

Polyherbal Gel Formulations and Investigations against Dermal Pathogens

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ABSTRACT:

Skin diseases are naturally occurring health problems that affects all ages from the infants to the elderly persons. Numerous skin problems cause adverse effect in innumerable ways. To conserve healthy body healthy skin is important. Different skin disease is reported that affect the skin adversely are cellulitis and cancer. Ancient documents state the use of different plants and their parts are used to cure skin problems. The curative properties of plants are exploited in formulation of natural drugs. In present scenario study on curative abilities of plants and plant products is one of the major thrust areas undertaken by researchers all over the world. Based on folklore knowledge in treating skin problems should be analyzed. Keeping this view a review of medicinal plants in formulation of gel and to evaluate the efficiency against skin diseases causing micro-organisms is attempted.

Keywords: Medicinal plants, Gel formulation, Natural drugs, Skin diseases, Microorganisms, Antimicrobial properties.

1. Introduction

Skin infections, caused by pathogens like *Propionibacterium acnes*, *Staphylococcus aureus*, *Trichophyton rubrum*, and *Candida albicans*, are widespread, leading to inflammation, discomfort, and diminished quality of life. While conventional treatments involving antibiotics and antifungal agents are widely used, they often result in antimicrobial resistance, adverse effects, and diminished efficacy over time. These challenges necessitate exploring safer, more effective alternatives. Herbal formulations, known for their bioactive compounds, biocompatibility, and minimal side effects, have emerged as promising options. Polyherbal gels, combining plant extracts, can offer synergistic effects, enhancing antimicrobial efficacy while combating drug-resistant strains. Such formulations represent an innovative, sustainable approach to addressing dermal infections and their associated challenges.

1.1 Overview of Skin Infections

Skin infections are common health issues affecting individuals of all ages, resulting from the invasion of various microorganisms, including bacteria, fungi, viruses, and parasites. These infections typically manifest as rashes, lesions, ulcers, or boils and can range from mild to severe, depending on the pathogen involved. Bacterial skin infections, such as *Staphylococcus aureus*-induced impetigo and cellulitis, are prevalent and often cause symptoms like redness, swelling, and pus formation (Buchholz et al., 2018). Fungal infections, including *Candida* and *Trichophyton rubrum*, can lead to conditions like athlete's foot,

ringworm, and candidiasis, often causing itching and scaling (Hay et al., 2014). Viral infections, such as herpes simplex and varicella-zoster, result in blisters and painful sores on the skin. Additionally, parasitic infections like scabies can cause intense itching and rashes (Ramos et al., 2021).

Infections can occur due to compromised skin barriers, such as cuts, wounds, or burns, as well as underlying conditions like diabetes or immunodeficiency. Poor hygiene, crowded living conditions, and inadequate sanitation further contribute to the spread of these infections. While some skin infections are self-limiting, others can cause significant discomfort, long-term scarring, or even life-threatening complications, particularly in immunocompromised individuals. Thus, effective management of skin infections is crucial for overall health.

1.2 Selection of Plant Species

The selection of plant species for polyherbal formulations is guided by their historical usage, phytochemical richness, and evidence-based antimicrobial efficacy. Referring to present study taken on account the plants *Eucalyptus globulus*, *Azadirachta indica* (Neem), *Bauhinia variegata*, *Mangifera indica* (Mango), and *Saraca asoca* those have shown promising dermatological applications with reference to literatures were considered to study the formulation and development of a novel polyherbal dermatological solution. *Eucalyptus globulus* is valued for its antimicrobial and anti-inflammatory properties (Ali et al., 2017). *Azadirachta indica* has been a cornerstone of traditional medicine for its broad-spectrum antimicrobial activities. *Bauhinia variegata* possesses wound-healing potential, while *Mangifera indica* is rich in antioxidants and phytochemicals beneficial for skin health. *Saraca asoca* is historically recognized for treating various skin disorders (Mahmood et al., 2021; Gupta et al., 2020). These plants collectively may offer a synergistic approach to combating skin pathogens and promoting dermal health.

2. Material and Methods

The materials and methods section delineates the systematic approach employed to achieve the objectives of this research, aimed at evaluating novel polyherbal gel formulations for their efficacy against dermal pathogens, including *Propionibacterium acnes*, *Staphylococcus aureus*, *Trichophyton rubrum*, and *Candida albicans*. This study incorporates a multidisciplinary methodology, encompassing plant-based phytochemical exploration, advanced formulation techniques, and microbial assessment protocols. Six plant species—*Eucalyptus globulus*, *Azadirachta indica*, *Bauhinia variegata*, *Mangifera indica*, and *Saraca asoca*—were selected for their known medicinal properties, particularly in the treatment of skin infections.

This integrative approach reflects the growing emphasis on plant-derived remedies for managing antimicrobial resistance and promoting safe, effective alternatives for dermatological care (Bhalodia & Shukla, 2011). The detailed protocols employed here are intended to standardize phytochemical-based formulations and optimize their therapeutic potential. The methodology followed in this study is discussed ahead in this chapter in details.

