containing 2 mL of ALE. The appearance of an orange to brown precipitate indicated a positive result for alkaloids.
2. **Flavonoid Detection (Shinoda Test)**: For flavonoid identification, 1 mL of ALE was mixed with 10% lead acetate solution in a test tube. The formation of a yellow precipitate confirmed the presence of flavonoids.
3. **Terpenoid Detection (Salkowski Test)**: For terpenoid detection, 2 mL of ALE was mixed with 2 mL of chloroform in a test tube, followed by the careful addition of 1 mL concentrated sulfuric acid. The formation of a reddish-brown interface layer confirmed the presence of terpenoids.
4. **Test for saponins (Froth Test):** To detect the presence of saponins, 2 mL of the ALE was diluted with 10 mL of distilled water in a test tube. The mixture was vortexed for 30 seconds and then allowed to stand undisturbed for 10–15 min. The formation of a stable, persistent froth layer (at least 1 cm in height) indicated the presence of saponin compounds.
5. **Detection of Tannins**: The presence of tannins was detected by adding 1 mL of ALE to 5 mL of bromine water in a test tube. A visible loss of colour in the solution indicated presence of tannins.

6. **Detection of Phenolic Compounds**: To identify phenolic constituents, 2 mL of the ALE was treated with a few drops of 5% ferric chloride solution. The appearance of a deep blue to black coloration indicated the presence of phenolic compounds.

5.2.2 Quantitative phytochemical analysis

GC-MS (Gas chromatography and mass spectrometry) analysis: The GC-MS analysis of *Artemisia vestita* leaf extract (ALE) was carried out using an Agilent 7010B GC/TQ system equipped with a VF-5 MS capillary column (60 m × 0.25 mm ID; film thickness 0.25 μm) operating in spitless mode. The system was coupled to a triple quadrupole mass detector (Agilent Technologies, Santa Clara, CA, USA). Helium served as the carrier gas at a constant flow rate of 1.0 mL/min. An injection volume of 0.8 μL was used with a split ratio of 1:80, and the injector temperature was maintained at 250°C throughout the analysis. The oven temperature was initially programmed at 40°C and held for 2 minutes, then gradually increased at a rate of 10°C/min to 150°C and held for 10 minutes. Subsequently, the temperature was ramped up to a final temperature of 280°C and maintained for another 10 minutes. The mass spectrometer operated under electron impact ionization (EI) at 70 eV, with the ion source temperature set at 260°C and the quadrupole temperature at 150°C. Spectral data were collected over a mass range of m/z 50–500 with a scan rate of 2 scans per second (Medeiros, 2018). Compound identification was achieved by comparing the obtained mass spectra and retention indices (RIs) to those of known standards and a homologous series of n-alkanes (C8–C40), analysed under identical conditions. Further verification was performed using co-injection of reference compounds when available. The spectral matching was conducted using the NIST Mass Spectral Library (Software Version 2.4; Data Version: NIST v20), accessible via http://www.nist.gov/srd/nist1a.cfm. Quantitative estimation of the constituents was determined by the area normalization method and expressed as the relative percentage of each component in the extract.

5.3 Antimicrobial Screening

5.3.1 Microbial Cultures and Preparation of Inoculum

The bacterial and fungal strains used in this study included *Bacillus subtilis* (MTCC 168), *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (MTCC 443), *Streptococcus pyogenes* (MTCC 1927), *Proteus mirabilis* (MTCC 425), and *Candida albicans* (MTCC 183). These strains were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) housed at the Institute of Microbial Technology (IMTECH), Chandigarh, India.

Upon receipt, all microbial strains were revived and cultured overnight to ensure optimal growth and viability before being subjected to antimicrobial activity assays. Bacterial cultures were grown in nutrient broth at 37°C under shaking conditions, while fungal cultures were maintained on Sabouraud dextrose agar at 28°C.

For precise evaluation of antimicrobial activity, it is crucial to use a standardized and consistent microbial inoculum. Variability in microbial density can impact the accuracy, reproducibility, and interpretation of experimental data. To maintain uniformity across all assays in the present investigation, microbial suspensions were standardized using the 0.5 McFarland turbidity reference.

To prepare the inoculum, a portion of each overnight microbial culture was suspended in sterile 0.5% sodium chloride (NaCl) solution. The suspension's turbidity was carefully adjusted to match that of the 0.5 McFarland standard, corresponding to an optical density (OD) of approximately 0.08 to 0.1 at 600 nm. This adjustment ensures a bacterial concentration close to 1×10^6 colony-forming units (CFU) per millilitre. The 0.5 McFarland standard was prepared by combining defined amounts of barium chloride ($BaCl_2$) and sulfuric acid (H_2SO_4), resulting in a turbid solution that visually resembles the density of bacterial cells.

Microbial suspensions were visually matched to the 0.5 McFarland standard to maintain uniform inoculum density and ensure reliable antimicrobial testing. The 0.5 McFarland standard corresponds to roughly 1.5×10^8 CFU/mL, which is typically diluted to about 10^6 CFU/mL for assays such as disc diffusion or broth dilution to achieve appropriate testing conditions (Jorgensen & Turnidge, 2015).

Preparation of the 0.5 McFarland Standard: The preparation involved mixing 0.05 mL of 1.175% (w/v) barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) with 9.95 mL of 1% (v/v) sulfuric acid (H_2SO_4). This chemical reaction generates a fine precipitate of barium

sulfate ($BaSO_4$), creating a uniform turbidity that mimics the light-scattering characteristics of a bacterial suspension at a known concentration (Jorgensen & Turnidge, 2015).

Adjustment of Microbial Suspensions: Bacterial and fungal cultures were incubated overnight to allow growth to the logarithmic (log) phase, ensuring high metabolic activity and viability. After incubation, cells were collected and resuspended in sterile 0.5% NaCl solution. The turbidity of each microbial suspension was visually compared with the 0.5 McFarland standard. Suspensions exhibiting higher turbidity were diluted with sterile saline, whereas less turbid suspensions were adjusted by adding more cells to achieve the desired standard.

Additionally, optical density readings were taken at 600 nm using a spectrophotometer for accurate confirmation. An OD value between 0.08 and 0.10 at 600 nm closely matches the turbidity of the 0.5 McFarland standard (Andrews, 2001).

Importance of Standardization: Proper adjustment of microbial density to match the 0.5 McFarland standard is crucial for:

- Ensuring consistent inoculum size across all experimental conditions.
- Reducing errors due to variations in microbial concentration.
- Facilitating reproducible and comparable antimicrobial susceptibility results.
- Minimizing experimental variability and enhancing overall data reliability.

Estimation of Colony-Forming Units (CFUs): After adjustment, microbial suspensions generally contain between 1×10^6 and 1×10^8 CFU/mL, depending on the specific organism. Colony-forming units represent the number of viable cells capable of forming visible colonies on solid media. Using McFarland standards provides a practical, indirect, and widely accepted method for estimating viable microbial counts (CLSI, 2018).

By maintaining a uniform microbial inoculum throughout the experimental procedures, this study ensured accurate and reproducible assessments of antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Proteus mirabilis*, and *Candida albicans*.

5.3.2 Agar well diffusion method

The agar well diffusion method is a widely utilized technique for evaluating the antimicrobial activity of various substances, including plant extracts, chemicals, and antibiotics. It is a simple, cost-effective, and reproducible assay commonly used in microbiology laboratories to screen for antimicrobial properties.

Principle of the Method

The agar well diffusion method involves placing the test compound into wells in an agar plate inoculated with a standardized microbial culture. The compound diffuses outward, forming a concentration gradient. If it is antimicrobial, a clear zone of inhibited growth appears around the well. The diameter of this zone is measured to assess its effectiveness.

Preparation of Microbial Inoculum: Fresh microbial cultures are prepared and standardized to the 0.5 McFarland turbidity standard, equivalent to approximately 1×10^6 CFU/mL for bacteria.

Inoculation of Agar Plates: To evaluate the antimicrobial activity of the ALE extract, the agar well diffusion method was employed. Sterile nutrient agar (Sigma Aldrich, USA) was used for bacterial cultures, while potato dextrose agar was utilized for fungal strains. Both media were autoclaved, poured into sterile Petri dishes under laminar airflow conditions, and allowed to solidify overnight.

The following day, 500 µL of overnight-grown bacterial cultures were evenly spread onto the surface of solidified nutrient agar using an L-shaped sterile spreader to achieve a uniform lawn. Subsequently, wells with a diameter of approximately 6 mm were carefully created in the agar using a sterile cork borer, ensuring the surrounding medium remained undisturbed (CLSI, 2020).

Each well was loaded with 100 µL of the test extract at three different concentrations (25, 50, and 100 µg/mL) in the first three wells, while a positive control azithromycin (10 µg/mL) for bacterial strains and fluconazole for fungal strains was introduced into the fourth well.

The prepared plates were incubated at 37°C for 24 hours for bacterial strains and at 28°C for 24–48 hours for fungal cultures. After incubation, the antimicrobial effect was

assessed by measuring the diameter of the clear inhibition zones (Zone of Inhibition, ZOI) around the wells in millimeters using a ruler or caliper. A larger zone indicated stronger antimicrobial activity. All experiments were conducted in triplicate to ensure reliability and reproducibility of the results.

The agar well diffusion method offers several advantages, including: ease of performance, simultaneous testing, quantitative comparison, applicability to various microorganisms**.** However, limitations include the fact that diffusion rates of different substances may vary, potentially affecting the zone size independently of antimicrobial potency (Balouiri et al., 2016).

5.3.3 Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentration (MBC)

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that prevents visible microbial growth after incubation. It is used to evaluate the potency of antibiotics or plant extracts and to guide appropriate antimicrobial therapy.

In laboratory practice, MIC is commonly determined using broth dilution methods (either microdilution or macrodilution) following standardized guidelines such as those provided by the Clinical and Laboratory Standards Institute (CLSI). In this method, serial two-fold dilutions of the antimicrobial agent are prepared in a suitable broth medium, inoculated with a standardized microbial suspension, and incubated under optimal conditions. The MIC is recorded as the lowest concentration at which no visible microbial growth (turbidity) is observed compared to the growth control (Wiegand et al., 2008; CLSI, 2020).

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) represents the lowest concentration of an antimicrobial agent that results in microbial death, defined as a 99.9% reduction in the initial inoculum. In other words, it is the lowest concentration capable of killing the microorganism rather than merely inhibiting its growth.

To determine the MBC, samples from the tubes or wells showing no visible growth in the MIC test are sub-cultured onto fresh, drug-free agar plates. After incubation, the MBC is identified as the lowest concentration at which no microbial colonies are observed. The MBC provides complementary information to the MIC, particularly useful in distinguishing between bacteriostatic and bactericidal agents (Pankey & Sabath, 2004).

The Minimum Inhibitory Concentration (MIC) of ALE was determined using the serial dilution method, following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) and as described by Eloff (1998). Different microbial strains were used for this study: *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Escherichia coli*, and *Proteus mirabilis* were cultured in nutrient broth (HiMedia, India), whereas *Candida albicans* were cultured using potato dextrose broth (HiMedia, India).

Initially, a range of ALE concentrations (10, 20, 30, and 40 µg/mL) was tested. However, no significant inhibition of bacterial or fungal growth was observed at these lower concentrations. Therefore, a second phase of testing was conducted using higher ALE concentrations of 100, 150, 200, 250, and 300 µg/mL.

Each test included a negative control without ALE to verify the ability of the microorganisms to grow uninhibited during the incubation period and to confirm the sterility of the culture media. After inoculation, all cultures were incubated at appropriate conditions for 24 hours.

Post-incubation, the cultures were examined visually for turbidity, which indicates microbial growth. The MIC was defined as the lowest concentration of ALE at which no visible turbidity was observed, signifying complete inhibition of microbial proliferation. All experiments were conducted in triplicate to ensure the reproducibility and accuracy of the results.

Two-phase concentration strategy for mic determination: The MIC assessment began with lower ALE concentrations (10–40 µg/mL) to evaluate antimicrobial activity at minimal doses. No growth inhibition was observed within this range, indicating that

the tested microorganisms were not affected at these lower levels or that the active phytochemical content was insufficient to produce an inhibitory effect at these concentrations.

As a result, a second phase of the experiment was designed, employing higher concentrations of ALE (100, 150, 200, 250, and 300 µg/mL). Incrementally increasing the concentration provided a more accurate identification of the minimum dose necessary to inhibit microbial growth. This progressive approach made it possible to pinpoint the threshold concentration where ALE shifted from being ineffective to exhibiting noticeable antimicrobial properties. Using a two-phase concentration approach allowed for accurate MIC determination by preventing underestimation caused by a narrow testing range. It also provided a clearer dose-response profile for ALE against the selected microorganisms. Such broader testing is especially important for plant extracts, where the levels of active compounds can vary. (Eloff, 1998; Clinical and Laboratory Standards Institute [CLSI], 2018).

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC): To determine the MBC/MFC, an aliquot (approximately 10 µL) from each broth tube showing no visible microbial growth at or above the MIC was aseptically inoculated onto fresh nutrient agar (for bacteria) or potato dextrose agar (for fungi) plates. The inoculated plates were incubated under appropriate conditions (37°C for 24 hours for bacteria and 28°C for 24–48 hours for fungi).

After incubation, the plates were examined for the presence or absence of microbial growth. The MBC or MFC was defined as the lowest concentration of ALE at which no visible colony formation occurred on the agar surface, indicating that 99.9% or more of the original microbial population had been killed.

The distinction between MIC and MBC/MFC is crucial:

- MIC represents the lowest concentration that inhibits visible growth (bacteriostatic/fungistatic effect).

- MBC/MFC represents the lowest concentration that results in microbial death (bactericidal/fungicidal effect).

All tests were performed in triplicate to ensure the reproducibility and reliability of the results.

5. 4 *In vitro* Pharmacological Evaluation

5.4.1 Cytotoxicity Assay

Cell line (HaCaT): The HaCaT cell line, an immortalized human keratinocyte model, is widely used for *in vitro* studies of skin biology, wound repair, and the effects of pharmacological and cosmetic agents. Originally isolated from adult human skin, these cells underwent spontaneous immortalization without the introduction of viral oncogenes, allowing them to maintain many features of normal keratinocytes (Boukamp et al., 1988).

HaCaT cells are capable of differentiating and producing important epidermal proteins such as keratin 1 and keratin 10. They display a consistent phenotype and largely normal karyotype, with only minor chromosomal changes, making them a dependable model for dermatological and toxicological research. A key benefit of HaCaT cells is their robust growth and simpler culture requirements compared to primary keratinocytes, which typically have limited proliferation and require more complex media. HaCaT cells are commonly grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS) and antibiotics, under standard conditions of 37°C and 5% CO_2. Because of their strong similarity to normal human keratinocytes in gene expression, differentiation ability, and responsiveness to stimuli, HaCaT cells are frequently used in studies of skin inflammation, oxidative damage, wound healing, aging, and cytotoxicity testing of bioactive compounds. For maintenance, HaCaT cells were subcultured every 2–3 days upon reaching 70–80% confluency. The cells were rinsed with sterile phosphate-buffered saline (PBS), gently detached using trypsin-EDTA (Gibco, USA), and reseeded into new culture flasks containing complete DMEM with 10% FBS and 1% antibiotics. All cultures were kept

in a humidified incubator at 37°C with 5% CO_2. It is important to avoid overexposing cells to trypsin, as excessive trypsinization can compromise cell membrane integrity.

In this study, HaCaT cells were utilized as an *in vitro* model to evaluate the cytoprotective and pharmacological properties of the plant extract under standardized laboratory conditions. The human keratinocyte cell line (HaCaT) was sourced from the National Centre for Cell Science (NCCS), Pune, India, and routinely cultured at the research laboratories of Lovely Professional University, Punjab, India. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA), supplemented with 10% fetal bovine serum (FBS) to provide essential nutrients and growth factors, along with 1% antibiotic solution containing penicillin (100 U/mL) and streptomycin (100 µg/mL) to inhibit bacterial growth. The cultures were kept at 37°C in a humidified incubator with 5% CO_2, replicating the physiological conditions of human cells. Regular monitoring under an inverted microscope was performed to observe cellular morphology and assess confluency. When the cells reached around 70–80% confluency, sub-culturing was carried out to maintain optimal growth and ensure healthy cell populations for experimental use. Ensuring standardized conditions during cell culture is key to obtaining reliable and reproducible outcomes in experimental assays (Freshney, 2016).

5.4.1.1 Cytotoxic activity of ALE on HaCaT cell line

The MTT assay is a widely utilized colorimetric method for assessing cell viability, proliferation, and cytotoxicity. The assay is based on the reduction of the yellow tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), into insoluble purple formazan crystals by mitochondrial dehydrogenase enzymes present in viable cells. The amount of formazan produced correlates directly with the number of metabolically active cells. Following incubation, the formazan crystals are solubilized using dimethyl sulfoxide (DMSO), and the resulting solution's absorbance is measured spectrophotometrically. The intensity of the absorbance provides a quantitative measure of cell viability and metabolic activity.

2.5.2 Methodology

The cytotoxic potential of Artemisia leaf extract (ALE) against the HaCaT human keratinocyte cell line was evaluated according to the standard protocol provided by the American Type Culture Collection (ATCC) (Cory et al. 1991; Shirwaikar et al. 2003; Wilkening et al. 2003).

HaCaT cells (5,000–8,000 cells per well) were seeded into 96-well plates and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. The cells were incubated under standard culture conditions (37°C, 5% CO_2) for 24 hours to facilitate attachment.

Following incubation, the medium was aspirated and replaced with fresh DMEM. Cells were then treated with ALE at concentrations of 31.25, 62.5, 125, 250, 500, and 750 µg/mL, with 5 µL of each concentration added to the appropriate wells. Treated plates were incubated for an additional 24 hours under identical conditions.

After treatment, the medium was removed, and 50 µL of MTT reagent (5 mg/mL) was added to each well. Plates were incubated for 4 hours at 37°C to allow viable cells to convert MTT into formazan crystals. Subsequently, 100 µL of DMSO was added to dissolve the formazan crystals, and the absorbance was measured at 550 nm with a reference wavelength of 660 nm using an iMark™ Microplate Reader (Bio-Rad, USA).

Untreated HaCaT cells were used as the negative control, and their absorbance values were considered to represent 100% viability. The percentage of cell viability for each ALE concentration was calculated using the following formula:

$$\%\text{age of viability} = \frac{\text{Mean absorbance of test sample} \times 100}{\text{Mean absorbance of control}}$$

The experiments were conducted in triplicate to ensure reproducibility. The percentage of viable cells was plotted against the different ALE concentrations to generate a dose-response curve. A decrease in absorbance relative to the control indicated cytotoxic effects of ALE on HaCaT cells.

5.4.2 Antioxidant activity

5.4.2.1 DPPH free radical-scavenging activity

Principle of the DPPH assay: The DPPH assay is based on the ability of antioxidants to donate electrons or hydrogen atoms to neutralize free radicals. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that has a deep violet color in solution due to its unpaired electron. When an antioxidant donates an electron or hydrogen to DPPH, it becomes a stable, non-radical molecule, and the solution's color fades from violet to yellow. This change in colour is directly related to the scavenging ability of the antioxidant compound. The decrease in absorbance at 517 nm, measured using a spectrophotometer, indicates the extent of the DPPH radical reduction. A greater decrease in absorbance means higher antioxidant activity of the tested sample. Thus, the DPPH assay provides a simple and effective method to estimate the free radical scavenging capacity of different substances (Brand Williams, 1995).

The DPPH assay is commonly employed to evaluate the antioxidant potential of samples by assessing their ability to neutralize free radicals. It operates on an electron transfer mechanism and was performed here with slight modifications based on earlier reported methods (Nithianantham et al. 2011; Zakaria et al. 2008). A stock solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared by dissolving the powder in methanol (or ethanol), resulting in a deep violet-colored solution, typically adjusted to a concentration of 0.2 mM. Different concentrations of the test sample (ALE) and the reference antioxidant (ascorbic acid) were prepared by dissolving them in ethanol.

For the assay, 10 µL of each sample concentration (ranging from 0 to 500 µg/mL) was added to 200 µL of 0.2 mM DPPH solution in the wells of a 96-well microplate.

The reaction mixtures were incubated at room temperature in the dark for 30 minutes to allow interaction between antioxidants and DPPH radicals, with light protection being essential to prevent DPPH degradation.

Following incubation, the reduction in absorbance was measured at 495 nm using a microplate reader (iMark, Bio-Rad, USA). Although DPPH absorbance is conventionally measured at 517 nm, readings at 495 nm were taken due to specific instrument optimization.

A control was prepared by replacing the sample with 20 µL of deionized water. The percentage of free radical scavenging activity was calculated using the formula. All tests were conducted in triplicate to ensure accuracy and reproducibility. Ascorbic acid (vitamin C) served as the positive control, absolute ethanol was used to blank the spectrophotometer, and a DPPH-only solution (without any sample) was used as the blank.

In this assay, absorbance was measured at 495 nm instead of the conventional 517 nm, as preliminary optimization showed better sensitivity and stability of DPPH absorbance at this wavelength under the experimental conditions."

Calculations:

DPPH scavenging activity (%) = $(Abs_{Control} - Abs_{Sample})/Abs_{Control}) \times 100$

where $Abs_{Control}$ = absorbance of the control, Abs_{Sample} = absorbance of the sample.

The IC_{50} value, which represents the concentration of the sample required to neutralize 50% of the DPPH radicals, was determined by plotting the percentage of radical scavenging activity against different sample concentrations. The IC_{50} was obtained from the resulting dose-response curve, where a lower IC_{50} value reflects stronger antioxidant potential. Lower the IC50 value greater the antioxidant activity. %age of inhibition was plotted against concentration and line obtained was used to get the IC50 value.

5.4.2.2 ABTS radical scavenging assay

The ABTS assay is a widely used method to assess the antioxidant capacity of various substances. The assay relies on the generation of the ABTS radical cation (ABTS•+), which exhibits a distinct blue-green color. This method allows the quantification of antioxidant activity by measuring the reduction in the intensity of the ABTS•+ radical cation.

Principle of the ABTS Assay: The ABTS assay works on the principle that antioxidants have the ability to neutralize the ABTS•+ radical cation, leading to a decrease in the blue-green color formed by the radical. The ABTS•+ radical is generated by oxidizing

the ABTS molecule with potassium persulfate ($K_2S_2O_8$) in the dark, at room temperature, or under light conditions. The ABTS•+ radical is stable and its concentration can be measured spectrophotometrically at 745 nm. The intensity of the color is directly proportional to the concentration of ABTS•+ radicals present, and the reduction in color intensity indicates the presence and activity of antioxidants in the sample (Scalzo et al. 2005).

First, dissolve 0.192 g of ABTS in 50 mL of methanol to prepare the ABTS stock solution. To generate the ABTS•+ radical, mix 2 mL of the ABTS stock solution with 2 mL of potassium persulfate (typically 2.45 mM in distilled water). Allow the mixture to incubate in the dark at 4°C for 12–18 hours. This incubation ensures the stable generation of ABTS•+ radicals. Prepare different concentrations of the sample (ALE) ranging from 0–500 µg/mL, using the appropriate solvent. These concentrations are selected to study the dose-dependent antioxidant activity of the ALE. **A**dd 2 mL of the ABTS•+ solution to each test sample containing the prepared concentrations of ALE. Incubate the samples at room temperature for a sufficient period to allow interaction between the ABTS•+ radicals and the antioxidants in the ALE. After incubation, measure the absorbance of the reaction mixture at 745 nm using a microplate reader (such as the i Mark from Bio-Rad, USA). The absorbance value gives an indication of the remaining concentration of ABTS•+ radicals in the solution. The reduction in the ABTS•+ radical concentration, reflected as a decrease in absorbance, indicates the antioxidant activity of the ALE. The percentage inhibition of the ABTS•+ radical by the test sample is calculated using the following formula:

$$\text{Scavenging activity (\%)} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / (\text{Abs}_{\text{Control}}) \times 100$$

where, $\text{Abs}_{\text{Control}}$=absorbance of the control and $\text{Abs}_{\text{Sample}}$ =absorbance of the sample.

A higher percentage of inhibition corresponds to stronger antioxidant activity in the sample. This method is effective for evaluating the antioxidant potential of various natural extracts and compounds and is often used in phytochemical studies to determine the bioactivity of plant-derived substances

5.4.2.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is a widely accepted method used to estimate the total antioxidant potential of a sample based on its ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. The underlying principle of this assay involves the electron-donating capacity of antioxidants, which reduces the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to its ferrous (Fe^{2+}) form, producing an intense blue color that can be quantitatively measured.

Principle: In the FRAP assay, antioxidants in the sample act as reducing agents by donating electrons to the ferric complex. The ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex, when reduced, forms a ferrous tripyridyltriazine (Fe^{2+}-TPTZ) complex, resulting in a blue-colored solution. The intensity of this blue color is directly proportional to the reducing power of the sample. The absorbance is measured spectrophotometrically at 593 nm, and an increase in absorbance indicates stronger reducing (antioxidant) activity (Benzie and Strain, 1996).

Materials and preparation of frap reagent: The FRAP reagent is freshly prepared by mixing the following components in a specific ratio:

- Acetate buffer (300 mM, pH 3.6) – 25 mL
- TPTZ (2,4,6-tripyridyl-s-triazine) solution (10 mM in 40 mM HCl) – 2.5 mL
- Fe $Cl_3 \cdot 6H_2O$ solution (20 mM in distilled water) – 2.5 mL

The freshly prepared reagent is pre-warmed at 37°C if required before use to enhance reaction kinetics. Different concentrations of the sample (ALE) are prepared, typically ranging from 0–500 µg/mL. On the day of the assay, 2.4 mL of the freshly prepared FRAP reagent is added to 100 µL of each concentration of ALE. The mixture is incubated in the dark at room temperature for 30 minutes to ensure complete reaction between the antioxidant components and the ferric-TPTZ complex. After incubation, the absorbance is recorded at 593 nm using a microplate reader (iMark, Bio-Rad, USA). An increase in absorbance correlates with an increased reducing ability of the sample. Ascorbic acid (Merck, India) is used as the positive control, providing a standard for antioxidant capacity comparison. Distilled water serves as the blank to zero the instrument and account for baseline absorbance. The higher the absorbance at 593 nm, the greater the antioxidant (reducing) capacity of the sample. In addition, the RP_{50} value

(the concentration required to achieve 50% of maximum reducing power) can be calculated. A lower RP_{50} value indicates stronger antioxidant potential. The FRAP assay provides a simple, rapid, and reproducible method to evaluate the total antioxidant power of plant extracts and other bioactive substances. It is particularly useful for screening natural products for their therapeutic antioxidant properties (Benzie and Strain, 1996; Prior et al., 2005).

