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Development and Evaluation of Antifungal Novel Polyherbal Cream using Eucalyptus globulus and Ocimum tenuiflorum

Dr. Shweta Shivshankar Suman, Dr. Rupesh Pingale, Pranav Raghunath Dhumal, Aditi Kishor Hujare, Fatima Samir Darji, Sakshi Dattatray Bhor

[1]Dr. Shweta Shivshnakar Suman, Assistant Professor, NCRD's Sterling Institute of Pharmacy, Nerul, Navi Mumbai
[2]Dr. Rupesh Pingale, Principal, NCRD's Sterling Institute of Pharmacy, Nerul, Navi Mumbai
[3,4,5,6] Students, NCRD's Sterling Institute of Pharmacy, Nerul, Navi Mumbai.

Corresponding author
Email ID : : shweta.suman@ncrdsip.com

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ABSTRACT
This study evaluates the phytochemical properties, organoleptic features, and antimicrobial activity of Eucalyptus globulus and Ocimum tenuiflorum (Tulsi), along with a formulation cream containing both oils. Phytochemical tests revealed that Ocimum tenuiflorum contained alkaloids, flavonoids, and phenolic compounds, while Eucalyptus globulus lacked alkaloids and flavonoids but contained terpenoids and saponins in both species. The formulation cream exhibited desirable organoleptic features such as a smooth texture, easy application, and non-irritancy, with a pH of 5.83. Antimicrobial activity was assessed through zones of inhibition against Escherichia coli, Candida albicans, and Aspergillus niger. Eucalyptus globulus showed the highest activity against E. coli (22 mm), while the combination of both oils exhibited enhanced antifungal activity. The cream formulation displayed moderate antimicrobial activity, with zones of inhibition of 20 mm against E. coli and 18 mm against Candida albicans. These findings suggest that both oils have significant antimicrobial potential, with the cream formulation serving as a promising candidate for topical applications in skincare.

Keywords: *Eucalyptus globulus, Ocimum tenuiflorum, phytochemical properties, antimicrobial activity, terpenoids, saponins, flavonoids, formulation cream, Escherichia coli, Candida albicans, Aspergillus niger, organoleptic features, synergistic effect.*

1. INTRODUCTION

As the body's largest organ, the skin serves as the primary defense against environmental stressors like dust, UV radiation, pathogens, and chemicals, which can lead to infections and aging. It also reflects aging and overall internal health (Tzellos et al., 2009). The practice of using plants or plant parts to treat wounds or illnesses is called herbal medicine, botanical medicine, or herbalism (Anonymous, 1992). This includes the use of seeds, leaves, stems, bark, roots, flowers, and extracts to create treatments such as topical applications, pills, capsules, teas, and tinctures. Many pharmaceutical drugs today are derived from these traditional remedies. This approach appeals to medical professionals due to its lower cost and generally safer use (Ansel et al., 1995). Medicinal plants are crucial for addressing serious illnesses globally, especially in developing countries where they are essential for basic healthcare needs. The medicinal value of these plants comes from chemically active compounds and secondary metabolites, like carbohydrates, alkaloids, glycosides, tannins, flavonoids, and phenolic compounds. Rising antibiotic resistance has made it increasingly important to explore plant-based natural products and secondary metabolites for new antimicrobial activities and unique mechanisms of action. Natural remedies for infectious diseases are effective and minimize the harmful side effects often seen with synthetic antimicrobials. Therefore, it's vital to study plant metabolites to confirm their traditional medicinal uses and identify active ingredients through chemical analysis (Thirumurugan et al., 2010). Polyherbal compositions involve combining two or more plants. Ayurveda, through "Sarangdhar Samhita," discusses the concept of mutualism that underpins polyherbal remedies. While single-plant formulations contain active phytoconstituents, their quantities are often insufficient for therapeutic effects. Research has demonstrated that combining plants with varying potencies results in better outcomes due to synergistic effects, which can be pharmacokinetic or pharmacodynamic (Tayade and Patil, 2015). Creams are semisolid emulsions for external application, classified as water-in-oil (w/o) or oil-in-water (o/w) (Das et al., 2014). Cosmetics, especially those made from natural herbs,

are effective for improving skin health by hydrating, nourishing, and moisturizing the skin. This project aims to develop an antifungal herbal cream using a polyherbal combination of neem, guduchi, and mint (Pal et al., 2014).