2.1 Collection of Plant Material

Collection of plant material in present study was done from the different localities of Bhopal. The plant material chosen in present study were considered based on recent researches and available literatures regarding their potential against microbial infections and their use as curative herbal remedies with reference to folks, regional knowledge and Ayurveda.

Table 2.1: List of Plant and Plant parts used for phytochemical extraction

S.N	Plants	Parts Used	Source Locality in Bhopal
1.	<i>Eucalyptus globulus</i>	Leaves	Barkatullah University Campus
2.	<i>Azadirachta indica</i>	Leaves	Saket Nagar
3.	<i>Bauhinia variegata</i>	Leaves	Shakti Nagar
4.	<i>Mangifera indica</i>	Leaves	Saket Nagar
5.	<i>Saraca asoca</i> .	Leaves	Barkatullah University Campus

2.2 Extraction of Phytochemical & Screening

The plant materials after collection, identification and approval were subjected to cleaning, shade drying and pulverization into fine powder before the process of extraction starts.

2.2.1 Defatting of plant material

Before applying extraction, the powdered plant materials were defatted by soaking it in petroleum ether at room temperature for 24 hours to remove any fatty, oily or lipid content from them. After defatting of plant material, the petroleum ether was removed by filtration and the crud drug is again dried that is to be extracted with distilled water and ethanol.

2.2.2 Soxhlet Extraction

A. Aqueous Extraction of Defatted Crud Drug

About 10-20 grams of defatted dried powder was subjected to Soxhlet extraction with 200 ml of distilled water as extraction solvent. Soxhlet process was carried out till the complete exhaustion of sample material at 80°C. The defatted fine powders of *Piper betle* leaves, *Myristica fragrans* nuts, *Psidium gunjuava* leaves, *Tinospora cardifolia* leaves and *Punica granatum* rinds were all subjected to soxhlet extraction with distilled water.

B. Ethanolic Extraction of Defatted Crud Drug

Like the aqueous extraction about 10-20 grams of defatted dried powder was subjected to Soxhlet extraction with 200 ml of 50% ethanol as extraction solvent till the complete exhaustion of sample material at 65°C. The defatted fine powders of *Eucalyptus globulus*, *Azadirachta indica*, *Bauhinia variegata*, *Mangifera indica*, and *Saraca asoca* were all subjected to Soxhlet extraction with 50% ethanol

2.2.3 Phytochemical Tests

A small portion of the dry extracts of each plant material used under present study were subjected to the phytochemical test using Harbourne's (1983) methods. The crude extracts were tested for presence of alkaloids, flavonoids terpenoids, tannins, saponins, and glycosides in this preliminary analysis.

Test for Alkaloids: About 1 ml of diluted extract was warmed with 2% H₂SO₄ for two minutes, filtered and few drops of Dragendorff's reagent added orange red precipitate indicates the presence of alkaloids.

Test for Flavonoids: 1 ml of diluted extract shaken with 5ml of distilled water and then a few drops of 10% lead acetate solution is added. A yellow or dirty white precipitate shows the presence of flavonoids.

Test for Terpenoids: About 1 ml of diluted extract was mixed with 2 ml chloroform (CHCl₃) and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface formed indicating the presence of terpenoids.

Test for Glycosides: The extracts hydrolyzed with HCl solutions and neutralized with NaOH solutions. A few drops of *Fehling* solution A and B were added. Red precipitate indicates the presence of glycoside. Another test use was *Benedict's test*, in which the filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for Tannins: Small quantity of extracts mixed with water, heated, filtered and ferric chloride added. A dark green solution indicates the presence of tannins.

Test for Saponins: About 1 ml of extract stock is shaken with 5ml of distilled water and then heated to boil frothing (appearance of creamy mix of small bubbles) shows the presence of saponins.\

2.3 Polyherbal Gel Preparation and Analysis

The polyherbal gel formulations were prepared using Carbopol-940 as the gel base, following modified protocols based on the investigations of Mohammad Haneefa KP et al. (2016), Muhammad Ubaid et al. (2016), and Krongrawa et al. (2018). A stock solution of crude phytochemical extracts was prepared at a concentration of 100 mg/ml in sterile distilled water, homogenized, and degassed using an ultrasonic bath. These stocks were subsequently incorporated into the gel base to achieve varied concentrations of extracts per gram of gel. The composition of the Carbopol-940 gel base is detailed in Table 3.2, comprising water (100 ml), Carbopol-940 (1 gm), methylparaben (200 mg), glycerol (1 ml), and plant extract as required. The gel formulations were developed with *Azadirachta indica* extract as a constant component due to its well-documented clinical properties and widespread application in skincare products. A 1:1 solution of 1 ml of 100 mg/ml stock extract from each test plant was prepared and added to the gel base in a volume of 1 ml per 100 ml of gel. The combinations of herbal extracts used for the polyherbal gel formulations are listed in Table 3.3, with formulations coded as Gel-A through Gel-D. These combinations included extracts of *A. indica*, *E. globulus*, *B. variegata*, *M.indica*, and *S. asoca*.