% Reducing power (RP) = $(Abs_{Control} - Abs_{Sample}) / (Abs_{Control}) \times 100$

5.4.3 Enzyme Inhibition Assay – Cyclooxygenase-2 (COX-2)

The Cyclooxygenase-2 (COX-2) inhibition assay is an essential method used to evaluate the anti-inflammatory potential of natural extracts or synthetic compounds. COX-2 is an inducible enzyme that plays a critical role in the biosynthesis of prostaglandins from arachidonic acid, which are mediators of inflammation, pain, and fever. Inhibiting COX-2 activity is a major therapeutic strategy for controlling inflammatory responses (Gierse et al., 1998; Smith et al. 2000).

Principle**:** This assay measures the ability of test substances to inhibit the enzymatic activity of COX-2. The principle is based on monitoring the oxidation of a chromogenic substrate, N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD), during the COX-catalyzed reaction. TMPD serves as a reducing co-substrate, which upon oxidation, produces a color change detectable at 595 nm. A decrease in absorbance in the presence of the sample indicates COX-2 inhibition.

Materials: *A. vestita* leaf extract (ALE) (0–500 µg/mL)**,** COX-2 enzyme**,** Tris-HCl buffer (pH 8.0), TMPD (N,N,N',N'-tetramethyl-p-phenylenediamine) solution, Arachidonic acid (substrate), Celecoxib (17 µM) as positive control, 96-well microplate, microplate reader (iMark, Bio-Rad, USA)

ALE was serially diluted to prepare different concentrations ranging from 0 to 500 µg/mL using Tris-HCl buffer (pH 8.0). Into each well of a 96-well plate, the following were added: Appropriate volume of ALE at different concentrations. Tris-HCl buffer to maintain the reaction environment. COX-2 enzyme solution. The enzymatic reaction was initiated by sequentially adding: 5 µL of the arachidonic acid substrate and 5 µL of

the TMPD solution. After addition of the substrates, the plate was incubated at room temperature for 10 minutes in the dark to allow the reaction to proceed. The absorbance was measured at 595 nm using a microplate reader. The color intensity corresponds to the enzymatic activity. A reduction in absorbance compared to the control (without extract) indicates inhibition of COX-2 activity by the ALE.

Positive Control: Celecoxib (17 μM), a selective COX-2 inhibitor, was used as a standard reference to validate assay performance. Negative Control: Reaction mixture without ALE (only buffer and enzyme) to represent 100% COX-2 activity.

Calculation of Inhibition Percentage: The inhibition of COX-2 activity was calculated using the formula: Percentage Inhibition= $(Abs_{Control} - Abs_{Sample})/Abs_{Control}) \times 100$

where $Abs_{Control}$ = absorbance of the control, Abs_{Sample} = absorbance of the sample.

This assay is critical in screening potential anti-inflammatory compounds from natural sources. The ability of ALE to inhibit COX-2 activity suggests its potential therapeutic application in managing inflammatory disorders.

5.4.4 *In vitro* scratch assay

The *in vitro* scratch assay, also known as the wound healing assay, is a simple and widely used technique to study cell migration and proliferation.

Principle of *in vitro* scratch (wound healing) assay: The scratch assay is a straightforward and cost-effective method for studying the migration ability of cells, which is crucial during processes like wound healing, tissue regeneration, and cancer metastasis (Rodriguez et al., 2005). The principle is based on creating a "wound" a mechanical gap in a confluent cell monolayer. Over time, cells migrate into the wound area to close the gap. By observing and quantifying the reduction in the wound area at specific time intervals, researchers can assess the effect of treatments on cell migration and proliferation (Liang et al., 2007; Cory, 2011).

In this study, the wound healing capacity of ALE) was evaluated using the scratch assay on HaCaT cells (human keratinocytes) as previously described method (Kumar et al. 2007; James and Friday, 2010). Cells were seeded at a density of 10,000 cells per well

into a 6-well plate containing Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% foetal bovine serum (FBS) and 1% antibiotic solution (penicillin and streptomycin). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 hours to allow the formation of a confluent monolayer. After 24 hours, the medium was carefully removed, and the cells were washed with phosphate-buffered saline (PBS) to eliminate non-adherent or dead cells. A uniform scratch was then made through the center of each well using a sterile 200 µL pipette tip. The detached cells were gently washed away with PBS.

Following the scratch, cells were treated with specific concentrations of ALE. A positive control was included, using Cipladine, a known wound healing agent (Shedoeva et al. 2019; Minda et al. 2022). Meanwhile, cells with no treatment were used as the negative control to establish baseline migration rates. Microscopic images of the wound area were taken immediately after scratching (0 hours) and after 24 hours of incubation using a phase-contrast microscope. The degree of wound closure was assessed by measuring the area of the gap at both time points. The analysis was performed using ImageJ software (National Center for Biotechnology Information, NCBI), which provides a quantitative evaluation of the cell migration by calculating the wound area reduction (Schindelin et al., 2012; Grada et al., 2017).

5.5 Preparation of Tragacanth gum-based hydrogel

Hydration of Tragacanth gum: A specific amount of Tragacanth gum powder was accurately weighed and added to distilled water. The mixture was allowed to rest for 30 minutes to enable the gum to swell, which aids in its hydration and the initial dispersion of the gum particles.

Homogenization: After the swelling period, the mixture was continuously stirred using a magnetic stirrer to ensure a uniform distribution of the gum particles. Stirring continued until a smooth, homogeneous, and viscous dispersion was achieved, free of lumps.

Preparation of preservative solution: In a separate beaker, propylene glycol was used as a solvent to dissolve the appropriate amounts of methyl paraben and propyl paraben.

The solution was stirred thoroughly at room temperature until the preservatives were completely dissolved, ensuring their even distribution throughout the final hydrogel.

Incorporation of preservatives and ALE: The preservative solution was added slowly to the Tragacanth gum dispersion while stirring gently to maintain uniformity. Following this, the required quantity of plant extract was introduced, and stirring continued to ensure thorough mixing and incorporation of all components.

Volume adjustment and pH modification: The total volume of the formulation was adjusted to 100 mL by adding more distilled water. The pH of the hydrogel was then measured using a calibrated pH meter. Triethanolamine (TEA) was added dropwise under stirring to bring the pH to the optimal range of 6.8–7.0, ensuring both the desired gel consistency and compatibility with skin or biological applications (Abdi et al. 2024; Ghayempour et al. 2015; Ghayempour et al. 2016).

Crosslinking (for enhanced stability): Mechanical strength and stability can be enhanced by incorporating an ionic crosslinking step. The hydrogel is immersed in a 0.1 M $CaCl_2$ solution for approximately 10–30 minutes to promote ionic bonding between polysaccharide chains. Crosslinking duration is controlled to increase structural integrity without causing excessive rigidity, ensuring the material remains biocompatible.

Final homogenization and storage: Once the formulation was adjusted and crosslinked (if applicable), the hydrogel was gently stirred to ensure uniformity. The final hydrogel formulation was transferred into airtight, sterile containers and stored at −20°C for long-term preservation, maintaining its physicochemical and biological properties until further use.

Crosslinking of Tragacanth Gum Hydrogel Using Calcium Chloride

For wound healing applications, Calcium chloride is preferred over glutaraldehyde as a crosslinker for wound-healing hydrogels because it is biocompatible, non-toxic, and supports natural tissue repair processes. It enables rapid gelation in materials like alginate, pectin, and tragacanth while preserving the activity of incorporated therapeutic agents and maintaining a moist healing environment. In contrast, glutaraldehyde is

cytotoxic and requires stringent detoxification, making it unsuitable for direct wound-care applications and complicating regulatory approval.

Materials required: Prepared Tragacanth gum hydrogel mixture, calcium chloride ($CaCl_2$), distilled water, magnetic stirrer, beakers, dropper or syringe, sterile containers

Procedure: To prepare the crosslinked Tragacanth gum hydrogel, a 0.1 M calcium chloride ($CaCl_2$) solution was first prepared by dissolving 11.1 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in one litre of distilled water (or 1.11 g in 100 mL for smaller volumes). The pre-formed Tragacanth gum hydrogel mixture was shaped as needed, such as by pouring into molds or Petri dishes. The shaped hydrogel was then carefully immersed in the 0.1 M $CaCl_2$ solution and allowed to crosslink at room temperature for 15–30 minutes. Gentle stirring or slow orbital agitation was employed to promote uniform crosslinking throughout the hydrogel. After the crosslinking period, the hydrogels were removed from the calcium chloride solution and rinsed thoroughly with sterile distilled water two to three times to eliminate any unbound calcium ions. The final crosslinked hydrogels were stored in sterile, airtight containers at 4°C for short-term use or at −20°C for extended preservation. It is important to note that the crosslinking time significantly influences the mechanical properties of the hydrogel; longer immersion typically results in a stiffer structure. Similarly, maintaining the $CaCl_2$ concentration within 0.05–0.1 M is crucial to achieve effective crosslinking without causing brittleness. This mild ionic crosslinking approach offers the key advantage of preserving the bioactivity of the incorporated extract while ensuring excellent compatibility for wound healing applications (Boateng et al. 2008; Firoozbahr et al. 2023).

Mechanism of $CaCl_2$ as crosslinker: Calcium ions (Ca^{2+}) bind to the carboxylate groups ($–COO^-$) present in the polysaccharide chains of Tragacanth gum, resulting in the formation of ionic crosslinks. This process leads to the development of a physically crosslinked hydrogel network. The ionic bonding not only enhances the structural stability of the hydrogel but also preserves its softness, flexibility, and biocompatibility—characteristics that are essential for effective wound healing applications.

5.5.1 Preformulation Studies

A pre-formulation evaluation was conducted to ensure the safety of the hydrogel composition. Such studies are essential for the development of formulations that are safe, effective, and stable (Rowe et al. 2009). They offer critical information regarding the stability, compatibility, physicochemical characteristics, and overall feasibility of the formulation, forming the groundwork for successful product development. The primary objective of this research was to assess the compatibility of the drug with selected excipients and to identify any newly emerging physical and chemical properties of the drug.

5.5.1.1 Drug excipients compatibility study

To ensure the long-term stability and effectiveness of the Tragacanth gum-based hydrogel, a drug-excipient compatibility study was performed. The objective of this study was to assess any potential physical interactions between the plant extract (active ingredient) and the excipients used in the formulation, including Tragacanth gum, propylene glycol, methyl paraben, and propyl paraben. Detecting and addressing any incompatibilities is crucial to achieving a minimum shelf life of one year for the final product.

The study involved evaluating the physical stability between the plant extract and each excipient individually. The extract was precisely weighed and thoroughly mixed with each excipient in separate, labelled vials. The drug-to-excipient ratios were matched to those in the final hydrogel formulation to replicate real-world conditions. Each mixture was manually homogenized to enhance molecular interaction and ensure uniform distribution.

By blending the plant extract with each excipient at formulation-equivalent concentrations, the study closely simulated the composition of the actual hydrogel product. Thorough mixing was employed to maximize molecular contact and increase the chances of detecting any potential incompatibilities. The resulting mixtures were then stored in clean, properly labelled vials and subjected to two distinct storage conditions:

1. **Accelerated stability testing**: The samples were stored at 40°C and 75% relative humidity for 15 days to simulate the stresses of long-term storage and to accelerate any potential degradation or interaction processes. The prepared drug-excipient mixtures were transferred into clean, dry, and labelled vials, then placed in a stability chamber maintained at these conditions. This storage protocol was selected based on the International Council for Harmonisation (ICH) guidelines for accelerated stability testing, allowing the prediction of long-term stability within a condensed timeframe.
2. **Room temperature observation**: Additional sets of drug-excipient mixtures were stored at ambient room temperature (25°C) for one month to replicate typical storage conditions.

Parameters Monitored:

1. **Visual inspection**: The samples were regularly observed for any changes in colour, appearance, phase separation, precipitation, or odor, which are indicative of physical instability or chemical interaction.
2. **Texture and consistency**: Any alteration in the texture or consistency of the mixtures was documented, as it could signal physical incompatibility or degradation.
3. **Preliminary chemical stability**: Although the primary focus was on physical compatibility, any signs of discoloration or precipitation were treated as potential indicators of chemical instability, warranting further investigation.

5.5.1.2 Physiochemical Characterization

1. **Evaluation of physical appearance:** The physical appearance of a formulated gel is a critical parameter influencing both its quality and user acceptance. Assessing characteristics such as colour, appearance, and transparency allows for the early detection of any formulation inconsistencies or physical instabilities that could impact the product's efficacy or aesthetic appeal.

   In the present study, the physical characteristics of the hydrogel formulated with *Artemisia vestita* leaf extract (ALE) incorporated into tragacanth gum were systematically evaluated. To ensure consistency, all samples were prepared in

identical containers. Colour evaluation was performed by trained observers, conducting triplicate assessments using standardized colour charts to enhance accuracy. The evaluations took place under controlled lighting conditions to eliminate environmental variability. For the assessment of appearance, the observers examined the samples for the presence of any visible particulates, uniformity, and surface texture, documenting their observations independently across three separate trials. To quantify clarity, a transparent grid was positioned behind each sample, and clarity was scored based on a numerical scale ranging from 1 to 5, with clearly defined criteria for each score (Table 2).

Additionally, the clarity of the hydrogel was objectively measured using a spectrophotometer at a wavelength of 600 nm. Turbidity levels or transmittance values were recorded, with higher transmittance (or lower turbidity) indicating greater clarity. This multi-parameter approach ensured a comprehensive evaluation of the hydrogel's visual and physical quality attributes.

Table 2: Scoring criteria for hydrogel samples.

Score	Description
1.	Completely opaque; no visibility of the grid behind the sample
2.	Very turbid; grid is barely visible through the sample
3.	Moderately turbid; grid is visible but blurred.
4.	Slightly turbid; grid is clearly visible with minimal distortion.
5.	Completely transparent; grid is sharply and clearly visible

2. **pH Measurement:** The pH of the *Artemisia vestita* leaf extract hydrogel (ALEH) was evaluated to ensure both the stability of the formulation and its compatibility with the skin's natural pH range. Measurements were carried out in triplicate using a calibrated Seven Excellence S400 pH meter (Mettler Toledo, India) to maintain accuracy and reproducibility.

For the pH assessment, 1 gram of the hydrogel was dispersed in 100 mL of distilled water. The dispersion was left to stand for 2 hours at room temperature to allow for complete equilibration between the hydrogel matrix and the aqueous medium, ensuring a stable and representative pH reading. Following this equilibration period, the pH of the solution was measured directly. All pH values were reported as mean ± standard error of the mean (SEM).

3. **Spreadability assessment**: The spreadability of the Artemisia vestita leaf extract hydrogel (ALEH) was evaluated to assess its ease of application and uniformity during topical use. A volume of 1 mL of the prepared hydrogel was carefully placed onto a clean glass plate using a sterile syringe. A calibrated glass plate was then gently positioned over the hydrogel. Weights of increasing mass (25, 50, 100, 200, 300, 400, and 500 g) were sequentially applied onto the top plate.

After placing each weight, a stabilization time of 20 seconds was allowed to enable uniform spreading of the hydrogel under the applied load. The radius of the spread hydrogel was then measured carefully. Each measurement was performed in triplicate at ambient room temperature to ensure the reliability and reproducibility of the results. The area covered by the hydrogel was calculated using the following formula: $P = \pi r^2$

where, P- the surface area covered by the ALEH (cm^2)

r- radius of the ALEH (cm)

Spreadability is a critical parameter for topical hydrogel formulations as it directly influences patient compliance, ease of application, and the evenness of drug delivery across the skin surface. A hydrogel with optimal spreadability ensures effective coverage with minimal effort, enhances aesthetic acceptability, and promotes better therapeutic outcomes.

4. **Rheological characterization**: Rheology examines how materials flow and deform when subjected to external stress or strain, making it essential for evaluating the physical characteristics of hydrogel systems. In this study, the Artemisia vestita leaf extract hydrogel (ALEH) was analyzed rheologically to determine its viscosity profile, flow properties, and viscoelastic behavior, all of which are critical for ensuring formulation stability, ease of application, and effective drug release.

Viscosity assessments were carried out using a Brookfield digital viscometer, maintained at a constant temperature of 24.8°C through a thermostated water jacket. The viscosity was measured at a rotation speed of 20 rpm after allowing the hydrogel sample to equilibrate for 5–6 minutes to eliminate air entrapment and achieve steady flow. To investigate shear-thinning characteristics — an advantageous property for topical applications — the shear rate was progressively increased, and the corresponding viscosity changes were recorded. Typically, hydrogels demonstrate non-Newtonian, pseudoplastic flow behavior, where viscosity declines with an increase in shear rate. This behavior promotes easy spreadability during application while retaining sufficient viscosity at rest to prevent dripping or flowing off the application site. A rheogram was plotted to visualize the relationship between shear rate and viscosity, offering insights into the flow dynamics of the formulation.

Furthermore, the hydrogel's mechanical strength and internal structure were explored by evaluating viscoelastic parameters such as the storage modulus (G') and loss modulus (G") through oscillatory rheological analysis. These measurements provide additional understanding of the formulation's elastic and viscous properties, contributing to the prediction of its long-term physical stability. Viscoelastic Properties: Storage Modulus (G') and Loss Modulus (G")

In addition to flow behavior, the viscoelastic properties of hydrogels offer valuable insight into their mechanical stability and structural organization. These properties are typically characterized by measuring the storage modulus (G') and loss modulus (G") using oscillatory rheological techniques. The storage modulus (G') reflects the elastic or solid-like behavior of the hydrogel, indicating the amount of energy stored during deformation and recovered when the stress is removed. A higher G' value suggests a more rigid and structured network within the hydrogel matrix. The loss modulus (G"), on the other hand, represents the viscous or liquid-like behavior, corresponding to the energy dissipated as heat during deformation. A higher G" value implies greater flowability and less structural integrity. The ratio between G' and G" provides an indication of the material's overall behavior; a hydrogel where G' exceeds G" behaves predominantly as an elastic, solid-like material, which is desirable for maintaining form and stability during application. Thus, analyzing G' and G" can further validate the

formulation's mechanical performance, offering predictive insights into its stability, spreadability, and user experience.

5. **Viscosity measurement:** The viscosity of the Artemisia vestita leaf extract hydrogel (ALEH) was evaluated using a Brookfield digital viscometer to assess its influence on spreadability, handling properties, and drug release behavior. The hydrogel sample was carefully loaded into the viscometer's flow jacket, and an appropriate spindle adapter was utilized for the measurements.

Viscosity readings were recorded at a rotational speed of 20 rpm. Throughout the measurement process, the temperature was maintained at 24.8°C by circulating water through a thermostatically controlled jacket to ensure consistency. Prior to recording the viscosity, the sample was allowed to settle undisturbed for 5–6 minutes to eliminate air bubbles and achieve a uniform flow behavior.

Viscosity was evaluated by applying different shear rates to assess the hydrogel's rheological behavior. Viscosity is critical for determining spreadability, application comfort, and structural stability. It also influences drug release, as higher viscosity can slow diffusion and support sustained delivery. Maintaining an appropriate viscosity range is therefore essential for stable formulation performance and effective therapeutic action.

6. **Extrudability assessment**: Approximately 10 g of the prepared hydrogel was carefully filled into eight collapsible aluminium tubes, which were then sealed at the ends by crimping. To evaluate the extrudability of the formulation, the initial weight of each filled tube was recorded. The tubes were then placed securely between two glass slides and clamped firmly to maintain stability. A 500 g weight was applied over the top slide to exert uniform pressure. After removing the tube cap, the hydrogel was allowed to extrude freely under the applied force. The extruded gel was collected and weighed to determine the amount released. The percentage of gel extruded was calculated using the following formula:

$$\text{Extrudability (\%)} = \frac{\text{Weight of gel extruded} \times 100}{\text{Total weight of gel loaded}}$$

Classification of Extrudability

1. Excellent Extrudability

Percentage Extruded: 90% and above. The hydrogel extrudes smoothly with minimal effort, demonstrating an ideal balance between viscosity and flow. It requires very little force for extrusion, ensuring ease of use and optimal application.

2. Good Extrudability

Percentage Extruded: 70% - 89%. The hydrogel extrudes with moderate effort, indicating good flow properties. Although slightly more force is required compared to the "Excellent" category, it remains easy to apply and performs well.

3. Fair Extrudability

Percentage Extruded: 50% - 69%. The gel extrudes with some difficulty, requiring noticeable force. While it still flows, it may not be as convenient for application, potentially causing discomfort during use.

4. Poor Extrudability

Percentage Extruded: Below 50%. The hydrogel shows poor extrudability, likely due to high viscosity or inadequate flow properties. Substantial force is needed for extrusion, making it difficult to apply effectively.

5.5.2 *In vitro* drug release study

The *in vitro* drug release study was designed to simulate how the drug would be released from the hydrogel formulation (ALEH) under different physiological conditions (temperature and pH). A Franz diffusion cell system was employed, which allows precise monitoring of drug diffusion across a semi-permeable membrane into a receptor solution over time. This method helps evaluate the release rate and cumulative release profile of the active compound.

Materials and setup: Franz diffusion cell apparatus (customized), dialysis membrane (molecular weight cutoff: 5000 da), ALEH (drug-loaded hydrogel), phosphate buffer

solutions at two pH levels: 4.5 and 6.8, incubators (for temperature control: 25°c, 37°c, and 40°c), UV-visible spectrophotometer (measuring absorbance at 276 nm).

Preparation of the diffusion system: The dialysis membrane was mounted securely between the donor and receptor compartments of the Franz diffusion cells. 1 gram of the ALEH formulation was placed in the donor compartment. The receptor compartment was filled with phosphate buffer solution at the selected pH (either 4.5 or 6.8). Gentle agitation at 50 rpm was maintained to ensure uniform mixing and avoid concentration gradients.

Temperature Variations: Experiments were conducted at three different temperatures:

- 25°C (ambient room temperature)
- 37°C (normal body temperature)
- 40°C (to simulate fever or elevated conditions)

Separate setups were maintained for each temperature condition.

At specified time intervals (0, 1, 2, 4, 6, 8, 10, and 12 hours), 1 mL samples were withdrawn from the receptor compartment via a lateral sampling tube. After each sampling, an equal volume of fresh buffer was added to the receptor compartment to maintain a constant volume and sustain sink conditions (where the concentration of drug remains far from saturation). Filtering was performed if necessary to remove any hydrogel particles or debris. The collected samples were analyzed using a UV-Visible spectrophotometer by recording absorbance at 276 nm, the λmax (wavelength of maximum absorbance) for the drug. A standard calibration curve of the drug was used to quantify the amount of drug released in each sample (Varshosaz and Dehghan, 2002; Franz, 1975).

The cumulative amount of drug released at each time point was calculated using the formula:

$$\text{Cumulative Release (\%)} = \frac{\text{Amount of drug released at time t}}{\text{Total drug loaded}} \times 100$$

Graphs were plotted showing cumulative drug release (%) versus time for each combination of pH and temperature, allowing comparison of release profiles.

The drug release behaviour under different conditions helps understand how pH and temperature influence the release kinetics of ALEH. A faster release at higher temperatures or acidic/basic pH could indicate sensitivity to environmental conditions, which is critical for predicting in vivo behaviour.

5.5.3 Kinetic Modeling and Drug Release Mechanism of ALEH Formulations

To understand the mechanism and the drug release behaviour from the ALE hydrogel formulations (F1–F8), *in vitro* drug release data were subjected to kinetic modelling. This evaluation was carried out using several well-established mathematical models, namely the Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models. These models were employed to determine the mechanism and rate of drug release under varying physiological conditions, including different pH values and temperatures (Aslani et al. 2010; Dash et al. 2010). All release experiments were performed in triplicate, and the formulation exhibiting the most desirable release characteristics was selected based on comparative kinetic fitting and statistical parameters (e.g., R^2 value).

Zero-order Model

The zero-order kinetic model describes a release process where the drug is released at a constant rate, independent of its concentration in the dosage form. The model is mathematically expressed as:

$$Q_t = K_0 . t$$

Where:

- Qt is the amount of drug released at time t,
- K_0 is the zero-order release rate constant (mg/h).

A plot of cumulative drug release versus time yields a straight line if the release follows zero-order kinetics. This model is ideal for sustained release systems where a consistent drug concentration is desired over time such as transdermal patches or matrix tablets.

First-Order model

The first-order model assumes that the drug release rate is proportional to the concentration of drug remaining in the formulation. The model is expressed as:

$\ln Q_t = \ln Q_0 - K_1 t$

or $Q_t = Q_0 * e^{(-K_1 t)}$

Where:

- Q_0 is the initial amount of drug,
- Q_t is the amount remaining at time t,
- K_1 is the first-order rate constant.

In this case, a plot of logarithm of the remaining drug versus time produces a straight line. This model is commonly observed in diffusion- and dissolution-controlled drug delivery systems (Costa & Lobo, 2001).

Higuchi Model

The Higuchi model is based on Fickian diffusion, describing drug release as a process driven by a concentration gradient. The equation is as follows:

$Q_t = K_H * t^{1/2}$ or as $Q_t = K_H * \text{sqrt}(t)$

Where:

- Q_t is the cumulative amount of drug released at time t,
- K_H is the Higuchi constant.

A linear plot of cumulative drug release versus the square root of time suggests that the release mechanism is primarily diffusion-controlled (Higuchi, 1963). This model is especially relevant for matrix-based systems, such as hydrogels.

Korsmeyer–Peppas Model

To further investigate the mechanism of release, especially in polymeric systems like hydrogels, the Korsmeyer–Peppas model was applied. This semi-empirical model is particularly useful when the release mechanism is not purely Fickian. The equation is expressed as:

$M_t/M \infty = K * t^n$

Where:

- Mt/M∞ is the fraction of drug released at time ttt,
- K is the release rate constant,
- n is the release exponent, indicative of the release mechanism.

By taking the logarithm of both sides, the equation becomes:

$\log (M_t/M \infty) = \log K + n \log t$

A plot of log (cumulative % drug release) versus log (time) yields a straight line, with the slope equal to n.

