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FORMULATION AND EVALUATION OF ANTI MICROBIAL POLYHERBAL SPRAY

Dr. M. Senthilraja*[1], Mr. K. Tholkappiyan[2], Ms. M. Abinaya[3], Ms. M. Deepika[4], Ms. S. Illakiya[5], Mr. K. Navarasan[6]

[1]Principal, Department of Pharmaceutical Chemistry, P.S.V College of Pharmaceutical Science and Research, Krishnagiri-635002, India.

[2,3,4,5,6]IV Year B.Pharm Students, Department of Pharmacognasy, P.S.V College of Pharmaceutical Science and Research, Krishnagiri-635002, India.

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***Corresponding Author**

Dr. M. Senthilraja

Principal, Department of Pharmaceutical Chemistry, P.S.V College of Pharmaceutical Science and Research, Krishnagiri-635002, India.

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ABSTRACT

This study developed two polyherbal antimicrobial sprays using *Pongamia pinnata*, *Azadirachta indica*, *Origanum vulgare and Clitoria ternatea*. Ethanolic (F1) and decoction (F2) extracts were prepared via Soxhlet and aqueous boiling, respectively. The aim was to compare their physicochemical properties and antimicrobial efficacy. Evaluations included organoleptic qualities, pH, evaporation time, spray angle, stability, and leak test. Antimicrobial activity was assessed gainst Staphylococcus aureus, Escherichia coli, and Candida albicans using disc diffusion. Both formulations were stable, skin-compatible (pH: F1=5.8, F2=6.4), non-sticky, and dried rapidly. Antimicrobial results showed that the decoction-based spray (F2) exhibited stronger antibacterial activity, with a 19 mm inhibition zone against S. aureus and 14 mm against E. coli. The ethanolic spray (F1) showed moderate broad-spectrum activity. Both displayed limited antifungal effect against C. albicans. In conclusion, the decoction-based polyherbal spray demonstrated superior antibacterial performance while maintaining optimal topical characteristics. This suggests aqueous extraction effectively harnesses antimicrobial phytoconstituents, positioning the formulation as a promising natural alternative to synthetic antimicrobials for managing skin infections and wound contamination. Further studies on long-term stability

and in vivo efficacy are warranted.

KEYWORDS: Pongamia pinnata, Azadirachta indica (Neem), Origanum vulgare (Oregano), Clitoria ternatea, Disc Diffusion Method, Topical Antimicrobial Spray.

1. INTRODUCTION

Skin infections and microbial contamination of wounds remain a significant concern in both hospital environments and community healthcare settings. Although the skin functions as a natural protective barrier, it is susceptible to invasion by pathogenic microorganisms when damaged. Minor injuries such as cuts, burns, abrasions, and surgical incisions can serve as entry points for microbes, potentially leading to infection, inflammation, and delayed und healing if not appropriately managed. In recent years, topical antimicrobial sprays have gained considerable attention due to their ease of application and enhanced patient compliance. These sprays are liquid formulations intended for application on the skin surface to inhibit or eliminate pathogenic microorganisms. One of the major advantages of topical sprays is their ability to deliver medication uniformly over the affected area without direct contact, thereby minimizing pain and reducing the risk of secondary contamination. Such formulations are commonly used for wound care, burns, post-surgical sites, and infected skin conditions.[1,2] Growing concerns regarding the safety and long-term use of synthetic topical antimicrobial agents have encouraged the development of herbal-based alternatives. Prolonged use of synthetic antimicrobials is often associated with adverse effects such as skin irritation, hypersensitivity reactions, delayed healing, and the development of antimicrobial resistance.[3] In contrast, herbal extracts contain multiple bioactive phytoconstituents that act synergistically to provide antimicrobial, anti-inflammatory, and antioxidant effects. This multi-target action reduces the likelihood of resistance development while promoting natural wound healing. Herbal topical antimicrobial sprays exert their effects through various mechanisms, including disruption of microbial cell membranes, inhibition of enzymatic activity, interference with protein synthesis, and suppression of essential metabolic pathways.[4] Additionally, certain plant-derived compounds help reduce inflammation and oxidative stress at the site of application, thereby supporting tissue repair and regeneration. The effectiveness of these sprays depends on factors such as extract concentration, formulation stability, contact time, and skin type. Topical antimicrobial sprays are widely used in hospitals, households, and first-aid settings. In clinical practice, they are employed for wound management and infection prevention, while in domestic use they are applied to minor

cuts, burns, and skin irritations.[5] Their non-greasy nature, rapid drying property, and convenience of use significantly improve patient comfort and adherence to treatment. From a pharmaceutical perspective, the formulation and evaluation of herbal topical antimicrobial sprays require careful consideration of parameters such as pH, viscosity, spray characteristics, stability, and antimicrobial efficacy.[6] Dermatological safety evaluation is essential to ensure that the formulation does not cause irritation or sensitization. Scientifically validating antimicrobial activity is crucial to justify therapeutic use. Therefore, herbal topical antimicrobial sprays represent a safe, effective, and environmentally friendly approach for managing skin infections. The present research focuses on the formulation, evaluation, and antimicrobial effectiveness of a herbal extract-based topical antimicrobial spray.[4,7]

1.1. Ideal Properties of a Topical Antimicrobial Spray

- **Broad-Spectrum Activity:** The formulation should be effective against a wide range of microorganisms, including bacteria and fungi commonly involved in skin infections.[1,2]
- **Safety and Non-Toxicity:** It should be non-irritating, non-allergenic, and safe for repeated topical application.[4]
- **Rapid Antimicrobial Action:** The spray should quickly reduce microbial load at the site of infection to prevent disease progression.[2,5]
- **Effective Penetration:** The formulation should adequately reach superficial infection sites, particularly in minor wounds and abrasions.[1,4]
- **Stability:** The product should remain physically and chemically stable during storage without loss of antimicrobial activity.[5]
- **Non-Greasy and Fast-Drying:** It should leave no sticky residue and provide comfort to the user.[2,5]
- **Eco-Friendly Nature:** Herbal formulations should ideally be biodegradable and environmentally safe.[7]