Table 1: Standard composition of Carbopol -940 gel base used for present study

S.N.	Composition	Amount
1.	Water	100 ml
2.	Carbopol -940	1 gm
3.	Methyl paraben	200 mg
4.	Glycerol	1 ml
5.	Plant extract	As required

Table 2: Combination of herbal extracts used to prepare Carbopol based formulate the polyherbal gel in present study.

S.N.	Formulation Code	Extract Combination
1.	Gel – A	<i>A. indica</i> + <i>E. Globulus</i>
2.	Gel – B	<i>A. indica</i> + <i>E. Globulus</i> + <i>B. variegate</i>
3.	Gel – C	<i>A. indica</i> + <i>E. Globulus</i> + <i>B. variegate</i> + <i>M. indica</i>
4.	Gel – D	<i>A. indica</i> + <i>E. Globulus</i> + <i>B. variegate</i> + <i>M. indica</i> + <i>S. asoca</i>

2.3.1 Antimicrobial Activity of Polyherbal Gel

The antimicrobial activity of the polyherbal gel formulations was evaluated against same standard microbial strains as above. The antimicrobial susceptibility assay was conducted for the selected polyherbal gel prepared.

For this, Mueller-Hinton Agar (MH) plates were prepared for bacterial cultures and Potato Dextrose Agar (PDA) plates for fungal cultures. Inoculum from the revived microbial cultures was seeded onto the

respective agar plates using sterile swabs. Approximately 50 µl of each polyherbal gel formulation was aseptically placed on the surface of pre-inoculated plates. The plates were incubated at 37°C for 24 hours for bacterial cultures and at 25 ± 3°C for 72 hours for fungal cultures.

The antimicrobial activity of the gel formulations was determined by measuring the zone of inhibition (ZOI) around each gel application site in millimeters. Observations were recorded, and the mean ZOI values were calculated for each formulation to assess its efficacy against the tested microbial strains.

3 Result and Discussion

3.1 Phytochemical Extraction & Qualitative Analysis

3.1.1 Yield of Extraction & Organoleptic Properties

The plant material chosen in present study were considered based on recent researches and available literatures regarding their potential against microbial infections and their use as curative herbal remedies with reference to folks, regional knowledge and Ayurveda (Kamboj, 2000; Grover *et al.*, 2002; Singh *et al.*, 2003; Fazal, *et al.*, 2014)

Determination of the percentage yield of extraction is one of the important parameters in this study since the cost of therapeutic substances or compounds largely depends on the quantity and availability of raw material and final product. Larger the quantity of phytochemical extracts presents less problems and efforts in further separation and purification of the constituent chemicals.

Table 1. Percentage yield and organoleptic properties of aqueous extracts of plant materials taken in present study.

S.N.	Plant Samples Used	% Yield	Organoleptic Properties			
			Colour	Texture	Smell	
1.	<i>Eucalyptus globulus</i>	32.26%	Chocolate Brown	Hard Sticky paste, rough texture	Intense, Aromatic, Unpleasant	
2.	<i>Azadirachta indica</i>	28.06%	Chocolate Brown	Hard Sticky paste, rough texture	Organic, Pleasant	
3.	<i>Bauhinia variegata</i>	32.13%	Dark Brown	smooth Sticky paste	Mild Organic, Pleasant	
4.	<i>Saraca asoca</i>	28.83%	Light Brown	smooth Sticky paste	Organic, Ethereal	
5.	<i>Mangifera indica</i>	27.23%	Chocolate Brown	smooth Sticky paste	Organic, Pleasant	

Table 2: Percentage yield and organoleptic properties of ethanolic extracts of plant materials taken in present study.

S.N.	Plant Samples Used	% Yield	Organoleptic Properties		
			Colour	Texture	Smell
1.	<i>Eucalyptus globulus</i>	27.41%	Dark brown	Hard sticky paste, smooth in appearance	Organic, Unpleasant

2.	<i>Azadirachta indica</i>	25.46%	Redish Brown	Hard sticky paste, rough texture	Organic, Pleasant
3.	<i>Bauhinia variegate</i>	27.53%	Brown	Smooth sticky paste,	Strong Organic, Pleasant
4.	<i>Saraca asoca</i>	26.53%	Redish brown	smooth sticky paste,	Organic, Etheral
5.	<i>Mangifera indica</i>	24.81%	Dark Brown	greasy sticky paste,	Organic, Pleasant

Quantitative Analysis of Phytoextracts and Polyherbal Gel Formulations

Quantitative analysis plays a pivotal role in evaluating the bioactive potential of plant extracts by determining the concentration of their Phyto-constituents.

Table:1. Estimation of total phenolic content in aqueous and ethanolic extracts for plant materials taken in present study.

