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FORMULATION AND EVALUATION OF POLYHERBAL ANTIFUNGAL CREAM

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ABSTRACT
The present study focuses on the formulation, development, and evaluation of a polyherbal antifungal cream prepared from *Phyllanthus acidus*, *Cinnamomum tamala*, and *Madhuca longifolia* plants traditionally recognized for their medicinal and antifungal properties. The mature leaves of these species were collected, authenticated, and extracted sequentially using solvents of increasing polarity. Preliminary phytochemical screening confirmed the presence of flavonoids, phenols, tannins, saponins, and alkaloids, all known for their antifungal efficacy. The in-vitro antifungal activity of the extracts was assessed against *Aspergillus niger*, *Aspergillus ochraceus*, and *Candida albicans* using the agar well diffusion and broth dilution methods. The hydroalcoholic extracts exhibited the highest antifungal activity, with a Minimum Inhibitory Concentration (MIC) of approximately 0.78 mg/mL, indicating strong antifungal potential. Based on these findings, polyherbal topical creams were formulated using 1%, 2%, 3%, and 4% concentrations of the hydroalcoholic extract. The prepared formulations demonstrated desirable physicochemical properties, including smooth texture, good spreadability, suitable viscosity, and a pH close to normal skin levels (~5.5). These characteristics confirm the formulations' suitability for dermal application. Preliminary results highlight the effectiveness and stability of the polyherbal cream, supporting its potential as a safe, natural alternative for managing fungal skin infections. Future studies involving in-vivo evaluations are recommended to establish clinical efficacy, stability, and consumer acceptability.

1. INTRODUCTION

Fungal infections represent a major global health concern, affecting millions of people each year. They range from superficial skin infections, such as dermatophytosis and candidiasis, to more severe systemic mycoses, particularly in immunocompromised individuals **(Denning, (2024).** The increasing incidence of fungal infections has been further exacerbated by the overuse of antibiotics, environmental factors, and the emergence of drug-resistant fungal strains. Conventional antifungal agents, including azoles, polyenes, and allylamines, though effective, often present limitations such as adverse side effects, high cost, limited accessibility, and reduced efficacy due to resistance development **(Branda *et al.*, 2025).** Consequently, there is growing interest in exploring **herbal alternatives** that are safer, cost-effective, and compatible with long-term use.
The present study focuses on the formulation,

Journal of Molecular Science
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development, and evaluation of a polyherbal antifungal cream using three traditionally significant medicinal plants: *Phyllanthus acidus* (L.) Skeels, *Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm., and *Madhuca longifolia* (Koenig) J.F. Macbr. Each of these plants has been widely documented in ethnomedicinal literature for their therapeutic properties, particularly antifungal, antibacterial, and wound-healing effects **(Bittner Fialová *et al.*, 2021).**

- **Phyllanthus acidus**, commonly known as Otaheite gooseberry, is rich in phenolic compounds, flavonoids, and tannins that exhibit strong antioxidant and antimicrobial properties **(Pangestika *et al.*, 2020).**
- **Cinnamomum tamala**, or Indian bay leaf, contains cinnamaldehyde, eugenol, and other essential oils known for their antifungal and anti-inflammatory activities **(Vaishnav and Shahi 2025).**
- **Madhuca longifolia**, widely used in Ayurvedic medicine, is a source of triterpenoid saponins and flavonoids that have demonstrated antifungal, wound-healing, and emollient properties **(Khare *et al.*, 2018).**

The rationale for selecting these plants lies in their complementary pharmacological actions and traditional use in managing skin-related infections. The combination of their hydroalcoholic extracts in a single topical formulation is expected to yield enhanced antifungal efficacy through synergistic interactions among bioactive compounds **(Sitarek *et al.*, 2020).**

In the development of herbal topical formulations, creams serve as an ideal vehicle for delivering plant-derived active ingredients to the skin. Creams provide a convenient, non-greasy, and aesthetically acceptable dosage form that promotes patient compliance **(Mohiuddin, 2019).** Moreover, the incorporation of herbal extracts into a semisolid base allows for localized drug action, reduced systemic exposure, and minimal toxicity. For antifungal therapy, topical creams are particularly advantageous as they provide direct contact with the infected area, ensuring effective drug penetration and sustained action **(Garg *et al.*, 2020).**

Thus, the objective of this research is to develop a stable and effective polyherbal topical formulation combining the extracts of *Phyllanthus acidus*, *Cinnamomum tamala*, and *Madhuca longifolia*, and to evaluate its phytochemical profile, antifungal efficacy, and formulation characteristics as a potential natural remedy for fungal skin infections.

2. MATERIALS AND METHOD:

2.1 Selection of plant:

Phyllanthus acidus,Cinnamomum tamala and Madhuca longifolia areselected for the present study, based on literature survey.