1.2. Advantages of Topical Antimicrobial Spray

- **Ease of Application:** Can be applied without direct contact with the wound, reducing the risk of contamination.[1,2]
- **Uniform Distribution:** Ensures even coverage over the affected skin area.[2,4]
- **Prevention of Secondary Infection:** Highly effective in reducing microbial growth in cuts, burns, and abrasions.
- **Improved Patient Compliance:** Non-greasy, painless, and quick-drying nature enhances

user acceptance.[4]

- **Safe for Repeated Use:** Herbal-based sprays are generally safe and suitable for repeated application.
- **Environmentally Safe:** Biodegradable herbal ingredients reduce environmental impact.[4,5]
- **Enhanced Healing:** Many plant-derived compounds promote healing through anti-inflammatory and antioxidant actions.[1,4]

1.3. Disadvantages of Topical Antimicrobial Spray

- **Limited Depth of Action:** May be less effective in deep-seated or chronic infections that require systemic therapy.[4,5]
- **Short Duration of Effect:** Antimicrobial activity may be temporary, necessitating frequent application.[2]
- **Formulation Variability:** Herbal extracts may show variation in composition, affecting consistency and stability.[1,3]
- **Risk of Allergic Reactions:** Certain individuals may exhibit sensitivity to specific herbal components.
- **Cost Consideration:** Herbal formulations may be slightly more expensive than conventional synthetic products.
- **Adjunct Therapy Requirement:** Severe infections may require additional systemic or topical treatment.[2,7]

1.4. FUNGAL INFECTION

Fungi are a diverse group of eukaryotic organisms that encompass yeasts, molds, and dimorphic species. They can be found in various natural habitats such as soil, plants, decaying organic matter, and even as part of the normal human microbiota. While the majority of fungi are non- pathogenic and contribute to the ecosystem in beneficial ways, there are a small number of species that have the potential to cause diseases in humans, known as mycoses. These infections can range from mild superficial skin conditions to severe, life-threatening systemic diseases. Unlike bacteria, fungi possess a true nucleus, chitinous cell wall, and membranes containing ergostero.[8] Depending on the extent of the infection, fungal diseases are broadly classified into categories such as superficial, subcutaneous, systemic, and opportunistic mycoses. Superficial mycoses primarily affect the skin, hair, and nails, while subcutaneous infections involve deeper layers of the skin.

Systemic infections target internal organs like the lungs and brain, whereas opportunistic infections occur when normally non-harmful fungi invade individuals with weakened immune systems.[9] Some of the key fungal pathogens of medical significance include Candida albicans, Aspergillus niger, Trichophyton rubrum, Microsporum gypseum, Epidermophyton floccosum, Cryptococcus neoformans, and Histoplasma capsulatum. Each of these organisms possesses unique characteristics, thrives in specific ecological niches, and manifests distinct disease symptoms in humans.[9]

1.4.1. Candida albicans

Candida albicans is a type of fungus that typically resides in the mouth, digestive tract, and vaginal lining without causing any harm. However, when the immune system is weakened, or in conditions like diabetes, pregnancy, or prolonged use of antibiotics, it can turn into a pathogen and lead to candidiasis.[10] This infection can manifest as oral thrush, vaginal yeast infection, skin candidiasis, or even a more serious systemic disease. C. albicans has the ability to transform between single cells and long, branching structures, which helps it invade tissues.[11] Its virulence is attributed to its capacity to stick to host cells, create biofilms, and produce enzymes like proteases and phospholipases.[12] Given that candidiasis is one of the most widespread fungal infections globally, Candida albicans continues to be a significant focus of both clinical and experimental research as a human pathogen.[13]

1.4.2. Aspergillus niger

Aspergillus niger, a common filamentous mold found in soil, organic matter, and indoor spaces, reproduces by generating small black conidia that can easily become airborne and be breathed in by people. While a healthy immune system can typically clear these spores, individuals with weakened immune systems or asthma are at risk of developing aspergillosis due to A. niger. This fungal infection often begins in the respiratory system and can lead to conditions such as pulmonary aspergillosis, sinusitis, or allergic bronchopulmonary aspergillosis. When cultured, A.niger forms distinct black colonies, and microscopic examination reveals conidial heads and septate hyphae. Certain Aspergillus species are capable of producing mycotoxins, which can worsen the severity of the associated diseases. Due to its widespread presence and prolific spore production, Aspergillus niger is a focal point of research in the medical and pharmaceutical fields.[14]

1.4.3. Trichophyton rubrum

Trichophyton rubrum is a dermatophytic fungus responsible for a large proportion of

superficial skin infections in humans. It colonizes keratinized tissues such as the stratum corneum of the skin, hair, and nails. The infections caused by this fungus include tinea pedis (athlete's foot), tinea corporis (ringworm), and onychomycosis (nail infection). *T. rubrum* invades keratinized cells by secreting keratinase enzymes that digest keratin, allowing the fungus to grow within the epidermis. Colonies typically appear white to pinkish with a red pigment on the reverse when cultured on Sabouraud dextrose agar. These infections are highly contagious but rarely invasive. Because of its prevalence and stable growth, *T. rubrum* is widely used in dermatological and antifungal susceptibility studies.[15]

1.4.4. Microsporum gypseum

Microsporum gypseum is a geophilic dermatophyte commonly found in soil. It can infect humans and animals through direct contact with contaminated soil or fomites. In humans, it causes tinea corporis, tinea capitis, and other cutaneous lesions characterized by circular, scaly, and itchy patches. Morphologically, *M. gypseum* produces rough-walled, spindle-shaped macroconidia and forms buff-colored, powdery colonies in culture. The infection is usually limited to the outer keratin layer of the skin and is not systemic. Because of its well- defined morphology and predictable infection pattern, *Microsporum gypseum* is a standard organism used in mycological and antifungal testing laboratories.[16]

