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FORMULATION AND EVALUATION OF ANTIMICROBIAL POLYHERBAL GEL FOR WOUND MANAGEMENT

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ABSTRACT

Wound infections are a significant healthcare challenge because they delay healing, promote microbial contamination, and are increasingly difficult to treat due to resistance to synthetic antimicrobial agents. This study aimed to formulate and evaluate an antimicrobial polyherbal gel for wound healing using medicinal plants such as *Caralluma fimbriata* and *Ocimum sanctum Linn.* (Tulsi), known for their antibacterial, antioxidant, and anti-inflammatory properties. Preliminary phytochemical screening of the plant extracts confirmed the presence of flavonoids, tannins, alkaloids, phenolics, and glycosides, which are beneficial for tissue repair and microbial inhibition.The gel was prepared using HPMC as a gelling agent, glycerin as a humectant, methyl paraben as a preservative, and citric acid for pH adjustment. The formulated gel was evaluated for physicochemical properties including appearance, homogeneity, spreadability, viscosity, pH, stability, and extrudability. Results showed satisfactory consistency, good spreadability, stability, and skin-compatible pH.Antimicrobial activity was tested by the agar well diffusion method against common wound pathogens such as *Escherichia coli* and *Staphylococcus aureus*. The gel exhibited significant zones of inhibition, indicating strong antibacterial potential. Additionally, the antioxidant and anti-inflammatory phytoconstituents may support faster wound contraction and tissue regeneration.In conclusion, the developed polyherbal gel demonstrated promising antimicrobial activity and suitable properties for topical wound care, suggesting it could serve as a safe, cost-effective, and effective herbal alternative for wound treatment.

KEYWORDS: Polyherbal gel, Wound management, *Caralluma fimbriata*, *Ocimum sanctum Linn.* (Tulsi), Antimicrobial activity, Wound healing.

INTRODUCTION

The integration of traditional medicinal knowledge with modern pharmaceutical technology has made the way for the development of innovative herbal drug delivery systems. The pharmaceutical business is paying a lot of attention to topical treatments, especially gels, because of their better spreadability, non-greasy texture, and increased penetration through the layers of skin. In order to address the growing global concern of antimicrobial resistance, this research focuses on creating a polyherbal gel using the medicinal potential of *Caralluma fimbriata* and *Ocimum sanctum linn.*

Due to the high susceptibility of open injuries to colonization by pathogenic microbes, proper wound treatment continues to be a major issue in contemporary clinical practice. These infections can cause serious systemic consequences in addition to impeding the body's natural healing process. Although synthetic antimicrobial agents such as chlorhexidine are widely used, their prolonged usage is often linked to negative consequences such as tissue irritation, discoloration, and the growing worldwide worry of bacteria resistance.[5,6] The creation of new medicinal compounds derived from plants is therefore desperately needed. A viable substitute is provided by polyherbal formulations, which make use of the synergistic potential of many phytochemicals to produce a broad-spectrum therapeutic impact with low toxicity.[1] India has long used the succulent plant *Caralluma fimbriata* also called *Caralluma adscendens var. fimbriata* for its many therapeutic uses. The plant is a rich source of "lead compounds," such as alkaloids, flavonoids, phenols, tannins, saponins, and triterpenoids including luteol and sitosterol, according to phytochemical screening.[3] Its notable pharmacological profile is a result of these components; in particular, the phenolic and flavonoid chemicals function as strong antioxidants and free radical scavengers, which are essential for lowering oxidative stress and encouraging cell restoration during tissue healing.[1] Additionally, C. fimbriata has shown strong antifungal activity against Candida albicans and Aspergillus niger as well as strong antibacterial efficacy against a range of pathogens, including Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus.[6]

In addition to *C. fimbriata, Ocimum sanctum Linn*, sometimes called Tulsi, is acknowledged as a "Queen of Herbs" in Ayurvedic medicine because of its wide range of therapeutic properties. The broad-spectrum antibacterial efficacy of tulsi against infections associated to the mouth and skin has been extensively documented.[4] According to research, O. sanctum ethanolic extracts are quite powerful against bacteria like Actinobacillus actinomycetemcomitans and Streptococcus mutans, and their effectiveness is frequently on par with common medicines like 0.2% chlorhexidine.[5]