S.N.	Plant Samples	Total phenolic content GAE (µg/mg of extract)	
		Plant Extracts	
		In Aqueous Extract	In Ethanolic Extract
1.	<i>Eucalyptus Globulus</i>	7.66	12.22
2.	<i>Azadirachta indica</i>	6.72	14.96
3.	<i>Bauhinia variegate</i>	9.92	14.16
4.	<i>Mangifera indica</i>	7.24	13.78
5.	<i>Saraca asoca</i>	8.64	17.06

The results confirm the suitability of the selected plants for antioxidant-based applications, with phenolic content correlating directly with potential bioactivity (Singleton et al., 1999). In earlier studies also, researchers conclude that higher content of polyphenolic compound is an indicator of having antioxidant potential also scientist found out that extracts prepared using methanol like highly polar solvents, shows high effectiveness as antioxidants (Tenguria et al., 2012; Altemimi, et al., 2017). This data is integral for evaluating the polyherbal gel formulations, as discussed in subsequent sections.

3.1.2 TPC and TFC estimation of prospected Polyherbal Topical Gel

The estimation of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) is crucial in evaluating the antioxidant potential and therapeutic efficacy of polyherbal formulations, such as topical gels. These phytochemicals are known for their wide range of biological activities, including anti-inflammatory, antimicrobial, and wound-healing properties (Saxena et al., 2013; Sharma et al., 2018). In the current study, both TPC and TFC were determined for a polyherbal gel formulation intended for topical application. The analysis of these bioactive compounds will provide valuable insight into the formulation's therapeutic properties and its potential for treating skin ailments (Brahmachari, 2017).

Table: 2. Results of estimation of total phenol and total flavonoid content within Gel-A to Gel-D formulations prepared in present study

S.N.	Formulations	TPC µg/ml	TFC µg/ml
1.	Gel-A	0.7134	0.1146
2.	Gel-B	0.7932	0.1346
3.	Gel-C	0.7885	0.1398
4.	Gel-D	0.8047	0.1386

The estimation of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in the polyherbal gel formulations (Gel-A to Gel-D) is crucial for determining their antioxidant and therapeutic properties. As shown in Table 4.9, the TPC and TFC values indicate the concentration of bioactive compounds, which contribute to the overall therapeutic efficacy of the formulations.

3.1.3. Antimicrobial Susceptibility Assay of Individual Extracts

The antimicrobial activity of aqueous and hydroethanolic extracts of five plant species (*Eucalyptus globulus*, *Azadirachta indica*, *Bauhinia variegata*, *Mangifera indica*, and *Saraca asoca*) was evaluated against four test microorganisms: *Staphylococcus aureus* (*S. aureus*), *Propionibacterium acnes* (*P. acnes*), *Candida albicans* (*C. albicans*), and *Trichophyton rubrum* (*T. rubrum*), as presented in Tables 4.10 and 4.11.

From the data, it is evident that *Eucalyptus globulus* demonstrated the highest antimicrobial activity among the plant extracts, with a zone of inhibition of 19 mm against both *S. aureus* and *C. albicans* in both aqueous and hydroethanolic extracts. This is in agreement with earlier studies that highlight the potent antimicrobial properties of *E. globulus*, attributed to its bioactive compounds such as eucalyptol and flavonoids (Cakir et al., 2004).

Table:3. Results of antimicrobial activity (Susceptibility) of individual phytochemical aqueous extracts against *Staphylococcus aureus* (ATCC-23235), *Propionibacterium acnes* (ATCC-11827), *Candida albicans* (MTCC-854) and *Trichophyton rubrum* (ATCC-28188) used in present study.

S.N.	Extract	Zone of inhibition (Φ in mm) against test microbes			
		<i>S. aureus</i>	<i>P. acnes</i>	<i>C. albicans</i>	<i>T. rubrum</i>
1.	<i>Eucalyptus globulus</i>	19 mm	10 mm	19 mm	Nil
2.	<i>Azadirachta indica</i>	13 mm	15 mm	09 mm	Nil
3.	<i>Bauhinia variegata</i>	08 mm	10 mm	13 mm	Nil
4.	<i>Mangifera indica</i>	18 mm	09 mm	12 mm	Nil
5.	<i>Saraca asoca</i>	08 mm	11 mm	10 mm	Nil

Table:4. Results of antimicrobial activity (Susceptibility) of individual phytochemical hydroethanolic extracts against *Staphylococcus aureus* (ATCC-23235), *Propionibacterium acnes* (ATCC-11827), *Candida albicans* (MTCC-854) and *Trichophyton rubrum* (ATCC-28188) used in present study.

S.N.	Extract	Zone of inhibition (Φ in mm) against test microbes			
		<i>S. aureus</i>	<i>P. acnes</i>	<i>C. albicans</i>	<i>T. rubrum</i>
1.	<i>Eucalyptus globulus</i>	19 mm	10 mm	19 mm	Nil
2.	<i>Azadirachta indica</i>	13 mm	15 mm	09 mm	Nil
3.	<i>Bauhinia variegata</i>	08 mm	10 mm	13 mm	Nil
4.	<i>Mangifera indica</i>	18 mm	09 mm	12 mm	Nil
5.	<i>Saraca asoca</i>	08 mm	11 mm	10 mm	Nil

Overall, these findings suggest that the tested plants possess antimicrobial potential, particularly *Eucalyptus globulus* and *Azadirachta indica*, which could be explored for the development of antimicrobial agents for skin infections and related conditions. Further studies are needed to isolate and characterize the active compounds responsible for these activities.