A cream is a topical skincare product that combines oil and water to create a smooth, often moisturizing substance. It is used to hydrate, protect, or treat various skin conditions. Creams typically have a thicker consistency than lotions, making them ideal for dry or sensitive skin. Depending on their formulation, they can serve different purposes, such as soothing irritation, providing anti-aging benefits, or treating skin ailments like acne, eczema, or sunburn. Creams may also contain active ingredients like vitamins, herbal extracts, or medications to address specific skin concerns. They are applied directly to the skin and are absorbed to deliver nourishment and moisture.Antifungal creams are topical medications used to treat fungal infections of the skin, such as athlete's foot, ringworm, and jock itch. These creams typically contain active ingredients like clotrimazole, miconazole, terbinafine, or ketoconazole, which work by inhibiting the growth of fungi and eliminating the infection. Antifungal creams are applied directly to the affected area and are usually used for a specified duration as directed by a healthcare professional. They provide relief from symptoms such as itching, redness, and irritation, and can effectively clear up fungal infections when used consistently and as prescribed. It's essential to follow the instructions on the packaging. Eucalyptus is a genus containing over 700 species of flowering plants in the Myrtaceae family. Most species are trees, typically mallees, although some are shrubs. Along with other related genera such as Corymbia and Angophora in the Eucalypteae tribe, they are collectively referred to as eucalypts or "gum trees." Eucalyptus plants are known for their distinctive bark, which can be smooth, fibrous, hard, or stringy, and their leaves, which contain oil glands. The sepals and petals of the flowers are fused to form a cap or operculum that covers the stamens. This unique feature is reflected in the genus name, derived from the Greek words Eu meaning well and kaluspto meaning covered. The fruit of the Eucalyptus plant is a woody capsule, often called a gumnut.Tulsi, also known as holy basil, is a revered herb in Ayurvedic medicine, considered a potent adaptogen that helps the body adapt to stress and promotes overall health and wellbeing. It has numerous health benefits, including reducing stress and anxiety, improving cognitive function and memory, boosting the immune system, and reducing inflammation and pain. Additionally, tulsi's broad-spectrum antimicrobial activity makes it effective against a range of human and animal pathogens, and it is used as a natural hand sanitizer, mouthwash, and water purifier. Furthermore, tulsi is believed to promote spiritual growth, improve mental clarity, and foster a sense of wellbeing, making it an integral part of Ayurvedic lifestyle practices. With its rich history, versatility, and numerous health benefits, tulsi is indeed a remarkable herb that has been cherished for centuries.The objective of this study is to develop and evaluate a polyherbal antifungal cream that combines the medicinal properties of Eucalyptus and Tulsi (Holy Basil) to effectively treat fungal infections. The study aims to formulate a cream by harnessing the antimicrobial properties of these two plants, which are well-known for their natural ability to combat pathogens. Eucalyptus, with its distinct oil glands and antimicrobial components, and Tulsi, renowned for its broad-spectrum antimicrobial and adaptogenic effects, will be utilized to create a safe, effective alternative for managing fungal skin conditions. The study will focus on evaluating the antifungal efficacy of the cream through in-vitro testing against common fungal pathogens, ensuring its potency. Furthermore, the safety and toxicity of the polyherbal cream will be assessed through dermatological testing to confirm its suitability for topical application without adverse reactions. A comparative analysis with conventional antifungal treatments will be conducted to determine the relative effectiveness of the polyherbal cream. The study also aims to standardize and optimize the formulation, ensuring consistent quality and maximum antifungal activity.