Bioactive substances like eugenol, which inhibit bacteria development, are primarily responsible for their antimicrobial qualities. Tulsi is a great option for topical dermatological applications because of its strong antibacterial properties as well as its notable anti-inflammatory and wound-healing properties.[2]

The goal of creating a polyherbal gel that combines the strong antibacterial and anti-inflammatory qualities of *Ocimum sanctum l* with the antioxidant and tissue-protective advantages of *Caralluma fimbriata*. By combining these extracts into a topical gel, the treatment can provide bioactive substances directly to the wound site for a prolonged period of time, accelerating healing and averting secondary infections. The formulation, characterisation, and assessment of this antibacterial polyherbal gel are the main objectives of this study, which aims to offer a natural, safe, and efficient substitute for complete wound care.

Plant Profile

Caralluma fimbriata

The edible succulent herb Caralluma fimbriata is native to India and has been used for generations as a "famine food" to increase endurance and stave off hunger. In terms of morphology, it is a branching plant that grows to a height of 20 to 30 cm. Its green, fleshy, four-angled stems have tiny leaves that sprout on immature branches for a short time before falling off. The plant's strong antioxidant activity is attributed to its abundance of phenolics and flavonoids, as well as the presence of pregnane glycosides, which are thought to prevent fat production.[9] Studies demonstrating that extracts from C. fimbriata considerably lessen pain in animal models demonstrate the plant's anti-nociceptive (analgesic) qualities, which have important medicinal utility. Its strong antioxidant and free radical scavenging properties also aid in the management of oxidative stress, which is essential for tissue protection during the healing process.[8,23]

Fig. 1: *Caralluma fimbriata Linn.*

Botanical Name: *Caralluma fimbriata*
Kingdom: Plantae
Order: Gentianales
Family: Apocynaceae
Genus: Caralluma
Species: C. fimbriata
Synonym: *Caralluma adscendens* var. *fimbriata*
Common Names: Caralluma, Appetite, Makad Shing

***Ocimum sanctum Linn*. (Tulsi)**

Ocimum sanctum Linn, also referred to as Holy Basil or Tulsi, is a highly regarded medicinal plant in the traditional Indian medical system. It is an upright, multibranched subshrub with hairy stems and intensely fragrant green or purple elliptical leaves. Eugenol, a phenolic molecule with strong antibacterial and antiseptic qualities that make it extremely effective against a wide range of wound-infecting microorganisms, is the main bioactive component of O. sanctum.[11,4]

The plant also contains a variety of flavonoids and ursolic acid, which have anti-inflammatory and wound-healing properties by speeding up wound contraction and epithelization. O. sanctum serves as the main antibacterial barrier when combined with a polyherbal gel, and its anti-inflammatory qualities combine with Caralluma fimbriata's analgesic qualities to offer complete wound treatment.[12,18]

Fig. 2: *Ocimum sanctum linn.*

Botanical Name: *Ocimum sanctum linn*
Kingdom: Plantae
Order: Lamiales
Family: Lamiaceae
Genus: Ocimum
Synonym: *Ocimum tenuiflorum*
Class: Magnoliopsida
Common Name: Tulsi, Holy Basil

EXPERIMENTAL DETAILS

A. Material and Method

Table no. 1: Ingredients and their role.

Ingredients	Role
Caralluma fimbriata	Antibacterial, antifungal, anti-inflammatory, antioxidant, wound healing, immunomodulatory
Ocimum sanctum Linn. (Tulsi),	Anti-inflammatory, antioxidant, antimicrobial, collagen synthesis, wound healing
HPMC	Gelling Agent
Xanthan gum	Gelling agent, Thickener
Glycerin	Humectant, penetration enhancer
Citric acid	PH adjuster
Methyl paraben	Preservative, antimicrobial
Distilled water	Solvent, vehicle

B. Collection and Drying

Caralluma Fimbriata steams Powder and *Ocimum sanctum linn* leaves powder were ordered from certified Manufacturer: KR IMPEX ENTERPRISES (SAI HERBS), Gulmarg Avenue, Amritsar, Punjab, India herbal suppliers.All plant materials were authenticated by a Certificate of Authentication (COA) provided by Sai herbs company.