Table: 5. Results of microbial activity (MIC) of *Eucalyptus globulus* leaf extract against *Staphylococcus aureus* (ATCC-23235), *Propionibacterium acnes* (ATCC-11827), *Candida albicans* (MTCC-854) and *Trichophyton rubrum* (ATCC-28188).

S.N.	Aqueous Extract Conc. (mg/ml)	Zone of inhibition (Φ in mm) against test microbes			
		<i>S. aureus</i>	<i>P. acnes</i>	<i>C. albicans</i>	<i>T. rubrum</i>
1.	100	19 mm	10 mm	19 mm	N/A
2.	50	14 mm	08 mm	14 mm	N/A
3.	25	11 mm	00 mm	09 mm	N/A
4.	12.5	09 mm	00 mm	00 mm	N/A
5.	6.25	00 mm	00 mm	00 mm	N/A

Table: 6. Results of microbial activity (MIC) of *Mangifera indica* leaf extract against *Staphylococcus aureus* (ATCC-23235), *Propionibacterium acnes* (ATCC-11827), *Candida albicans* (MTCC-854) and *Trichophyton rubrum* (ATCC-28188).

S.N.	Aqueous Extract Conc. (mg/ml)	Zone of inhibition (Φ in mm) against test microbes			
		<i>S. aureus</i>	<i>P. acnes</i>	<i>C. albicans</i>	<i>T. rubrum</i>
1.	100	18 mm	09 mm	12 mm	N/A
2.	50	15 mm	00 mm	00 mm	N/A
3.	25	12 mm	00 mm	00 mm	N/A
4.	12.5	10 mm	00 mm	00 mm	N/A
5.	6.25	08 mm	00 mm	00 mm	N/A

Table: 7. Results of microbial activity (MIC) of *Azadirachta indica* leaf extract against *Staphylococcus aureus* (ATCC-23235), *Propionibacterium acnes* (ATCC-11827), *Candida albicans* (MTCC-854) and *Trichophyton rubrum* (ATCC-28188).

S.N.	Aqueous Extract Conc. (mg/ml)	Zone of inhibition (Φ in mm) against test microbes			
		<i>S. aureus</i>	<i>P. acnes</i>	<i>C. albicans</i>	<i>T. rubrum</i>
1.	100	13 mm	16 mm	09 mm	N/A
2.	50	09 mm	10 mm	00 mm	N/A
3.	25	00 mm	00 mm	00 mm	N/A
4.	12.5	00 mm	00 mm	00 mm	N/A
5.	6.25	00 mm	00 mm	00 mm	N/A

Table: 8. Results of microbial activity (MIC) of *Bauhinia variegata* leaf extract against *Staphylococcus aureus* (ATCC-23235), *Propionibacterium acnes* (ATCC-11827), *Candida albicans* (MTCC-854) and *Trichophyton rubrum* (ATCC-28188).

S.N.	Aqueous Extract Conc. (mg/ml)	Zone of inhibition (Φ in mm) against test microbes			
		<i>S. aureus</i>	<i>P. acnes</i>	<i>C. albicans</i>	<i>T. rubrum</i>
1.	100	08 mm	10 mm	13 mm	N/A
2.	50	09 mm	08 mm	08 mm	N/A
3.	25	00 mm	00 mm	00 mm	N/A
4.	12.5	00 mm	00 mm	00 mm	N/A
5.	6.25	00 mm	00 mm	00 mm	N/A

Table: 9. Results of microbial activity (MIC) of *Saraca asoka* leaf extract against *Staphylococcus aureus* (ATCC-23235), *Propionibacterium acnes* (ATCC-11827), *Candida albicans* (MTCC-854) and *Trichophyton rubrum* (ATCC-28188).

S.N.	Aqueous Extract Conc. (mg/ml)	Zone of inhibition (Φ in mm) against test microbes			
		<i>S. aureus</i>	<i>P. acnes</i>	<i>C. albicans</i>	<i>T. rubrum</i>
1.	100	08 mm	11 mm	09 mm	N/A
2.	50	00 mm	00 mm	00 mm	N/A
3.	25	00 mm	00 mm	00 mm	N/A
4.	12.5	00 mm	00 mm	00 mm	N/A
5.	6.25	00 mm	00 mm	00 mm	N/A

The MIC results in the current study indicate that the effectiveness of the plant extracts is concentration-dependent, with higher concentrations resulting in larger zones of inhibition. The plants *Eucalyptus globulus* and *Mangifera indica* demonstrated the strongest antimicrobial effects, while *Bauhinia variegata* and *Saraca asoka* showed only moderate activity, which aligns with their susceptibility assay results. These findings emphasize the importance of considering both susceptibility and sensitivity assays in evaluating the therapeutic potential of plant extracts. The observed variations in antimicrobial effectiveness could be

attributed to the differences in chemical composition and the active constituents present in the extracts, such as flavonoids, terpenoids, and alkaloids, which are known for their antimicrobial properties (Nostro et al., 2000).