1.4.5. Epidermophyton floccosum

Epidermophyton floccosum is another dermatophytic fungus that causes superficial infections of the skin and nails. It is responsible for tinea cruris (jock itch), tinea pedis, and tinea unguium, but it does not infect the hair. The organism is anthropophilic, meaning it primarily infects humans, and transmission occurs via direct contact or contaminated objects such as towels and clothing. Colonies of *E. floccosum* are olive-green or khaki-colored with a suede-like texture, and it produces smooth, club-shaped macroconidia. The infections are generally mild but can be persistent if untreated. Because of its clinical frequency and ease of culture, *Epidermophyton floccosum* is one of the principal species used in laboratory evaluation of antifungal compounds.[17]

1.4.6. Cryptococcus neoformans

Cryptococcus neoformans is an encapsulated yeast that causes cryptococcosis, a potentially fatal systemic fungal infection. It is found in soil contaminated with pigeon or bird droppings and is acquired mainly through inhalation of infectious spores. After entering the lungs, the fungus can disseminate to the central nervous system, resulting in cryptococcal meningitis.

This disease is particularly common in patients with advanced HIV/AIDS or those receiving immunosuppressive therapy. *C. neoformans* is distinguished by its thick polysaccharide capsule, which protects it from phagocytosis and contributes to its virulence. Microscopically, it appears as round or oval budding yeast cells surrounded by a clear capsule visible with India ink staining. Because of its importance in opportunistic infections, *C. neoformans* serves as a key model organism in studies of fungal pathogenesis.[18]

1.4.7. Histoplasma capsulatum

Histoplasma capsulatum is a dimorphic fungus that exists as a mold in the environment and as a yeast in host tissues. It causes histoplasmosis, a respiratory infection acquired by inhalation of spores from soil contaminated with bird or bat droppings. In healthy individuals, the infection may be mild or asymptomatic, resembling influenza, but in immune compromised patients it can become disseminated, affecting multiple organs including the liver, spleen, and bone marrow. The transition from mold to yeast form within the host is a crucial factor in its pathogenicity. In culture, it produces septate hyphae with microconidia and tuberculate macroconidia. Because *H. capsulatum* demonstrates both environmental and parasitic forms, it is used extensively in research on fungal dimorphism and systemic mycoses.[19]

1.5. BACTERIAL INFECTION

Bacterial infections are caused by the invasion and multiplication of pathogenic bacteria inhe human body, leading to tissue damage, inflammation, and disease. Bacteria are microscopic, single-celled organisms that exist everywhere in the environment, including oil, water, air, and the human body. While many bacteria are harmless or beneficial and form part of the normal flora of the skin, intestine, and respiratory tract, certain species possess virulence factors that enable them to cause infections. Bacterial infections occur when the natural defence mechanisms of the body, such as intact skin, mucous membranes, immune cells, and antimicrobial secretions, are compromised. This may happen due to cuts, burns, wounds, surgical procedures, poor hygiene, chronic diseases, malnutrition, or weakened immunity. Once bacteria enter the body, they adhere to tissues, multiply rapidly, and release toxins or enzymes that damage host cells, resulting in clinical symptoms.[20,21] The severity of a bacterial infection depends on several factors, including the type and virulence of the bacteria, the number of organisms invading the body, the site of infection, and the immune status of the individual. Bacterial infections may be localized, such as boils or abscesses, or

systemic. Common manifestations include redness, swelling, pain, pus formation, fever, and delayed wound healing. In recent years, bacterial infections have become more difficult to treat due to the increasing problem of antibiotic resistance. Many bacteria have developed resistance mechanisms such as enzyme production, altered drug targets, and efflux pumps, making conventional antibiotics less effective. This has increased the need for preventive strategies, alternative therapies, and novel antimicrobial agents.[22,23]

1.5.1. Gram-Positive Bacteria

Gram-positive bacteria are characterized by a thick peptidoglycan layer in their cell wall and the absence of an outer membrane. This structure allows them to retain the crystal violet stain during Gram staining, appearing purple under a microscope. Gram-positive organisms are commonly found on the skin and mucous membranes and are among the most frequent causes of skin and soft tissue infections.[24]

These bacteria often cause infections when they gain entry through minor skin injuries, hair follicles, or surgical wounds. Their ability to produce toxins, enzymes, and adhesins contributes to their pathogenicity.[25]

1.5.1.1. Staphylococcus aureus

Staphylococcus aureus is one of the most important and commonly encountered bacterial pathogens in humans. It is normally present on the skin and in the nasal passages butbecomes pathogenic when it enters deeper tissues. This organism produces several virulence factors, including coagulase, hemolysins, and toxins, which enable it to evade the immune system and cause tissue destruction. Clinically, *S. aureus* is responsible for a wide range of skin infections such as boils, furuncles, carbuncles, abscesses, impetigo, cellulitis, and infected wounds. A hallmark feature of *S. aureus* infection is pus formation due to intense inflammatory response. In severe cases, the bacteria may spread into the bloodstream, leading to bacteremia, septicemia, or toxic shock syndrome.[26] The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has further complicated treatment and increased hospital-acquired infections.[27]

1.5.1.2. Streptococcus pyogenes

Streptococcus pyogenes is a highly virulent Gram-positive bacterium that primarily infects the skin and soft tissues. It produces enzymes such as streptokinase, hyaluronidase, and exotoxins that allow rapid spread through tissue layers. Unlike *Staphylococcus aureus*,

streptococcal infections often produce less pus but cause extensive tissue inflammation. This organism is responsible for skin infections such as erysipelas, impetigo, cellulitis, and necrotizing fasciitis. Necrotizing fasciitis, also known as flesh-eating disease, is a life-threatening condition characterized by rapid tissue destruction, severe pain, swelling, and systemic toxicity. Early diagnosis and prompt treatment are essential to prevent complications.[28]