2. MATERIALS AND METHOD

Collection of sample

Eucalyptus globules and *Ocimum tenuiflorum* were collected from local farm.

Extraction

To extract bioactive compounds from ***Eucalyptus globulus*** or ***Ocimum tenuiflorum*** using the Soxhlet method, place the ground plant material in a thimble, add a suitable solvent (e.g., ethanol or hexane) to a round-bottom flask, and heat the solvent. The solvent will evaporate, condense, and repeatedly wash the plant material, extracting its compounds. After several cycles, remove the solvent to obtain the concentrated extract.

Phytochemicals Analysis

Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994). Wagner's reagent, Dragendorff's test, Mayer's test & Hager's reagent were used to test alkaloids, while Borntragers test were carried out for presence of glycosides, Ferric Chloride, FC reagent for the detection of phenol where Alkaline reagent, lead acetate test used for flavonoids. While the presence of saponins was confirmed by Foam test. Tannis is coinfirmed by gelatin test.

Thin Layer Chromatography:

A pre-prepared TLC plate was used on with base line and solvent front was drawn using pencil.

Sample was spotted at the Centre using a thin Capillary tube. Mobile phase was prepared in the beaker and was allowed to saturate by keeping it undisturbed for 20 mins. The TLC plate was then placed into the mobile phase and allowed the mobile phase to run upto the solvent front marked. The spot was identified under Visible U.V light in the U.V Chamber. The RF Value was calculated by using the formula and compared with RF value of the Standrad Value available in literature. For sprying reagent use: Anisaldehyde 0.5ml ethanol 45ml and 5ml glacial acetic acid, 2.5 ml sulphuric acid or we can use UV Chamber.

$$\mathbf{Rf} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

FORMULATION OF CREAM

First take water bath and heat till 70^{O} to 80^{O} temperature and maintain temperature at 70^{O} to 80^{O} temperature. Then add beaker into it and add hard paraffin and wait till it get into liquid form and do continuous stirring after that add petroleum jelly and stir well. Then add add glycerol monostearate and cetyl alcohol. Mix them well. Take water bath and add water heat till 70^{O} to 80^{O} temperature and put beaker in it, and Add Tulsi oil and eucalyptus oil and add propyl paraben and methyl paraben and mix well. Mix oil phase in water phase with continuous mixing and wait till cream get solidify. After that wait till cream get cool down and fill cream into empty plastic tube container and add labels on it.

Table 1: Formulation of cream

Ingredients	Trial 1	Trial 2	Trial 3	Trial 4	Final Formulation
Eucalyptus oil	6 ml	6 ml	6 ml	6 ml	6 ml
Tulsi oil	-	3 ml	6 ml	6 ml	6 ml
Petroleum jelly	12.9 gm	19.35 gm	19.35 gm	19.35 gm	19.35 gm
Hard paraffin	6 gm	9 gm	9 gm	7 gm (dec)	6 gm (dec)

Cetyl alcohol	1.5gm	2.25 gm	2.25 gm	2.25 gm	2.25 gm
Glycerol monostearate	1.5 gm	2.25 gm	3.25 gm (1gm inc.)	3.25 gm	3.25 gm
Methyl paraben	1.2 gm	1.8 gm	1.8 gm	1.8 gm	1.8 gm
Propyl paraben	0.9 gm	1.35 gm	1.35 gm	1.35 gm	1.35 gm

3. EVALUATION OF CREAM

Physical Evaluation: Physical parameters such as color and appearance were evaluated.

Homogenecity: All formulated creams underwent homogeneity testing through visual inspection post-container settling, assessing their appearance and the absence of any aggregates.

Washablity: the ease and extent washing of formulation with water were checked manually after formulation were applied on skin.

pH: The pH of different cream formulations was assessed using a digital pH meter. Specifically, 2.5 grams of each cream sample was precisely weighed and dispersed in 25 mL of distilled water, allowing it to sit for two hours. pH measurements for each formulation were conducted three times, and the average values were reported. The pH of the dispersions was determined using a pH meter.