The value of the release exponent (n) helps determine the drug release mechanism:

- n=0.45: Fickian diffusion (Case I)
- 0.45<n<0.89: Anomalous transport (non-Fickian; a mix of diffusion and polymer relaxation)
- n=0.89: Case II transport (relaxation-controlled, zero-order)
- n>0.89: Super Case II transport (swelling or erosion-controlled systems)

This model is particularly important for systems where polymer swelling, degradation, or relaxation significantly influences the release profile (Siepmann & Peppas, 2001; Costa and Lobo, 2001).

5.5.4 Antimicrobial activity of ALEH

The antimicrobial activity was evaluated using the agar well diffusion method following Bauer et al. (1966). Sterilized nutrient agar (for bacteria) and potato dextrose

agar (for fungi) were poured into petri dishes and allowed to solidify. Bacterial suspensions (500 µL) were spread evenly, and 6 mm wells were made using a sterile cork borer. Different concentrations of ALEH (25, 50, 100 µg/mL) were added to the wells, alongside positive controls (azithromycin for bacteria, fluconazole for fungi at 10 µg/mL). Plates were incubated at 37°C (bacteria) and 28°C (fungi) for 24 hours. Zones of inhibition (ZOI) were measured in millimeters, and antimicrobial activity was compared to that of the controls. All experiments were performed in triplicate. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the broth dilution method, based on CLSI guidelines (Eloff, 1998; CLSI, 2014). ALEH concentrations ranging from 10 to 300 µg/mL were tested in nutrient broth (for bacteria) and potato dextrose broth (for fungi). After 24-hour incubation, turbidity was visually inspected: the MIC was defined as the lowest concentration without visible growth, and the MBC as the lowest concentration killing 99.9% of the inoculum. Negative controls without ALEH confirmed growth and media sterility. All tests were conducted in triplicate.

5.5.5 *In vitro* Wound Healing (Scratch) Assay for ALE Hydrogel

The wound healing activity of *Artemisia vestita* leaf extract hydrogel (ALEH) was assessed using the scratch assay method, following the protocol described by (Liang et al. 2007), on HaCaT cells. Briefly, HaCaT cells were seeded at a density of 10,000 cells per well in a 6-well plate and incubated for 24 hours in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO_2 humidified atmosphere.

After achieving 70–80% confluency, the cells were gently washed with phosphate-buffered saline (PBS) and a uniform scratch was made across the cell monolayer using a sterile 200 µL pipette tip. Care was taken to avoid disturbing the adjacent cells. Following the scratch, the cells were treated with ALEH at a concentration of 125 µg/mL. CiplaDine (5 µg/mL), a standard wound healing agent (Kumar et al. 2007; James and Friday, 2010), served as the positive control, while untreated cells were used as the negative control.

The plates were then incubated under standard culture conditions. Images of the wounded area were captured at 0- and 24-hours post-scratch to monitor cell migration and wound closure. The remaining gap area was quantified using ImageJ software version 1.54. The extent of wound closure among the untreated, hydrogel-treated, and positive control groups was compared to determine the efficacy of ALEH in promoting cell migration and wound healing.

5.6 Data Analysis

All experimental procedures were conducted in triplicate to ensure the accuracy, reproducibility, and consistency of the results. Independent experiments were performed three times under identical conditions.

The data obtained from each set of experiments were averaged, and results are presented as the mean ± standard deviation (SD). This format highlights both the central tendency and the variability within each experimental group.

Statistical analysis was carried out using GraphPad Prism software, version 6.0 (GraphPad Software, San Diego, CA, USA). For the comparison of multiple groups, one-way analysis of variance (ANOVA) was applied. To identify statistically significant differences between groups, Tukey's post hoc test was subsequently performed.

A p-value of less than 0.05 was considered indicative of a statistically significant difference. In graphical representations, error bars correspond to the standard deviation (SD), providing a visual indication of data variability.

CHAPTER 6

RESULTS

AND

DISCUSSION

Chapter-6

6.1 Plant material collection and preparation

Fresh leaves of *Artemisia vestita* were carefully harvested during its peak blooming period in April and May of 2021 to maximize the phytochemical yield. Following collection, the leaves were subjected to shade drying for a duration of 15 days. This method was chosen specifically to preserve the integrity of their bioactive compounds by minimizing degradation caused by direct sunlight. Once adequately dried, the leaves were ground into a fine powder, and 25 grams of this powdered material were used for extraction. The extraction was performed using a Soxhlet apparatus, which yielded approximately 10.95 grams of crude extract. Prior to the extraction process, the identity of the plant material was verified and authenticated by expert botanists at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, located in Solan, Himachal Pradesh. The voucher specimen was deposited in the university herbarium under Accession Number 13916.

6.2. Phytochemical Analysis

6.2.1 Qualitative screening

A preliminary phytochemical screening of the crude extract revealed the presence of several key secondary metabolites. These included alkaloids, flavonoids, terpenoids, saponins, tannins, and phenolic compounds, as outlined in Table 3. These findings suggest the potential of *A. vestita* as a source of bioactive phytochemicals for further pharmacological investigation.

Table 3: Preliminary phytochemical screening.

Constituents	Tests	Observations	Result
Alkaloids	Dragendorff's test	Orange-brown precipitate	+
Flavonoids	Shinoda test	Yellow color precipitate	+
Terpenoids	Salkowski test	Reddish brown coloration	+
Saponins	Foam test	Foams were observed	+

Tannins	Tannic acid test	Brown discoloration	+
Phenolic compounds	5% $FeCl_3$ solution	Deep blue-black color	+

The preliminary phytochemical screening of the crude extract derived from *A. vestita* leaves was conducted using standard qualitative methods to identify the presence of various secondary metabolites. The presence of alkaloids was confirmed by Dragendorff's test, which resulted in the formation of an orange-brown precipitate. Flavonoids were detected through the Shinoda test, indicated by the appearance of a yellow precipitate. Terpenoids were identified using the Salkowski test, which produced a reddish-brown coloration upon reaction. The foam test confirmed the presence of saponins by the formation of stable froth. Tannins were detected through the tannic acid test, where a brown discoloration was observed. Lastly, phenolic compounds were confirmed using a 5% ferric chloride solution, which yielded a deep blue-black coloration. These results collectively suggest that the extract is rich in a variety of bioactive phytoconstituents, warranting further investigation into its pharmacological properties.

6.2.2 Quantitative phytochemical analysis

GC-MS *(*Gas chromatography and mass spectrometry) analysis: Phytochemicals are naturally occurring chemical substances produced by plants during their metabolic activities. These compounds, commonly referred to as secondary metabolites, play important roles in plant defense and ecological interactions. In the present study, the primary objective was to analyse and characterize the chemical constituents of *Artemisia vestita* leaf extract (ALE) using Gas Chromatography–Mass Spectrometry (GC-MS).

The GC-MS analysis led to the identification of 22 distinct chemical compounds, collectively comprising 94.89% of the total extract composition. These constituents were identified based on the interpretation of their chromatographic peaks and were matched with known spectra, retention times, and molecular profiles available in the

NIST (National Institute of Standards and Technology) database (http://www.nist.gov/srd/nist1a.cfm) represented in Figure 8.

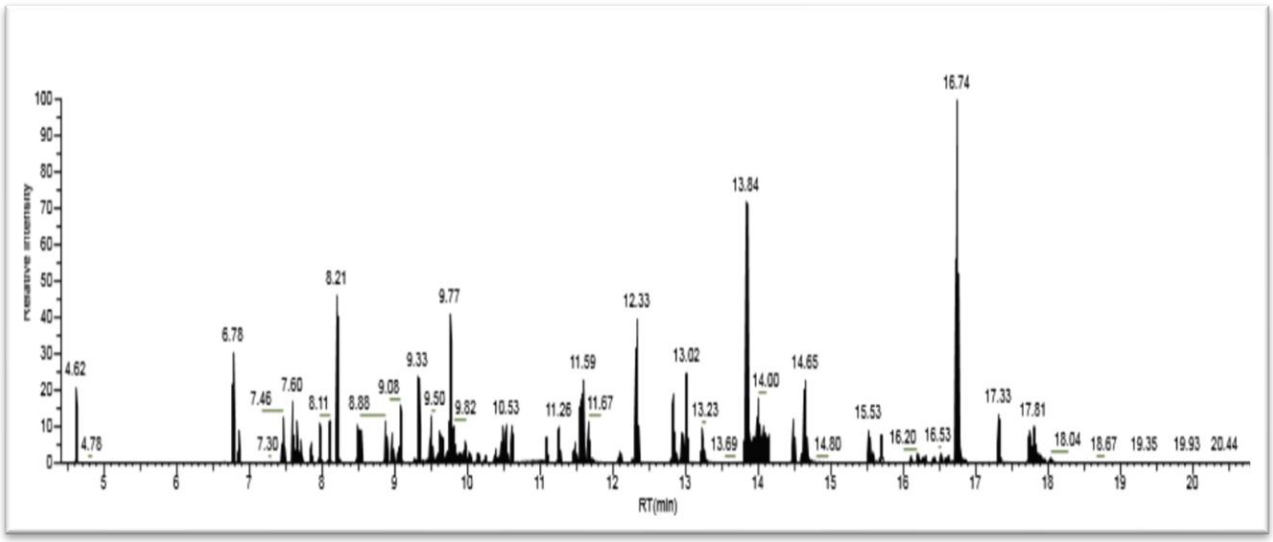


Figure 8: Gas chromatography mass spectrometry of *Artemisia vestita* leaf extract (ALE) from HP (India).

The chromatogram displayed above represents the Gas Chromatography–Mass Spectrometry (GC-MS) analysis of the leaf extract of *A. vestita*. The x-axis denotes the retention time (in minutes), while the y-axis represents the relative intensity or abundance of the detected ions, which correlates with the concentration of individual compounds present in the extract.

Each peak on the chromatogram corresponds to a specific phytochemical compound, with its height indicating the relative abundance. In this analysis, a total of 22 prominent peaks were identified, indicating the presence of 22 different bioactive constituents. These peaks were matched with standard mass spectral data from the NIST database for accurate identification.

The compound eluting at 13.84 minutes shows the highest peak, suggesting that it is the most abundant component in the extract. This peak corresponds to grandisol, which constitutes approximately 28.45% of the total composition. Similarly, other major peaks are observed at retention times 9.77 min (1,8-cineole), 12.33 min (borneol), and 16.74 min (β-caryophyllene), all of which are known for their significant

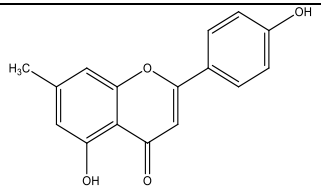
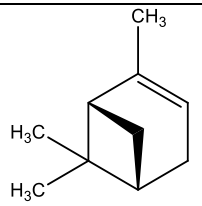
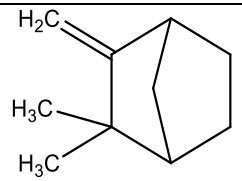
pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant effects.

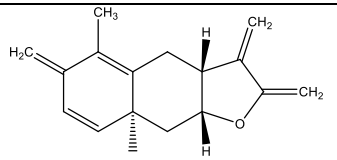
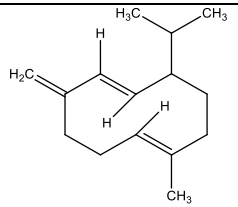
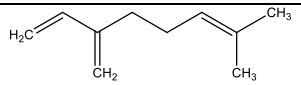
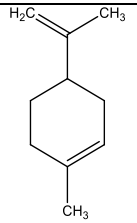
The distribution of peaks across the retention time axis indicates a diverse range of compounds varying in polarity and volatility, reflecting the complex chemical nature of the plant extract. The presence of both early- and late-eluting compounds further confirms the extraction of a wide spectrum of secondary metabolites.

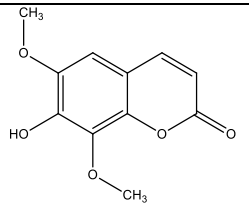
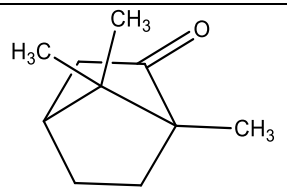
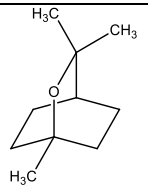
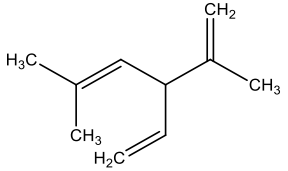
This chromatographic profile validates the richness of *A. vestita* in therapeutically relevant phytochemicals and supports its potential application in herbal medicine and pharmaceutical formulations.

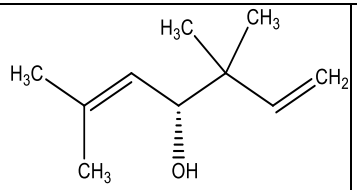
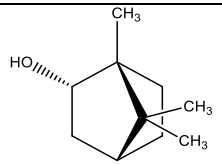
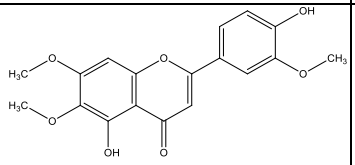
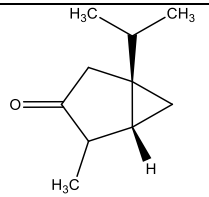
The detailed information including their molecular weights, retention times, percentage area, molecular formulas, structural representations, and known biological of all identified compounds are compiled and summarized in Table 4. This comprehensive chemical profiling underscores the therapeutic potential of *A. vestita* leaf extract and supports its traditional use in herbal medicine.

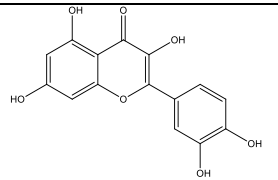
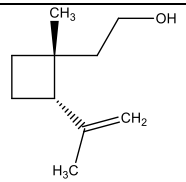
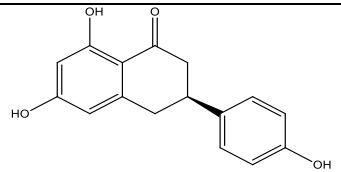
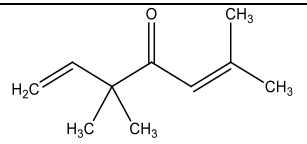
Table 4: Phytochemical compounds of *Artemisia vestita* leaf extract (ALE) from HP (India) detected by using GC-MS analysis and their biological activity.

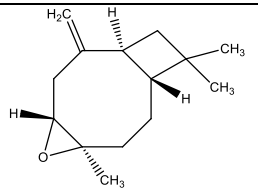
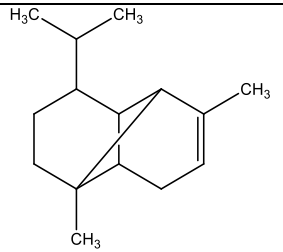
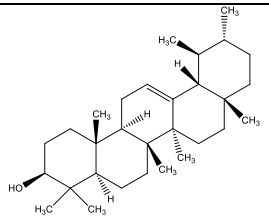
S No.	Retention time (min)	Name of the compound	Molecular formula	Molecular weight g/mol	Peak area (%)	Structure of compounds	Biological activity
1.	4.62	Apigenin	$C_{15}H_{10}O_5$	270.237	0.35		Antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, and antiproliferative activities
2.	6.78	alpha-Pinene	$C_{10}H_{16}$	136.2340	8.77		Antimicrobial, apoptotic and antibiotic
3.	7.46	Camphene	$C_{10}H_{16}$	136.2340	2.29		Antioxidant, antimicrobial, apoptosis

4.	7.60	Yomogin	$C_{15}H_{16}O_3$	244.29	2.74		Anti-inflammatory
5.	8.11	Germacrene–D	$C_{15}H_{24}$	204.3511	4.85		Antimicrobial
6.	8.21	β-Myrcene	$C_{10}H_{16}$	136.2340	1.6		Analgesics, anti-diabetic, antioxidant, anti-inflammatory
7.	8.88	Limonene	$C_{10}H_{16}$	136.23	0.26		Antitumor, antiviral, anti-inflammatory, and antibacterial

8.	9.33	Isofraxidin	$C_{11}H_{10}O_5$	222.1941	2.21		*Anti-bacterial, anti-oxidant, anti-depressive, and anti-inflammatory*
9.	9.50	Camphor	$C_{10}H_{16}O$	152.2334	1.2		Antimicrobial, antifungal, bactericidal, and antiparasitic properties
10.	9.77	1,8-cineol	$C_{10}H_{18}O$	154.2493	11.35		Anti-inflammatory, antioxidant, antimicrobial
11.	10.53	Santolina triene	$C_{10}H_{16}$	136.2340	0.7		Antimicrobial, anti-inflammatory

12.	11.59	Artemisia alcohol	$\underline{C_{10}H_{18}O}$	154.25	5.62		Antimicrobial
13.	12.33	Borneol	$C_{10}H_{18}O$	154.2493	11.12		Antioxidant, anti-inflammatory
14.	13.02	Cirsilineol	$C_{17}H_{14}O_7$	344.0896	2.25		Antioxidant, anticancer, antibacterial, and immunosuppressive activity
15.	13.23	Thujone	$C_{10}H_{16}O$	152.2334	0.12		Antioxidant, anti-inflammatory

16.	13.69	Quercetin	$C_{15}H_{10}O_7$	302.2357	0.39		Antioxidant
17.	13.84	Grandisol	$C_{10}H_{18}O$	154.25	28.45		Antimicrobial
18.	14.65	Naringenin	$C_{15}H_{12}O_5$	272.257	0.53		Antidiabetic, gastroprotective, anticancer, antiobesity, immunomodulator, cardioprotective, antimicrobial, nephroprotective
19.	15.53	Artemisia ketone	$C_{10}H_{16}O$	*152.233*	3.12		Antimicrobial

20.	16.74	β-caryophyllene	$C_{15}H_{24}$	204.36	5.67		Antibacterial, antioxidant, gastroprotective, anxiolytic, anti-inflammatory
21.	17.33	Copaene	$C_{15}H_{24}$	204.36	1.41		Cytotoxicity, antiviral, antibacterial, anti-inflammatory
22.	17.81	α-amyrin	$C_{30}H_{50}O$	426.7174	0.1		Antinociceptive and anti-inflammatory

The chemical profile of *A. vestita* leaf extract was investigated using Gas Chromatography–Mass Spectrometry (GC-MS), resulting in the identification of 22 phytochemical constituents. These compounds represented a cumulative 94.89% of the total chemical composition. Each compound was characterized based on its retention time, molecular formula, molecular weight, and peak area percentage. Identification was confirmed by comparing mass spectra, retention indices, and structural data with those in the NIST library database. The compounds identified span a broad range of bioactive chemical classes, including terpenoids, flavonoids, and phenolics, each with recognized pharmacological properties. Notably, grandisol exhibited the highest peak area at 28.45%, followed by 1,8-cineole (11.35%) and borneol (11.12%), indicating their abundance in the extract.

Key compounds and their biological activities include:

- **Apigenin** (Rt: 4.62 min): A flavonoid known for its antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, and antiproliferative properties.
- **α-Pinene** (Rt: 6.78 min): Exhibits antimicrobial, apoptotic, and antibiotic effects.
- **Camphene** (Rt: 7.46 min): Acts as an antioxidant and has antimicrobial and pro-apoptotic effects.
- **Yomogin** (Rt: 7.60 min): Recognized for its anti-inflammatory activity.
- **Germacrene-D** (Rt: 8.11 min): A sesquiterpene with potent antimicrobial action.
- **β-Myrcene** (Rt: 8.21 min): Demonstrates analgesic, antidiabetic, antioxidant, and anti-inflammatory properties.
- **Limonene** (Rt: 8.88 min): Known for its antitumor, antiviral, antibacterial, and anti-inflammatory effects.
- **Isofraxidin** (Rt: 9.33 min): Displays antibacterial, antioxidant, antidepressant, and anti-inflammatory activity.

- **Camphor** (Rt: 9.50 min): Possesses antimicrobial, antifungal, bactericidal, and antiparasitic properties.
- **1,8-Cineole** (Rt: 9.77 min): A major component with strong antioxidant, antimicrobial, and anti-inflammatory activity.
- **Santolina triene** (Rt: 10.53 min): Known for antimicrobial and anti-inflammatory effects.
- **Artemisia alcohol** (Rt: 11.59 min): Contributes primarily to antimicrobial properties.
- **Borneol** (Rt: 12.33 min): Possesses significant antioxidant and anti-inflammatory effects.
- **Cirsilineol** (Rt: 13.02 min): A polymethoxyflavone with antioxidant, anticancer, antibacterial, and immunosuppressive activities.
- **Thujone** (Rt: 13.23 min): Exhibits antioxidant and anti-inflammatory actions.
- **Quercetin** (Rt: 13.69 min): A well-known flavonoid with strong antioxidant potential.
- **Grandisol** (Rt: 13.84 min): The most abundant compound in the extract, primarily contributing to antimicrobial activity.
- **Naringenin** (Rt: 14.65 min): Exhibits diverse biological activities including antidiabetic, anticancer, gastroprotective, and cardioprotective effects.
- **Artemisia ketone** (Rt: 15.53 min): Known for its antimicrobial properties.
- **β-Caryophyllene** (Rt: 16.74 min): A sesquiterpene with antibacterial, antioxidant, gastroprotective, anxiolytic, and anti-inflammatory effects.
- **Copaene** (Rt: 17.33 min): Demonstrates cytotoxic, antiviral, antibacterial, and anti-inflammatory actions.
- **α-Amyrin** (Rt: 17.81 min): A triterpenoid with antinociceptive and anti-inflammatory effects.

The chemical composition of the methanolic leaf extract of *Artemisia vestita* was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). The analysis identified 22 bioactive compounds, collectively accounting for 94.89% of the total extract composition. The identification was based on retention times, molecular weights, and mass spectra, corroborated with the National Institute of Standards and Technology (NIST) library (version 2.4; data version: NIST v20).

The major constituents included:

- **Grandisol** (28.45%): Known for its antimicrobial properties.
- **1,8-Cineole** (11.35%): Exhibits anti-inflammatory and antimicrobial activities.
- **Borneol** (11.12%): Recognized for antioxidant and anti-inflammatory effects.
- **β-Caryophyllene** (5.67%): Possesses antibacterial and gastroprotective properties.

Other notable compounds such as apigenin, quercetin, and naringenin were also detected, each contributing to the extract's pharmacological potential.

Comparative Phytochemical Analysis with Other *Artemisia* Species

To contextualize the phytochemical profile of *A. vestita*, a comparative analysis with other *Artemisia* species was conducted, highlighting both similarities and distinctions in their chemical compositions.

***Artemisia* absinthium:** GC-MS analysis of *A. absinthium* ethanolic extract revealed 45 compounds, with yangambin (34.44%) and mome inositol (11.83%) being predominant. The extract also contained significant amounts of phytosterols like γ-sitosterol (9.55%) and stigmasta-5,22-dien-3-ol (3.34%), contributing to its anti-inflammatory and anticancer activities (Kauser et al. 2023).

***Artemisia annua*:** In *A. annua*, high-performance liquid chromatography (HPLC) coupled with diode-array detection (DAD) and mass spectrometry identified key flavonoids such as casticin and isorhamnetin, along with sesquiterpenes like artemisinin. The content of these compounds varied geographically, with artemisinin

levels ranging from 0.03% to 1.5% of dry leaf weight, indicating environmental influence on phytochemical accumulation (Fu et al. 2020)

***Artemisia dracunculus* and *Artemisia abrotanum*:** The essential oil of *A. dracunculus* was dominated by estragole (approximately 80%), while *A. abrotanum* contained high levels of davanone and its derivatives. These species exhibited distinct volatile profiles, reflecting their unique phytochemical compositions (Obistioiu et al. 2014).

***Artemisia sieberi*, *Artemisia judaica*, and *Artemisia monosperma*:** GC-MS analysis of these species from Saudi Arabia revealed diverse phytochemical profiles. *A. sieberi* exhibited the highest total phenolic content, while *A. judaica* showed the highest flavonoid content. The variation in phytochemical constituents among these species underscores the influence of genetic and environmental factors on secondary metabolite production (Salih et al. 2023).

The GC-MS analysis of *A. vestita* leaf extract demonstrates a rich composition of bioactive compounds, many of which are shared with other *Artemisia* species. The presence of compounds like 1,8-cineole and borneol aligns with findings in *A. vulgaris* and *A. absinthium*, suggesting conserved biosynthetic pathways within the genus. However, the high concentration of grandisol in *A. vestita* distinguishes it from its congeners, indicating potential for unique therapeutic applications. Comparative analyses highlight the diversity of phytochemicals across *Artemisia* species, influenced by factors such as geography, climate, and extraction methods. Understanding these variations is crucial for the development of standardized herbal formulations and for harnessing the full therapeutic potential of each species.

6.3 Antimicrobial activity of *Artemisia vestita* leaf extract (ALE)

The antimicrobial efficacy of the *A. vestita* leaf extract (ALE) of *A. vestita* was evaluated against various bacterial and fungal pathogens commonly associated with skin infections. The assessment employed the agar well diffusion method, targeting microbes including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Proteus mirabilis* and *Candida albicans*. The extract's potency was measured by the diameter of the zone of inhibition (ZOI), comparing it to

standard antibiotics azithromycin for bacteria and fluconazole for fungi. Additionally, the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined. As presented in Figure 9 and Table 5, the *A. vestita* extract demonstrated antimicrobial activity against all tested microorganisms responsible for skin infections. Notably, ALE exhibited comparable effects to synthetic drugs in many cases. The extract showed particularly strong activity against *E. coli*, *S. pyogenes*, and *C. albicans*, with maximum ZOI values of 17.6 mm, 17.3 mm, and 17.6 mm, respectively. Moderate inhibition zones were observed for *S. aureus* (14.2 mm), *B. subtilis* (13.1 mm). The lowest sensitivity was noted in *P. mirabilis*, with a ZOI of 9.4 mm.

The study further revealed a concentration-dependent antimicrobial response, with the largest zones of inhibition recorded at the highest tested concentration of 100 µg/mL. The MIC values ranged from 100 to 250 µg/mL for bacteria, while fungi showed MIC/MFC values between 100 and 300 µg/mL. The ranking of antibacterial effectiveness based on ZOI was: *E. coli* > *S. pyogenes* > *S. aureus* > *B. subtilis* > *P. mirabilis*, and for antifungal activity: *C. albicans*. These MIC and MBC/MFC ranges compare favourably with the standard drugs azithromycin and fluconazole. The findings align with previous research where the essential oil of *A. vestita*, particularly its main component grandisol, showed promising antimicrobial properties against *S. pyogenes*, with MIC and MBC values between 130 and 200 µg/mL (Yang et al. 2015). Key constituents such as α-pinene, thujone, nerol, 1,8-cineole, terpineol, and other oxygenated monoterpenes are believed to contribute significantly to this antibacterial activity (Sahoo and Banik. 2018). Overall, the data indicate that *A. vestita* leaf extract possesses strong antimicrobial potential, making it a promising candidate for the treatment of various bacterial and fungal skin infections including acne, boils, and wound infections. These encouraging results have paved the way for further investigations into its wound healing, antioxidant, anti-inflammatory, and cytotoxic properties *in vitro*.

