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Design and development of polyherbal cream for fungal infection

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Abstract
The purpose of this study was to create and assess a polyherbal antifungal cream by combining the beneficial effects of Ajwain (*Trachyspermum ammi*), Tulsi (*Ocimum sanctum*), Cumin (*Cuminum cyminum*), and Neem (*Azadirachta indica*). Using a standardized process, extracts of these medicinal plants—known for their antifungal properties—were added to the cream's formulation. A range of physicochemical properties, including pH, viscosity, spread ability, and stability, were assessed in order to guarantee the cream's quality and effectiveness. The polyherbal cream that was created had favorable physical and chemical characteristics, such as a pH that was appropriate for skin application, ideal viscosity, and good spreadability. With inhibitory zones comparable to those of conventional antifungal drugs, the cream showed strong antifungal efficacy against a variety of pathogenic fungal species. In addition, the cream exhibited no symptoms of skin irritation or unfavorable reactions in animal models, suggesting that it is safe for topical application.

Keywords: Antifungal, polyherbal formulation, cream, herbal cream

Introduction
The skin, being the largest organ in the body, serves as the body's final barrier against environmental stressors such dust, UV radiation, transmissible viruses, and chemical agents that can lead to infections and aging. Skin can be used to assess overall internal health and ageing [1]. Herbal medicine, often known as botanical medicine or herbalism, is the practice of treating wounds or ailments with plants or plant components [2]. The plant parts—seeds, leaves, stems, bark, roots, flowers, and so on—as well as their extracts that are used to make topical applications, tablets, capsules, teas, tinctures, and other formulations in herbal therapy. A few of these conventional plants are extracted and sold as medicinal drugs. The cheaper price, and frequently use, has attracted many medical professionals [3]. Plant matter is a valuable resource for combating a number of grave illnesses across the globe. Traditional medical practices, particularly the application of medicinal herbs as pastes, powders, etc. In impoverished nations, medicinal herbs are crucial for meeting basic health demands. The chemically active chemicals found in plants and secondary metabolites that can provide a specific physiological function to the human body are what give these herbs their therapeutic worth. Of such plant bioactive substances, carbohydrates, alkaloids, glycosides, tannins, flavonoids, phenolic compounds, etc. are the most important. Antibiotic resistance effects have become an ever-growing therapeutic problem as a result of the significant rise and spread of diseases in recent years. Higher plant natural products and secondary metabolites may have new antibacterial activity sources and maybe unique mechanisms of action. Natural remedies for infectious diseases are effective and reduce many of the harmful side effects associated with synthetic antimicrobials. Thus, it's critical to screen and study plant metabolites to confirm their application in traditional medicine and identify the active ingredient by chemical constituent separation and characterisation [4]. Polyherbal compositions consist of two or more plant combinations. The pharmacological formulation in Ayurveda is based on two principles and is administered as a single medication. A polyherbal composition uses a variety of medications. Ayurveda's "Sarangdhar Samhita" addresses the concept of mutualism that forms the basis of polyherbal remedies. Although active phytoconstituents are present in single-plant formulations, the levels present are often insufficient to produce the desired therapeutic benefits. Studies have shown that combining plants with varying degrees of potency yields better results than doing so alone. Positive interactions between herbs produce synergism. Synergistic effects can also be pharmacokinetic or pharmacodynamic [5].

Cream is a term used to describe semisolid emulsions that are meant to be applied externally. They can be water-in-oil (w/o) or oil-in-water (o/w). Cream is divided into two phases: water-in-oil and oil-in-water [6]. The best option for lessening skin issues is cosmetics. Cosmetics are used to improve one's beauty on the outside as well as one's health by lowering skin problems. Skincare products made from natural herbs hydrate, nourish, and moisturize the skin. The current project seeks to create a herbal cream with antifungal properties. Present a polyherbal cream containing medications such as Tulsi, Ajwain, Cumin, and Neem [7].

Neem

Neem is a kind of tree in the Meliaceae family, Azadirachta indica. The primary growing region for neem (Azadirachta indica) is the Indian subcontinent. It is said that neem has been used extensively by people throughout history to treat a wide range of ailments. The World Health Organization claims that in developing countries, traditional medicine is practiced by the entire populace. In the Indian subcontinent, the Azadirachta indica tree has been used for medicinal purposes for over 4500 years. Medicine is made from the seeds, bark, and leaves. The fruit, flower, and root are all utilized, but less commonly. Neem leaf is applied topically to leprosy, eye conditions, bleeding noses, intestinal worms, upset stomachs, appetite loss, skin ulcers, heart and blood vessel problems (cardiovascular disease), fever, diabetes, gingivitis, and liver problems.

