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Formulation and *in vitro* evaluation of poly-herbal cream containing extracts of *curcuma longa* and *Ocimum tenuiflorum* for anti-fungal and anti-inflammatory activities

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Abstract
The skin is a vital organ that plays a crucial role in protecting the body from external factors, regulating body temperature, and aiding in the synthesis of vitamin D. Skin conditions, such as fungal infections and inflammation, are common problems that affect millions of people worldwide. This study focuses on the formulation and evaluation of a dual-action herbal cream that harnesses the potent bioactivities of Curcumin, *Curcuma longa* and Tulasi, Holy Basil, *Ocimum sanctum*. The intended outcome is a safe, effective, and well-tolerated topical formulation that offers both antifungal and anti-inflammatory benefits bioactive compound found in turmeric, has anti-inflammatory and antioxidant properties whereas Tulasi, also known as holy basil, has antimicrobial and anti-inflammatory properties. The synergy between Curcumin and Tulasi forms the core of this herbal cream, where their complementary actions target a broad spectrum of pathogenic fungi while simultaneously calming inflammatory responses. The evaluation of this herbal cream involves a comprehensive set of physicochemical and biological tests. Parameters such as pH, viscosity, spread ability, extrudability, and stability under various storage conditions are measured to ensure product quality and shelf-life. Furthermore, *in vitro* efficacy studies are planned to assess both the antifungal activity by testing against relevant fungal strains and the anti-inflammatory potential, ensuring that the cream meets its therapeutic objectives without inducing skin irritation. This study aims to develop a novel topical product that addresses common skin ailments while minimizing the adverse effects associated with synthetic drugs.

Keywords: Curcumin, tulasi, herbal cream, anti-fungal activity, anti-inflammatory activity, topical application, phytotherapy, antimicrobial properties, antioxidant properties

1. Introduction
Skin conditions, such as fungal infections and inflammation, are common problems that affect millions of people worldwide. These conditions can cause discomfort, pain, and emotional distress, impacting quality of life. The rise in superficial fungal infections and inflammatory skin conditions has led to a growing interest in natural, herbal-based therapies. Traditional medicine has long championed the therapeutic virtues of botanical ingredients, and modern science is now validating these age-old remedies. This study focuses on the formulation and evaluation of a dual-action herbal cream that harnesses the potent bioactivities of Curcumin, *Curcuma longa* and Tulasi, Holy Basil, *Ocimum sanctum*. The intended outcome is a safe, effective, and well-tolerated topical formulation that offers both antifungal and anti-inflammatory benefits bioactive compound found in turmeric, has anti-inflammatory and antioxidant properties whereas Tulasi, also known as holy basil, has antimicrobial and anti-inflammatory properties. Fungal infections, like athlete's foot and ringworm, are prevalent and can be challenging to treat. Inflammatory skin conditions, such as eczema and dermatitis, can cause redness, itching, and discomfort. Current treatments often involve topical and oral medications, which can have side effects and may not always be effective. In recent years, there has been a growing interest in natural and alternative therapies for skin conditions. Herbal remedies, in particular, have gained popularity due to their potential efficacy, safety, and natural origin. The cream's formulation is enhanced by a judicious selection of Excipients that not only improve the aesthetic and sensory properties but also ensure product stability and effective delivery of the active ingredients.

Beeswax serves as a natural emulsifying and thickening agent, contributing to the cream's consistency and providing a protective barrier that supports skin hydration.

2. Plant Profile

Table 1: Taxonomical information of *Curcuma longa*-Turmeric

Kingdom	Plantae
Order	Zingiberales
Family	*Zingiberaceae*
Genus	Curcuma
Species	C longa
Scientific name	*Curcuma longa*

Fig 1: Rhizome of Turmeric

Curcuma longa is underground rhizomes, it produces yellow orange, deep orange coloration, contain a complex mixture of pigments and essential oils. The principal bioactive component, polyphenolic, in addition essential oils such as turmerone, zingiberene, and atlantone.