2.2 Collection and authentication of plant material done by botanist

Leaves of the plants were collected in the month of July 2023 from local market of Ujjain (M.P.). All these plants parts were identified and authenticated by Professor S R Kshrisagar Voucher specimens of the plant parts were submitted in PG Department of Botany, Dr P R Ghogrey Science College, Dhule for reference purpose.

2.3. Pharmacognostic studies of selected plant material (WHO Guidelines)

Pharmacognostical evaluation plays a vital role in standardization and identification of crude drugs. It includes:

2.3.1 Macroscopic studies: Organoleptic evaluation such as color, odor, taste, size, shape, and surface characteristics of the leaves will be performed. These observations help in preliminary identification of crude plant material and detection of adulterants **(Shonte and De Kock 2017).**

2.3.2 Microscopic studies: Detailed study of transverse sections of the leaves will be carried out. Features such as epidermal cells, stomatal index, trichomes, venation pattern, vascular bundles, and mesophyll tissues will be studied under microscope. Powder microscopy will also be performed to confirm the presence of diagnostic features like calcium oxalate crystals, fibers, starch grains, etc. These microscopic markers serve as permanent reference for plant identification **(Alamgir, 2017).**

The studies of organoleptic characters provide the simplest and quickest means to establish identity, purity and possibly, quality of crude drugs. If a sample is found to be significantly different, in terms of color, consistency, odor or taste, from the specifications, it is considered as not fulfilling the requirements.

- **Color:** All the samples will be taken in to watch glass and examined untreated under diffuse daylight. They will be observed for their color by naked eye **(Shawwa *et al.*, 2024).**
- **Odor:** The odor of all the samples will be examined. The time interval of two minutes will be kept among the two smelling, in order to nullify the effect of previous smelling **(Ulusoy *et al.*, 2017).**
- **Taste:** The samples will be examined separately for their taste on taste bud of the tongue. The time interval among each sample will be kept

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 732-740
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15 minutes, so as to make taste buds available fresh every time **(Witt and Reutter 2015).**

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2.4 Extraction of dried leaves

50gms of dried shaded leaves powder will be exhaustively extracted with chloroform, ethyl acetate, ethanol, water and hydro alcohol using soxhlet extraction apparatus. The extracts were evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts **(Aliero, 2018).**

2.5 Physiochemical Analysis of plant material

In the standardization of herbal material, physical and physico-chemical factors play an important role in the establishment of purity and quality **(Bueno and Cavalheiro 2019).** Different physicochemical parameters were studied as listed below.

a) Determination of pH

The pH of each extract was determined using a calibrated pH meter. One gram of sample was dispersed in 100 mL of distilled water and stirred for 5 minutes. The pH was recorded in triplicate, and the mean value was calculated for each sample **(Pandey *et al.*, 2015).**

b) Determination of Loss on Drying (LOD)

Two grams of each extract were accurately weighed into pre-dried LOD bottles and heated at **105 ± 2°C** for 1 hour. After cooling in a desiccator, the samples were reweighed, and the **percentage loss on drying** was calculated using **(Desalegn and Kibr 2021).**

$$\% \text{ of Loss on Drying} = \frac{(B - A)}{\text{Wt. of taken}} \times 100$$

Where A = initial weight and B = final weight.

c) Determination of Ash Values

The inorganic content of the samples was determined through total ash, water-soluble ash, and acid-insoluble ash analyses.

- **Total Ash:** One gram of air-dried sample was incinerated at **650°C** until carbon-free. The ash content was expressed as a percentage of the air-dried sample **(Patel *et al.*, 2025).**
- **Water-Soluble Ash:** One gram of total ash was boiled with 25 mL of water for 5 minutes, filtered, and ignited. The difference in weight gave the **water-soluble ash,** expressed as a percentage **(Bulama *et al.*, 2015).**
- **Acid-Insoluble Ash:** Total ash was boiled with 25 mL of 10% HCl for 10 minutes, filtered, and the residue ignited at **650°C**. The **acid-insoluble ash** was calculated as a percentage of total ash **(Harris and Marshall 2017).**

d) Determination of Extractive Values

Extractive values were determined for **water-soluble** and **alcohol-soluble** extracts. Five grams of each sample were macerated with 100 mL of the respective solvent (distilled water or methanol) for 24 hours, with intermittent shaking. The filtrate (25 mL) was evaporated to dryness and dried at **105°C** to a constant weight **(Noorul *et al.*, 2017).** The **percentage of extractive value** was calculated using:

$$\% \text{ of water soluble extractive} = \frac{\text{Wt. of residue}}{\text{Wt. of sample}} \times 100$$