1.5.1.3. Corynebacterium Species

Corynebacterium species are part of the normal skin flora and usually exist without causing harm. However, under conditions such as poor hygiene, excessive sweating, open wounds, or compromised immunity, they may become opportunistic pathogens. These bacteria can cause mild skin infections, inflammation of sweat glands, and wound contamination. Although infections caused by *Corynebacterium* species are generally less severe compared to other Gram- positive bacteria, they can delay wound healing and contribute to secondary infections, particularly in hospitalized patients.[29]

1.5.2. Gram-Negative Bacteria

Gram-negative bacteria differ structurally from Gram-positive bacteria due to the presence of a thin peptidoglycan layer and an outer membrane containing lipopolysaccharides (LPS). This outer membrane acts as a protective barrier, making these bacteria more resistant to antibiotics, disinfectants, and environmental stress. Gram-negative bacteria are commonly associated with hospital-acquired infections, chronic wounds, burns, and infections in immune compromised individuals. Their endotoxins play a major role in causing inflammation and systemic complications.[30]

1.5.2.1. Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium widely found in water, soil, and moist environments. It commonly infects burn wounds, surgical sites, ulcers, and moist skin areas. The organism produces pigments such as pyocyanin, giving infected wounds a greenish-blue color and a distinctive foul odor. This bacterium is highly resistant to many antibiotics and is capable of forming biofilms, which protect it from immune responses and antimicrobial agents. Infections caused by *P. aeruginosa* are often chronic and difficult to treat, especially in hospitalized or critically ill patients.[31]

1.5.2.2 Escherichia coli

Escherichia coli is a normal inhabitant of the gastrointestinal tract and plays a role in maintaining gut health. However, when it contaminates wounds or enters sterile body sites, it can cause infections. *E. coli* is commonly involved in secondary wound infections, urinary tract infections, and abdominal infections. Certain pathogenic strains of *E. coli* produce oxins that cause severe inflammation, tissue damage, and systemic illness. In wound infections, *E. coli* may contribute to delayed healing and increased risk of complications.[32]

1.5.2.3. Klebsiella and Proteus Species

Klebsiella and *Proteus* species are opportunistic Gram-negative bacteria frequently ssociated with hospital-acquired infections. These organisms commonly infect wounds, burns, and surgical sites, particularly in patients with weakened immune systems or prolonged hospital stays. *Klebsiella* species are known for their thick capsule, which protects them from phagocytosis, while *Proteus* species exhibit swarming motility that aids in tissue invasion. Both organisms show increasing resistance to multiple antibiotics, making their infections challenging to manage.[32]

2. DRUG PROFILE

2.1. Pongamia Pinnata

Description: Pongamia pinnata (L.) Pierre, also known as Indian beech or Karanja in Hindi, is a medium-sized perennial tree belonging to the Fabaceae family. It is widely distributed in India, Australia, Bangladesh, and China. This tree has been valued in traditional medicinal systems for its ability to treat various human ailments and provide nutritious food. Pongamia pinnata contains a variety of beneficial compounds such as alkaloids, flavonoids, tannins, hormones, glycosides, karangin, glabrin, kanugin, and fixed oils, among other phytoconstituents. Traditionally, it has been used in Ayurveda and Siddha, the Indian medicinal systems, for its anti-inflammatory, antioxidant, and analgesic properties, as well as its ability to treat conditions such as diarrhea, fungal infections, and ulcers. In addition to its medicinal uses, Pongamia pinnata is also valued for its multipurpose advantages and is considered a potential source of biodiesel. The seeds of this tree contain approximately 28-34% oil, which is high in polyunsaturated fatty acids. This makes it a promising candidate for sustainable biofuel production.[33,34,35]

2.1.1. Active Constituents

Major phytochemical constituents isolated from *Pongamia pinnata* include

- Flavonoids: Pongamol, Karanjin, Pinnatin, Kanugin, Pongapin, and Glabrin
- Furanoflavones: Karanjin and Pongapin (main bioactive markers)
- Fixed oil: Pongam oil (contains oleic, linoleic, stearic, and palmitic acids)
- Other components: Sterols, tannins, triterpenoids, saponins, and alkaloids.[34,38]

Fig. 1: *Pongamia pinnata.*

2.1.2. General Medicinal Use

Anti-inflammatory Activity, Antimicrobial Properties, Skin-relatedUses, Antidiabetic Potential, Hepatoprotective Activity, Anti-inflammatory & Analgesic Uses, Gastrointestinal Uses, Antioxidant Effects, Antiparasitic Action, Wound Healing & Tissue Repair.[33,34,36]

2.1.3. Pharmacological Properties

- **Anti Microbial Activity**

The ethanolic extract of ***Pongamia pinnata*** exhibits significant antimicrobial activity against a broad range of microorganisms. Studies have reported strong inhibitory effects against Gram- positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, as well as Gram-negative bacteria including *Escherichia coli* and *Pseudomonas aeruginosa*. The antifungal activity of the ethanolic extract has also been demonstrated against *Candida albicans* and *Aspergillus* species. This enhanced antimicrobial efficacy is mainly attributed to the presence of flavonoids and furanoflavones such as **karanjin, pongamol, kanugin, and glabrin**, which disrupt microbial cell membranes and interfere with enzyme systems.[37] In contrast, the **aqueous extract (water decoction)** of *Pongamia pinnata* shows **moderate to mild antimicrobial activity**. The activity is primarily due to water-soluble phytoconstituents such as tannins, glycosides, and phenolic compounds. Although less potent than ethanolic extracts, aqueous preparations still exhibit inhibitory effects against certain Gram-positive bacteria and are traditionally used for external applications and mild infections.[38]

2.2. CLITORIA TERNATEA

Clitoria ternatea L., commonly known as Butterfly Pea or Aparajita, is a perennial herbaceous plant belonging to the family Fabaceae. It is native to tropical regions of Asia, particularly India, and is widely used in traditional systems of medicine such as Ayurveda and Unani. The plant is known for its distinctive blue flowers and its diverse pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and wound-healing effects.[39,40]

Fig. 2: *Clitoria ternatea.*

2.2.1. General Medicinal Use

Anxiolytic, Antidepressant, Antioxidant, Anti-inflammatory, Antidiabetic, Antimicrobial, Analgesic, Anticonvulsant, Hepatoprotective, Diuretic, Wound-healing, Anti-asthmatic, Neuroprotective.