Taxonomy Classification: [8]

Table 1: Taxonomy Classification

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	Azadirachta
Species	A. indica

Ajwain

The erect annual herb known as ajwain, or *Trachyspermum ammi* (L.) Sprague, has a striate stem and is native to eastern Persia and India. The little fruit that resembles caraway is the most commonly used portion of ajwain and is especially well-liked in Indian savory recipes, savory pastries, and snacks. Its antispasmodic, stimulant, tonic, and carminative qualities make it a useful herb in Ayurvedic therapy. Since ancient times, aromatherapy has made extensive use of the scent compounds found in spices, which may have some positive health effects in addition to their delicious flavor. They also prevent other unfavorable alterations to food that could damage its flavor, texture, and nutritional value.

Taxonomy Classification: [9-13]

Table 2: Taxonomy Classification

Kingdom	Plantae
Division	Magnoliopsida
Class	Magnoliopsida
Order	Apiales
Family	Apiaceae
Genus	Trachspermum
Species	T. ammi

Cumin

The world's second most popular spice, *cuminum cyminum* L., is grown mostly in Arabia, China, India, and the nations bordering the Mediterranean Sea. Black pepper is the most popular spice worldwide. Cumin is used medicinally as a stimulant, carminative, astringent, and to treat diarrhea, gas, and indigestion.

Taxonomy Classification: [14-15]

Table 3: Taxonomy Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Apiales
Family	Apiaceae
Genus	*Cuminum* L
Species	*Cuminum Cyminum* L

Tulsi

Tulsi is considered to be the most sacred plant in India. In Ayuveda, the usage of Ocimum sanctum, or Tulsi, as an aromatic plant is well-documented. It is a member of the Labiateae family. It is cultivated in subtropical and tropical regions, such as India. It is ubiquitous in every Indian field. It's a tall herb with a pleasant smell. The Sanskrit name "Tulsi" means "the incomparable one". The entire plant is utilized as a medicinal source. Two types of tulsi are more popular in India: Rama tulsi (light) and Shyama (Krishna) tulsi. The former is frequently used for worship and has more medical value. Many more species, such as *O. canum, O. basilicum, O. kilimandscharicum, O. ammericanum, and O. camphora*, and *O. micranthum*. are also frequently found in India.

Taxonomy Classification: [16-19]

Table 4: Taxonomy Classification

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	Ocimum
Species	O. tenuiflorum

Material and Instruments

a) Instruments used for work

Table 5: Instruments used for work

Sr.no.	**Name of Instrument**
1.	Soxhlet Apparatus
2.	Electronic weighing balance
3.	pH meter
4.	Brookfield viscometer (LVDV-60)
5.	Heating mantle
6.	Electronic waterbath

b) Chemicals used for work

Experimental Methods

Pharmacognostic Investigation

Collection and Authentication

Collection of Neem, Ajwain, Cumin, Tulsi powders from Aditya herbals, Kolhapur.

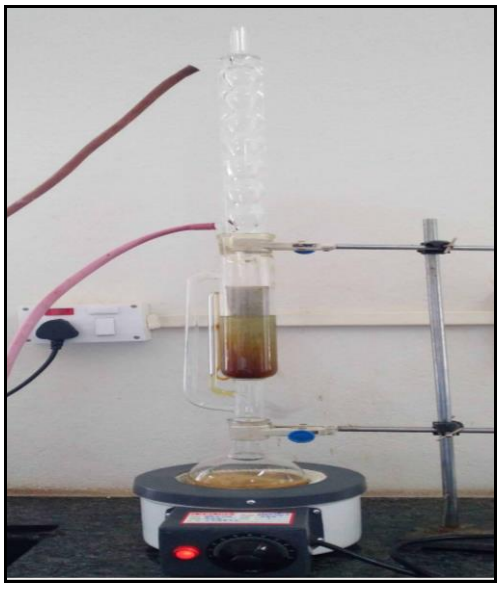
Table 6: Chemicals used for work

Sr. No.	Chemicals
1.	Ethanol
2.	Stearic acid
3.	Cetyl alcohol
4.	Alomond oil
5.	Triethanolamine
6.	Benzyl alcohol
7.	Glycerine
8.	Propyl paraben
9.	Methyl paraben
10.	Distilled water

Extraction

Polyherbal ethanolic extract preparation.