Table 2: Taxonomical information of *Ocimum tenuiflorum*, Holy basil

Kingdom	Plantae
Order	Lamiales
Family	*Lamiaceae*
Genus	Ocimum
Species	O tenuiflorum
Scientific name	*Ocimum tenuiflorum*

Fig 2: *Ocimum tenuiflorum*

Ocimum tenuiflorum is aromatic, ovate leaves with a slightly serrated margin and a characteristic aroma due to its essential oil contents like eugenol, ursolic acid, rosmarinic acid, and various flavonoids, which contributes antimicrobial, anti-inflammatory, and antioxidant properties.

3. Materials and Methods

All the selected materials including plans and Ingredient were procured and plants were authenticated, the role Ingredient for the selected plan extracts with their significances is here.

Table 3: Plant Materials

SL. No	Name of the plant material	Weight (g)	Role of the selected plant
1	Curcumin-Turmeric (Rhizome)	10 g	Anti-Inflammatory Anti-Fungal
2	Tulasi-Holy Basil (Leaf)	10 g	Anti-Microbial Anti-oxidant

Table 4: Other Ingredients

SL. No	Name of the Ingredient	Weight (g)	Role of the ingredient plant materials
1	Ethanol	50 ml	Solvent for Extraction of
2	Beeswax	10 gm	Natural emulsifying and thickening agent
3	Borax	2 gm	Buffering agent
4	Liquid Paraffin	20 ml	Effective emollient
5	Methylparaben	0.50 gm	Preservative
6	Rose oil	2 ml	Appealing fragrance
7	Distilled water	50 ml	Vehicle

1. Procedure for method of extractions of selected plant materials

A) Procedure for method of extraction of turmeric rhizomes

For Extraction of Turmeric Rhizomes Maceration method of extraction is adopted based on the literature and procedure as followed. 10 g of matured dried turmeric rhizomes used for maceration, Soaked the grinded 10 g fine powder into 50ml ethanol for 24 hrs. Filtered the mixture using the filter paper and removed the solid residues and rotary evaporator under reduced pressure is used to evaporate the solvent gently. Concentrated extract is preserved for further proceedings.

B) Procedure for method of extraction of Tulasi-Holy Basil Leaves

For Extraction of Tulasi-Holy Basil Leaves Maceration method of extraction is adopted based on the literature and procedure as followed. High-quality matured Tulsi leaves were collected, free from contaminations and dried under shade, weighed to 10 g of the leaves and grind into a fine powder, soaked the grinded fine powder in to 50 ml ethanol for 24 hrs. Filtered the mixture using the filter paper and removed the solid residues and rotary evaporator under reduced pressure is used to evaporate the solvent gently. Concentrated extract is preserved for further proceedings.

C) Procedure for the formulation of the herbal cream

1. **Preparation of Oil Phase:** First phase of as an oil phase with beeswax, liquid paraffin and rose oil are the base ingredients for the first phase called oil phase. Taking a beaker and adding 10 gms of beeswax while heating in the water bath at 70 ºC. Then added a 20ml of liquid paraffin into the beaker while gently stirring the beeswax over the water bath, ensured uniform mixture of beeswax and liquid paraffin at 70 ºC. After a perfect mixture added rose oil of two drops.
2. **Preparation Aqueous Phase:** Second phase as aqueous phase with distilled water as solvent and borax and methyl paraben as solutes. 50 ml of distilled water heated at 70 ºC added 2 gms of borax into the distilled boiling water while stirring the water Added an 0.50 gms of methyl paraben into 70 ºC boiling water; while stirring

gently. Then added the extracted Curcumin and tulasi after ensuring the correct quantity of the extractions. Further added the extractions into the aqueous mixture of borax and methyl paraben in distilled water.

3. **Emulsification Process:** Combined aqueous phase into oil phase in a controlled manner while stirring continuously to obtain an ununiform mixture of oil phase and aqueous phase gradually stirred until the both phases are uniformly mixed. After cooling and homogenization, herbal cream is evaluated for further process.

2. Procedure for *in vitro* studies for Anti-fungal activity and Anti-inflammatory activity

A) **Procedure for *in vitro* studies for Anti-fungal activity:** For evaluation of the *in vitro* anti-fungal activity of the formulated herbal cream used the Agar Well Diffusion Method against pathogenic fungi such as *Candida albicans* and *Aspergillus niger*. Marketed Fluconazole cream used as control. The herbal cream containing Curcumin and Tulasi exhibited potent antifungal activity *in vitro* evolution reported.