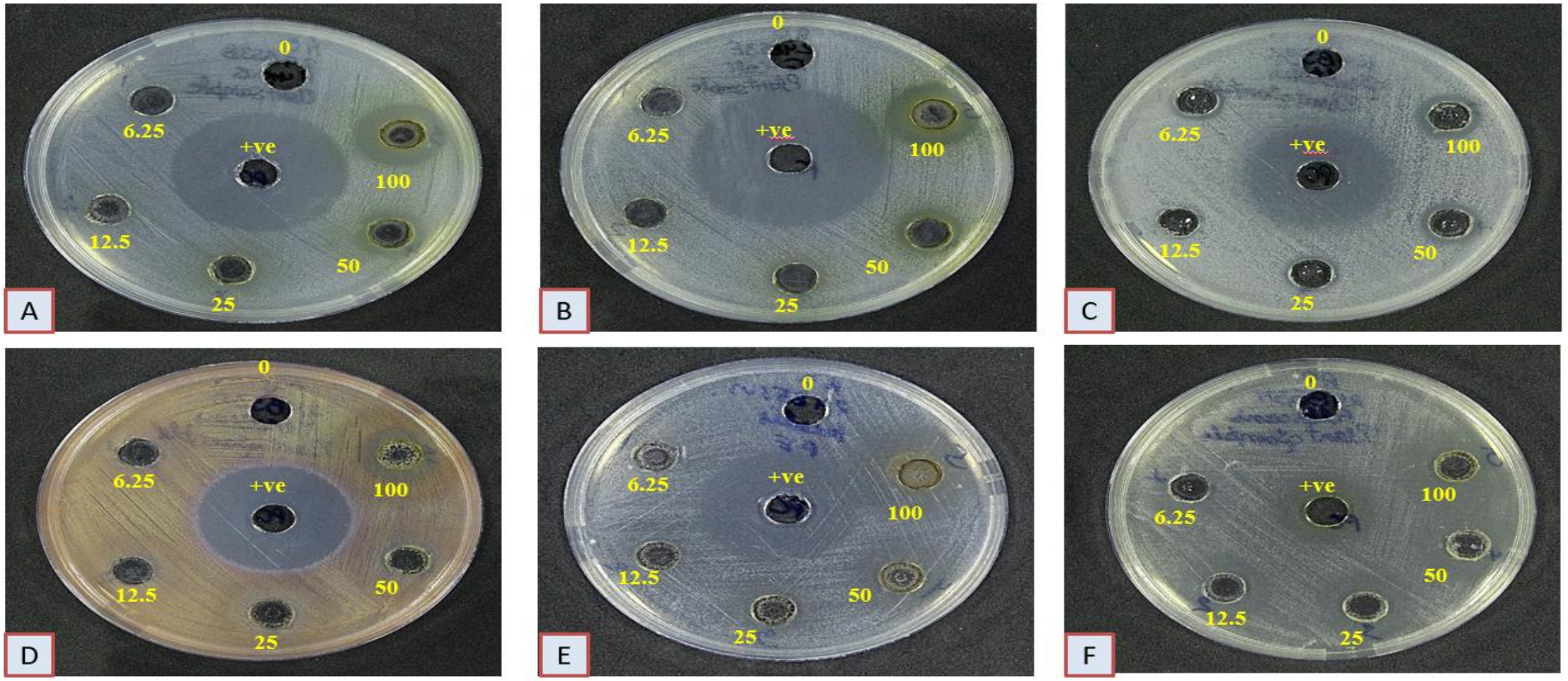


Figure 9: Antimicrobial activity of *Artemisia vestita* leaf extract at various concentrations (25, 50, 100 mg/mL); positive control azithromycin (bacteria)/fluconazole (fungus) against (A1, A2, A3, A4) *Staphylococcus aureus*; (B1, B2, B3, B4) *Escherichia coli*; (C1, C2, C3,C4) *Bacillus subtilis*; (D1, D2, D3, D4) *Streptococcus pyogenes*; (E1, E2, E3, E4) *Proteus mirabilis*; (F1, F2, F3, F4) *Candida albicans* respectively.

Table 5: Antimicrobial activity of *Artemisia vestita* leaf extract (ALE).

Microbes	Zone of inhibition (mm) from ALE				MIC and MBC/MFC (µg/mL) of ALE	
	25(µg/mL)	50(µg/mL)	100(µg/mL)	Standard Drug (Azithromycin/Fluconazole)	MIC (µg/mL)	MBC/MFC (µg/mL)
S. aureus	9.2 ± 1.53	11.24 ±0.53	14.2 ±0.28	18.4 ±0.25	100	240
E. coli	9.4±1.84	14.5 ±0.29	17.6 ±0.52	20.1± 0.51	200	250
B. subtilis	5.47 ±1.17	6.47 ±0.41	13.1 ±0.37	16.4±0.26	150	200
S. pyogenes	8.12 ±0.93	12.1 ±0.37	17.3 ±0.64	20.1± 0.21	200	250
P. mirabilis	5.21±0.87	7.28 ±0.63	9.4 ±0.56	15.2 ±0.12	100	200
C. albicans	10.2±0.36	12.1±0.32	17.6±0.11	20.1±0.25	250	300

MIC: Minimum inhibitory concentration; **MBC:** Minimum bactericidal concentration; **MFC:** Minimum fungicidal concentration; **ALE:** *A. vestita* leaf extrac

6.4 *In vitro* Pharmacological Evaluation

6.4.1 Cytotoxicity Assay

The graph presents the viability percentage of HaCaT cells exposed to increasing concentrations of *Artemisia vestita* leaf extract, ranging from 31.25 µg/mL to 750 µg/mL, alongside two controls: untreated cells and cells treated with DMSO (vehicle control). At lower concentrations (31.25 µg/mL and 62.5 µg/mL), cell viability remains high, slightly reduced from the untreated control but still above 97%, indicating minimal cytotoxic effect. This suggests that the extract is largely non-toxic at these levels and maintains good biocompatibility with human skin cells.

As the concentration increases from 100 to 125 µg/mL and 250 µg/mL, a gradual decline in cell viability is observed, although viability still exceeds 96%, reflecting only mild cytotoxicity. At 500 µg/mL, the viability drops closer to 95%, and at the highest concentration of 750 µg/mL, cell viability reaches approximately 94.5% represented in Figure 10.

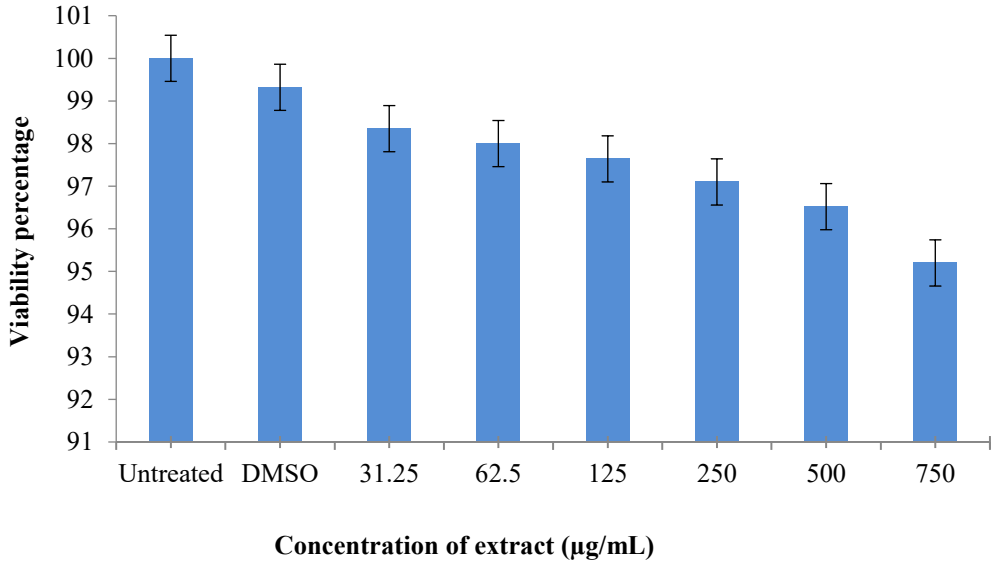


Figure 10: The cytotoxic effect of *Artemisia vestita* leaf extract on HaCaT cells.

While there is a dose-dependent decrease in cell viability, all concentrations tested maintain over 90% viability, indicating that the extract is relatively safe and well tolerated by HaCaT cells. The small reduction in viability at higher doses may be due to the presence of active phytochemicals exerting mild cytostatic effects. These results suggest that *A. vestita* leaf extract is biocompatible with HaCaT keratinocyte cells at all tested concentrations, with only slight cytotoxic effects observed at higher doses. This supports its potential use in topical wound healing formulations, particularly when used at lower concentrations where cell viability remains nearly equivalent to untreated controls.

6.4.2 Antioxidant activity

6.4.2.1 DPPH free radical-scavenging activity

The DPPH scavenging assay was conducted using ALE to evaluate its antioxidant capacity. The IC_{50} value, which represents the concentration needed to inhibit 50% of free radicals, serves as an indicator of antioxidant strength—a lower IC_{50} denotes greater activity. In this study, the IC_{50} was determined using a specialized software module designed for IC_{50}/IC_{90} calculations. The findings revealed that ALE demonstrated strong DPPH radical scavenging activity, though it was less potent than the reference antioxidant, ascorbic acid.

The antioxidant activity results for DPPH, ABTS, and FRAP assays are illustrated in Figure 11 and summarized in Table 4.

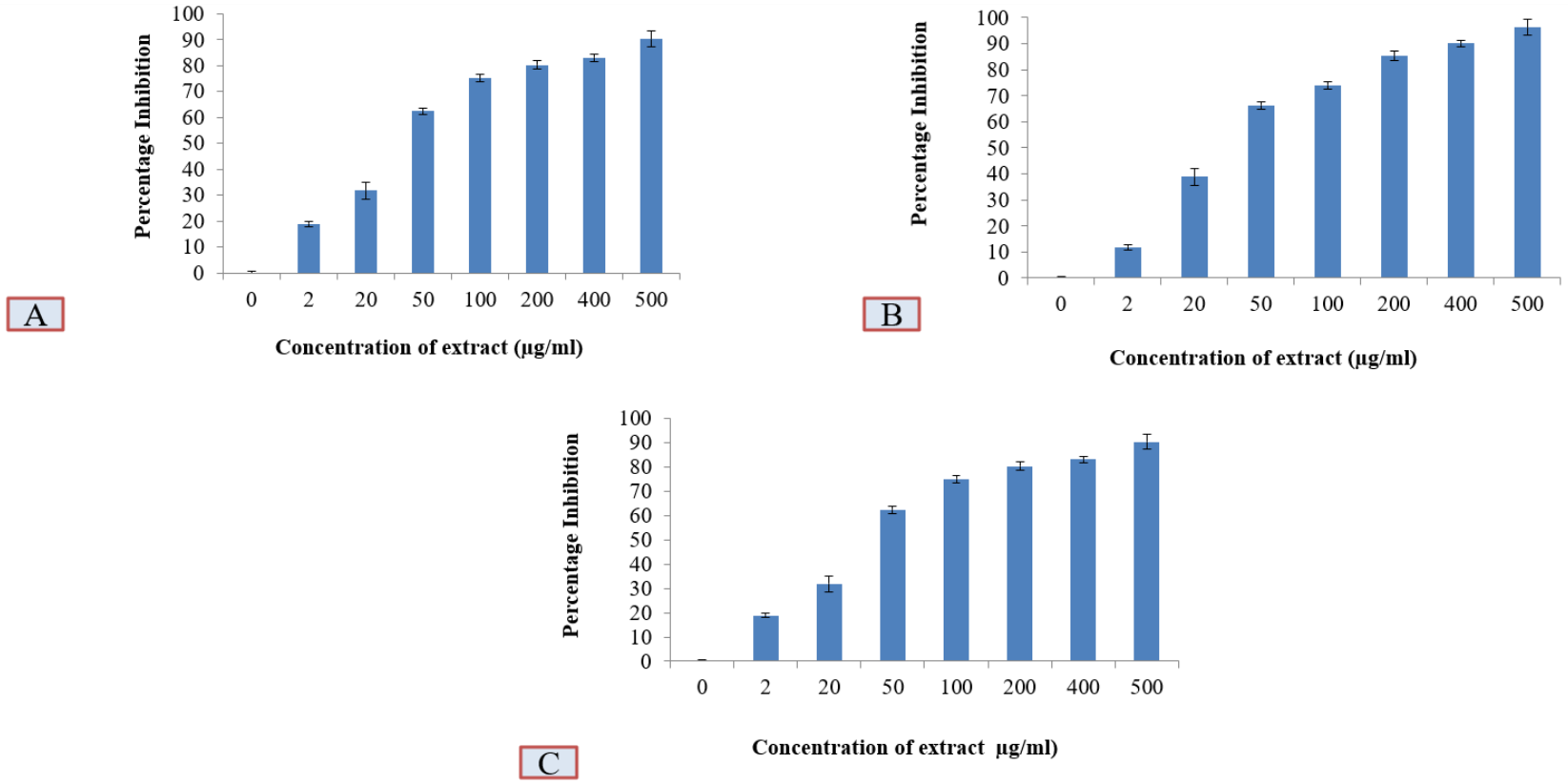


Figure 11: Scavenging activity of *Artemisia vestita* leaf extract on **(A)** DPPH, **(B)** ABTS and **(C)** FRAP assay respectively.

The bar graph (Figure 11 (A)) displays the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of ALE at various concentrations (µg/mL), as represented by percentage inhibition. As the concentration of the extract increases, the percentage inhibition also increases. This indicates a dose-dependent antioxidant activity—higher concentrations of the extract are more effective in scavenging free radicals. Even at a low concentration of 2 µg/mL, there is detectable activity (~15% inhibition), suggesting the extract has a measurable antioxidant effect even at minimal doses. A sharp increase is noted between 20 µg/mL (~30%) and 50 µg/mL (~65%), indicating a strong antioxidant response within this range. After 100 µg/mL (~75%), the increase in inhibition becomes more gradual, reaching about 95% at 500 µg/mL. This suggests the extract is nearing its maximum scavenging capacity. The presence of small error bars indicates that the results are consistent and reproducible, with low variability. The extract exhibits strong antioxidant potential, as evidenced by its ability to inhibit DPPH radicals in a concentration-dependent manner.

The bar graph (Figure 11 (B)) represents the ABTS radical scavenging activity of ALE at various concentrations, showing the percentage inhibition of ABTS radicals. The scavenging effect of ALE increases consistently with rising concentrations, indicating a dose-dependent antioxidant response. At 2 µg/mL, the inhibition is low but detectable, while at 500 µg/mL, inhibition reaches nearly 95%, suggesting strong antioxidant action at higher doses. A noticeable increase in activity is observed between 20 and 50 µg/mL, where the extract's radical-scavenging efficiency becomes more pronounced. This suggests that ALE begins to exhibit significant antioxidant activity from moderate concentrations onward. From 100 µg/mL upwards, the rate of increase in inhibition slows, indicating the extract is nearing its maximum scavenging capacity.

The bar graph (Figure 11 (C)) illustrates the ferric reducing antioxidant power (FRAP) of a plant extract (ALE) at various concentrations, based on the percentage inhibition observed. The reducing power of ALE increases progressively with rising concentration. At low concentrations (2–20 µg/mL), the extract displays moderate activity, while at higher concentrations (400–500 µg/mL), it shows strong ferric-reducing capability. A sharp rise in reducing power is evident from 20 to 100 µg/mL, where percentage inhibition increases significantly. This suggests that key antioxidant

compounds in ALE are more active in this range. Beyond 200 µg/mL, the percentage inhibition begins to plateau, indicating that the extract is reaching its maximum reducing efficiency. The Table 6 compares the antioxidant activity of ALE with ascorbic acid (vitamin C), a well-known standard antioxidant, using three different assays:

Table 6: The antioxidant activity of ALE with ascorbic acid.

Assay	**Sample**	
	Ascorbic acid (µg/mL)	**ALE (µg/mL)**
DPPH scavenging activity/IC50 (mg/mL) [a]	3.11	32.474
ABTS scavenging activity/IC50 (mg/mL) [a]	6.085	28.82
Ferric reducing assay power /RP50 (mg/mL) [b]	2.572	39.11

a: Sample concentration required to inhibit the 50% radical formation

b: Sample concentration required to reduce 50% ferric to ferrous ions

- IC_{50} (Inhibitory Concentration 50%): The concentration at which 50% of free radicals (DPPH or ABTS) are scavenged. Lower IC_{50} = higher antioxidant activity.
- RP_{50} (Reducing Power 50%): The concentration at which 50% of the maximal ferric reducing power is observed. Lower RP_{50} = stronger reducing (antioxidant) capability.

1. DPPH Scavenging Activity (IC_{50}): Ascorbic acid: 3.11 µg/mL and ALE: 32.474 µg/mL

Ascorbic acid is about 10 times more potent than ALE in scavenging DPPH free radicals. However, ALE still demonstrates notable antioxidant activity.

2. ABTS Scavenging Activity (IC_{50}): Ascorbic acid: 6.085 µg/mL and ALE: 28.82 µg/mL

ALE performs better in the ABTS assay compared to DPPH and FRAP, needing about 4.7 times more concentration than ascorbic acid to reach 50% scavenging. This suggests ALE may be more effective against certain types of radicals ($ABTS^{+}$) than others.

3. Ferric Reducing Power (RP_{50}): Ascorbic acid: 2.572 µg/mL and ALE: 39.11 µg/mL

Ascorbic acid shows stronger reducing power, being about 15 times more effective than ALE. However, ALE has moderate ferric-reducing ability, which contributes to its antioxidant potential. Among the three assays, ALE followed the antioxidant effectiveness order: FRAP > DPPH > ABTS. In the DPPH assay, ALE showed a significant ability to neutralize free radicals, achieving 50% inhibition at a concentration of 32.474 µg/mL, indicating its capability to reduce the purple-colored DPPH radical to the yellow-colored DPPH-H form.

In the ABTS assay, ALE recorded a slightly lower IC_{50} value of 28.82 µg/mL, suggesting a moderate scavenging effect. For the FRAP assay, ALE demonstrated its reducing potential by converting ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions at an IC_{50} of 39.11 µg/mL. These outcomes align with previous research on other *Artemisia* species, which displayed a similar antioxidant activity pattern of FRAP > DPPH > ABTS (Cavar et al. 2012).

6.4.3 Enzyme Inhibition Assay – Cyclooxygenase-2 (COX-2)

The bar graph represented in Figure 12 shows the COX-II (Cyclooxygenase-2) enzyme inhibition activity of ALE across different concentrations, with a comparison to a positive control (PC), likely a standard anti-inflammatory agent. The inhibitory effect of the extract on COX-II enzyme activity increases with concentration, indicating a clear dose-response relationship. At the lowest concentration (1 µg/mL), inhibition is minimal but present (~25%), while at 500 µg/mL, inhibition exceeds 80%, demonstrating strong enzyme suppression at higher doses. The most significant jump in inhibition occurs between 10 µg/mL and 100 µg/mL, where activity increases from ~45% to ~70%, showing the extract becomes biologically active at moderate concentrations. At 250 µg/mL, the inhibition reaches around 80%, and at 500 µg/mL, it slightly improves, suggesting the extract approaches maximum inhibitory capacity

within this range. The positive control (PC) shows the highest inhibition (~95%), indicating the standard compound is more potent. However, the extract's performance at high concentrations is comparable, which supports its potential as a natural COX-II inhibitor. The extract demonstrates notable COX-II inhibitory activity, which strengthens with increasing concentration. Although slightly less effective than the positive control, it shows substantial potential, particularly at doses above 100 µg/mL. These findings suggest that the ALE could serve as a natural anti-inflammatory agent by inhibiting COX-II, an enzyme closely associated with inflammation and pain processes.

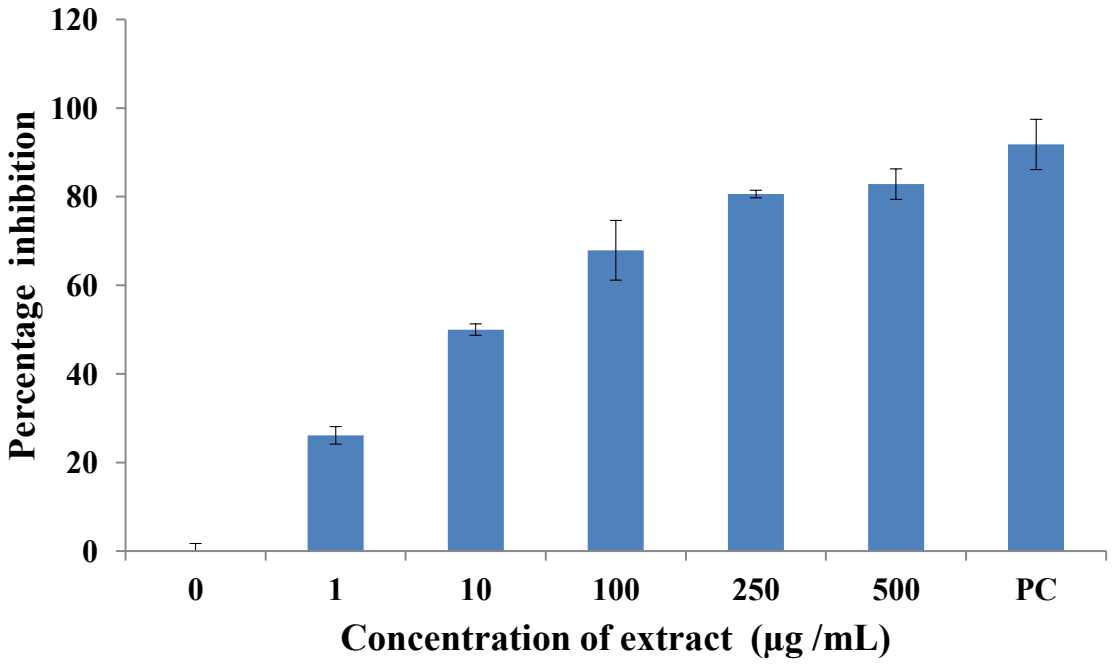


Figure 12: Inhibition of COX-II enzyme at different concentration of *Artemisia vestita* leaf extract with IC_{50} value 11.46 µg/mL.

ALE demonstrated notable anti-inflammatory potential, with an IC_{50} value of 11.46 µg/mL as shown in Figure 4, indicating its possible application in both nutritional and therapeutic contexts. Compounds such as apigenin, cirsilineol, and 6-methoxytricin, which are flavones found in *A. vestita*, have been reported to possess anti-inflammatory and immunosuppressive properties (Yin et al. 2008). Various *Artemisia* species have long been used in traditional Chinese and Tibetan medicine to manage inflammatory

conditions like sepsis, allergic skin reactions (contact dermatitis), and rheumatoid arthritis (Gupta and Tandon. 2004; Nazemizadeh and Masoudi. 2017; Qiangba et al. 2002).

6.4.4 *In vitro* scratch assay

The scratch assay has become a commonly used *in vitro* method to evaluate the wound healing potential of bioactive plant compounds. In this study, HaCaT cells were exposed to 125 µg/mL of ALE for 24 hours. Images were captured at the start (0 h) and after 24 hours, and the extent of wound closure was quantified using ImageJ software (NCBI). Findings revealed that ALE treatment led to 94.625% wound closure within 24 hours. The comparative wound closure percentages at 0 and 24 hours for both Cipladine-treated and ALE-treated cells are shown in Figure 13 provides microscopic evidence of *in vitro* wound healing, where (A, B) depict untreated cells with no noticeable changes, (C, D) represent cells treated with Cipladine (positive control), and (E, F) illustrate the effects of *Artemisia vestita* extract at the beginning and after 24 hours, respectively.

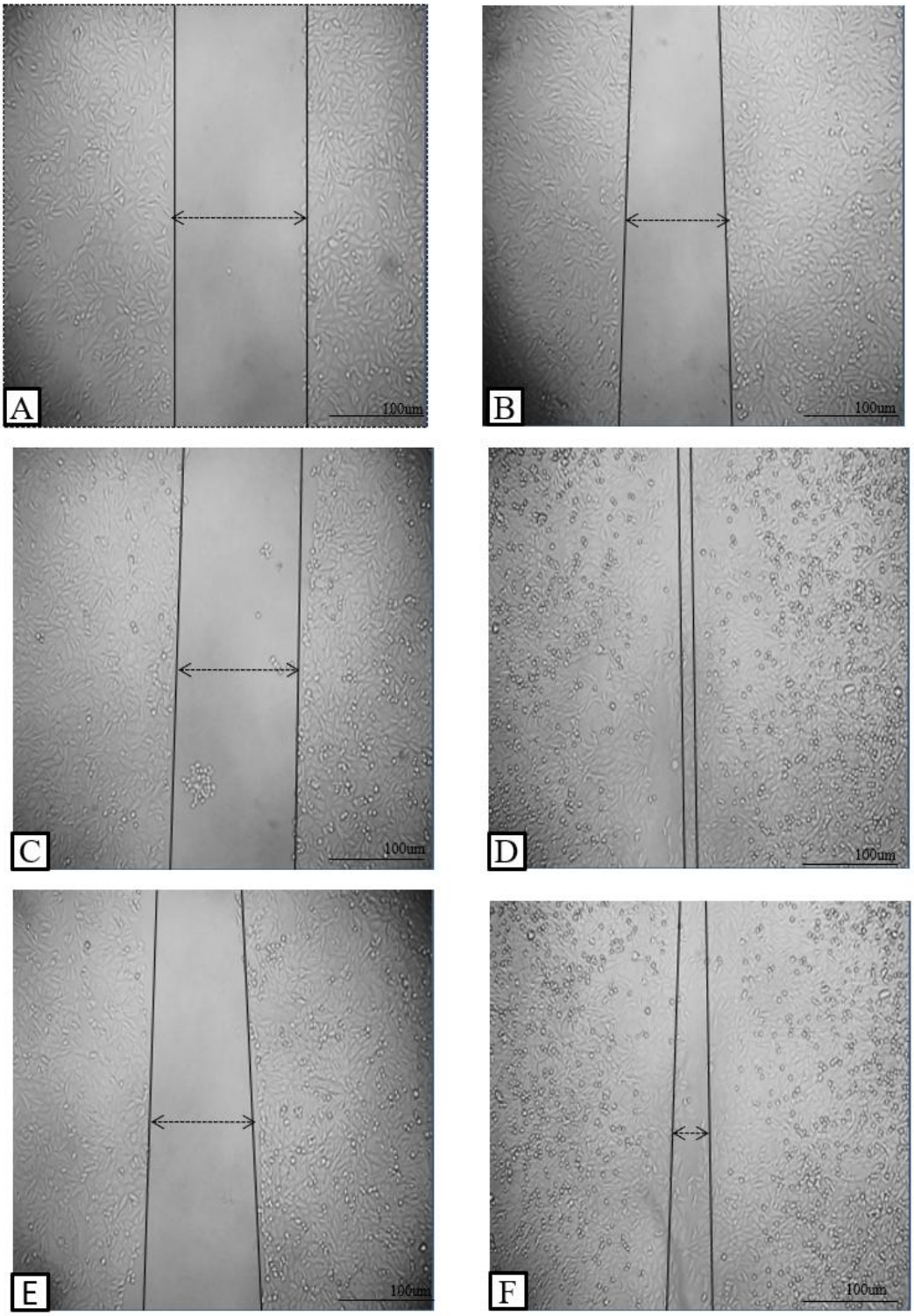


Figure 13: Microscopical images representing the *in vitro* wound healing nature **(A, B)** No treatment; **(C, D)** Cipladine (Positive control); **(E, F)** *Artemisia vestita* extract treatment at 0 and 24 h respectively.