Table no. 2: Analytical Parameters of *Caralluma fimbriata* powder.

Test Parameter	Specification	Results
Description	Brown fine powder	Complies
Identification	Positive for *Caralluma fimbriata*	Complies
Odour	Characteristic	Conforms
Loss on Drying (% w/w)	NMT 10.0%	6.8%
Total Ash (% w/w)	NMT 5.0%	3.2%
Acid Insoluble Ash (% w/w)	NMT 2.0%	0.7%
Water Soluble Extractive	NLT 10%	12.5%
Alcohol Soluble Extractive	NLT 8%	10.4%

Pregnane Glycosides Content	NLT 5%	6.7%
Foreign Matter	NMT 2.0%	0.15%
Total Plate Count	NMT 10^5 cfu/g	2.3×10^3 cfu/g
Yeast & Mold Count	NMT 10^3 cfu/g	1.2×10^2 cfu/g
E. coli	Absent	Absent
Salmonella	Absent	Absent
Heavy Metals	Within Permissible Limits	Complies
Lead (Pb)	NMT 10 ppm	< 1.0 ppm
Arsenic (As)	NMT 3 ppm	< 0.5 ppm
Cadmium (Cd)	NMT 0.3 ppm	< 0.1 ppm
Mercury (Hg)	NMT 1 ppm	< 0.05 ppm
Aflatoxin B1	NMT 5 ppb	< 1 ppb
Pesticide Residues	Within Permissible Limits	Complies

Table no. 3: Analytical Parameters of *Ocimum sanctum linn* Powder.

Test Parameter	**Specification**	**Results**
Description	Brown fine powder	Complies
Identification	Positive for *Ocimum sanctum*	Complies
Odour	Characteristic	Conforms
Loss on Drying (% w/w)	NMT 10.0%	7.3%
Total Ash (% w/w)	NMT 5.0%	3.1%
Acid Insoluble Ash (% w/w)	NMT 2.0%	0.8%
Water Soluble Extractive	NLT 10%	12.6%
Alcohol Soluble Extractive	NLT 8%	10.5%
Foreign Matter	NMT 2.0%	0.21%
Total Plate Count	NMT 10^5 cfu/g	2.7×10^3 cfu/g
Yeast & Mold Count	NMT 10^3 cfu/g	1.4×10^2 cfu/g
E. coli	Absent	Absent
Heavy Metals	Within Permissible Limits	Complies
Lead (Pb)	NMT 10 ppm	< 1.0 ppm
Arsenic (As)	NMT 3 ppm	< 0.5 ppm
Cadmium (Cd)	NMT 0.3 ppm	< 0.1 ppm
Mercury (Hg)	NMT 1 ppm	< 0.05 ppm
Aflatoxin B1	NMT 5 ppb	< 1 ppb
Pesticide Residues	Within Permissible Limits	Complies

C. Extraction Procedure of Plant Materials

The cold maceration method was used to create ethanolic extracts from both plant sources using 1:5 ratio. In a closed container, 25 g of precisely weighed powdered plant material (C. fimbriata stem powder and O. sanctum leaf powder) were macerated separately with 125mL of 95% ethanol for 72 hours at room temperature with stirring.[17] Whatman No. 1 filter paper was used to filter the combination, To produce a semisolid residue, the combined filtrates were concentrated using a rotary evaporator at a temperature below 60°C under low pressure.[5,6]

D.Phytochemical Screening

Standard qualitative tests were used to perform preliminary phytochemical screening of the ethanolic extracts of C. fimbriata and O. sanctum.

The following phytochemical constituents were tested:

Alkaloids: Mayer's test and Dragendroff's test were performed.

Flavonoids: Ferric Chloride Test

Tannins: lead acetate test.

Saponins: Foam test.

Steroids: Salkowski test and Liebermann-Burchard test

Glycosides: Legal test and Liebermann-Burchard test.[20,21]

E.Formulation of Polyherbal Gel

HPMC was used in the formulation of the polyherbal gel as the main gelling agent. Three gel formulations were created: F1 (gelling agent conc low), F2 (gelling agent conc medium), and F3 (gelling agent conc high). The formulations' composition is shown in Table 1.[22]

Table 4: Composition of Polyherbal Gel Formulations.