3.1.4. Antimicrobial Susceptibility Assay of Polyherbal Topical Gel

The data presented in Table 4.17 illustrates the antimicrobial activity of four polyherbal gel formulations against four test microorganisms: *Staphylococcus aureus* (S. aureus), *Propionibacterium acnes* (P. acnes), *Candida albicans* (C. albicans), and *Trichophyton rubrum* (T. rubrum). The zone of inhibition is measured to assess the efficacy of each gel formulation in inhibiting microbial growth.

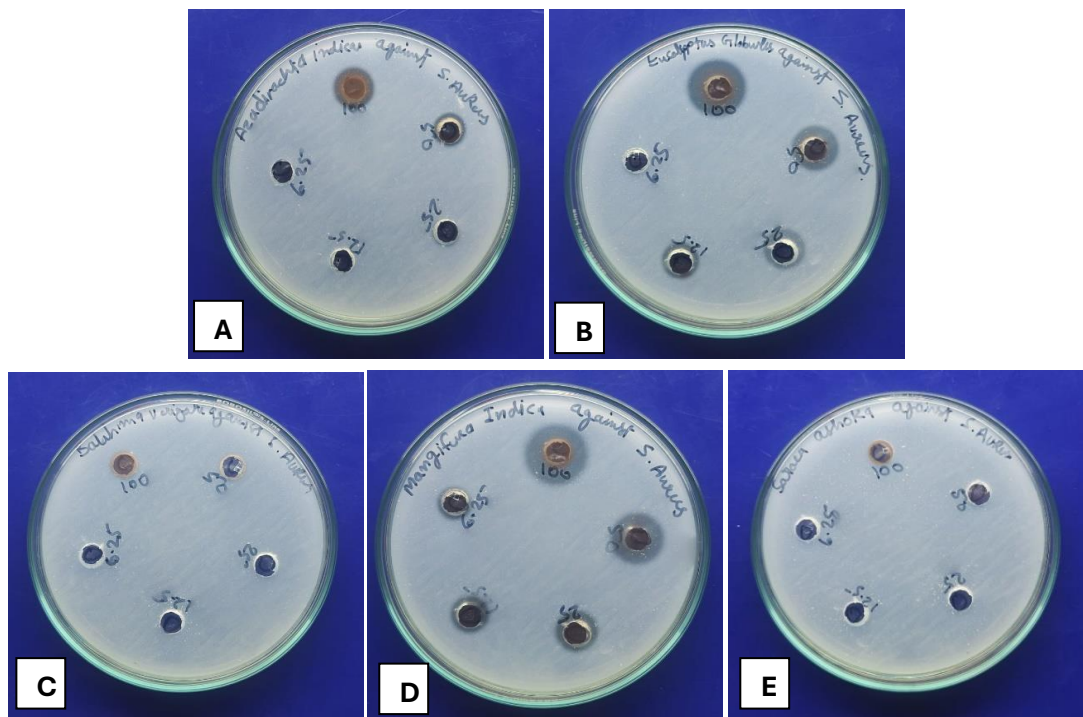


Image 4.4: Antimicrobial activity (MIC) of aqueous extracts of test plant materials considered for further experiments against *S. aureus* bacteria: **A.** *Azadirachta indica*, **B.** *Eucalyptus globulus*, **C.** *Bauhinia variegata*, **D.** *Mangifera indica*, and **E.** *Saraca asoca*.