◄—►: represents wound closure in images

- C0: Control group at 0 hours
- T0: Treated group (ALE) at 0 hours
- C24: Control group at 24 hours
- T24: Treated group (ALE) at 24 hours

At 0 hours, both groups (C0 and T0) show relatively low wound healing activity, which is expected as no time has passed for healing to occur.

C0 starts at around 85%, and T0 is slightly lower at around 75%.

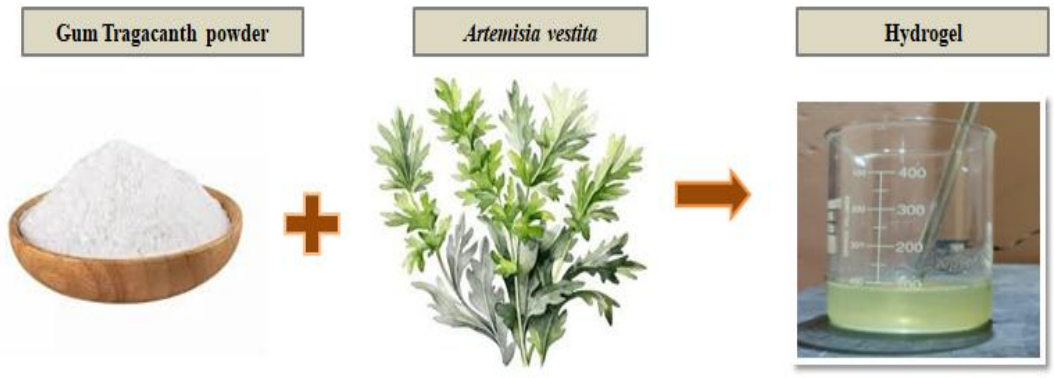
At 24 hours, both groups exhibit a significant increase in wound healing activity. C24 (control, likely with standard drug like Cipladine) shows nearly 100% wound closure, confirming its strong healing capability. T24 (ALE-treated group) reaches approximately 95–96%, indicating a substantial wound healing effect, very close to the standard control.

The results demonstrate that ALE significantly enhances wound healing *in vitro*, showing 94–96% wound closure within 24 hours. Though slightly less effective than the standard control, it still presents strong therapeutic potential for promoting cell migration and tissue repair. Figure 13 illustrates that treatment with ALE resulted in significant wound closure, achieving 94% healing (image F) within 24 hours, which is comparable to the standard drug Cipladine, showing 99.05% closure (image D). These findings demonstrate promising healing potential. Previous literature has also highlighted the therapeutic value of traditional Chinese medicine, including *Artemisia vestita*, in managing wounds and reducing inflammation and bleeding (Jung et al. 2018). Flavonoids are recognized as key contributors to the wound repair process. Other species in the *Artemisia* genus, such as *A. scoparia* Waldst. Kit and *A. parviflora*, are traditionally used for their wound-healing properties. Herbal decoctions prepared from these plants have been commonly employed in Pakistan to treat skin conditions, injuries, and wounds (Tareen et al. 2010; Negahban et al. 2006).

6.5 Preparation of Tragacanth gum-based hydrogel

This study set out to extend the shelf-life of *A. vestita* leaf extract (ALE) and strengthen its antimicrobial and wound-healing performance by transforming the extract into gel formulations. Earlier investigations had already catalogued ALE's pharmacological effects, but here the extract was embedded in a tragacanth-gum hydrogel to improve its stability. The resulting gels were rigorously evaluated for visual appearance, pH, spreadability, extrudability, viscosity, accelerated stability, in-vitro release kinetics, antimicrobial effect, and wound-repair potential. Collectively, these tests aimed to produce formulations that offer prolonged stability, enhanced antimicrobial and wound-healing activity, and controlled drug release features that position them as promising candidates for dermatological and wound-care use**.**

Hydrogel preparation with extract and tragacanth gum: Tragacanth gum, serving as the gelling agent, was used at various concentrations during the formulation process, resulting in the creation of 8 batches of gels containing *A. vestita* extract.



Gel formulations were done according to the standardized composition represented in Table 7.

Table 7: Quantitative composition of *Artemisia vestita* extract using Tragacanth gum for hydrogel.

Composition	F1	F2	F3	F4	F5	F6	F7	F8
Leaves extract (g)	1	1	1	1	1	1	1	1
Gum Tragacanth (g)	0.25	0.5	0.75	1.0	1.25	1.5	1.75	2.0
Propylene glycol (mL)	10	10	10	10	10	10	10	10
Methyl paraben (mL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Triethanol amine (mL)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water (mL)	100	100	100	100	100	100	100	100

F: formulation

6.5.1 Physical observations of compatibility study

To evaluate the physical appearance, pH, spreadability, and extrudability of 8 different formulations, samples of formulations were withdrawn after a one-month time interval, which helps to monitor the quality and stability of the formulation over time. After storing the excipients with the drug extract for one month, we did not observe any noticeable physical changes. There is no discoloration, no caking, and no liquification initially at day 1 and results remain consistent after 30 days (Table 8 and Figure 14).

Table 8. Compatibility study of drug-excipients involved in ALEH preparation.

Batch	Discoloration		Caking		Liquification	
	Initial	After 30 days	Initial	After 30 days	Initial	After 30 days
ALE	No	Consistent	No	Consistent	No	Consistent
Tragacanth gum	No	Consistent	No	Consistent	No	Consistent
ALE + Tragacanth gum	No	Consistent	No	Consistent	No	Consistent

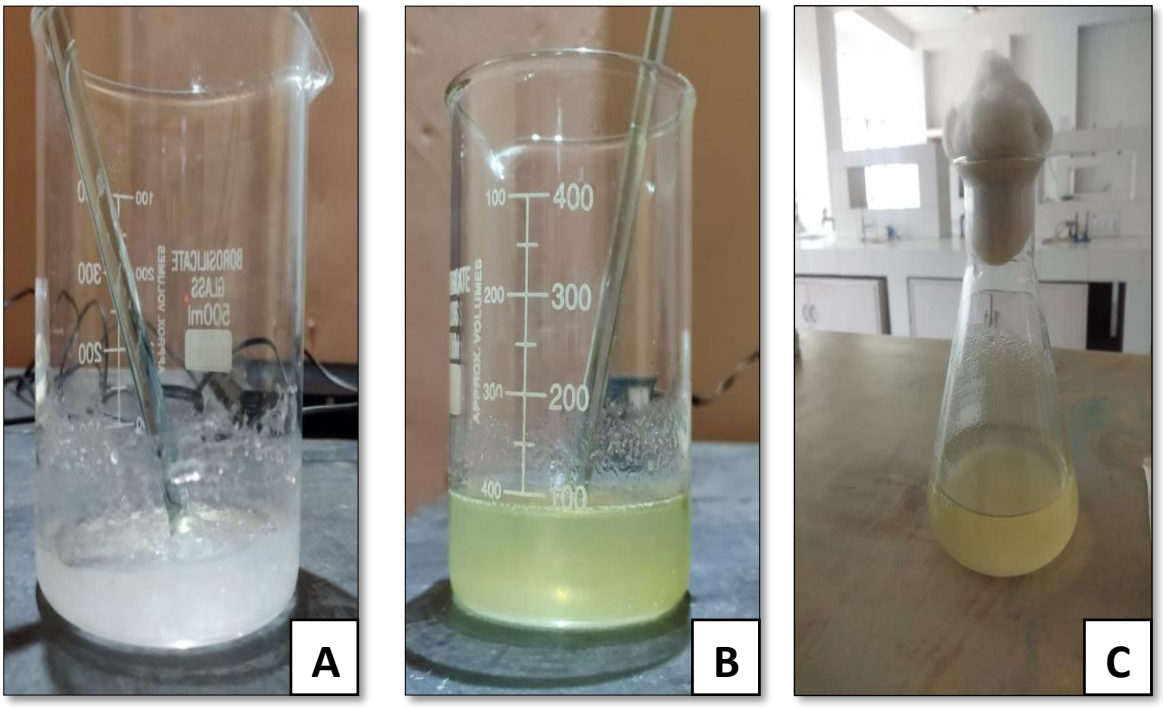


Figure 14. Images illustrating the compatibility study **(A)** Tragacanth gum without extract **(B)** Formulated gel with extract at 0 day and **(C)** Formulated gel with extract after 30 days.

Preformulation studies are vital in the development of hydrogel formulations as they offer valuable insights into the physicochemical properties, stability, and compatibility of the formulation. These studies are crucial for ensuring the safety and efficacy of the hydrogel for therapeutic applications. By thoroughly investigating factors such as

solubility, pH, viscosity, and compatibility with other ingredients or excipients, preformulation studies help in optimizing the formulation to meet the desired requirements and performance criteria. This proactive approach early in the development process can help identify potential issues and pave the way for a successful formulation with enhanced therapeutic outcomes.

6.5.1.1 Physical observations of compatibility study

All formulations were exempt from the presence of clumps and particles. Tragacanth gum was found to have a translucent appearance. Good homogeneity was observed (F1-F8) in all the prepared gels with the absence of lumps and they appeared clear and transparent. All formulation batches (F1-F8) were found to be homogeneous light yellow gel preparations. A homogeneous appearance indicates the uniform distribution of ingredients throughout the gel, which is crucial for ensuring consistent dosage and efficacy. The light-yellow colour suggests that the formulations are visually consistent and likely meet the intended specifications. This visual inspection result provides confidence in the quality and uniformity of the gel formulations, which is a positive outcome in the formulation development process.

6.5.1.2 Measurement of pH

Skin pH is influenced by various factors, including external factors (such as detergents, dermatological drugs, and cosmetics) and internal factors (e.g., age, sex, sweat composition, and sebum secretion intensity) (Freshney. 2015; Liang et al. 2007). Maintaining an acidic pH on the skin surface, is advantageous for the physiology of the epidermis, which is the outer layer of the skin, and the skin microflora (the community of microorganisms that naturally inhabit the skin). It helps to preserve the integrity of the stratum corneum which is the outermost layer of the epidermis and the lipid barrier, both of which are essential for maintaining skin hydration and protection. The acidic character of the skin contributes to the antimicrobial response and process of skin barrier regeneration (Kumar et al. 2007). Table 9 represents the pH values depicted for different batches of formulation, and all of which demonstrate satisfactory results. In this study, the lowest pH values recorded for the ALEH formulations were 6.83±0.05,

6.86±0.1, 6.9±0.10, and 6.90±1.08, while the highest values observed for the ALEH formulations were 6.93±0.15, 7.03±0.11, and 7.16±0.05. Overall, the pH values of the ALEH formulations suggest that they are suitable for application on the skin without irritating. The closeness of these pH values to the natural skin pH indicates that the formulations are compatible with skin physiology.

6.5.1.3 Spreadability

Spreadability plays an important role in ensuring the uniform distribution of a product on the skin. It's determined by factors like the expansion of the hydrogel's surface area when pressure is applied. The smoother the spread, the more likely patients are to apply it consistently and evenly. Thus, easy spreading enhances patient compliance and promotes uniform application across the skin. The spreadability of *A. vestita* extract gel formulation was evaluated and all formulations exhibited acceptable spreadability (Table 9). It was observed that ALEH had the highest spreadability of 15.75±0.19 and lowest at 11.63±0.07gm.cm^{-2}. ALEH formulations could be grouped in ascending order based on their spreadability, as follows: F6<F7<F5<F3<F8<F2<F1<F4.

6.5.1.4 Rheological study

The viscosities of the prepared hydrogels were assessed in triplicate using a Brookfield viscometer (LV-61 spindle), and the average of three measurements was documented (Table 9).

Table 9. Characterization of formulated hydrogels.

Formulation Code	pH	Spreadability (gm.cm^{-2})	Viscosity (centipoise)
F1	6.86±0.1	15.08±0.085	2318±3.78
F2	6.93±0.15	14.75±0.16	2540±2.51
F3	6.83±0.05	13.03±0.09	2463±5.13

F4	**7.03±0.11**	**15.75±0.19**	**2450±2.08**
F5	7.16±0.05	12.84±0.08	2637±2.08
F6	6.90±1.08	11.63±0.07	2429±3.21
F7	6.83±0.05	12.83±0.11	2318±2.08
F8	6.9±0.10	14.31±0.11	2411±1.15

6.5.1.5 Extrudability

Achieving the right consistency in gel formulations is critical for ensuring easy extrusion from collapsible aluminium tubes, particularly during application. Gels with low consistency tend to flow too quickly, potentially leading to wastage, while highly viscous gels may struggle to be extruded from the tube, causing inconvenience to the user. In this context, the extrudability of tragacanth gum was found to be satisfactory. This indicates that tragacanth gum, likely used as a thickening or gelling agent in the formulation, effectively balances viscosity to allow for smooth extrusion from the tube without being too thin or too thick. This finding is significant as it contributes to the usability and practicality of the gel formulation, ensuring a seamless application experience for the end-user. Formulations F4, F6 and F7 demonstrated excellent extrudability, with a high percentage of the gel being successfully extruded from the tubes. Formulations F2, F3, and F8 showed good extrudability, indicating that a substantial amount of gel was extruded, though slightly lower than those with excellent extrudability. Formulations F1 and F5 exhibited fair extrudability, suggesting that a moderate amount of gel was extruded, which needs improvement in terms of ease of extrusion.

6.5.1.6 Optimization

According to the ICH guidelines, gels were prepared and packaged in collapsible (aluminium) tubes. After a one-month interval, samples of the gels were taken for evaluation, focusing on pH, spreadability, extrudability, and physical appearance. Among the formulations, code F4 demonstrated favourable results (on the basis of

spreadability, pH and viscosity value) following batch optimization, indicating its suitability for further development or use. Formulation batch 4 was used for further evaluation of anti-microbial activity and *in vitro* wound healing activity.

6.5.2 Drug Release study

An *in vitro* drug release study was conducted on batches F1-F8 using Franz diffusion cells provided valuable information about the release kinetics and mechanisms of the formulations. The results have been shown in Table 10 and Figure 15 as cumulative percentage of drug release over time. The time required for the release of 50% of the total drug content from each formulation (F1-F8) is determined.

Table 10: Drug release study of eight distinct formulations at 25℃.

Time (H)	**Cumulative % Drug Release (25℃)**							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.25	7.982	6.390	12.588	3.878	3.291	13.593	5.553	10.578
0.5	12.664	9.282	17.362	11.995	8.969	23.831	10.940	22.096
1	29.078	24.540	25.912	17.259	16.349	31.840	25.395	32.333
2	46.067	32.814	38.144	24.633	21.613	42.010	39.883	40.337
4	60.369	50.540	52.285	33.069	42.721	57.061	53.896	56.109
6	68.155	61.488	63.093	47.778	52.014	72.312	69.515	75.699
8	80.596	75.400	78.201	61.512	70.020	84.246	77.050	82.176
10	88.988	84.709	86.468	76.508	75.468	87.848	83.787	88.752
12	98.440	93.254	94.200	86.587	84.431	92.244	91.636	96.267

F1 to F8 = Formulation1 to Formulation 8

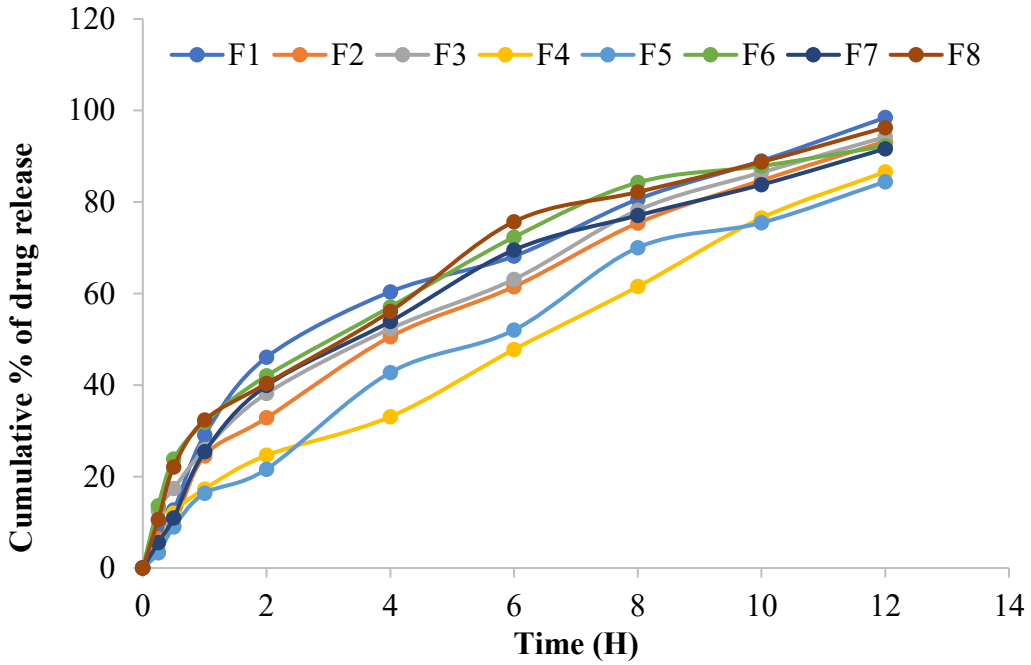


Figure 15: Drug release study of eight distinct formulations (Formulations: F1, F2, F3, F4, F5, F6, F7, and F8).

Drug release and swelling of the hydrogel were observed to be greater at pH 6.8 when compared to pH 4.5 at 37℃ and 40℃ with an order of pH 6.8> 4.6 presenting pH-responsive nature of hydrogel (Table 11 and Figure 16). The polymer volume fractions were lower at high pH, indicating the hydrogel's maximum swelling capacity at higher pH. Thus, we can conclude that prepared hydrogel could be applied externally for the controlled delivery of drugs.

Table 11: Drug release of F4 formulation at different pH and temperatures.

Time (H)	Cumulative % of drug release (F4)			
	pH 4.5 Temp 37℃	pH 4.5 Temp 40℃	pH 6.8 Temp 37℃	pH 6.8 Temp 40℃
0	0.000	0.000	0.000	0.000
0.25	4.811	1.604	1.815	11.028
0.5	11.248	9.510	5.961	15.542
1	18.702	14.781	15.818	22.267
2	23.784	23.031	22.083	33.138
4	32.819	29.448	29.361	40.785
6	42.532	34.260	43.272	50.274
8	49.760	40.792	47.879	65.198
10	64.329	51.677	68.147	74.411
12	74.380	54.656	81.044	79.110

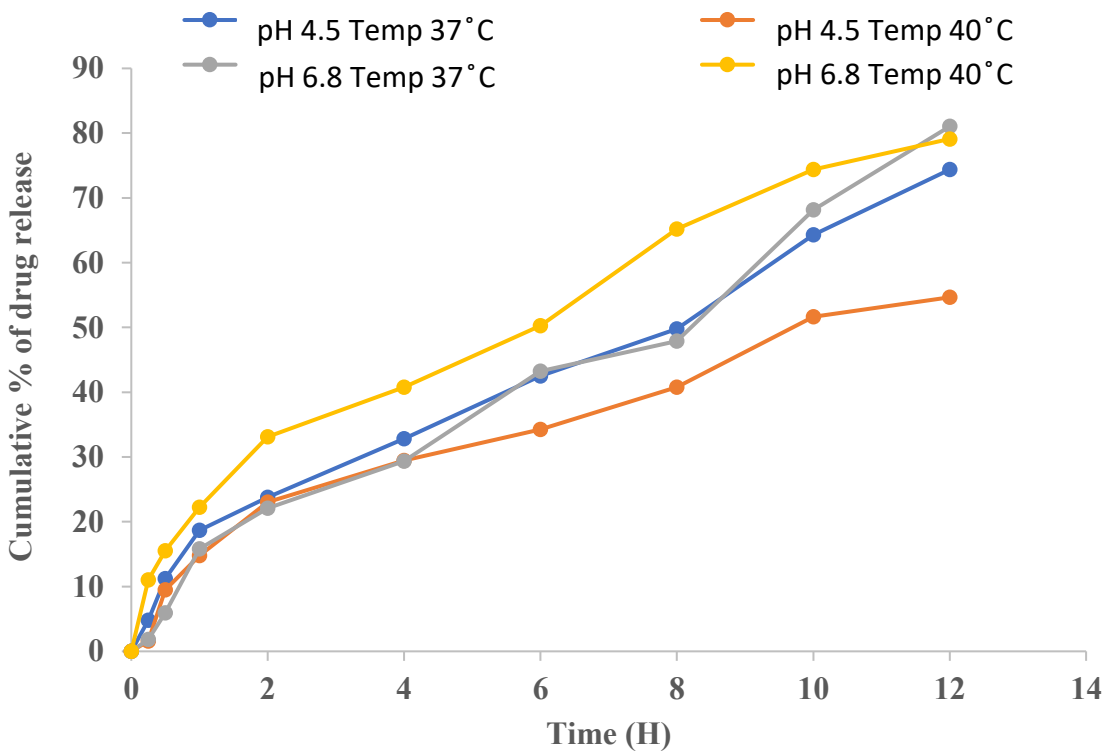


Figure 16: Graphical representation of the drug release (F4 formulation) at different pH and temperatures.

The drug release data from batches F1-F8 were analysed by fitting them to different kinetic models, including Zero-order kinetics, First-order kinetics, the Higuchi model, and the Korsmeyer-Peppas kinetic model (Figure 17). This allows for the identification of the most suitable model that best describes the release behaviour of the formulations. The best-fit drug release model and mechanism were confirmed by regression coefficient (R^2 value). Drug release data showed an R^2 value of 0.9143 for zero order, 0.9902 for the first order, and 0.9926 for the Higuchi model. To assess the drug release mechanism from the gels, *in vitro* drug release data were fitted into the Korsmeyer-Peppas equation. Analysis showed all (F1-F8) formulations exhibited non-fickian diffusion, indicating a complex release mechanism involving both diffusion and polymer relaxation.

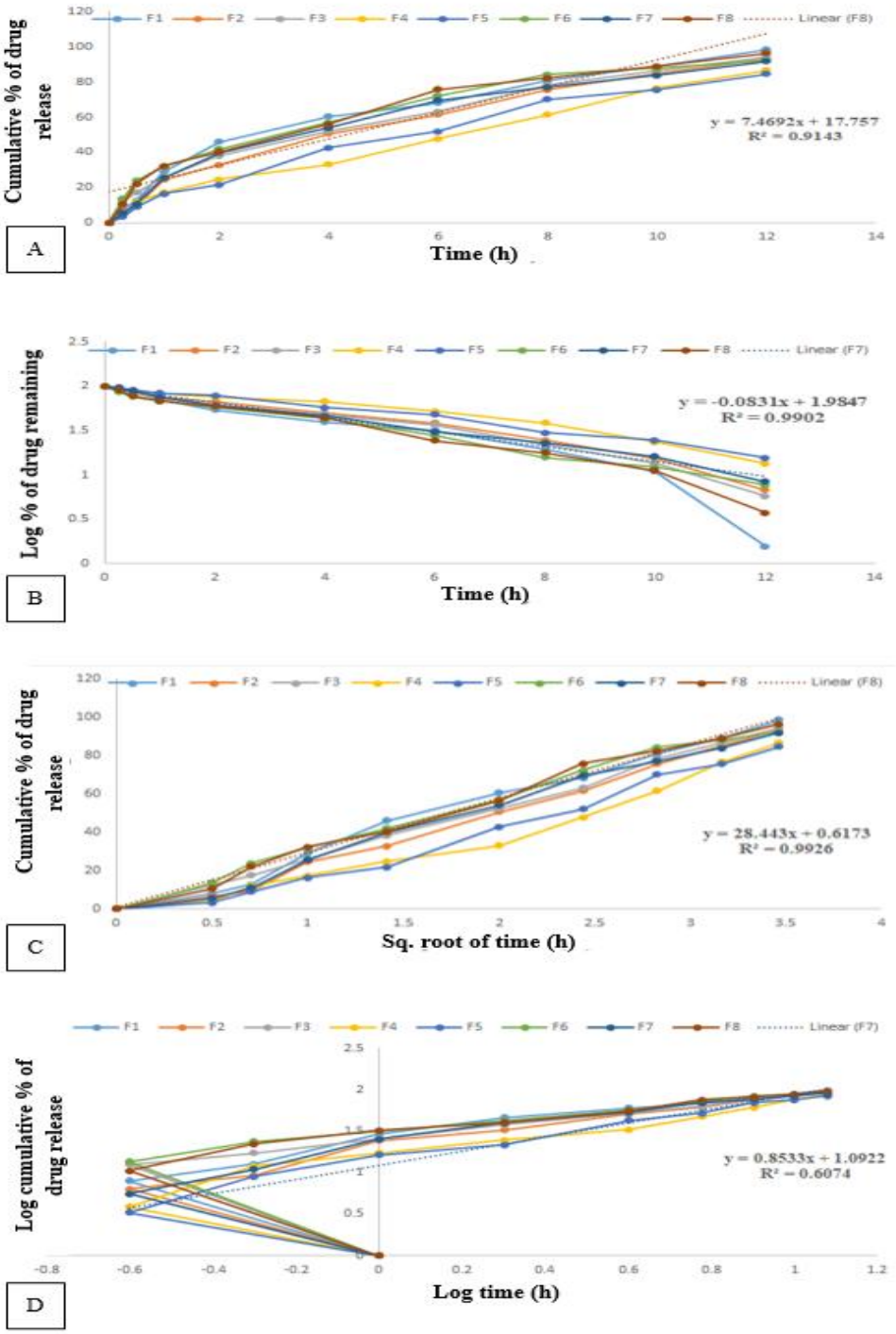


Figure 17. *In vitro* drug release data from batches F1-F8 are fitted to various kinetic **(A)** Zero order kinetics, **(B)** First order kinetics, **(C)** Higuchi model, and **(D)**Korsmeyer-Peppas kinetic model.