Ingredient	F1	F2	F3	Function
C. fimbriata ethanolic extract	0.30	0.30	0.30	Active ingredient
O. sanctum ethanolic extract	0.30	0.30	0.30	Active ingredient
HPMC	0.30	0.45	0.60	Gelling agent
Glycerin	1.50	1.50	1.50	Humectant
Xanthan gum	0.30	0.45	0.60	Thickener
Methylparaben	0.06	0.06	0.06	Preservative
Citric acid	q. s	q. s	q. s	pH adjuster
Distilled water	30ml	30ml	30ml	Solvent/vehicle

1) Preparation Procedure

a) Preparation of Dispersion

Raise the temperature of 40–50% of the water to 80°C. Stirring constantly, add the HPMC to the boiling water. This inhibits early swelling and permits initial dispersion.

To create a smooth paste (slurry), xanthan gum in glycerin was dispersed in different container. To avoided clumping When water was introduced.

b) Mixing and Hydration:

Stir constantly while adding the remaining cold water to the HPMC dispersion until the HPMC cools and hydrates, creating a clear, viscous solution.Stir the Xanthan Gum slurry into the HPMC solution until it is uniform.

c) Mixing and Hydration:

The mixture was stirred constantly while the remaining cold water was added to the HPMC dispersion until the HPMC cooled and hydrated, forming a clear, viscous solution. The Xanthan Gum slurry was then stirred into the HPMC solution until a uniform mixture was obtained.

d) Preservation

The preservatives was incorporated into the gel base.

e) Finalization

Then finally both active extract were added, and water was used to modify the overall weight. To create a clear, smooth, and uniform gel, the gel was either let to stand or bubbles were eliminated using a magnetic stirrer.[24]

F. Evaluation of Polyherbal Gel

The formulated polyherbal gel was evaluated for the following parameters

1. Organoleptic Characteristics

The produced gels were visually inspected for texture, homogeneity, color, odor, and appearance.

2. pH Determination

A digital pH meter that has been calibrated was used to determine the pH of each composition. Before measuring pH, 2.5 g of gel was dissolved in 25 mL of clean water and allowed to settle for two hours. Triplicate measurements were made, and average results were noted. For topical administration, the acceptable pH range is 6.0–7.4 .Result was shown in table below.[16]

3. Spreadability

Measured by sandwiching gel between two glass slides under a predetermined weight (100 g) for one minute, then calculating the spread diameter.it spread uniformly .

4. Washability test:

The dorsal surface of the hand was treated with a tiny amount of gel before being cleaned with tap water. The degree of removal ease was evaluated subjectively.

5. Viscosity

A Brookfield viscometer with spindle number two operating at 50 rpm at room temperature was used to measure the viscosity of the gel compositions. The unit of measurement for viscosity was centipoise (cP). Each measurement was done three times. Result was shown in table below.

G. Nutrient Broth Composition

Table no.5: Components for the composition of Nutrient Agar.

Components	Quantity (g/100 mL)
Nutrient Broth	2.4gm
Agar	6gm
Distilled water	100 mL

H. Antimicrobial activity

The agar well diffusion method was used to assess the gel formulation's in vitro antibacterial efficacy against the following test organisms:

Staphylococcus aureus – Gram-positive bacterium

Escherichia coli – Gram-negative bacterium[13]

Procedure

Fresh broth cultures were created and the bacterial strains were kept on nutrient agar slants. A sterile swab was used to prepare and inoculate culture media Petri plates for bacteria by uniformly spreading the turf. A sterile cork borer was used to drill 8 mm-diameter wells into the agar. One hundred microliters of the test gel formulation were put into each well. Negative control wells were given the gel base, and positive control wells were given azithromycin, a common antibiotic. In order to facilitate diffusion, the plates were pre-incubated at 4°C for two hours. After that, the bacterial cultures were incubated at 37°C for twenty-four hours. A digital vernier caliper was used to measure the zones of inhibition in millimeters, including the well diameter.[5,16]

RESULTS

A. Collection

Collection *Caralluma Fimbriata* steams Powder and *Ocimum sanctum linn* leaves powder were ordered from certified Manufacturer: KR IMPEX ENTERPRISES (SAI HERBS), Gulmarg Avenue, Amritsar, Punjab, India herbal suppliers.