- The Fine powder were carefully chosen.
- A 43-mesh sieve was used to filter the fine powder, which was then placed in an airtight container for later usage.
- Using the hot extraction method and the Soxhlet apparatus, about 50 gm of powdered material were extracted with ethanol as a solvent.
- The extraction was carried out until the solvent in the thimble turned clear, at which point a few drops were collected in the test tube during the cycle's conclusion and the solvent's chemical composition was tested.
- The extract was dried in a rotary vacuum evaporator following each extraction.
- Moreover, some part of the extract was preserved for preliminary Phytochemical screening [20].



IMG 1: Extraction Process

Preliminary Phytochemical Investigation

The ethanolic extract was subjected to qualitative chemical investigation. The following procedures were adopted to test for the presence of various phytochemical constituents in the extract. Most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins, saponins and glycosides. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs. The following procedures were adopted to test for the presence of various chemical constituents in extract.

Test

- **Test for Saponins**

Foam test

A small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of Saponin.

- **Test for Alkaloids**

a) Mayer's test: 2-3 ml of filtrate with few drops of Mayer's reagent gives ppt.

b) Wagner's test: 2-3 ml of filtrate with few drops of Wagner's reagent gives Reddish brown colour.

- **Test for Tannins**

Ferric chloride test

To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of Tannins.

- **Test for Steroids**

Liebermann's reaction

Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of conc. H2SO4. Blue color appears.

- **Test for Flavanoids**

Alkaline reagent test

Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

- **Test for Terpenoids**

Salkowski reaction

To 2 ml of extract, add 2 ml chloroform and 2 ml of conc. H2SO4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

- **Test for Reducing Sugar**

Benedict's test

Mix equal volume of Benedict's reagent and test extract in test tube. Heat in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

- **Test for Proteins**

Biuret test

a) Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins.

b) To 3 ml. extract add 4% NaOH and few drops of 1% CuSO4 solution. Violate or pink colour appears [21].

Preformulation study

Preformulation studies are needed to ensure the development of a stable as well as effective and safe dosage form. It is a stage of development during which the pharmacist characterizes the physic-chemical properties of the drug substances and its interaction with various formulation components. Goals of Preformulation study:

- To determine the necessary physicochemical parameter of a new drug substance.
- To establish its incompatibility with excipients of formulation.

Experimental Design:

Formulation of Herbal Cream

Preparation of herbal Cream

Selection of excipients

Neem, Ajwain, Cumin, Tulsi is collected from Aditya herbals, Kolhapur. The raw materials and chemicals were taken from Ashokrao mane institute of pharmacy, AMBAP, kolhapur.

Method of preparation

Oil in water (O/W) emulsion, (semisolid formulation) is formulated. The stearic acid cetyl alcohol and almond oil are dissolved in the oil phase and these are heated To 75°C. This is part A. The water-soluble components like methylparaben, triethanolamine, propylparaben, extract dissolved in an aqueous phase and it is heated up to 75°C. This is Part B. After heating, the aqueous phase was added in portions to the oil phase with Continuous stirring until the cooling of the emulsifier took place [22].

Table 7: Formulation table

Sr.no.	Ingredients	Batches			Role of ingredient
		A1	A2	A3	
1	Ethanolic extract	0.5 gm	0.5 gm	0.5 gm	Therapeutic agent
2	Almond oil	0.5 ml	0.5 ml	0.5 ml	Emollient
3	Benzyl alcohol	0.2 ml	0.2 ml	0.2 ml	Preservative
4	Stearic acid	1 gm	1 gm	1 gm	Emollient
5	Cetyl alcohol	0.5 gm	0.5 gm	0.5 gm	Emollient, Co-emulsifier
6	Triethanolamine	0.2 ml	0.2 ml.	0.2 ml	Neutralizer
7	Glycerine	0.5 ml	0.5 ml	0.5 ml	Vehicle
8	Propyl paraben	0.2 gm	0.2 gm	0.2 gm	Preservative
9	Methyl Paraben	0.2 gm	0.2 gm	0.2 gm	Preservative
10	Distilled water	qs	qs	qs	Vehicle

Evaluation of Cream

1. Physical Evaluation

Physical parameters such as color and appearance were evaluated.

2. Homogenecity

All developed creams were tested for homogeneity by visual inspection after the creams have been set in the container for their appearance and presence of any aggregates.