6.6 Antimicrobial activity of *Artemisia vestita* leaf extract hydrogel (ALEH)

The antimicrobial activity of the leaf extract of *Artemisia vestita* (ALEH) was evaluated against various bacterial and fungal strains using the agar well diffusion method. The results demonstrated a dose-dependent increase in the zone of inhibition (ZOI), with the highest concentrations (100 µg/mL) showing the most significant antimicrobial effects. Notably, *Streptococcus pyogenes* and *Escherichia coli* exhibited the largest ZOI of 20.3 mm and 19.6 mm, respectively, indicating strong susceptibility. Among fungi, *Candida albicans* showed a substantial ZOI of 19.4 mm. Conversely, *Proteus mirabilis* was the least sensitive, with a maximum ZOI of 9.4 mm at 100 µg/mL (Figure 18 and Table 12). The MIC values ranged from 100 to 200 µg/mL, while MBC/MFC values varied between 180 and 250 µg/mL, reflecting effective antimicrobial potential of ALEH against both bacteria and fungi. These findings suggest that ALEH possesses promising antimicrobial properties, comparable to standard drugs such as azithromycin and fluconazole.

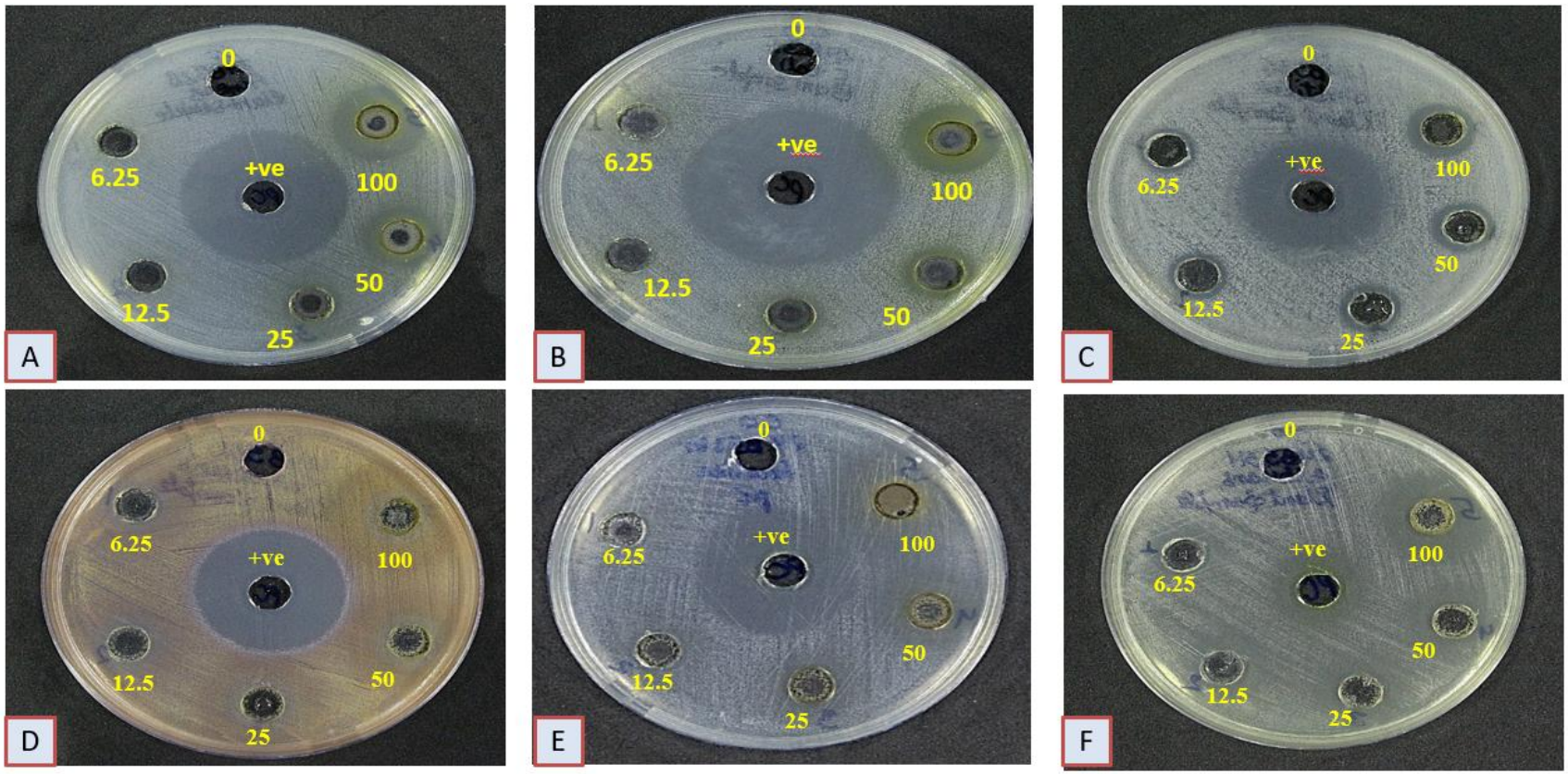


Figure 18: Antimicrobial activity of *Artemisia vestita* leaf extract hydrogel at various concentrations (25, 50, 100 mg/mL); positive control azithromycin (bacteria)/fluconazole (fungus) against (A1, A2, A3, A4) *Staphylococcus aureus*; (B1, B2, B3, B4) *Escherichia coli*; (C1, C2, C3,C4) *Bacillus subtilis*; (D1, D2, D3, D4) *Streptococcus pyogenes*; (E1, E2, E3, E4) *Proteus mirabilis*; (F1, F2, F3, F4) *Candida albicans* respectively.

Table 12: Antimicrobial activity of *Artemisia vestita* leaf extract hydrogel (ALEH).

Microbes	Zone of inhibition (mm) from ALEH				MIC and MBC/MFC (µg/mL) of ALE	
	25(µg/mL)	50(µg/mL)	100(µg/mL)	Standard Drug (Azithromycin/Fluconazole)	MIC (µg/mL)	MBC/MFC (µg/mL)
S. aureus	5.2 ± 1.43	12.14 ±0.52	15 ±0.26	20.4 ±0.22	110	220
E. coli	6.4±1.84	14.5 ±0.29	19.6 ±0.52	22.1 ± 0.31	200	240
B. subtilis	10.47 ±1.1	13.47 ±0.40	15.1 ±0.17	17.4 ±0.23	160	200
S. pyogenes	12.12 ±0.9	18.1 ±0.70	20.3 ±0.60	23.1 ± 0.20	200	250
P. mirabilis	5.21±0.87	7.28 ±0.63	9.4 ±0.56	14.2 ±0.10	100	180
C. albicans	10.2±0.36	12.1±0.32	19.4±0.01	22.1±0.24	200	250

MIC: Minimum inhibitory concentration; **MBC:** Minimum bactericidal concentration; **MFC:** Minimum fungicidal concentration; **ALEH:** *A. vestita* leaf extract hydrogel

The antimicrobial efficacy of both ALE and ALEH was evaluated against a panel of microbial strains, including both Gram-positive and Gram-negative bacteria, as well as a fungal pathogen (*Candida albicans*). The analysis included measurements of the zone of inhibition, along with MIC and MBC/MFC values, to assess their inhibitory and bactericidal/fungicidal potential. The zone of inhibition is an indicator of the extract's ability to suppress microbial growth. A larger zone reflects stronger antimicrobial activity. ALE demonstrated moderate inhibition across tested strains, with the highest inhibition observed at 100 µg/mL, especially against *C. albicans* (17.6 mm), *E. coli* (17.6 mm), and *S. pyogenes* (17.3 mm).

ALEH, on the other hand, generally produced larger inhibition zones at the same concentrations, suggesting enhanced antimicrobial activity. For example, at 100 µg/mL: *S. pyogenes* showed the greatest sensitivity (20.3 mm), *C. albicans* had a higher zone (19.4 mm) than ALE, *E. coli* and *B. subtilis* also responded better to ALEH compared to ALE. This trend indicates that ALEH possesses improved antimicrobial potential in comparison to ALE, particularly at higher concentrations

MIC represents the lowest concentration at which microbial growth is visibly inhibited. For ALE, MIC values ranged from 100 to 250 µg/mL where *S. aureus* and *P. mirabilis* were most susceptible (MIC = 100 µg/mL), while *C. albicans* required the highest concentration (MIC = 250 µg/mL). ALEH exhibited slightly better or equivalent MIC values in most cases. Notably: *S. aureus* and *P. mirabilis* retained low MICs (110 µg/mL and 100 µg/mL respectively), *C. albicans* showed a lower MIC (200 µg/mL) compared to ALE, indicating greater antifungal efficacy. MBC/MFC values indicate the lowest concentration needed to completely eliminate the microbes. With ALE, MBC/MFC values were generally higher than MICs, ranging from 200 to 300 µg/mL. ALEH, however, showed slightly lower or equivalent MBC/MFC values, suggesting its superior killing capability. For instance: *C. albicans* had an MFC of 250 µg/mL with ALEH, compared to 300 µg/mL with ALE. *P. mirabilis* required only 180 µg/mL with ALEH, versus 200 µg/mL with ALE. While both ALE and ALEH were less potent than the standard

antibiotics (Azithromycin/Fluconazole), ALEH consistently approached the activity levels of these drugs more closely than ALE. This was particularly evident in the inhibition zones for *S. pyogenes*, *E. coli*, and *C. albicans*. According to published studies, the use of a combination of tragacanth gum and amphotericin B (TG-AAC-AmB) has been found to decrease swelling in mice infected with *C. albicans* (Mohamed et al. 2018).

6.7 *In vitro* Wound Healing (Scratch) Assay for ALE Hydrogel

Currently, the *in vitro* technique scratch assay is widely adopted for gaining a better understanding to assess wound closure and cell migration dynamics. In this study, HaCaT cells were exposed to ALEH (*A. vestita* leaf extract hydrogel) for 24 h. The migration of cells was observed at both the start (0 h) and end (24 h) of the experiment, and the extent of wound closure was measured using NCBI Image J software version 1.54. Overall, the finding revealed that hydrogel at a concentration of 125 µg/mL, resulted in approximately 95% gap closure within 24 h. Figure 19 illustrates the results, showcasing the percentage of wound closure at the final state (24 h) relative to the initial state (0 h), both the positive control cipladine-treated cells and the ALEH-treated cells were compared.

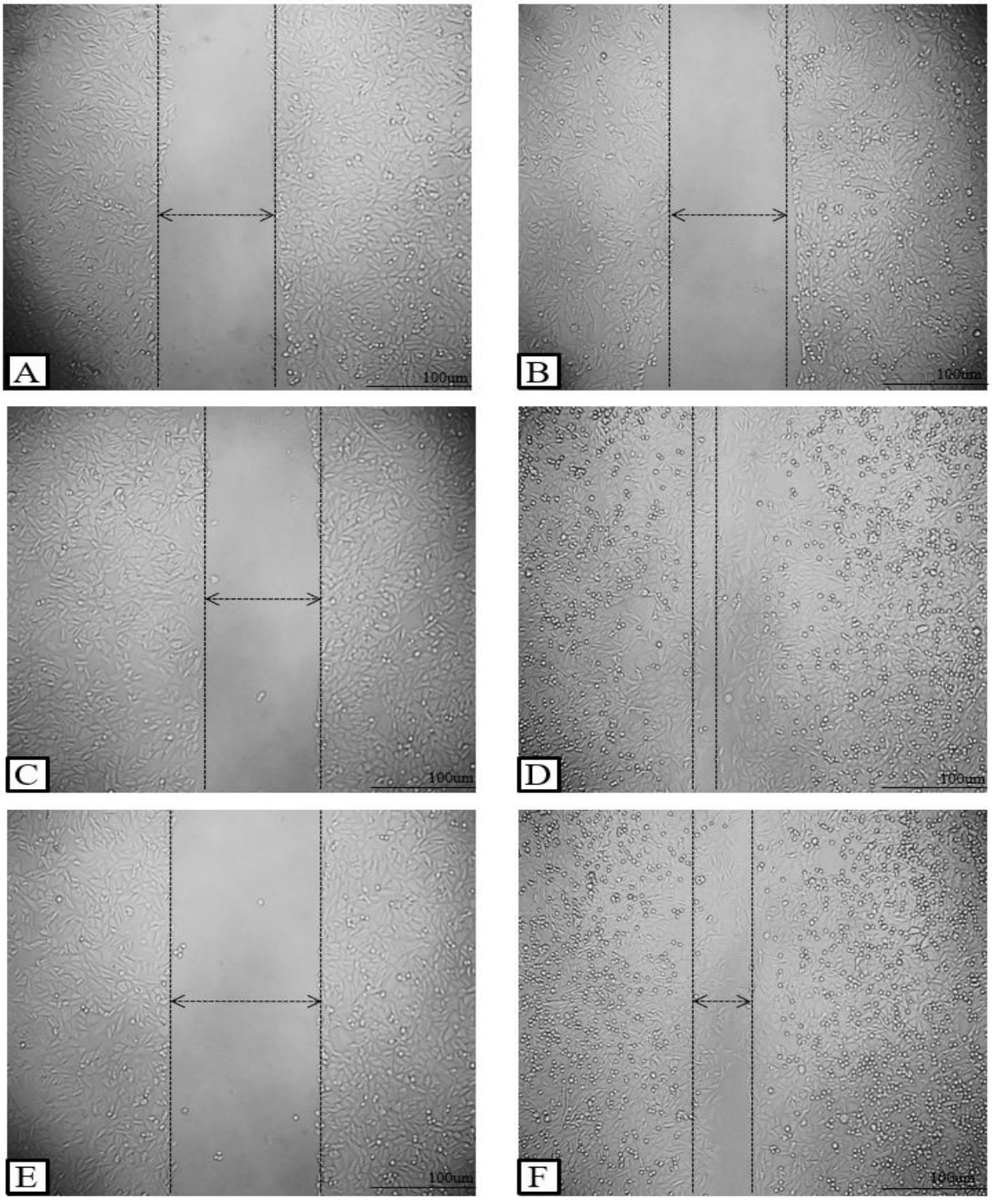


Figure 19 Microscopic images illustrating the *in vitro* wound healing properties **(A, B)** No treatment; **(C, D)** Positive control: Cipladine; **(E, F)** ALEH treatment at 0 and 24 h respectively. ◄---►: Depicts wound healing progression through visual images.

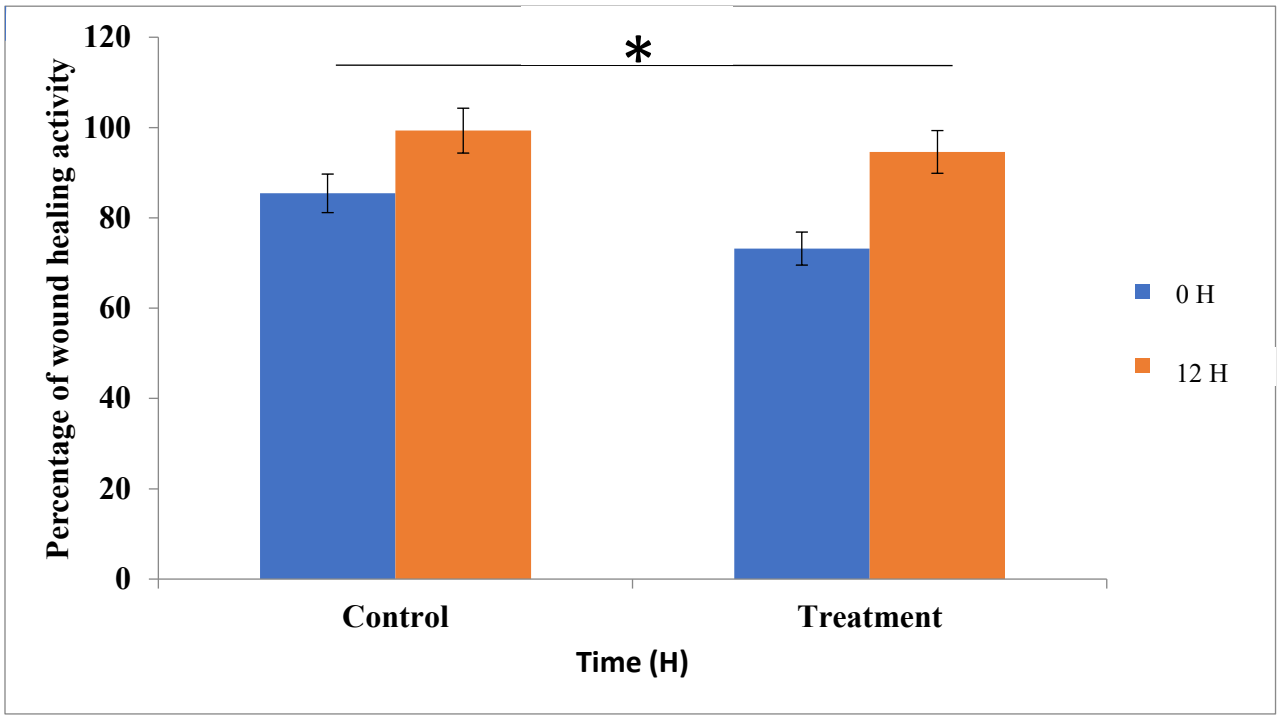


Figure 20: Wound healing percentage of control and ALEH treatment at 0 and 24 h (*: p-value = 0.05).

As illustrated in Figure 20, treatment with ALE resulted in approximately 94% wound closure (Image F) within 24 hours, closely approaching the 99.05% healing observed with the reference drug Cipladine (Image D), thus reflecting favourable healing outcomes. At the 24-hour mark, ALE-treated wounds showed significantly improved healing compared to the untreated control group (p = 0.05). Existing studies have highlighted the promising potential of combining tragacanth gum (TG) with silver nanoparticles to enhance antimicrobial effects. Silver nanoparticles, known for their broad-spectrum antibacterial action, when integrated with TG, could lead to innovative wound care solutions targeting bacterial infections (More et al.2023; Baumgardner. 2012).

TG has been recognized as a delivery vehicle for various bioactive agents, including anti-inflammatory, antimicrobial, antioxidant, and anticancer (Taghavizadeh et al. 2021). In one

study, TG-based dressing materials were applied to treat burn wounds in rats, leading to rapid and complete wound healing—results that surpassed those seen in the control group. Specifically, a full-thickness wound in rats was treated twice daily with TG solution for 10 consecutive days, demonstrating notable therapeutic benefits (Fayazzadeh et al. 2014). These effects are attributed to the presence of key bioactive components like tragacanthin and bassorin, which contribute significantly to tissue repair and wound contraction.

The study also describes a hydrogel formulation developed for topical use and drug delivery, based on a natural polymer—tragacanth gum—and herbal extract from *A. vestita*. Designed to promote wound healing, this hydrogel offers an innovative approach with potential for long-term storage stability. Traditionally, *A. vestita* has been employed in folk medicine for treating conditions such as fever, inflammation, skin infections, sepsis, and contact dermatitis, particularly in regions like China and Tibet. It is also used to alleviate stomach pain, with crushed leaf paste applied externally to stop bleeding.

Incorporating natural polysaccharide-based gums such as tragacanth gum enhances the hydrogel's properties by offering emulsifying, thickening, and stabilizing functions. Apart from TG (from *Astragalus gummifer*), other plant-derived gums such as guar gum (*Cyamopsis tetragonoloba*), gum acacia (*Acacia arabica*), xanthan gum (*Xanthomonas campestris*), psyllium husk polysaccharides (*Plantago ovata*), gum ghatti (*Anogeissus latifolia*), and locust bean gum (*Ceratonia siliqua*) may be used for gel formulations. Additives such as preservatives (e.g., methyl, ethyl, and butyl parabens), humectants (e.g., glycerin and propylene glycol), and pH adjusters (e.g., sodium hydroxide, potassium hydroxide, and triethanolamine) are incorporated to improve product stability and usability. In such hydrogel systems, tragacanth gum plays a central role by maintaining structural integrity and enhancing the formulation's functional performance

To maintain the appropriate pH balance in hydrogel formulations, stabilizing agents such as triethanolamine, sodium hydroxide, and potassium hydroxide are commonly employed. Tragacanth gum plays a multifaceted role in hydrogel systems by enhancing structural integrity, viscosity, hydration, biocompatibility, and enabling controlled drug release. A

slight variation in viscosity, ease of extrusion, and spreadability of the gel—despite a substantial increase in the concentration of tragacanth gum—can be explained by the unique characteristics of this polysaccharide and the general behaviour of hydrogel materials. As the concentration of tragacanth increases, it promotes the formation of a more defined gel network, thereby improving the gel's stability and viscosity. Nevertheless, the rheological features of the gum ensure that the hydrogel remains easily extrudable and spreadable even at higher levels of gum content. Its notable water-retaining ability keeps the formulation hydrated and pliable. Stabilization of the gel matrix is further supported by molecular interactions, including hydrogen bonds and electrostatic forces. Additionally, synergistic interactions help reinforce the hydrogel's structural and functional stability, mitigating the effects of concentration changes.

In ALEH, a hydrogel incorporating *Artemisia vestita* leaf extract (ALE), the porous structure of the tragacanth-based matrix facilitates the sustained release of active compounds. To comprehensively evaluate tragacanth gum-based hydrogels, various physical and chemical analytical techniques are applied. Physical assessments often include rheological analysis, which reveals the viscoelastic and mechanical properties of the gels, alongside swelling studies that measure water uptake and structural integrity (Joshi et al. 2022).

Conducting preformulation studies is critical for the successful development of reliable and safe drug delivery systems. These studies aim to investigate the physicochemical characteristics of a new drug compound and ensure its compatibility with the excipients used in the formulation. Hydrogels—typically composed of more than 90% water—create an ideal moist environment for wound sites (Zhao et al. 2020). They assist in vital healing processes such as tissue granulation, epidermal regeneration, and removal of necrotic tissue. Their ability to crosslink at the application site prevents drying of the wound and promotes autolytic debridement of damaged tissue (Singh and Singh. 2021). Furthermore, hydrogels can be loaded with diverse therapeutic agents and antimicrobials, supporting

processes like cell encapsulation. Their low adherence to the wound surface simplifies dressing changes and reduces pain and infection risk (Xiang et al. 2020).

The antimicrobial effect of ALEH is consistent with prior findings on *A. vestita* extracts. Notably, *A. vestita* extract exhibited strong antimicrobial efficacy against *Escherichia coli*, *Streptococcus pyogenes*, and *Candida albicans*, with zones of inhibition (ZOIs) of 17.6, 17.3, and 17.6 mm, respectively. Moderate ZOI values included 14.2 mm for *Staphylococcus aureus*, 13.1 mm for *Bacillus subtilis*, 15.3 mm. Similarly, ALEH displayed enhanced antimicrobial properties, with ZOIs reaching 19.6 mm (*E. coli*), 20.3 mm (*S. pyogenes*), and 19.4 mm (*C. albicans*), respectively. Intermediate values included 15 mm for *S. aureus*, 15.1 mm for *B. subtilis*, all measured at a concentration of 100 µg/mL.

In terms of wound healing capability, ALEH performed comparably to ALE. The application of ALE at 125 µg/mL led to 94.625% wound closure within 24 hours. After incorporation into a hydrogel matrix, the ALEH formulation demonstrated an almost identical healing efficiency of around 95% under the same experimental conditions. This consistency suggests that tragacanth gum effectively supports the therapeutic potential of the extract without impairing its wound healing function.

Topical drug delivery plays a crucial role in managing various skin-related disorders and dermatological conditions. Hydrogels formulated with tragacanth gum present numerous benefits in this context. These hydrogels exhibit strong mucoadhesive capabilities, enabling them to adhere effectively to the skin, enhance drug residence time, and support better penetration through the stratum corneum (Taghavizadeh et al. 2021). In addition, their excellent biocompatibility, biodegradability, and water-retaining capacity make them ideal candidates for extended drug release and prolonged therapeutic action (Li and Mooney. 2016). In topical applications, drug release can be finely controlled by incorporating nanosized carriers such as drug-loaded nanoparticles, lipid vesicles, or microparticles within the hydrogel matrix. These strategies contribute to improved drug stability, predictable release patterns, and enhanced permeation into deeper skin layers. Recent developments in tragacanth gum-based topical hydrogels have demonstrated

considerable promise across multiple therapeutic uses (Fayazzadeh et al. 2014). For instance, their utility in wound care (Ghorbani et al. 2021) has resulted in accelerated tissue repair, reduced infection rates, and better regeneration of damaged tissue (Zagórska-Dziok and Sobczak. 2020). Moreover, the inclusion of bioactive compounds such as plant extracts or growth-promoting factors has shown positive effects in managing chronic inflammatory skin conditions like atopic dermatitis and psoriasis (Homayun et al. 2019).

Emerging studies also highlight the potential of tragacanth gum-based hydrogels in oral drug delivery. Innovations such as incorporating nanoparticles or designing stimuli-responsive systems have facilitated site-specific drug targeting and improved therapeutic results (Xia et al. 2023). The intrinsic characteristics of tragacanth gum-based hydrogels—such as high moisture absorption, compatibility with biological systems, and tunable gelation behavior—render them particularly suitable for pharmaceutical delivery applications (Taghavizadeh et al. 2021). These hydrogels are capable of protecting delicate drug molecules from premature breakdown, ensuring controlled and sustained drug release, and allowing targeted delivery to specific tissues or regions (Gao et al. 2016).