2.2.2. Active Constituents

The plant contains a variety of bioactive phytochemicals such as flavonoids (kaempferol, quercetin, myricetin), triterpenoids, saponins, anthocyanins (ternatins), and phenolic compounds. These compounds are primarily responsible for its pharmacological activities, especially antimicrobial and antioxidant properties.[38,40]

2.2.3. Pharmacological Properties

- **Antimicrobial Activity**

Several studies have demonstrated that Clitoria ternatea exhibits broad-spectrum antimicrobial activity. The ethanolic extract of its leaves has shown significant inhibition against common skin pathogens, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. The presence of flavonoids and phenolic compounds is believed to cause disruption of microbial cell walls, leading to leakage of cellular contents and inhibition of microbial growth.[42] Aqueous decoction extracts exhibit inhibitory activity against both Gram-positive and Gram-negative bacteria.[40,41]

2.3. Azadirachta Indica (Neem)

Azadirachta indica A. Juss., commonly known as Neem, is a rapidly growing evergreen tree that is native to the Indian subcontinent and is widely celebrated for its remarkable medicinal**,** agricultural, and environmental significance. It belongs to the family eliaceae and has been utilized for centuries in traditional systems of medicine such as Ayurveda, Siddha, and Unani. Neem is often referred to as a village pharmacy due to ts extensive therapeutic potential, encompassing antimicrobial, antifungal, antiviral, antipyretic, anti-inflammatory, and antioxidant properties. All parts of the tree—leaves, bark, seeds, fruits, flowers, and roots— are harnessed for their medicinal and industrial benefits. Additionally, Neem is highly prized for its capacity to cleanse the air, manage ests, and enhance soil fertility. This makes Neem an invaluable resource for diverse applications, benefiting both human health and the environment.[43,44]

Fig. 3: ***Azadirachta indica.***

2.3.1. Active Constituents

- **Limonoids:** Azadirachtin, Nimbin, Nimbidin, Nimbidol, Salannin, Meliantriol
- **Flavonoids:** Quercetin, Kaempfero
- **Tannins and Phenolic Compounds:** Gallic acid, Catechin
- **Sterols:** β-Sitosterol, Stigmasterol
- **Saponins and Alkaloids:** Margosine, Gedunin, Mahmoodin
- **Fixed Oil:** Neem oil rich in oleic, stearic, palmitic, and linoleic acids.[44,45]

2.3.2. General medicinal use

Antibacterial, Antifungal, Antiviral, Anti-inflammatory, Antioxidant, Antidiabetic, Antimalarial, Anticancer, Antipyretic, Analgesic, Hepatoprotective, Immunomodulatory, Antiparasitic, Gastroprotective, Wound-healing, Anti-ulcer, Antiseptic, Insecticidal, Anti-plaque / Dental protective.

2.3.3. Pharmacological Properties

- Antimicrobial Activity

Neem leaf extracts, particularly ethanolic and aqueous preparations, exhibit potent antimicrobial effects against a wide range of Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The activity is attributed mainly to compounds like nimbidin and nimbin, which disrupt bacterial cell membranes and inhibit enzyme systems. Studies have demonstrated neem leaf extracts as effective natural alternatives to synthetic antibiotics for topical and oral infections.[46]

- Antifungal Activity

The antifungal potential of neem leaf extract has been reported against dermatophytes and pathogenic fungi such as *Candida albicans*, *Aspergillus niger*, and *Trichophyton rubrum*. The active limonoids and flavonoids interfere with fungal cell wall synthesis and ergosterol production, leading to inhibition of fungal growth. Neem-based formulations are commonly used in the treatment of skin fungal infections, athlete's foot, and dermatophytosis.[47]

2.4. Origanum Vulgare

Origanum vulgare, commonly known as Oregano or Wild Marjoram, is a perennial aromatic herb belonging to the family Lamiaceae. It is native to the Mediterranean region and Western Asia, and now cultivated worldwide for its culinary and medicinal value. The plant grows up to 30–90 cm in height with erect, branched, and quadrangular stems, a typical feature of the mint family. The leaves are opposite, simple, ovate to elliptic, and covered with fine hairs; they contain numerous glandular trichomes that secrete essential oils responsible for oregano's strong aromatic and spicy fragrance. The flowers are small, purple or pink, and borne in terminal clusters during the summer season. The fruit is a tiny brown nutlet, containing small seeds used for propagation. Oregano thrives well in sunny, dry climates and well-drained soils, and it is drought-tolerant. Phytochemically, oregano is rich in essential oils such as carvacrol, thymol, p-cymene, and γ-terpinene, along with flavonoids (apigenin, luteolin, quercetin) and phenolic acids (rosmarinic acid, caffeic acid). These compounds contribute to its strong antimicrobial, antifungal, antioxidant, anti-inflammatory, and antiviral activities. In traditional and modern medicine, oregano leaves and their essential oil are used for treating respiratory tract infections, skin diseases, digestive problems, and fungal infections. Due to its potentantimicrobial and preservative nature, oregano is also widely used

in herbal formulations, antiseptic sprays, and cosmetic preparations.[48,54]

Fig. 4: *Origanum vulgare.*

2.4.1. Active Constituents

Oregano leaves are rich in volatile and phenolic compounds, primarily: Essential oils: Carvacrol, Thymol, p-Cymene, γ-Terpinene, Linalool, Borneol Phenolic acids: Rosmarinic acid, Caffeic acid, Ferulic acid.