Plant materials was found to be free of moisture as confirmed by COA provided by company.

Fig. 3:- *Caralluma Fimbriata* steams Powder.

Fig. 4:- *Ocimum sanctum l* leaves powder.

B. Maceration and Extraction

The maceration of *Caralluma Fimbriata* steams Powder and *Ocimum sanctum linn* leaves powder powders using 95% ethanol for 72 hours yielded dark greenish extract for *Caralluma Fimbriata* and brown extract for *Ocimum sanctum linn*.

Fig. 5: Extraction process by cold maceration method.

*C.*Phytochemical Screening

The phytochemical analysis of the ethanolic extracts of both plant species revealed the presence of a diverse array of secondary metabolites. The results are presented in Table 6.

Table 6: Phytochemical Screening of Ethanolic Extracts of C. fimbriata and O. sanctum.

S.No.	Phytochemical Constituent	C. fimbriata Extract	O. sanctum Extract
1	Alkaloids	+ (Present)	+ (Present)
2	Flavonoids	+ (Present)	+ (Present)
3	Tannins	+ (Present)	+ (Present)
4	Saponins	+ (Present)	+ (Present)
5	Glycosides (Pregnane/Cardiac)	+ (Present)	+ (Present)
6	Steroids	+ (Present)	+ (Present)

Fig. 6: Phytochemical Screening test of Ethanolic Extracts of C. fimbriata.

Fig. 7: Phytochemical Screening test of Ethanolic Extracts of O. sanctum.

D. Preparation of Gel

The polyherbal gel was successfully prepared using HPMC as the gelling agent with the incorporated extracts of *Caralluma Fimbriata* and *Ocimum sanctum linn*. The gel appeared as a smooth, semi-translucent, light-greenish semisolid with a characteristic herbal odor. The addition citric acid neutralized the HPMC and resulted in a clear, viscous gel of appropriate consistency.

Fig. 8: Final gel Formulation.

F. Evaluation of Polyherbal Gel

Table 7: Results of Evaluation Tests for the Formulated Polyherbal Gel

Sr.No	Parameter	F1 Batch	F2 Batch	F3 Batch
1.	Colour	Greenish colour	Light greenish	Dark greenish brown
2.	Odour	Aromatic	Aromatic	Aromatic
3.	Texture	Smooth	Smooth	Smooth
4.	State	Semisolid	Semisolid	Semisolid
5.	pH determination	5.60	6.52	7.20
6.	Spreadability	17.80 sec	20.15 sec	24.2 sec
7.	Washability	Easily washable	Easily washable	Easily washable
8.	Appearance	Homogeneous	Homogeneous	Homogeneous
9.	Viscosity(cP)	31244.4 cP	32561.1 cP	35672.7 cP

G. Antimicrobial Activity Results

Table 8: Zone of Inhibition (mm) of Polyherbal Gel Against Test Organisms

Test Organisms	Sample Tested	Zone of Inhibition
S. aureus (mm)	Gel Formulation	Right zone (control)-9mm Left zone (F2 batch)-15mm
E. coli (mm)	Gel Formulation	Right zone (control)-10mm Left zone (F2 batch)-18mm

Fig. 9: Preparation of culture media and Pouring nutrient broth in petri plates.

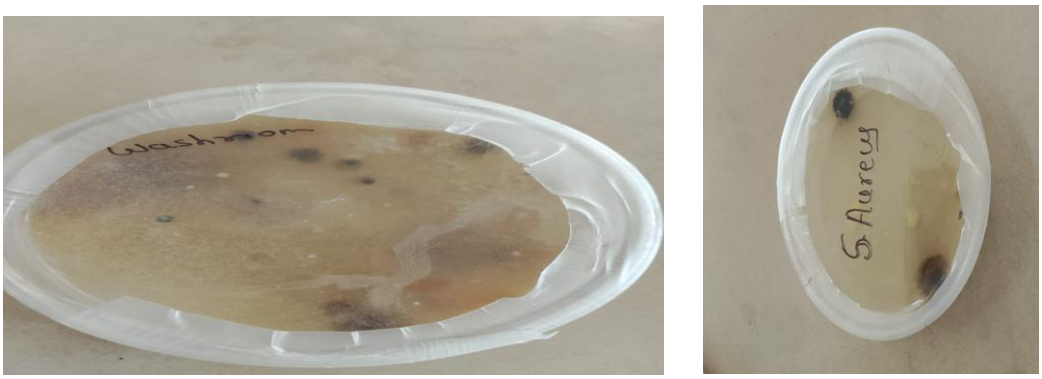

Fig. 9: The Bacterial sample collection form master plates for inoculation in petri plates.