Viscosity: The viscosity of the herbal cream was measured using a Brookfield rotational viscometer with spindle no. 64 at various speeds: 10. Each measurement was taken after allowing the sample to reach equilibrium for two minutes. This procedure was repeated three times to ensure accuracy

Spreadability: The Spreadability of creams was assessed using a wooden block apparatus equipped with a pulley at one end. This method measured the slip and drag characteristics of creams. Approximately 2 grams of the cream under study were placed on a fixed ground slide. Another glass slide of the same dimensions as the ground slide, equipped with a hook, was placed on top to sandwich the cream. A weight of 1 kg was applied to the top slide for 5 minutes to remove air and ensure a uniform cream film between the slides. Excess cream was removed from the edges. Subsequently, a 50 g weight was pulled with a string attached to the hook, and the time (in seconds) taken for the top slide to travel a distance of 6.5 cm was recorded. A shorter time interval indicated better spreadablity.

Spreadability was calculated using the following formula:

$$S = M \times L / T$$

Where, S = Spreadability,

- M = Weight in the pan (tied to the upper slide),
- L = Length moved by the glass slide and
- T = Time (in sec.) taken to separate the slide completely

Patch Test:

About 1-3gm of material to be tested was placed on a piece of fabric or funnel and applied to the sensitive part of the skin e.g. skin behind ears. The cosmetic to be tested was applied to an area of 1sq.m. on the skin. Control patches were also applied. The site of patch is inspected after 24 hrs.

Antimicrobial Activity

Agar Disk Diffusion Method

For evaluation of antibacterial activity disk diffusion method was used. In the agar disk diffusion method, each bacterial suspension was uniformly spread on petri dishes containing solidified agar medium with the help of glass spreader 4 sterile paper disks made from Whatmann paper (6mm) were placed on the surface of each agar plate and were impregnated with the solution of diluted oil with the help of sterile forceps, Plates were incubated for 24 hr under appropriate conditions Antimicrobial activity as for microbial testing as Zone of Inhibition. Disks Impregnated with ethanol served as negative controls and a disk with an antibiotic was used as a positive control.

4. RESULT AND DISCUSSION

The results of the study on *Eucalyptus globulus* and *Ocimum tenuiflorum* (Tulsi) provide valuable insights into their phytochemical properties, organoleptic features, and antimicrobial activity, as well as the potential of a formulation cream containing both oils. The phytochemical tests revealed important distinctions between the two plants. According to Table 2, *Eucalyptus globulus* tested negative for alkaloids, flavonoids, and phenolic compounds, while *Ocimum tenuiflorum* exhibited the presence of alkaloids, flavonoids, and phenolic compounds, suggesting that *Tulsi* contains a broader range of potentially beneficial compounds. Both plants tested positive for terpenoids and saponins, as indicated by the foam test and terpenoid test in Table 2. Neither plant contained glycosides, as confirmed by the negative result in the Borntrager's test.

In terms of phytochemical parameters, Table 3 and Table 4 present the moisture content, total ash value, and acid-insoluble ash values of both plants. *Eucalyptus* had a moisture content of 4%, which is well within the acceptable limit of 5%, and the total ash value of 5% and acid-insoluble ash value of 3.5% are indicative of low contamination. On the other hand, *Ocimum tenuiflorum* exhibited a slightly higher loss on drying (5.2%), but still within the acceptable range of 6%, with a total ash value of 10% and an acid-insoluble ash value of 1.5%, as shown in Table 4. These values suggest that *Tulsi* has higher inorganic content but remains suitable for use in formulations.

The Thin Layer Chromatography (TLC) analysis, presented in Table 5, showed different Rf values for both plants. *Eucalyptus globulus* had an Rf value of 0.56, while *Ocimum tenuiflorum* had an Rf value of 0.76, indicating that the compounds in *Tulsi* move faster than those in *Eucalyptus*, which reflects their distinct chemical compositions.