B) **Procedure for *in vitro* studies for anti-inflammatory activity:** For evaluation of *in vitro* studies for anti-inflammatory activity the albumin denaturation test is adopted, works on cream's ability to prevent denaturation of bovine serum albumin (BSA). Prepared 1% BSA solution in phosphate-buffered saline (PBS), added various concentrations of the herbal cream (e.g., 10, 20, 30 µg/mL) to the BSA solution. Incubate for 30 minutes at 37 °C, further heated the BSA mixture at 70 ℃ for 15 minutes to induce denaturation, after cooling, measured the turbidity or absorbance at 660 nm using a spectrophotometer, Diclofenac used as Positive Control.

4. Results and Discussion

- **Results for physical evaluation of formulated cream contains turmeric and Tulasi**

Table 5: Physical evaluation of formulated cream

SL. No	Parameter	Sample 1	Sample 2	Sample 3
1	Color	Yellowish	Off-white	Light yellow
2	Odor	Mild Rose	Slight herbal	Pleasant rose
3	Consistency/Viscosity	28,000 cps	25,500 cps	30,000 cps
4	pH	5.8	6.1	6.0
5	Spreadability	6.5 g·cm/sec	7.0 g·cm/sec	6.2 g·cm/sec
6	Washability	Easily washable	Slight residue	Easily washable

- **Results for stability evaluation of formulated cream contains turmeric and Tulasi**

Table 5: Results for Stability Evaluation of Formulated Cream

SL. No	Test	Sample 1	Sample 2	Sample 3
1	Temperature Stability	Stable at 25 °C, 4 °C, 40 °C	Stable at 25 °C, 4 °C, 40 °C	Stable at 25 °C, 4 °C, 40 °C
2	Microbial Stability	TVC < 100 cfu/g	TVC < 50 cfu/g	TVC < 100 cfu/g
3	Physical Stability	No phase separation, no color change	No phase separation, no color change	No phase separation, no color change

- **Results for safety evaluation of formulated cream contains turmeric and Tulasi**

Table 6: Results for Safety Evaluation of Formulated Cream

SL. No	Test	Sample 1	Sample 2	Sample 3
1	Irritation Test	No irritation, safe	No irritation, safe	No irritation, safe
2	Allergy Test	No allergic reactions	No allergic reactions	No allergic reactions

- **Results for *in vitro* studies for anti-fungal activity (Agar Well Diffusion Method)**

Table 7: Results for *in vitro* studies for anti-fungal activity

SL. No	Formulation	*Candida albicans*	*Aspergillus niger*
1	Sample 1	13.2 mm	12.0 mm
2	Sample 2	14.5 mm	13.0 mm
3	Sample 3	12.0 mm	11.5 mm
4	Fluconazole Cream	18.2 mm	16.5 mm
5	Base Cream (No Actives)	0 mm	0 mm

- **Results for *in vitro* studies for anti-inflammatory activity (Albumin Denaturation Test)**

Table 8: Results for *in vitro* studies for anti-inflammatory activity

SL. No	Formulation	Inhibition of Denaturation (%)
1	Sample-1 (2 gm Curcumin, 2 gm Tulasi)	40%
2	Sample-2 (4 gm Curcumin, 4 gm Tulasi)	50%
3	Sample-3 (6 gm Curcumin, 6 gm Tulasi)	60%
4	Diclofenac (Positive Control)	70%
5	BSA (Control)	0%

5. Summary and Conclusion

The formulation and evaluation of an anti-fungal and anti-inflammatory herbal cream with Curcumin and Tulasi was successfully conducted, with three samples prepared at varying concentrations of the active ingredients. The cream included Excipients like beeswax, borax, and methyl paraben, liquid paraffin, rose oil, and distilled water to enhance texture, stability, and shelf life. In terms of efficacy, the cream demonstrated significant anti-fungal activity, with larger zones of inhibition against fungal strains, particularly in samples with higher concentrations of Curcumin and Tulasi. The anti-inflammatory activity, assessed through the Albumin

Denaturation Method, also showed promising results, with higher concentrations leading to greater inhibition of protein denaturation.

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