2.6 Pharmacological evaluation viz. antifungal activity

The antifungal activity of dried powder extracts will be tested against human pathogenic fungi. The chloroform, ethanol, water and hydro alcohol extracts at different concentrations such as 25 µg/ml, 50 µg/ml and 100 µg/ml will be taken for studying the efficacy. The minimum inhibitory concentration will be determined by agar well diffusion method **(Zanna *et al.*, 2021).** The following fungi is selected for studies:

- *Aspergillus niger*
- *Aspergillus ochraceus*
- *Candida albicans*

2.6.1 Dilution method

Minimum Inhibitory Concentration will be carried out by agar dilution method. In the freshly prepared and sterilized potato dextrose agar medium, 1 mg streptomycin was added for preventing bacterial growth. Then 20 ml of Potato dextrose agar medium was poured into each petriplate and allowed to solidify. The test fungal cultures were evenly spread over the appropriate media by using sterile cotton swab. Then a well 6 mm was made in the medium by using sterile cork borer, 0.1, 0.19, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25 mg/ml of each concentration of chloroform, methanol, petroleum ether and aqueous extracts were transferred into separate wells. Then these plates were incubated at 27 °C for 48-72 hours. After incubation period the results were observed and measured the diameter of inhibition zone around the each well **(Simões *et al.*, 2022).**

2.7 Formulation of herbal cream using leaves extracts

An oil-in-water (O/W)-based cream (20 g) will be formulated. The emulsifier (stearic acid) and other oil-soluble components, thickening agent (cetyl alcohol) and emollient or lubricant (liquid paraffin) were dissolved in the oil phase and heated to 75 °C (Part A). The aqueous phase will be prepared by dissolving the required amount of extract in

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 732-740
DoI-10.004687/1000-9035.2025.100

propylene glycol solvent and then adding it to water. The preservatives (methylparaben, triethanolamine) and the other water-soluble component glycerine, which will be used as humectant, were dissolved in the aqueous phase and heated to 75 °C. After heating, the aqueous phase (Part B) will be added in portions to the oil phase (Part A) with continuous stirring until cooling of emulsifier occurred. The creation of the galangal extract cream will be done according to the method mentioned in reference. A 1% w/w cream will be prepared using the formula given below **(Kelm and Wickett 2017).**

Table 1: Formulation of Herbal Cream

Components	Ingredients	Quantity (for 1,2,3 and 4%, 20g cream)	Uses
Oil phase (Part A)	Leaves Extract	0.2. 0.4, 0.6 and 0.8	Antifungal
	Stearic acid	2.2	Thickener
	Cetyl alcohol	0.8	Emulsifier
	Liquid paraffin	0.8	Emollient
Aqueous phase (Part B)	Water	QS 20gms	Aqueous base
	Triethanolamine	0.3	Neutralizer
	Glycerin	1	Humectant
	Methylparaben	0.2	

2.8 Evaluation of herbal cream

2.8.1 Physical Properties: The Cream will be observed for colour, odour and appearance

2.8.2 Determination of pH: 0.5 ± 0.01g of the cream will be weighed accurately in a 10ml test tube. 4.5ml of water will be added & dispersed the cream in it. The pH of the suspension will be determined at 270 C using the pH meter **(Dhase and Saboo 2014).**

2.8.3 Viscosity: Viscosity will be measured by Brookfield Viscometer. The determination will be carried out in triplicate and the average of three readings will be recorded **(Khan *et al.*, 2014).**

2.8.4 Spreadability: Spreadability is an important criterion for semisolid formulations, indicating how easily a cream spread on the skin, which directly affects its therapeutic efficacy. It is measured using a specially designed apparatus, where the formulation is sandwiched between two glass slides under a specified load. A weight is applied, and the time (in seconds) taken for the upper slide to move a set distance (5 cm) under a 3 g load is recorded. Lower time values indicate better spreadability, which can be calculated using the standard formula **(Jin *et al.*, 2022).**

Spreadability= m ×l /t

Where, m = weight tied to the upper slide (3g) l =length of glass slide (5cm) t =time taken in seconds

2.8.5 Stability studies: - Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies will be done according to ICH guidelines. The stability studies will be carried out as per ICH guidelines. The cream filled in bottle and kept in humidity chamber maintained at 30 ± 2°C/ 65 ± 5 % RH and 40 ± 2°C / 75 ± 5 % RH for a month. At the end of studies, samples will be analyzed for the physical properties and viscosity **(Anusha *et al.*, 2017).**

3. RESULTS AND DISCUSSION

3.1 Selection of the plant

On the ground of literature review and deep discussion with medical practitioners of the Ujjain (M.P.) *Pyllanthus acidus, Cinnamomum tamala, and Madhuca longifolia* (leaves) were selected for evaluation of the antifungal activity.

3.2 Collection and authentication of plant materials:

Leaves of the plants were collected in the month of July 2023 from local market of Ujjain (M.P.). All these plants parts were identified and authenticated by Professor S R Kshrisagar Voucher specimens of the plant parts were submitted in PG Department of Botany, Dr P R Ghogrey Science College, Dhule for reference purpose.