Flavonoids: Quercetin, Apigenin, Luteolin Tannins and Terpenoids.[50]

2.4.2. General Medicinal Use

Antimicrobial, antioxidant, cough, cold, flu, sore throat, respiratory health by reducingcongestion and clearing mucus, reduces bloating, anti-inflammatory, analgesic.

2.4.3. Pharmacological Significance

- Antifungal Activity

The essential oil and ethanolic extract of oregano are highly effective against various fungal species including *Candida albicans*, *Aspergillus niger*, *Trichophyton mentagrophytes*, and *Microsporum gypseum*. Carvacrol and thymol alter the integrity of fungal cell walls by interacting with ergosterol and membrane lipids, leading to cell lysis and inhibition of spore germination. Hence, oregano extract is often used in topical antifungal formulations for skin infections such as dermatophytosis and candidiasis.[51,52]

- Antimicrobial Activity

Oregano leaf extracts exhibit strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. The active components carvacrol and thymol disrupt bacterial cell membranes, causing leakage of ions and inhibition of enzyme systems. Studies have demonstrated significant inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes*. Ethanol and methanol extracts of oregano leaves have shown superior antibacterial activity compared to

aqueous extracts. The decoction of *Origanum vulgare* shows strong antimicrobial activity due to water-soluble phenolics like rosmarinic acid and flavonoids. It effectively inhibits bacteria such as *Staphylococcus aureus* and *E. coli*, and shows antifungal action against *Candida* species. The decoction also provides mild antiviral and antiparasitic effects, reduces microbial biofilm formation, and enhances immune response. Its anti- inflammatory properties further help relieve symptoms of infections, making it a useful natural remedy.[53]

3. FORMULATION

Table No. 5: Formulation.

Ingredient	Ethanolic Formulation (F1)	Decoction Formulation (F2)
Extract of *Pongamia pinnata*	5.00 mL	5.00 mL
Extract of *Azadirachta indica*	5.00 mL	5.00 mL
Extract of *Origanum vulgare*	5.00 mL	5.00 mL
Extract of *Clitoria ternatea*	5.00 mL	5.00 mL
Ethanol (95 %)	40.00 mL	40.00 mL
Propylene glycol	10.00 mL	10.00 mL
Glycerine	5.00 mL	5.00 mL
Polysorbate 80	2.00 mL	2.00 mL
Sodium benzoate	0.10 g	0.10 g
Ascorbic acid	0.05 g	0.05 g
Lavender oil	0.1 ml	0.1 ml
Distilled water (q.s.)	23.00 mL	23.00 mL

3.1. FORMULATION PROCEDURE

3.1.1. Procurement of material

The leaf were selected for the study having anti microbial property. Fresh parts of *Pongamia pinnata*, *Azadirachta indica*, *Origanum vulgare* and *Clitoria ternatea* were obtain from the local market.

3.1.2. FORMULATION NO: 1 (F1)

- **Prepare Ethanolic Extract**

The plant materials—*Pongamia pinnata, Azadirachta indica, Origanum vulgare,* and *Clitoria ternatea*—were first shade-dried, coarsely powdered, and 10–20 g of each powdered sample was accurately weighed. The powdered drug was placed into a cellulose thimble and inserted into the Soxhlet extractor. A round-bottom flask containing a hydroalcoholic solvent system of ethanol and water (210 ml:90 ml) was connected to the extractor, and a condenser was attached with continuous water circulation. The flask was heated using a heating mantle, allowing the ethanol to vaporize, condense, and percolate through the plant material. The extraction was continued for 6–10 cycles, or until the siphoned solvent appeared almost

colorless, indicating exhaustive extraction of ethanol-soluble phytoconstituents. After completion, the apparatus was allowed to cool and the extract was collected from the round-bottom flask. The solvent was then removed by evaporation on a water bath maintained below 50 °C to obtain a thick, concentrated ethanolic extract. The final extract was transferred into an airtight container and stored for further formulation studies.

Fig. 6: Ethanolic Plant Extract

- **Formulation Of Herbal Spray**

The formulation was prepared by first developing the aqueous and ethanolic phases separately. In the aqueous phase, propylene glycol and glycerine were mixed in a clean beaker, and sodium benzoate and ascorbic acid were added with continuous stirring until completely dissolved. For the ethanolic phase, equal volumes (2.5 mL each) of the four ethanolic plant extracts were combined in another beaker, followed by the addition of ethanol (40 mL) and polysorbate 80 (2 mL), and the mixture was stirred thoroughly to obtain a uniform solution. The aqueous phase was then slowly added to the ethanolic phase under constant stirring using a magnetic or mechanical stirrer for 10–15 minutes. Distilled water was gradually added to adjust the final volume to 100 mL, and the formulation was stirred gently for an additional 5 minutes to ensure homogeneity. Finally, the formulation was filtered through muslin cloth or Whatman No. 1 filter paper and transferred into sterilized spray bottles, which were appropriately labeled with the batch number, date of preparation, and storage instructions, including “Shake well before use.

3.1.3. FORMULATION NO: 2 (F2)

- **Prepare of the Decoction**

Take coarsely powdered plant materials in equal or desired proportions. Add water in a **1:10 ratio** (drug: water). Boil gently until the volume reduces to **one-third**. Cool and filter through

muslin cloth. Concentrate slightly to enhance potency if needed.

Fig. 7: Decoctions of plant extract.