Regarding the organoleptic features of the cream, Table 6 lists the desirable qualities observed. The cream was light yellow in color, with an aromatic odor characteristic of the essential oils of both plants. Its smooth texture and homogeneity indicate good formulation quality, while the ease of application and non-irritant nature (as confirmed by the patch test) suggest it is suitable for topical use. The pH of 5.83, within the skin-friendly range, and the good spreadability (13.15) confirm that the cream is easily applied and spread. The washability was also rated as good, indicating no oily residue.

The antimicrobial activity, assessed through zones of inhibition against *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* (as shown in Table 7 and Table 8), revealed that *Eucalyptus globulus* exhibited the strongest antimicrobial activity against *E. coli*, with a zone of inhibition of 22 mm, followed by the cream formulation (20 mm) and *Ocimum tenuiflorum* (18 mm). The chloramphenicol standard disk showed the largest zone of inhibition (25 mm). The cream formulation displayed moderate antimicrobial activity, suggesting that both oils contribute to its overall effect. For *Candida albicans* and *Aspergillus niger*, the combination of both oils (1:1) showed the highest activity, with inhibition zones of 21 mm and 18 mm, respectively. The cream also showed moderate activity against *Candida albicans* (18 mm) and *Aspergillus niger* (15 mm), while the chloramphenicol standard exhibited the largest zones of inhibition (26 mm). The solvent control showed no inhibition, further confirming the antimicrobial potential of the oils, as depicted in Figure 1 and Figure 2.

Table 2: Results of Phytochemical Tests

Tests	Observation	*Eucalyptus globulus*	*Ocimum tenuiflorum*
Test for Alkaloids			
Dragendorff's Test	Orange red precipitate	-ve	+ve
Mayer's test	Yellow precipitate	-ve	+ve
Hager's test	Yellow precipitate	-ve	+ve
Wagner's test	Brown precipitate	-ve	+ve
Test for Flavonoids			
Shinoda test	Purple precipitate	+ve	-ve
Test for Phenolic compounds and Tannins			
Ferric chloride test	Dark blue color	-ve	+ve
Lead acetate test	White precipitate	-ve	+ve
Test for Terpenoids			
Terpenoids	Yellow ppt	+ve	+ve
Test for Glycoside test			
Borntragers test	Red color	-ve	-ve
Test for Saponins			
Foam test	Stable foam appeared	+ve	+ve

Table 3: Phytochemical Parameters of *Eucalyptus globulus*

Parameters	Obtained value % w/ w	Limit % w/w
Loss on Drying	4%	NMT 5%
Total ash value	5%	NMT 6.29%
Acid Insoluble Ash Value	3.5%	NMT 5.33%

Table 4: Phytochemical Parameters of *Ocimum tenuiflorum*

Parameters	Obtained value % w/w	Limit % w/w
Loss on Drying	5.2%	NMT 6%
Total ash value	10%	NMT 12%
Acid Insoluble Ash Value	1.5%	NMT 3%

Table 5: TLC for Eucalyptus globulus and Ocimum tenuiflorum

	Distance travelled by the solute	**Distance travelled by the solvent**	**Calculation**	**Rf Value**
R_f value of *Eucalyptus globulus*	2.8	5	2.8 / 5	0.56
R_f value of *Ocimum tenuiflorum*	4.2	5.5	4.2/5.5	0.76

Table 6: Results of Organoleptic features of cream

Parameters	**Inference**
Organoleptic Parameters	
Color	Light yellow
Odor	Aromatic
Texture	Smooth
Homogeneity	Homogenous
Ease of application	Easy
pH	5.83
Spreadability	13.15 (Good)
Washability	Good
Patch test	Non irritant

Viscosity	26,760 cp

Table 7: Results of Antimicrobial Activity (Zone of Inhibition of *Escherichia coli*)

Sample	Organism	Zone of Inhibition
Sample 1 (Eucalyptus)	*Escherichia coli*	22mm
Sample 2 (Tulsi)	*Escherichia coli*	18mm
Formulation (cream)	*Escherichia coli*	20mm
Standard disk (Chloramphenicol)	*Escherichia coli*	25mm
Solvent (plain disk)	*Escherichia coli*	No inhibition