Fig. 10:The inoculation of bacterial culture and then formulated gel.

Fig 11:- The antimicrobial activity of gel against test microorganism after the incubation for 24-48 hrs. at 37^0C

DISCUSSION

The current study focused on the formulation and evaluation of an antimicrobial gel using the ethanolic extracts of *Ocimum sanctum* leaves and *Caralluma fimbriata* stems. These plants are traditionally used for treating infections and wounds, which prompted their selection for this herbal formulation. The ethanolic extracts were successfully incorporated into an HPMC gel formulation. The prepared formulation was smooth, non-greasy, and easy to apply, indicating good patient acceptability. The gel's physical analysis showed that it had favorable qualities like a consistent look, a pH of about 6.52, good spreadability, and an appropriate viscosity. Topical preparations must have these characteristics in order to be stable and useful.

The most important factor, antibacterial activity, was measured with the agar well diffusion method. Both Staphylococcus aureus (a Gram-positive bacterium) and Escherichia coli (a Gram-negative bacterium) were significantly inhibited by the gel. The zones of inhibition was observed 18 mm for E.coli and 15mm for S. aureus were similar to those of conventional antibiotics, indicating the efficacy of the phytoconstituents found in Caralluma fimbriata and Ocimum sanctum, primarily flavonoids, tannins, alkaloids, and glycosides, which are recognized for their wound-healing and antimicrobial qualities.[14,15] These findings are in alignment with previous research studies that have reported the antimicrobial and therapeutic potential of *Ocimum sanctum* and *Caralluma fimbriata*. The consistency and pH stability of the gel throughout the storage period also indicated that the formulation was stable.[19]

Combining the potent antimicrobial and anti-inflammatory qualities of Ocimum sanctum with the tissue-protective and antioxidant advantages of Caralluma fimbriata was the aim of developing a polyherbal gel. These extracts can be included into a topical gel to give bioactive compounds directly to the wound site over an extended period of time, promoting healing and preventing subsequent infections. The primary goals of this study were to formulate, characterize, and assess this antibacterial polyherbal gel in order to provide a safe, effective, and natural substitute for full wound treatment.

This study emphasizes the potential of using medicinal plants as an effective and safer alternative to synthetic antimicrobial agents. The formulation developed could be a promising candidate for commercial herbal gel products, especially for individuals seeking natural skin infection treatments.

CONCLUSION

The antimicrobial activity of the gels made with extracts from Ocimum sanctum leaves and Caralluma fimbriata stems was assessed. Out of the three formulations (F1, F2, and F3), the F2 batch showed better antibacterial efficacy against the microorganisms that were tested. The F2 formulation's ideal concentration of excipients and plant extracts, which enhanced the active phytoconstituents' diffusion and bioavailability, may be the cause of this increased activity.In this study, different gel formulations containing extracts of Caralluma fimbriata stems and Ocimum sanctum leaves (F1, F2, and F3) were prepared and evaluated for their antimicrobial activity.[14,15] Among these formulations, F2 exhibited the highest antimicrobial activity i.e- 18 mm for E.coli and 15mm for S . aureus. This superior performance may be due to the most suitable concentration of plant extracts and excipients in the F2 batch, which enhanced the diffusion and bioavailability of the phytochemical constituents present in the gel.The phytoconstituents present in Caralluma fimbriata and Ocimum sanctum are well known for their antimicrobial properties.[14] The F2 formulation allowed these active constituents to act more effectively in combination and also demonstrated a synergistic effect between the two plant extracts. Therefore, the F2 batch shows promising potential for further development as an effective polyherbal topical antimicrobial gel for the treatment of skin infections.

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