- **Formulation Of Herbal Spray**

The formulation was prepared by first developing the aqueous and decoction phases separately. In a clean beaker, propylene glycol and glycerine were mixed, and sodium benzoate and ascorbic acid were added with continuous stirring until completely dissolved to form the aqueous phase. In another beaker, the decoction phase was prepared by combining equal volumes (2.5 mL each) of the four plant decoction extracts, followed by the addition of ethanol (40 mL) and polysorbate 80 (2 mL), and the mixture was stirred thoroughly to obtain a uniform solution. The aqueous phase was then slowly added to the decoction phase under constant stirring using a magnetic or mechanical stirrer for 10–15 minutes. Distilled water was added gradually to adjust the final volume to 100 mL, and the formulation was stirred gently for an additional 5 minutes to ensure homogeneity. Finally, the formulation was filtered through muslin cloth or Whatman No. 1 filter paper and transferred into sterilized spray bottles, which were appropriately labeled with the batch number, date of preparation, and storage instructions, including “Shake well before use.

Fig. 8: Ethanolic formulation (F-1) and Decoction Formulation (F-2).

4. Physicochemical Evaluation

4.1. Organoleptic evaluation

The formulation was carried out by selecting parameters such as colour, odour, appearance, and clarity. A small quantity of the formulation was placed in a clean, transparent container and observed under natural daylight to note the colour. The odour was assessed by gently

smelling the sample at a safe distance and recorded as characteristic or non-characteristic. he appearance of the formulation was visually examined for uniformity, presence of particulate matter, and any phase separation. The clarity of the sample was evaluated by observing it against both white and black backgrounds to detect any turbidity or lack of transparency.

4.2. Determination of PH

The pH of the formulation was determined using a calibrated digital pH meter. The instrument was first standardized using buffer solutions of pH 4.0, 7.0, and 9.2. A sufficient quantity of the formulation was taken and allowed to reach room temperature. The glass electrode was rinsed with distilled water, gently dried, and immersed into the sample. After allowing the reading to stabilize, the pH value was noted.

4.3. Determination of Evaporation time

The evaporation time of the formulation was determined by spraying a fixed quantity of the sample onto a clean, dry glass slide or marked skin-simulating surface maintained at room temperature. The time required for the formulation to completely evaporate, leaving no visible wetness, was recorded using a stopwatch. The test was carried out in triplicate under identical conditions, and the average evaporation time was calculated and reported as the evaporation time of the formulation.

4.4. Stickness Test

The stickiness of the spray formulation was evaluated by spraying a fixed quantity of the formulation onto a clean, dry glass slide or marked skin-simulating surface. After allowing the formulation to dry completely at room temperature, the surface was gently touched with a fingertip to assess the presence or absence of tackiness. The test was performed in triplicate, and the formulation was considered **non-sticky** if no adherence or discomfort was felt upon touch.

4.5. Spray Angle

The spray angle test is carried out by mounting the nozzle vertically on a suitable stand and connecting it to a pressurized fluid supply. A flat target screen or white sheet is placed at a fixed distance from the nozzle, perpendicular to the spray direction. The required operating pressure is set using a pressure regulator, and the fluid is allowed to flow until a steady spray is obtained. The spray is directed onto the target surface for a few seconds to produce a clear and uniform spray pattern. After stopping the flow, the outer edges of the spray pattern are

marked and the maximum width of the spray is measured. The distance between the nozzle tip and the target surface is also noted. The test is repeated several times under the same conditions to ensure accuracy, and the average values are used to calculate the spray angle. Spray angle (θ)=2tan−1(r\d) where **r** is the radius of the spray pattern and **d** is the distance between the nozzle and the paper. The test was performed in triplicate, and the average spray angle was calculated and recorded.

4.6 Leak Test

The leak test was performed to evaluate the integrity of the spray container. The filled spray containers were tightly closed and cleaned externally to remove any adhered formulation. Each container was then kept in an inverted position at room temperature for 24 hours. The containers were visually examined at regular intervals for any signs of leakage, wetness, or loss of contents. The absence of leakage indicated that the spray container passed the leak test.

5. Microbiological Evaluation

Nutrient agar for bacterial culture and Sabouraud dextrose agar for fungal culture were prepared according to standard compositions, adjusted to the appropriate pH, and sterilized by autoclaving. The sterilized media were aseptically dispensed into Petri dishes and stored under refrigeration until use. Microorganisms were inoculated onto the agar plates using the spread plate technique with a sterile L-shaped glass rod, followed by incubation at optimal temperature and duration for bacterial and fungal growth. Antimicrobial activity of the formulation was assessed using the disc diffusion assay. Sterile filter paper discs impregnated with the test formulation were placed on inoculated agar plates alongside positive and negative controls. Following incubation, zones of inhibition surrounding the discs were measured in millimeters. The diameter of these zones was used to determine the antimicrobial efficacy of the formulation against bacterial and fungal strains, with results categorized as sensitive, moderately sensitive, or resistant.

6. RESULT AND DISCUSSION

6.1. Physicochemical Evaluation

6.1.1. Organoleptic Evaluation

The spray formulation was found to be aesthetically acceptable with a uniform and clear appearance. No particulate matter or phase separation was observed, indicating good formulation stability.

Table No. 6: Organoleptic Evaluation.

Parameter	F1	F2
Colour	Light brown / pale yellow	Light brown / pale yellow
Odour	Lavender	Lavender
Appearance	Uniform	Uniform
Clarity	Yes	NO
Phase separation	Absent	Absent

6.1.2. Determination Of PH

The pH of the formulation was within the acceptable range for topical application, indicating skin compatibility.

Table 7: pH of Formulation.

SI.NO	FORMULATION	pH
1	F1	5.8
2	F2	6.4

Fig. 9: PH of F-1.

Fig. 10: PH of F-2

6.1.3. Evaporation Time

The formulation showed rapid evaporation, which is desirable for topical sprays.

Table 8: Evaporation Time.

SI.NO	FORMULATION	TIME
1	F1	100 sec
2	F2	120 sec

6.1.4. Stickiness Test

The formulation was found to be non-sticky after drying, indicating better patient acceptability.

Table 9: Stickiness Test.

SI.NO	FORMULATION	Result
1	F1	Non-sticky
2	F2	Non-sticky

6.1.5. Spray Angle

The spray pattern was uniform with an acceptable spray angle, indicating proper dispersion.