Furthermore, the incorporation of bioactive agents or nanocarriers into the gel network enhances drug-loading capacity and overall treatment efficiency. One notable feature of tragacanth gum hydrogels is their pH-sensitive nature, which allows them to respond to environmental pH changes. In controlled drug delivery systems, this swelling behavior at different pH levels supports the timed release of therapeutic substances (Singh et al. 2020). For instance, at pH 6.8 and temperatures of 37°C and 40°C, the hydrogel exhibited increased drug release and swelling in comparison to conditions at pH 4.5, indicating effective response in mildly alkaline environments. The observed reduction in polymer volume fractions at higher pH levels further confirmed the hydrogel's optimal swelling behavior under such conditions. These findings suggest that the designed hydrogel system is well-suited for external applications requiring controlled and sustained drug delivery.

In summary, this study introduces an innovative hydrogel formulation incorporating *A. vestita* extract and tragacanth gum, aimed at therapeutic applications with a focus on skin

care and wound treatment. The hydrogel demonstrated notable effectiveness, particularly in promoting faster wound closure and enhanced scratch healing, highlighting its potential role in tissue regeneration and repair. Furthermore, qualitative evaluations of cellular orientation, structure, and alignment at the wound margins offer valuable insight into how the formulation influences cellular dynamics and behaviour during the healing process. Additionally, other Artemisia species found in India may exhibit similar therapeutic properties and warrant investigation in future research.

CHAPTER 7

SUMMARY

&

CONCLUSION

Chapter 7

Summary

Medicinal plants have long been recognized as valuable sources of novel therapeutic agents and represent a rich reservoir for drug discovery. This study presents the first in-depth evaluation of the *Artemisia vestita* leaf extract (ALE), collected from Shimla, Himachal Pradesh, India, assessing its antimicrobial, antioxidant, anti-inflammatory, cytotoxic, and wound-healing properties. Preliminary phytochemical analysis identified the presence of key bioactive constituents, including alkaloids, flavonoids, terpenoids, tannins, glycosides, and phenolic compounds, with further confirmation provided by GC-MS profiling.

Both ALE and ALEH exhibited significant antimicrobial and antifungal properties, with ALEH generally showing superior performance across most tested criteria. Specifically, ALEH demonstrated:

- Larger zones of microbial inhibition.
- Lower or comparable minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) values.
- Greater effectiveness, particularly against *Streptococcus pyogenes*, *Candida albicans*, and *Escherichia coli*.

These results imply that the herbal enhancement or advanced extraction method used in ALEH likely boosted the availability or concentration of bioactive phytochemicals, thereby improving its antimicrobial efficacy. At a concentration of 100 µg/mL, ALE proves optimal for wound healing purposes. It delivers strong antimicrobial action against primary wound-infecting organisms while maintaining high host cell viability (~98%), indicating minimal cytotoxicity. Importantly, this concentration remains below the MIC/MBC levels for most pathogens, suggesting its therapeutic viability.

Although ascorbic acid consistently displayed higher antioxidant capacity across all assays, ALE still showed moderate antioxidant effects. Its most notable performance occurred in

the ABTS assay, indicating that while it is less potent than ascorbic acid, ALE still holds promise as a natural antioxidant. This potential likely arises from the presence of polyphenols, flavonoids, or other phytochemical constituents. The findings underscore the need for further research into the antioxidant components of ALE and its potential applications in therapeutic or preservative contexts. In antioxidant assays, ALE's activity increased sharply at lower concentrations and plateaued at higher doses—a typical response pattern. This supports its prospective use as a natural antioxidant agent in pharmaceutical, nutraceutical, or food-related applications. ALE demonstrated concentration-dependent ABTS radical scavenging activity, reinforcing its value as a natural antioxidant. The consistency of results across multiple assays suggests its potential role in alleviating oxidative stress by neutralizing free radicals. Furthermore, FRAP assay outcomes confirmed ALE's strong electron-donating capacity, as evidenced by its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in a dose-dependent manner. These results further validate ALE's antioxidant potential, especially at higher concentrations, by highlighting its capacity to counteract oxidative agents through reduction-based mechanisms.

Cytotoxicity testing via the MTT assay indicated that ALE is non-toxic to HaCaT human keratinocyte cells at lower doses, although slight cytotoxic effects were observed at elevated concentrations. Notably, ALE enhanced wound closure with a 94.6% healing rate within 24 hours—closely approximating the 99.05% achieved with the positive control, Cipladine—likely due to its flavonoid content.

Further, this study examined a hydrogel formulation incorporating ALE and TG, evaluating its phytochemical composition, antimicrobial activity, and *in vitro* pharmacological properties. The formulation retained a broad spectrum of bioactive molecules, as identified through GC-MS. The resulting hydrogel demonstrated effective antimicrobial action against skin pathogens, as well as significant antioxidant and anti-inflammatory properties. MTT assay results confirmed the hydrogel's biocompatibility, with only mild cytotoxicity observed at higher doses. Importantly, the hydrogel facilitated wound healing comparable

to standard medical treatments, reinforcing its potential application in topical skin care and wound management.

Conclusion

It is estimated that approximately 80% of the world's population relies on herbal remedies for primary healthcare. According to local healers and community members, *Artemisia vestita* leaf extract (ALE) has traditionally been applied directly as a paste to fresh wounds for rapid healing. Although the therapeutic benefits of ALE are widely recognized in folk medicine, there is a need to enhance its shelf life, which can be achieved by developing it into more stable gel formulations. One of the primary objectives of this study was to scientifically validate the traditional use of ALE. In line with this, preliminary efforts have been made to formulate it into a gel-based product.

Formulations containing ALE show strong potential for use in personal care, particularly in managing minor injuries, skin irritation, redness, bleeding, and infections caused by microbes. Given its broad pharmacological properties, ALE can be developed into hydrogel-based products for dermatological purposes, offering a natural, economical, and safer alternative to synthetic topical agents. Historically valued for its healing properties, *A. vestita* appears well-suited for integration into modern skincare and wound-care hydrogel technologies.

The hydrogel formulated from ALE not only promotes rapid wound closure but also displays substantial antimicrobial activity against skin-related pathogens. Moreover, it maintains its stability for up to one year, making it a practical option for long-term storage and use. Being plant-derived, this hydrogel poses a lower risk of allergic reactions compared to animal-based materials such as collagen. Its low immunogenic profile, combined with affordability and environmental friendliness, makes it highly appealing for use in both cosmetic and therapeutic product development.

Recommendations

This research presents a novel bioactive hydrogel scaffold composed of Tragacanth gum integrated with *Artemisia vestita* extract, designed to overcome key limitations in skin tissue engineering. With its biodegradable nature, potent antimicrobial properties, and ability to provide controlled drug release, the hydrogel emerges as a highly promising candidate for wound care applications. This innovative formulation signifies a substantial advancement in skin regeneration strategies, offering a safer and more effective therapeutic approach. Future research efforts should focus on scaling up production, validating its clinical potential, and exploring the integration of cutting-edge technologies like 3D bioprinting.

Additionally, the 3D skin models developed within the framework of this study show immense potential to revolutionize wound healing approaches and enhance *in vitro* modelling for skin tissue repair. To date, this is the first study to demonstrate the synergistic therapeutic effects of *A. vestita* leaf extract in combination with Tragacanth gum within a hydrogel matrix designed for dermatological applications. Further clinical investigations are necessary to explore and confirm its potential for broader medical use.

1. **Clinical trials and safety evaluation**: Future investigations should involve in vivo studies and human clinical trials to assess the hydrogel's safety, therapeutic efficacy, and optimal dosage for clinical applications.
2. **Mechanism elucidation**: Advanced molecular research is needed to uncover the detailed mechanisms by which the hydrogel promotes wound healing and combats microbial infections.
3. **Formulation refinement**: Additional studies should focus on enhancing the hydrogel's physicochemical properties—such as viscosity, ease of application, and shelf-life—to make it suitable for large-scale manufacturing.

4. **Use of 3D skin models**: Employing 3D skin tissue models will offer a more accurate and biologically relevant assessment of the hydrogel's regenerative effects.
5. **Wider antimicrobial profiling**: Expanded screening is advised to evaluate the hydrogel's action against a broader spectrum of pathogens and to investigate its potential to minimize scar formation.
6. **Commercial product development**: Given its beneficial properties, the hydrogel can be further developed into an affordable, plant-based, and environmentally friendly product for managing minor wounds, infections, and inflammatory skin conditions.

CHAPTER 8

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Zhu XX, Yang L, Li YJ, Zhang D, Chen Y, Kostecká P, Kmoníčková E, Zídek Z (2013) Effects of sesquiterpene, flavonoid and coumarin types of compounds from *Artemisia annua* L. on production of mediators of angiogenesis. Pharmacol Rep 65:410–420

APPENDICES

(A) List of paper publications

Paper published

1. Dogra S, Yadav D, Koul B, Rabbee MF (2025) Plant-based polysaccharide gums as sustainable bio-polymers: focus on tragacanth gum and its emerging applications. Polymers 17(23):3163
2. Dogra S, Koul B, Singh J, Mishra M, Rabbee MF (2024) Formulation and in vitro assessment of tragacanth gum-based hydrogel loaded with *Artemisia vestita* leaf extract for wound healing. Processes 12(12):2750
3. Dogra S, Koul B, Singh J, Mishra M, Yadav D (2024) Phytochemical analysis, antimicrobial screening and in vitro pharmacological activity of *Artemisia vestita* leaf extract. Molecules 29(8):1829
4. Dogra S, Singh J, Koul B, Yadav D (2023) Artemisia vestita: a folk medicine with hidden herbal fortune. Molecules 28(6):2788
5. Dogra S, Singh J, Vashist HR (2021) Extraction, isolation and pharmacognostical characterization of components from *Artemisia vestita* Wall ex Besser. Natural Volatile and Essential Oils Journal 8:12955–12976
6. Dogra S, Singh J, Vashist HR (2021) Anthology of pharmacological activities from folklore medicine *Artemisia.* Natural Volatile and Essential Oils Journal 8:3678–3693

1.

polymers

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Review

Plant-Based Polysaccharide Gums as Sustainable Bio-Polymers: Focus on Tragacanth Gum and Its Emerging Applications

Shivani Dogra [1], Dhananjay Yadav [2], Bhupendra Koul [3,*] and Muhammad Fazle Rabbee [4,*]

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Abstract

Plant-based natural polymers are gaining attention as ecofriendly alternatives to synthetic materials with applications in food, biomedical, pharmaceutical, and environmental science. Tragacanth gum (TG), a natural exudate obtained from *Astragalus* species, represents a unique polysaccharide with a complex molecular structure and distinctive rheological properties. It has been traditionally used for centuries as a stabilizer and emulsifier. Recent advances highlight its potential as a multifunctional biopolymer with industrial and biomedical potential. This review explores the structural characteristics, physicochemical properties, and modification strategies of TG, comparing it with other plant derived gums. Special emphasis is given to its applications in drug delivery, tissue engineering, wound healing, biodegradable packaging, and functional food formulation. Strengths such as biocompatibility and gel-forming ability but challenges remain including variability in quality, limited standardization, and issues with large scale production. Emerging trends, such as nanoformulations, hybrid polymer composites, and smart hydrogels, are also discussed. By positioning TG within the broader context of sustainable biomaterials, this review identifies key research gaps and proposes future directions to advance its role in the green polymer economy.

Keywords: tragacanth gum; biocompatible; drug delivery; wound healing; nanocomposites

Academic Editors: Longgang Wang and Haiyan Xiao

Received: 15 October 2025
Revised: 24 November 2025
Accepted: 25 November 2025
Published: 27 November 2025

Citation: Dogra, S.; Yadav, D.; Koul, B.; Rabbee, M.F. Plant-Based Polysaccharide Gums as Sustainable Bio-Polymers: Focus on Tragacanth Gum and Its Emerging Applications. *Polymers* 2025, *17*, 3163. https://doi.org/10.3390/polym17233163

1. Introduction

Plant-derived polysaccharide gums have emerged as important candidates for replacing synthetic polymers due to their renewability, biodegradability, and structural versatility [1–3]. These natural polymers contribute significantly to the green bioeconomy by offering safe and sustainable alternatives across diverse sectors. *Astragalus gummifer* (Labill.) [4], a thorny perennial shrub of the family Fabaceae, is the botanical source of the natural polymer known as 'Tragacanth Gum', 'Gond Katira', or 'Gum Katira'. Since ancient times, it has been incorporated into foods and pharmaceuticals as a stabilizer, emulsifier, and thickening agent [5–8]. In traditional systems of medicine, it has been employed to manage a wide range of conditions, including respiratory problems, gastrointestinal disorders, metabolic irregularities, and general weakness [1,9–11]. In recent years, research has

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Article

Formulation and In Vitro Assessment of Tragacanth Gum-Based Hydrogel Loaded with *Artemisia vestita* Leaf Extract for Wound Healing

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Abstract: *Artemisia vestita* Wall. ex Besser, a traditional medicinal plant with healing properties, is receiving significant attention as a potential therapeutic agent for wound healing. In this study, eight *Artemisia vestita* leaf extract hydrogel formulations (F1–F8) were carefully designed and studied. The hydrogel formulations contained *A. vestita* leaf extract, tragacanth gum, humectants, preservatives, pH stabilizers, and Milli-Q water. A preformulation study was conducted to ensure safety and efficacy. Moreover, various experiments assessed the potential application and characteristics of *A. vestita* leaf extract hydrogel (ALEH). Drug release and swelling studies were conducted at different pH levels and temperatures. The best drug release model was identified based on the regression coefficient (R^2). Antimicrobial efficacy was assessed using the agar well diffusion method, and wound healing in HaCat cells was assessed using the scratch assay. ALEH exhibited non-Fickian diffusion, with higher drug release noted at pH 6.8 than at pH 4.5, indicating pH-responsive behavior. It exhibited significant antimicrobial activity against various strains and achieved 95% wound closure after 24 h in vitro, indicating strong wound healing properties. It also had a long shelf life; therefore, it could have pharmaceutical and medical applications. Our study is the first to report the potential applications of ALEH in skincare and wound management.

Keywords: *Artemisia vestita*; tragacanth gum; antimicrobial; wound healing; hydrogel

Citation: Dogra, S.; Koul, B.; Singh, J.; Mishra, M.; Rabbee, M.F. Formulation and In Vitro Assessment of Tragacanth Gum-Based Hydrogel Loaded with *Artemisia vestita* Leaf Extract for Wound Healing. *Processes* **2024**, *12*, 2750. https://doi.org/10.3390/pr12122750

Academic Editors: Vijaya Raghavan and Paolo Trucillo

Received: 11 September 2024
Revised: 4 November 2024
Accepted: 2 December 2024
Published: 3 December 2024

1. Introduction

Wound healing is a complex biological process. It involves a sequence of intricate events that occur in response to skin injury, followed by the actions of signaling molecules, various cell types, and extracellular matrix components [1–3]. The wound healing process involves three main phases. In the inflammatory phase (0–3 days), inflammatory cells migrate toward the wound site to remove debris and pathogens [4,5]. In the cellular proliferation phase (2–12 days), new tissue formation is initiated. In this phase, fibroblasts generate collagen, while epithelial cells multiply and move across the wound surface to cover it [6,7]. Between days 12 and 90, the scar continues to mature, becoming smoother, softer, and less noticeable. Although the scar tissue gains strength during this time, it may still be more susceptible to injury or trauma than intact skin. In the remodeling phase (3–6 months), the newly formed tissue undergoes maturation and remodeling [8,9]. In this phase, wound size decreases, collagen fibers are reorganized, and the healed tissue gradually gains functionality and strength. However, during the healing process, there are

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Article

Phytochemical Analysis, Antimicrobial Screening and In Vitro Pharmacological Activity of *Artemisia vestita* Leaf Extract

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Abstract: *Artemisia vestita* Wall. Ex Besser is a folklore medicinal plant that belongs to Asteraceae family and a treasure trove of drugs. The aim of this research study was to investigate the phyto-constituents, antimicrobial activity, antioxidant, anti-inflammatory, cytotoxicity and wound healing potential of *A. vestita* leaf extract (ALE). Phytochemical analysis of the ALE was carried out by Soxhlet extraction and GCMS (gas chromatography–mass spectrometry) analysis. Antimicrobial activity was performed by the agar well diffusion method against selected bacterial and fungal strains. Free radical scavenging potential was evaluated by DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and FRAP (Ferric reducing antioxidant power) assays. Anti-inflammatory activity was performed by enzyme inhibition assay–COXII. The cytotoxicity of ALE on HaCaT cells was studied via MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. An in vitro scratch assay was performed for the evaluation of the wound healing property of ALE. It showed satisfactory antimicrobial activity against *Staphylococcus aureus* (14.2 ± 0.28 mm), *Escherichia coli* (17.6 ± 0.52 mm), *Bacillus subtilis* (13.1 ± 0.37 mm), *Streptococcus pyogenes* (17.3 ± 0.64 mm), *Proteus mirabilis* (9.4 ± 0.56 mm), *Aspergillus niger* (12.7 ± 0.53 mm), *Aspergilus flavus* (15.3 ± 0.25 mm) and *Candida albicans* (17.6 ± 0.11 mm). In ALE, 36 phytochemicals were detected by GCMS analysis, but 22 were dominant. Moreover, the ALE was effective in scavenging free radicals with different assays and exhibited reasonable anti-inflammatory activity. The MTT assay revealed that ALE had a cytotoxic effect on the HaCaT cells. The scratch assay showed 94.6% wound closure (after 24 h incubation) compared to the positive control Cipladine, which is remarkable wound healing activity. This is the first report on the wound healing property of *A. vestita*, which can serve as a potential agent for wound healing and extends knowledge on its therapeutic potential.

Keywords: *Artemisia vestita*; antimicrobial; cytotoxicity; anti-inflammatory; antioxidant; wound healing

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Citation: Dogra, S.; Koul, B.; Singh, J.; Mishra, M.; Yadav, D. Phytochemical Analysis, Antimicrobial Screening and In Vitro Pharmacological Activity of *Artemisia vestita* Leaf Extract. *Molecules* **2024**, *29*, 1829. https://doi.org/10.3390/molecules29081829

Academic Editors: Xingfeng Shao, Jun Tian, Hong Zeng and Yongxin Li

Received: 5 March 2024
Revised: 3 April 2024
Accepted: 15 April 2024
Published: 17 April 2024

1. Introduction

Proper care of wounds is an important aspect in promoting rapid regeneration of skin, early healing and prevention of infections. It involves proper management. Folklore medicines are our native heritage. They have been used since time immemorial for maintaining a healthy life as mentioned in Unani, Ayurveda, Homeopathy, Traditional Chinese medicine and other traditional medicinal regimes [1–4]. These medicines are derived from traditional herbs by taking clues from their traditional uses. Novel potential bioactive compounds can be extracted, drug formulations can be prepared and more evidence regarding

4.

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Review

Artemisia vestita: A Folk Medicine with Hidden Herbal Fortune

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Abstract: Traditional medicines are nature's gift and our native heritage, which play a vital role in maintaining a disease-free life. *Artemisia vestita Wall. ex Besser* (family: Asteraceae), popularly known as "Kubsha" or "Russian wormwood", is a highly enriched folklore medicine with wound-healing, antiphlogistic, antifebrile, antifeedant, anti-helminthic, antimicrobial, antiviral, antitumor, and antiproliferative potential attributed to the presence of various volatile and non-volatile secondary metabolites. A systematic and extensive review of the literature on *A. vestita* was carried out via the Web of Science, PubMed, INMEDPLAN, EMBASE, Google Scholar, and NCBI, as well as from several websites. The highly relevant literature contained in 109 references was selected for further inclusion in this review. A total of 202 bioactive compounds belonging to different chemical classes such as terpenoids, coumarins, flavonoids, alkaloids, acetylenes, tannins, carotenoids, and sterols have been reported in *A. vestita*, which are responsible for different pharmacological activities. The chemical structures obtained from the PubChem and Chem Spider databases were redrawn using the software Chem Draw® version 8.0. This review paper summarizes the distribution, botanical description, phytochemistry, pharmacological activities, and conservation of *A. vestita*, which will assist scientists for further investigation. Extensive studies on the active constituents, pharmaceutical standardization, mode of action, and sustainable conservation of *A. vestita* are needed to further explore its wound-healing and allied medicinal properties.

Keywords: *Artemisia vestita*; traditional medicine; ethnopharmacology; phytoconstituents; cytotoxicity

Citation: Dogra, S.; Singh, J.; Koul, B.; Yadav, D. *Artemisia vestita*: A Folk Medicine with Hidden Herbal Fortune. *Molecules* **2023**, *28*, 2788. https://doi.org/10.3390/molecules28062788

Academic Editor: Soran Maria Loredana

Received: 27 January 2023
Revised: 12 March 2023
Accepted: 15 March 2023
Published: 20 March 2023

1. Introduction

The world is enriched with a treasure trove of traditional medicinal herbs that are of global importance for health security. India harbours four mega-biodiversity hotspots and is highly enriched with 17,500 species of medicinal plants used effectively against multiple disorders [1–3]. These medicinal herbs have been time-tested and recommended by saints, maharishis, vaidyas, and ayurvedic acharyas and have strong credence in different traditional medicinal systems such as ayurveda, unani, siddha, homeopathy, naturopathy, allopathy, and traditional Chinese medicine for treating ailments related to both humans and animals. Moreover, these medicines are safe, heal the cause of the ailment, and have less or no side effects compared to allopathic drugs [4]. For centuries, plant essential oils have played a provocative role for mankind. The people of Egypt were known to be skilled perfumers and taught the art of perfumery to Hebrews around 5000 years ago [5].

Earlier naturalized or wild plants provided social security to people in the form of supplements, fuel, fodder, raw material for companies, and an additional good income source. According to the WHO, approximately 80% of people are reliant on herbal remedies in developing nations. A total of 90% of herbal species used in India are brought from the western Himalayas, which is well known for its rich plant diversity, with 1748 medicinal species used in various fields such as pharmacological research, chemistry, clinical therapeutic studies, and pharmacognosy. Unfortunately, the traditional knowledge of

5.

Nat. Volatiles & Essent. Oils, 2021; 8(4): 12955-12976

NVEO
Natural Volatiles & Essential Oils

Extraction, Isolation And Pharmacognostical Characterization Of Components From Artemisia Vestita Wall Ex Besser

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Abstract

The aim of present study "Extraction, Isolation and Pharmacognostical characterization of components from Artemisia vestita Wall ex Besser". The whole plant collected from wild forest of 'Kotgarh' HP (India). Collected plant authenticated at YS Parmar University of Horticulture and forestry, Solan (HP) and submitted sample linked to UHF-Herbarium no. 13916. Ash values determination for total ash, water soluble and acid insoluble ash were calculated, Triplet study of plant material done for Loss on drying (LOD) and extractive value calculated for Distilled water, Methanol, Chloroform and Petroleum ether respectively. 10.95g and 2.36g crude extract obtained for Distilled water and methanol solvents by soxhlet extraction. Hydrodistillation method performed for extraction of volatile from dried powdered drug. Results concluded after pharmacognostical characterization, phytochemical, spectroscopical analysis specify purity of the plant material and Distilled water and methanol as suitable solvent from which artemisinin and thujon identified as main components and presence of flavonoids, terpenoids, alkaloids, volatile oil.

Key Words Artemisia vestita, Physicochemical, TLC, Spectroscopical screening, Artemisinin, Thujon

1. Introduction

Artemisia is a large diverse genus of plant with near 500 species belonging to family Asteraceae. Various species from the genus commonly known as mugwort, wormwood and sagebrush. The genera mainly distributed in the northern temperate regions of the world. Different species of Artemisia have been used in various treatments since ancient time as folklore medicines [1]. Different species of plant are commonly used in the treatment of gastrointestinal problems, in anorexia, indigestion, antispasmolytic, antirheumatic, in bronchitis, asthma, antibacterial, antifungal, antimalarial, in cold and cough and many more [2].

6.

Nat. Volatiles & Essent. Oils, 2021; 8(5): 3678 - 3693

NVEO
Natural Volatiles & Essential Oils

Anthology of Pharmacological activities from folklore medicine Artemisia

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Abstract

The name of Artemisia is derived from Artemis, the Greek mythological goddess of the moon and hunting. Artemisia is a genus comprising of over 20000 species belongs to the family of Asteraceae. Artemisia is a hardy herbaceous shrub known for valuable secondary metabolites in their plant. Common names for various species involve mugwort, southern worts, sagebrush, and wormwood. Presence of several bioactive ingredient participate in its broad spectrum bioactive activities through various mode of action. Because of its widespread pharmacological activity, it has been used in folklore medicine from ancient time as an antibacterial, antispasmodic, antiarthritic, anthelminthic agent and for the treatment of cancer, inflammation, malaria, menstrual related disorders and in hepatitis. This review includes the traditional uses and pharmacological activities of different Artemisia species.

KeyWords: Artemis, Artemisia, Secondary metabolites, Broad spectrum, Pharmacological activity, Folklore medicine.

Introduction

Artemisia is included in the tribe Anthemideae the wild Asteraceae family and comprises of over 500 species which are mainly found in the Asia, North America, Europe and Southern Africa [Bishop JF et al,1996, Abad MJ et al, 2013, Bora KS et al, 2011]. A large member of Asteraceae tribe members are important as flower, ornamental and used for essential oil extraction which exhibit several medicinal properties used in folk and modern remedy. The plants of this family are gaining good reputation in cosmetic and pharmaceutical industries [Teixeira da Silva J.A. et al, 2004]. Near 400 species of Artemisia have aromatic and bitter taste, main herb used in vermouth which is identified to give the characteristic bitter taste [Willcox M, 2009].

Globally, genus artemisia is widely used as traditional medicine with well known medicinal values. The therapeutic involves anti-malarial, antimicrobial, anti-inflammatory, antitumor, antioxidant, antispasmodic and many other [Maria, M. N., et al, 2019]. The volatile oil fraction of artemisia mainly involve 1,8-cineole, β-pinene, thujone, artemisia ketones, camphor, caryophyllene, camphene and germacrene D [Pande A.K.et al, 2017] which act by different mechanism. Solubility of these components had been described in different solvents like]i) 1,8-cineole is soluble in water miscible with ether, alcohol, glacial acetic acid, oil, soluble in ethanol, ethyl ether, slightly soluble in carbontetrachloride. ii) β- pinene is monoterpene colourless liquid soluble in alcohol but not in water. iii) Thujone is soluble in water. iv) Artemisia ketone is practically insoluble in water. v) Camphor is slightly soluble or insoluble in water and have anti-inflammatory and analgesic.vi) Caryophyllene is soluble in ethanol, oils and ether but insoluble in water. vii) Camphene is insoluble in water. viii) Germacrene D is sesquiterpene and soluble in chloroform, dichloromethane, DMSO, ethyl acetate.