3. pH

The pH of various creams formulations were determined by using digital pH meter. 2.5gm of cream was accurately weighed and dispersed in 25ml of distilled water and stored for two hours. The measurement of pH of each formulation was carried out in triplicate and the average values are represented. The pH of dispersions was measured using pH meter.

4. Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadability was measured on the basis of slip and drag characteristics of creams. An excess of creams (about 2 g) under study was placed on this ground slide. The cream was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. Weight of 1 kg was placed on the top of the slide for 5 minutes to expel air and to provide a uniform film of the cream between the slides. Excess of the cream was scrapped off from the edges. The top plate was then subjected to pull of 50 g. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 6.5 cm be noted. A shorter interval indicates better spreadability.

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 each other.

5. Viscosity

Viscosity of herbal cream was determined by using Brookfield rotational viscometer at 5, 10 20, 30 and 50 rpm using spindle no.64. Each reading was taken after equilibrium of the sample at the end of two minutes. The viscosity determination of samples was repeated three times [23, 24].

6. Stability study

The optimized cream formulations were prepared; packed in aluminum collapsible tubes and subjected to stability studies at 40 ^{0}C/75% RH for a period of 1 month as per ICH Guidelines. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance, pH, rheological properties, spreadability.

Result and Discussion

Extraction of Herbs

Table 8: Extractive values of Herbs

Sample	Extraction method	Solvent used	Wt. of sample	Extraction value (%w/w)
Neem	Soxhlet extraction	Ethanol	100 gm	10% w/w
Ajwain	Soxhlet extraction	Ethanol	100 gm	10% w/w
Cumin	Soxhlet extraction	Ethanol	100 gm	10% w/w
Tulsi	Soxhlet extraction	Ethanol	100 gm	10% w/w

Physicochemical evaluation of Cream:

Physical Appearance

Table 9: Physical appearance of cream

Sr. no.	Batch	Color	Appearance
1	A1	Light Green	Green
2	A2	Green	Green
3	A3	Dark Green	Green

All formulation batches were found to be homogeneous Green cream preparations

Homogeneity
All developed creams were tested for homogeneity by visual inspection after the creams have been set in the container.

Table 10: Homogeneity of formulation

Sr. No.	Batch	Homogenecity
1	A1	Homogeneous
2	A2	Homogeneous
3	A3	Homogeneous

Measurment of pH
The pH values of all prepared formulation ranged from 6-7 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Spreadability
The time in seconds require to separate the two slides was taken as measure of spreadability.

Table 11: pH and Spreadability of extracts formulation.

Sr. No.	Batch	pH	Spreadability (gm.sm/sec)
1	A1	6.8 /±0.03	16.15/±0.005
2	A2	7.0/±0.03	15.50/±0.005
3	A3	7.2/±0.03	15.35/±0.005

Viscosity
Viscosity of cream was determined by using Brookfield rotational viscometer at 5, 10, 20, rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times.

Table 12: Viscosity value of herbal cream

Sr. No.	rpm	Viscosity (Cps)
1	5	3615±0.20
2	10	3710±0.21
3	20	4027±0.11

Optimization Batch
The batches were optimized by checking, and by studying physical evaluation to their pH, viscosity, Spreadability, greasiness, homogenicity, washability and Stability study of all formulation batches. By studying the evaluation parameters off all batches, batch A2 from leaves extract cream formulation were be optimized.

Conclusion
In the realm of natural cosmetics and dermatology, the creation of a polyherbal antifungal cream that makes use of the synergistic effects of neem, tulsi, cumin, and ajwain offers a promising approach. Because these herbs each contain special bioactive components that have strong antifungal, antibacterial, and anti-inflammatory qualities, they are useful substances in the fight against different types of fungal skin infections. The compounds azadirachtin and nimbin from neem, eugenol and rosmarinic acid from tulsi, cuminaldehyde from cumin, and thymol from ajwain work together to produce a wide range of antibacterial activities, as well as to reduce inflammation and encourage skin healing. The polyherbal antifungal cream offers efficacy against fungal pathogens and potential benefits in reducing related symptoms like itching and redness by mixing various botanical elements into a synergistic composition. In addition, using natural components lowers the possibility of side effects that are frequently linked to synthetic antifungal agents, making it a safer substitute for long-term beauty routines. To confirm the effectiveness and safety of this polyherbal formulation in a wider patient group, additional clinical research is necessary. The polyherbal antifungal cream, which harnesses the power of nature's pharmacy to promote skin health and well-being, offers a viable path toward the development of comprehensive and effective treatments for fungal skin infections.

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