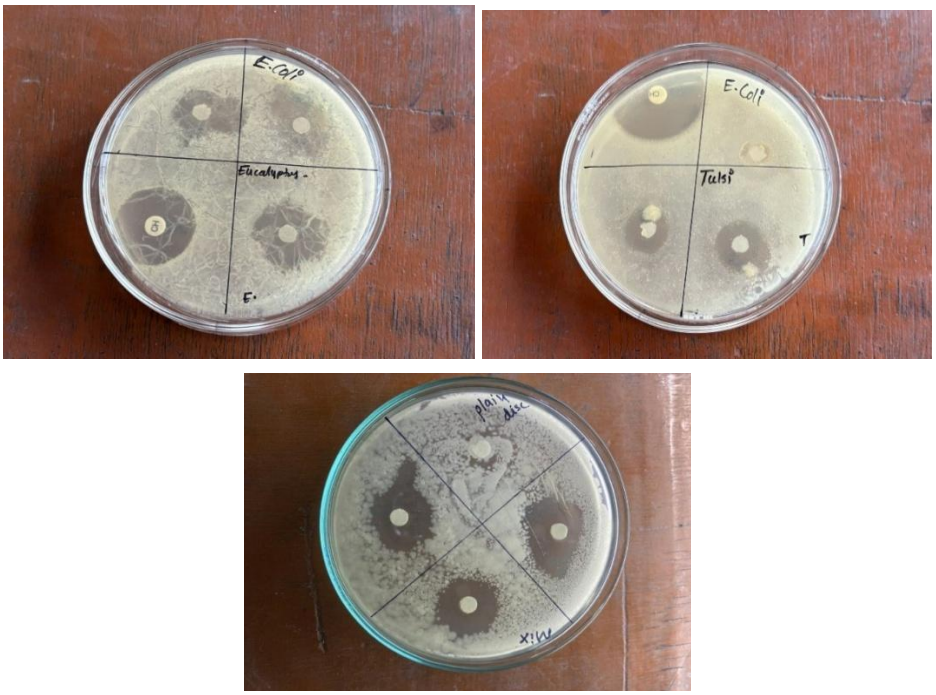

Figure 1: Antibacterial Study of Eucalyptus oil, Tulsi oil and Formulation against *E Coli*

Table 8: Zone of Inhibition of *Candida albicans* and *Aspergillus niger*

Sample	Organism	Zone of Inhibition
Sample 1 (Eucalyptus)	*Candida albicans*	18mm
	Aspergillus niger	13mm
Sample 2 (Tulsi)	*Candida albicans*	17mm
	Aspergillus niger	16mm
Sample 3(Mixture) (1:1) Eucalyptus + Tulsi	*Candida albicans*	21mm
	Aspergillus niger	18mm
Formulation (cream)	*Candida albicans*	18mm
	Aspergillus niger	15mm
Standard disk (Chloramphenicol)	*Candida albicans*	26mm
Solvent (plain disk)	*Candida albicans*	No inhibition
	Aspergillus niger	No inhibition

Figure 2: Antifungal study on *Candida albicans* and *Aspergillus niger*

5. CONCLUSION

Both *Eucalyptus globulus* and *Ocimum tenuiflorum* demonstrate significant antimicrobial properties, with *Eucalyptus* showing superior activity against *E. coli*, while the combination of both oils exhibits a synergistic effect, enhancing their antifungal activity. The phytochemical screening revealed that *Tulsi* contains a broader range of bioactive compounds than *Eucalyptus*, while both plants contain terpenoids and saponins, which contribute to their therapeutic potential. The formulation cream containing both oils exhibited promising organoleptic properties and moderate antimicrobial activity, making it a potential candidate for topical applications. The results suggest that these oils, either alone or in combination, could be effectively used in the development of antimicrobial creams.

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