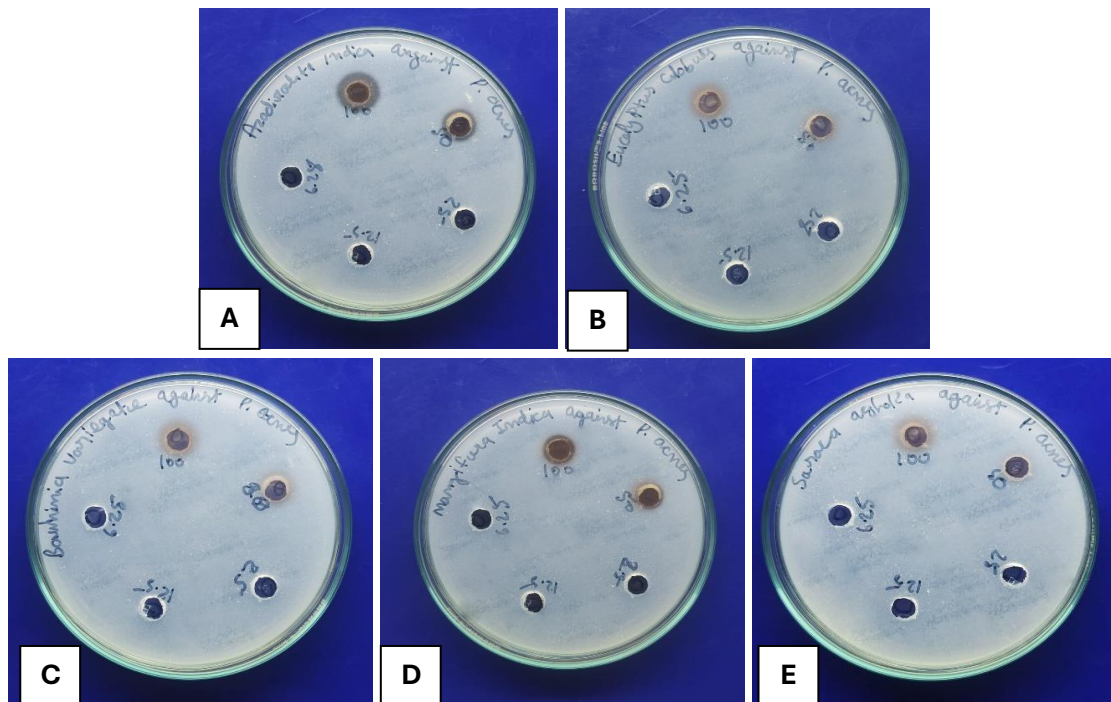


Image 4.5: Antimicrobial activity (MIC) of aqueous extracts of test plant materials considered for further experiments against *P. acnes* bacteria: **A.** *A. indica*, **B.** *E. globulus*, **C.** *B. variegata*, **D.** *M. indica*, and **E.** *S. asoka*

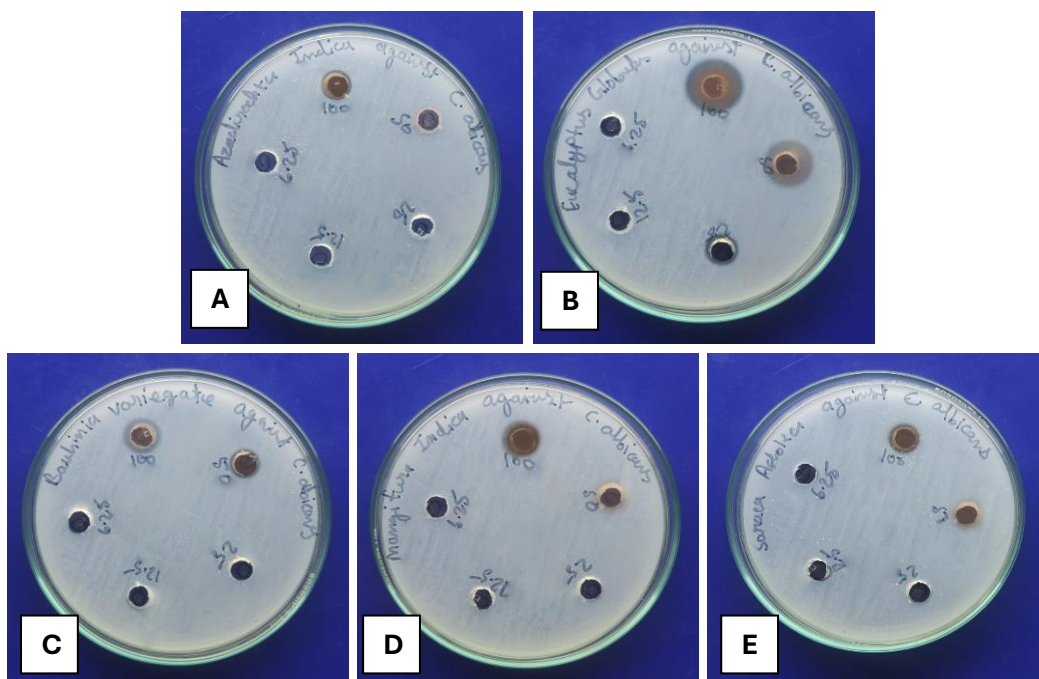


Image 4.6: Antimicrobial activity (MIC) of aqueous extracts of test plant materials considered for further experiments against *C. albicans*: **A.** *A. indica*, **B.** *E. globulus*, **C.** *B. variegata*, **D.** *M. indica*, and **E.** *S. asoka*