Table 10: Spray Angle.

SI.NO	Formulation	Distance from Nozzle (L) (mm)	Spray Width (W) (mm)	Calculated Spray Angle (°)
1	F1	110	90	33.4
2	F2	110	85	38.6

6.1.6. Leak Test

No leakage was observed during the 24-hour test period, confirming the integrity of the container.

Table 11: Leak Test.

SI.NO	FORMULATION	OBSERVATION TIME	RESULT
1	F1	24 HRS	No leakage
2	F2	24 HRS	No leakage

Fig. 11: Leak test of F-1.

Fig. 12: Leak test of F-2.

6.2. Biological Evaluation

The spray formulation exhibited effective antimicrobial activity against both bacterial and fungal strains.

Table No. 12: Antimicrobial Activity (Zone of Inhibition)

S. No.	Microorganism	Type	F 1 (Zone of nhibition, mm)	F 2 (Zone of nhibition, mm)	Positive Drug (mm)	Negative (m)
1	*Staphylococcus aureus*	Gram-positive (+)	8 ± 0.8	19 ± 0.7	27 ± 0.5	0mm
2	*Escherichia coli*	Gram-negative (−)	10 ± 0.6	14 ± 0.5	30 ± 0.6	0mm
3	*Candida albicans*	Fungi	8 ± 0.7	5 ± 0.6	18 ± 0.4	0mm

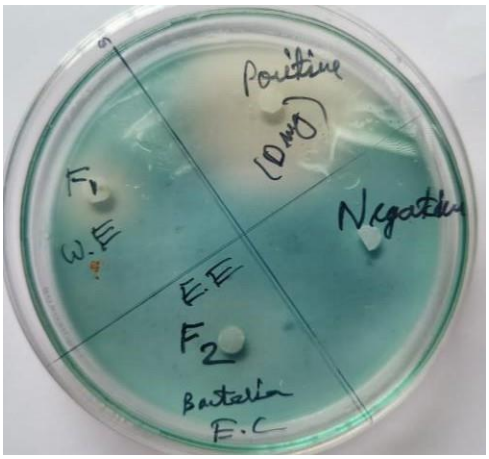

Fig. 13: *Staphylococcus aureus.*

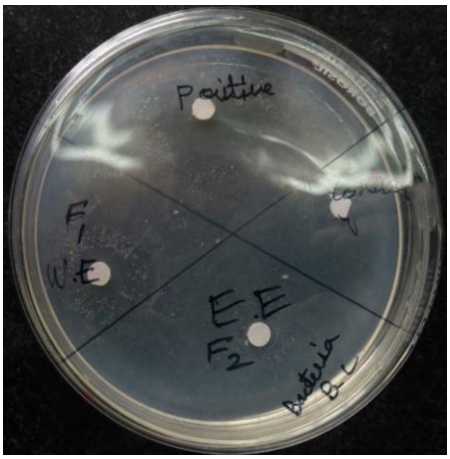

Fig. 14: *Escherichia coli.*

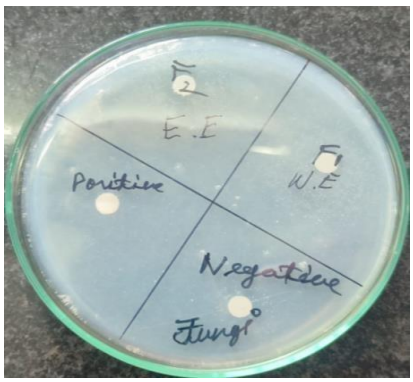

***Fig.15: Candida albicans* (Fungi).**

The antimicrobial activity Of formulation 1 And formulation 2 was evaluated by the disk diffusion method against *staphylococcus aureus*, *escherichia coli*, and *candida albicans*. formulation 1 showed mild antimicrobial activity with zones of inhibition of **8 ± 0.8 mm**

against *staphylococcus aureus*, **10± 0.6 mm** against *escherichia coli*, and **8 ± 0.7 mm** against *candida albicans*. Formulation 2 exhibited comparatively better antibacterial activity against *staphylococcus aureus* (**19 ± 0.7 mm**) and *escherichia coli* (**14 ± 0.5 mm**), but lower antifungal activity against *candida albicans* (**5 ± 0.6 mm**). The standard drug showed the highest zones of inhibition against all tested microorganisms, whereas the control showed **no zone of inhibition**, confirming that the antimicrobial activity was due to the active constituents present in the formulations.

7. CONCLUSION

In this study, we prepared two herbal spray formulas: an ethanolic formula (F1) and a decoction-based formula (F2). We used extracts from Pongamia pinnata, Azadirachta indica, Origanum vulgare, and Clitoria ternatea. Both formulations were stable, visually appealing, and suitable for skin application based on various evaluation factors, including appearance, pH, evaporation time, stickiness, spray angle, and leak tests. The pH levels of both formulations fell within the safe range for skin use, indicating good compatibility. Their quick evaporation and non- sticky texture suggest they are user-friendly and likely to encourage patient compliance. The even spray pattern and lack of leakage further supported their suitability for topical delivery. Microbial tests showed that both formulations had antimicrobial effects against the bacterial and fungal strains tested. Formulation 1 showed mild antimicrobial properties against Staphylococcus aureus, Escherichia coli, and Candida albicans. In contrast, Formulation 2 exhibited stronger antibacterial effects against Staphylococcus aureus and Escherichia coli, but it had lower antifungal activity against Candida albicans. The lack of inhibition in the negative control confirmed that the observed antimicrobial effects resulted from the active plant compounds in the formulations. Overall, the study concludes that the herbal spray formulations, especially the decoction-based one (F2), show promising antimicrobial properties and good physicochemical characteristics. These formulations could serve as effective natural options for topical antimicrobial use. However, further research, including stability testing, toxicity assessment, and clinical trials, is needed to confirm their safety, effectiveness, and therapeutic use.

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