The solubility of artemisinin had been studied in 12 different organic solvents ethanol, butanol, acetone, ethyl acetate, isopropyl acetate acetonitrile, hexane, heptane, 2-butanone, methyl tert- butyl-ether and toluene as well as in three binary solvent mixtures of ethyl acetate+ethanol, ethyl acetyate +acetone, and ethanol +acetone within the temperature range of 284.10 and 323.15 K [Gybaah, JN et al, 2010]. Similarly, the solubility of artemisinin been reported in seven different solvents including methanol, ethyl acetate, acetone, acetonitrile, cyclohexane, toluene and chloroform over the temperature range from (283.15 to 323.15) K at atmospheric pressure. The solubility of artemisinin was then reported to increase with the increase in

(B) List of patents published/filed

Patent published

1. Shivani Dogra, Bhupendra Koul (2023). A novel composition of hydrogel containing *Artemisia vestita* and process thereof. Patent application no.- 202311069887
2. Shivani Dogra, Bhupendra Koul, Joginder Singh (2024). Enhancement of the wound healing capability of *Artemisia vestita* leaf extract using biodegradable Tragacanth gum-based hydrogel. Patent application no.-202311087841

Patent filed

1. Shivani Dogra, Bhupendra Koul, Joginder Singh. Treatment of alcohol related liver diseases by leaf extracts of Artemisia vestita. Patent application no.- 202411102608
2. Shivani Dogra, Bhupendra Koul. Tragacanth gum based bioactive scaffold enriched with Kubsha extract for enhanced skin tissue regeneration. Patent application no.- 202511039931
3. Shivani Dogra, Bhupendra Koul. Fabrication of 3D-printed Tragacanth gum and *Artemisia vestita* extract-based hydrogel scaffolds for tissue engineering. Patent application no.- 202511043757

Patent published- 1

(12) PATENT APPLICATION PUBLICATION

(19) INDIA

(22) Date of filing of Application :16/10/2023

(21) Application No.202311069887 A

(43) Publication Date : 24/11/2023

(54) Title of the invention : A NOVEL COMPOSITION OF HYDROGEL CONTAININGARTEMISIA VESTITA AND PROCESS THEREOF

(51) International classification	:A61K0009000000, A61K0036282000, A61K0047140000, A61Q0019000000, A61K0047320000
(86) International Application No Filing Date	:NA :NA
(87) International Publication No	: NA
(61) Patent of Addition to Application Number Filing Date	:NA :NA
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(57) Abstract :
The present invention describes the novel hydrogel composition featuring an aqueous extract of Artemisia vestita has been developed, consisting of natural gums, humectants, preservatives, pH stabilizers, and a suitable vehicle, either alone or in combination. Eight distinct formulations (F1 to F8) were meticulously crafted in the experimental design. Pre-formulation assessments were conducted to ensure the composition's safety and efficacy, establishing critical physical and chemical parameters as well as compatibility with drug excipients. This novel hydrogel, designed exclusively for external use, exhibits remarkable wound-healing properties when applied topically. Notably, it boasts a shelf life exceeding one year, making it a cost-effective and industrially applicable invention with substantial potential in the pharmaceutical and medical fields.

No. of Pages : 32 No. of Claims : 8

Patent published- 2

(12) PATENT APPLICATION PUBLICATION

(19) INDIA

(22) Date of filing of Application :21/12/2023

(21) Application No.202311087841 A

(43) Publication Date : 23/02/2024

(54) Title of the invention : ENHANCEMENT OF THE WOUND HEALING CAPABILITY OF ARTEMISIA VESTITA LEAF EXTRACT USING BIODEGRADABLE TRAGACANTH GUM-BASED HYDROGEL

(51) International classification	:A61F 13/00, A61K 17/18 , A61K 36/282, A61K 9/00, A61L 15/42, A61L 27/52, A61P 17/02
(86) International Application No	:NA
Filing Date	:NA
(87) International Publication No	: NA
(61) Patent of Addition to Application Number	:NA
Filing Date	:NA
(62) Divisional to Application Number	:NA
Filing Date	:NA

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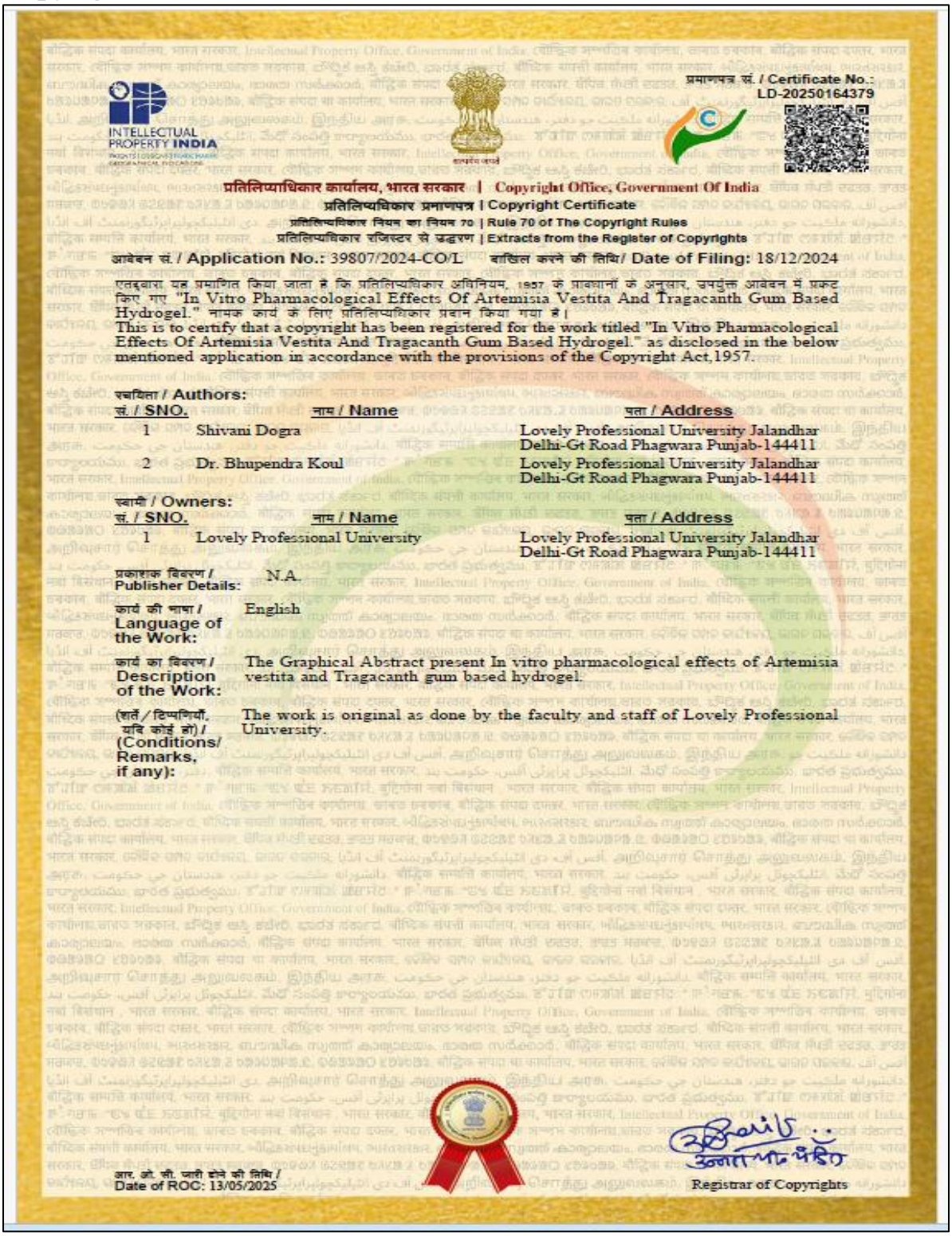
(57) Abstract :
ENHANCEMENT OF THE WOUND HEALING CAPABILITY OF ARTEMISIA VESTITA LEAF EXTRACT USING BIODEGRADABLE TRAGACANTH GUM-BASED HYDROGEL Folklore medicine Artemisia vestita is treasure trove of new drug. Leaves extract of plant is used for treating skin infections and wound healing. It showed remarkable antimicrobial activity, anti-oxidant, anti-inflammatory, cytotoxicity and wound healing capacity due to the presence of sesquiterpenoids, flavanoids and phenolic compounds. It has antioxidant and antimicrobial properties which promote wound healing process with in a short span can heal the cause of the ailment, safe and have less or no side effects compared to allopathic drugs. It can be proved best herbal antibiotic skin ointment for the wound regeneration. A. vestita revealed remarkably best antioxidant activity with IC50 value 19.11µg/mL as compared to the standard ascorbic acid IC50 value 22.474 µg/mL. Artemisia vestita extract exhibited great potential for anti-inflammatory activity with IC50 value 11.46 µg/ml.

No. of Pages : 22 No. of Claims : 8

(C) List of Copyright

1. *In vitro* pharmacological effects of *artemisia vestita* and Tragacanth gum-based hydrogel (Diary No.- 39807/2024-CO/L).
2. Development and assessment of hydrogel based on *Artemisia vestita* leaf extract and Tragacanth (Diary No.: 40806/2024-CO/L).

Copyright- 1



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LD-20250164379

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आवेदन सं. / Application No.: 39807/2024-CO/L दाखिल करने की तिथि/ Date of Filing: 18/12/2024

एतद्द्वारा यह प्रमाणित किया जाता है कि प्रतिलिप्याधिकार अधिनियम, 1957 के प्रावधानों के अनुसार, उपर्युक्त आवेदन में प्रकट किए गए "In Vitro Pharmacological Effects Of Artemisia Vestita And Tragacanth Gum Based Hydrogel." नामक कार्य के लिए प्रतिलिप्याधिकार प्रदान किया गया है।
This is to certify that a copyright has been registered for the work titled "In Vitro Pharmacological Effects Of Artemisia Vestita And Tragacanth Gum Based Hydrogel." as disclosed in the below mentioned application in accordance with the provisions of the Copyright Act,1957.

रचयिता / Authors:

सं. / SNO.	नाम / Name	पता / Address
1	Shivani Dogra	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411
2	Dr. Bhupendra Koul	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411

स्वामी / Owners:

सं. / SNO.	नाम / Name	पता / Address
1	Lovely Professional University	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411

प्रकाशक विवरण / Publisher Details: N.A.

कार्य की भाषा / Language of the Work: English

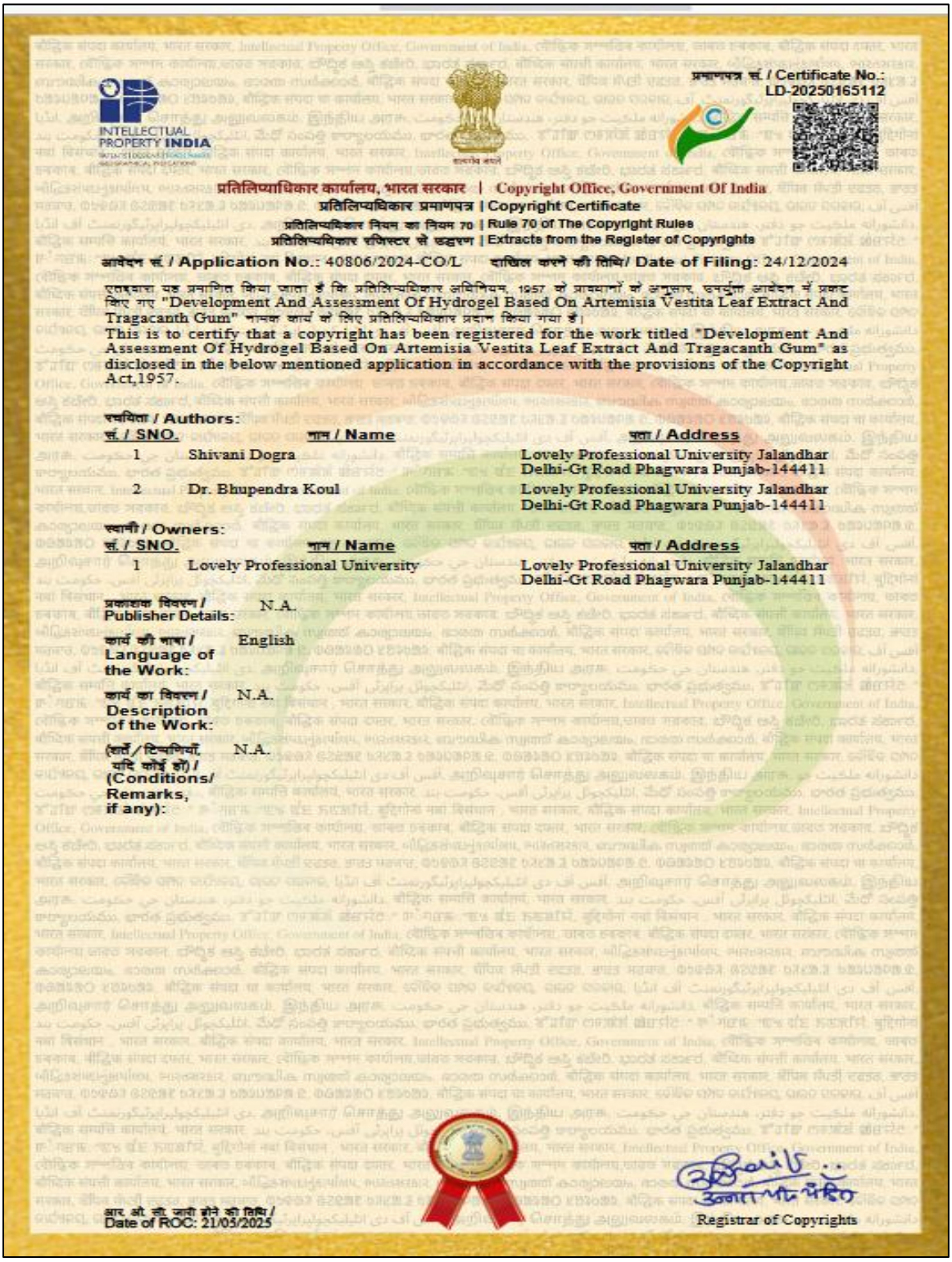
कार्य का विवरण / Description of the Work: The Graphical Abstract present In vitro pharmacological effects of Artemisia vestita and Tragacanth gum based hydrogel.

(शर्तें / टिप्पणियाँ, यदि कोई हों) / (Conditions/ Remarks, if any): The work is original as done by the faculty and staff of Lovely Professional University.

आर. ओ. सी. जारी होने की तिथि / Date of ROC: 13/05/2025

Registrar of Copyrights

Copyright- 2



प्रमाणपत्र सं. / Certificate No.:
LD-20250165112

प्रतिलिप्याधिकार कार्यालय, भारत सरकार | Copyright Office, Government Of India
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प्रतिलिप्यधिकार रजिस्टर से उद्धरण | Extracts from the Register of Copyrights

आवेदन सं. / Application No.: 40806/2024-CO/L दाखिल करने की तिथि/ Date of Filing: 24/12/2024

एतद्द्वारा यह प्रमाणित किया जाता है कि प्रतिलिप्यधिकार अधिनियम, 1957 के प्रावधानों के अनुसार, उपर्युक्त आवेदन में प्रकट किए गए "Development And Assessment Of Hydrogel Based On Artemisia Vestita Leaf Extract And Tragacanth Gum" शीर्षक कार्य के लिए प्रतिलिप्यधिकार प्रदान किया गया है।
This is to certify that a copyright has been registered for the work titled "Development And Assessment Of Hydrogel Based On Artemisia Vestita Leaf Extract And Tragacanth Gum" as disclosed in the below mentioned application in accordance with the provisions of the Copyright Act,1957.

रचयिता / Authors:

सं. / SNO.	नाम / Name	पता / Address
1	Shivani Dogra	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411
2	Dr. Bhupendra Koul	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411

स्वामी / Owners:

सं. / SNO.	नाम / Name	पता / Address
1	Lovely Professional University	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411

प्रकाशक विवरण / Publisher Details: N.A.

कार्य की भाषा / Language of the Work: English

कार्य का विवरण / Description of the Work: N.A.

(शर्तें / टिप्पणियाँ, यदि कोई हों) / (Conditions/ Remarks, if any): N.A.

आर. ओ. सी. जारी होने की तिथि / Date of ROC: 21/05/2025

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(D) Conference (s)/ Training

Total 6 conferences and 1 research facility training programme attended and awarded with 3rd prize.

Conference- 1

A National Conference Sponsored by

Himachal Pradesh Technical University (HPTU)

on

Herbal Drugs : "The Present & Future Scenario: An Overview"

12th-13th February 2020

Certificate

This is to certify that Dr./Mr./Ms. SHIVANI DOGRA of RESEARCH SCHOLAR, LOVELY PROFESSIONAL UNIVERSITY, JAL. (Pb) Participated as Keynote Speaker/ Resource Person /Evaluator/ Paper Presenter (Oral / Poster) / Delegates in the National Seminar organized by " Himachal Institute of Pharmaceutical Education & Research, Nadaun, Himachal Pradesh.

Chairman
Mr. Ashok Sharma

Organizing Secretary
Dr. Rohini Diwedi

Conference- 2

Certificate of
PARTICIPATION

This certifies that

Prof./Dr./Mr./Ms. SHIVANI DOGRA

Has Actively Participated In

INDO-AFRICAN CONFERENCE

Theme: "Recent Advances and Future Scenario of Pharmaceutical and Allied Health Sciences"

at Nootan College of Pharmacy, Narsinhgaon, Kavathemahankal, Sangli, Maharashtra

organized by APP Maharashtra State Branch and APP Ethiopian International Branch

on the 13th day of September 2019.

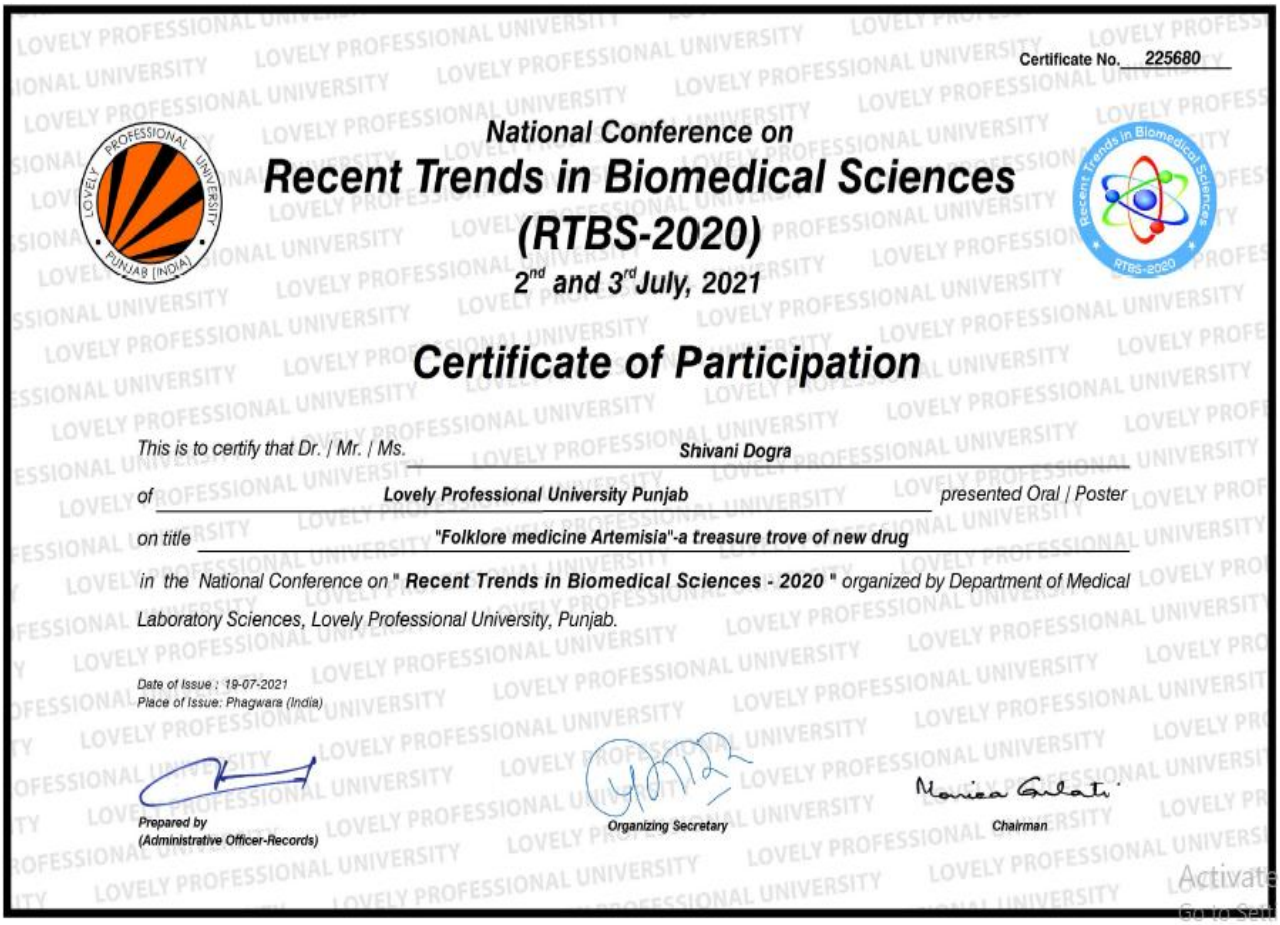
Dr. Mrunal K. Shirsat
ORGANIZING SECRETARY & PRINCIPAL
NOOTAN COLLEGE OF PHARMACY
NARSINHGAON, KAVATHEMAHANKAL, SANGLI

Dr. Sawani S. Yarnalkar
DIRECTOR
NATIONAL HEALTH LAW RESEARCH INSTITUTE
NARSINHGAON, KAVATHEMAHANKAL, SANGLI

Dr. Rajiv Dahiya
PRESIDENT APP
THE UNIVERSITY OF THE WEST INDIES
TRINIDAD & TOBAGO

APP: ALWAYS FOR THE PHARMACY PROFESSION & PROFESSIONALS

Conference- 3



Certificate No. 225680

National Conference on

Recent Trends in Biomedical Sciences (RTBS-2020)

2nd and 3rd July, 2021

Certificate of Participation

This is to certify that Dr. / Mr. / Ms. **Shivani Dogra**

of **Lovely Professional University Punjab** presented Oral / Poster

on title **"Folklore medicine Artemisia"-a treasure trove of new drug**

in the National Conference on " **Recent Trends in Biomedical Sciences - 2020** " organized by Department of Medical Laboratory Sciences, Lovely Professional University, Punjab.

Date of Issue : 19-07-2021
Place of Issue: Phagwara (India)

Prepared by
(Administrative Officer-Records)

Organizing Secretary

Chairman

Conference certificate- 4

LOVELY PROFESSIONAL UNIVERSITY
Transforming Education Transforming India

INSTITUTE OF FOREST PRODUCTIVITY

Certificate No. 238897

Certificate of Participation

This is to certify that ***Prof./Dr./Mr./Ms. Shivani Dogra*** *of* ***Lovely Professional University*** *has participated in* ***Oral Presentation*** *on the topic entitled* ***Artemisia-Hidden Herbal*** *in International Conference on* ***Sustainability: Life on Earth 2021 (ICS-LOE 2021)*** *held on 17-18 December 2021 organized by Department of Botany and Zoology, School of Bio-engineering and Biosciences, and Institute of Forest Productivity, Ranchi, Jharkhand, at Lovely Professional University, Punjab.*

Date of Issue: 18-02-2022
Place of Issue: Phagwara (India)

Prepared by
(Administrative Officer-Records)

Dr. Joydeep Dutta
Organizing Secretary, ICS-LOE, 2021

Dr. Neeta Raj Sharma
Convener, ICS-LOE, 2021

Conference certificate- 5

3ICP2022ORAL PRESENTATION 03

Serial No. _____

LOVELY PROFESSIONAL UNIVERSITY

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Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. **Shivani Dogra** has successfully participated as Delegate & Presented Poster/ Oral Presentation on **The Magical Herb- "Artemisia vestita"** in the 3rd International Conference of Pharmacy (ICP-2022) on the Theme of "Practice, Promotion & Publication of Innovation : A Way of Transforming Health" held on 09th & 10th November 2022 organized by School of Pharmaceutical Sciences in a collaboration with Indian Pharmaceutical Association (IPA) at Lovely Professional University, Punjab.

Mr. Suresh Khanna
National Hon. Gen. Secretary, IPA

Dr. Bimlesh Kumar
Organizing Secretary

Dr. T.V. Naryana
National President IPA

Dr. Monica Gulati
LOC Chairperson

Sri Herbasia Biotech

Tishk International University, Iraq

Conference certificate- 6

Certificate No.257757

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

Bioclues Organization

Certificate of Participation

This is to certify that **Prof./Dr./Mr./Ms. Shivani Dogra** of **Lovely Professional University** has participated in **Oral Presentation** on the topic entitled **Phytochemical analysis & in vitro pharmacological effects of Artemisia vestita Wall. ex Besser leaf extract using hydrogel** in the International Conference on **Bioengineering and Biosciences (ICBB-2022)** held on **18-19 November 2022** organized by the Department of Biotechnology, School of Bio-engineering and Biosciences in association with the Society of Bioinformatics for Experimenting Scientists (Bioclues) organized at Lovely Professional University, Punjab.

Date of Issue : 07-12-2022

Place: Phagwara (Punjab), India

Prepared by
(Administrative Officer-Records)

Dr. Himanshu Singh
Organizing Secretary, ICBB, 2022

Dr. Neeta Raj Sharma
Convener, ICBB, 2022

Training- 1

S.A.S. NAGAR

आज़ादी का अमृत महोत्सव

SERB INDIA

One Day Research Facility Training Programme

This is to certify that ..... Shivani Dogra ..... has participated in Quiz Competition held during One Day Research Facility Training Programme entitled "Tools & Techniques in Protein Science" under Scientific Social Responsibility (SSR) Policy of SERB (Science & Engineering Research Board) organized on 25.05.2022 at NIPER S.A.S. Nagar. He/She was awarded the..... Third .....prize.

Ipsita Roy
Prof. Ipsita Roy
Coordinator

Prof. Arvind K Bansal
Dean

Dulal Panda
Prof. Dulal Panda
Director