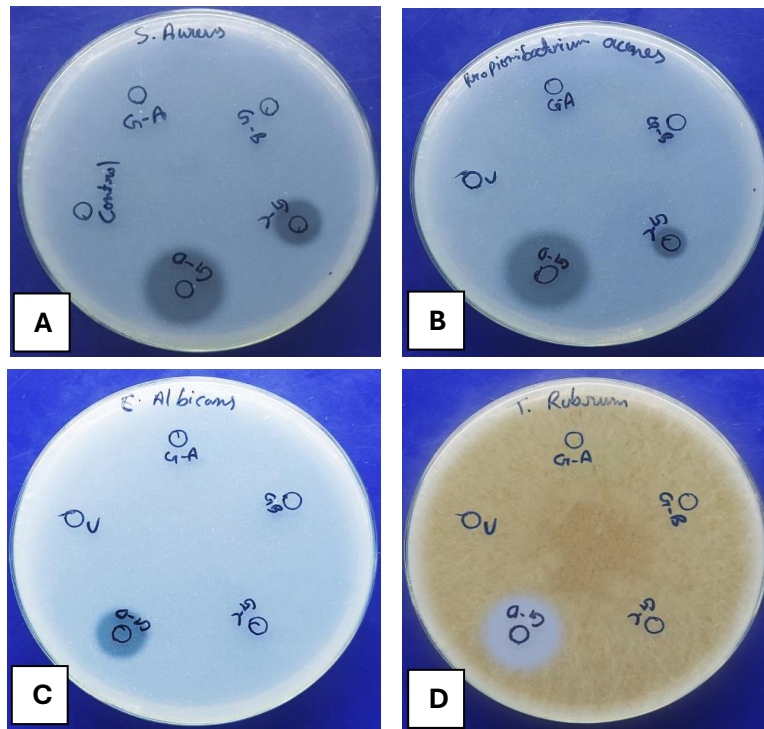


Image 4.6: Antimicrobial Susceptibility test of 4 different Polyherbal gels prepared out of aqueous extracts of leaves of test plants *A. indica*, *E. globulus*, *B. variegata*, *M. indica*, and *S. asoca* against the dermal pathogenic microbes: **A.** *S. aureus*, **B.** *P. acnes*, **C.** *C. albicans* and **D.** *T. rubrum*.

Conclusion

This study has significantly advanced the understanding and application of phytochemicals in the development of plant-based topical gels for treating skin infections caused by bacterial and fungal pathogens. By focusing on six selected plant species, the research provided a comprehensive analysis of their phytochemical profiles and demonstrated their potential in combating common pathogens. The formulations developed through this work not only exhibited promising antimicrobial activity but also addressed the need for sustainable and eco-friendly alternatives to conventional treatments.

The phytochemical analysis revealed the presence of key bioactive compounds, including alkaloids, flavonoids, tannins, and phenols, which are known for their potent antimicrobial and anti-inflammatory properties. These compounds played a pivotal role in the efficacy of the formulated gels, which were tested against skin pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*. The findings underscore the relevance of integrating plant-based solutions in addressing contemporary challenges, such as antimicrobial resistance and environmental sustainability.

The development of topical gels further highlighted the potential for combining phytochemicals in stable formulations with desirable physical and sensory properties. This aspect enhances user acceptability and ensures practical applications. The study bridges traditional knowledge of medicinal plants with modern scientific methodologies, creating a pathway for innovation in phytomedicine.

Key Conclusions:

- 1. Phytochemical Potential:** The selected plant species exhibited rich phytochemical profiles, with significant amounts of bioactive compounds such as alkaloids, flavonoids, tannins, and phenols, which

are directly linked to antimicrobial efficacy.

2. **Effective Antimicrobial Action:** The topical gel formulations demonstrated strong antimicrobial activity against bacterial and fungal pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*.
3. **Eco-Friendly Formulations:** The gels were developed using natural plant extracts and sustainable practices, minimizing the use of synthetic additives and promoting environmentally responsible alternatives to conventional therapies.
4. **Physical and Sensory Properties:** The gels exhibited favourable characteristics such as spreadability, stability, and adherence, ensuring their suitability for practical use as topical applications.
5. **Addressing Antimicrobial Resistance:** The plant-based formulations present a viable alternative to conventional antibiotics and antifungal agents, contributing to the fight against antimicrobial resistance.
6. **Integration of Traditional and Modern Knowledge:** The research successfully combined traditional ethnobotanical knowledge with advanced formulation techniques, underscoring the importance of interdisciplinary approaches in drug development.
7. **Scope for Clinical Validation:** The results provide a strong foundation for future clinical trials to validate the efficacy, safety, and practical applications of the developed gels *in vivo*.

Future Directions

Despite these accomplishments, there are areas for future exploration. Clinical trials will be essential to validate the efficacy and safety of the formulations *in vivo*. Additionally, assessing the stability of the gels under various environmental conditions will ensure their long-term usability and reliability. Expanding the spectrum of tested pathogens could further enhance the therapeutic relevance of the formulations and their potential applications in other skin-related conditions, such as eczema or psoriasis.

Future studies could also explore optimizing the extraction and formulation processes to improve yield and maximize the synergistic effects of bioactive compounds. Advancements in nanotechnology could be leveraged to enhance the bioavailability and targeted delivery of the active ingredients within the gels, ensuring higher therapeutic efficacy.

This research has laid a strong foundation for the development of plant-based topical therapies, bridging traditional knowledge and modern scientific methods. It contributes significantly to the fields of phytomedicine, formulation science, and antimicrobial therapy, offering a sustainable and promising solution to contemporary healthcare challenges. These findings encourage further exploration and innovation in developing natural remedies for skin infections and beyond, highlighting the relevance of phytochemical-based interventions in modern medicine.

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