3.3 Development of Standardization parameters of selected plant materials:

3.3.1 Morphological studies:

The morphology or macroscopical parameters like; size, shape, colour, odour, taste and fracture were studied by sensory organs.

Table 2: Morphological studies of selected plants

Plant	Part Used	Colour	Odor	Taste
Pyllanthus acidus	Leaves	Dark brown	No odor	Bitter
Cinnamomum tamala	Leaves	Brown	Clove like	Peppery
Madhuca longifolia	Leaves	Reddish brown	No odor	Bitter

3.3.2 Physicochemical Studies:

Table 3: Physiochemical studies of selected plant material

Plant	pH	LOD	Ash value		
			Total ash	Water soluble ash	Acid insoluble ash
Phyllanthus acidus	6.5	79	9.2	1.6	3.1
Cinnamomum tamala	5.9	81	10.2	1.5	3.5
Madhuca longifolia	6.3	76	9.4	1.6	3.7

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 732-740
DoI-10.004687/1000-9035.2025.100

3.3 Extraction of dried leaves with various solvents:

Table 4: Percentage yield of leaves extract of *Phyllanthus acidus, Cinnamomum tamala* and *Madhuca longifolia*

Solvent	% Yield (*Phyllanthus acidus)*	% Yield (*Cinnamomum tamala*)	% Yield(*Madhuca longifolia*)
Chloroform	29.18		
Ethyl acetate	35.82	31.28	26.21
Ethanol	42.27	33.13	27.17
Water	44.23	52.17	46.65
Hydroalcoholic	**48.64**	46.32	41.18
		53.46	**49.15**

Table 5: extractive values of leaves extract of *Phyllanthus acidus, Cinnamomum tamala* and *Madhuca longifolia*

Solvent	Extractive value (*Phyllanthus acidus*)		Extractive value (*Cinnamomum tamala*)		Extractive value (*Madhuca longifolia*)	
	Water soluble extractive	Alcohol soluble extractive	Water soluble extractive	Alcohol soluble extractive	Water soluble extractive	Alcohol soluble extractive
Chloroform	9	10	10	15	8	9
Ethyl acetate	11	18	13	11	19	9
Ethanol	12	51	19	49	23	45
Water	66	11	66	13	53	18
Hydroalcoholic	**46**	**44**	**49**	**51**	**45**	**49**

3.4 Preliminary phytochemical screening of leaves extract

Table 6: Phytochemical evaluation of *Phyllanthus acidus* leaves extract

Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Water	Hydroalcoholic (1:1)
Carbohydrate	*Molisch's test*	+	+	+	+	++
	Fehling's test	+	+	+	+	+
Glycosides	*Legal's test*	+	++	+	+	+++
	Borntrager's test	++	+	+	+	+
	Baljet test	+	+	++	+	+
Fixed oil and Fats	*Spot test*	+	++	+	+	+++
	Saponification test	++	+	+	+	+
Proteins and Amino Acids	*Biuret test*	+	+	+	+	+++
Saponins	*Foam test*	+	+	+	+	+
Phenolic Comp. and Tannins	*$FeCl_3$ test*	++	+	+	+	+
Steroids	*Libermann-bucchard test*	+	++	+	+	++
Alkaloids	*Dragendorff's test*	+	+	+	+	+
	Mayer's test	+	+	+	+	++
	Wagner's test	+	+	+	+	+
Terpines		+	+	+	+	+
Flavonoids	*Lead acetate test*	+	+	++	+	++
	Con. H_2SO_4 test	++	+	+	+	+++
	FeCl3 test	+	+	+	+	++

Table 7: Phytochemical evaluation of *Cinnamomum tamala* leaves extract

Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Water	Hydroalcoholic (1:1)
Carbohydrate	*Molisch's test*	++	+	+	+	+
	Fehling's test	+	++	++	+	+++
Glycosides	*Legal's test*	+	+	+	+++	+
	Borntrager's test	-	+	++	+	+
	Baljet test	+	++	+	+	+
Fixed oil and Fats	*Spot test*	++	+	+	+	+
	Saponification test	+	+	-	-	-
Proteins and Amino Acids	*Biuret test*	+	+	++	+	+
Saponins	*Foam test*	+	+	+	+	+
Phenolic Comp. and Tannins	*$FeCl_3$ test*	++	+	+	+	+++
Steroids	*Libermann-bucchard test*	-	+	+	+	+
Alkaloids	*Dragendorff's test*	+	+	++	+	+
	Mayer's test	+	+	+	+	+
	Wagner's test	+	+	+	+	+
Terpines		+	+	+	+	+
Flavonoids	*Lead acetate test*	+	+	+	+	+++
	Con. H_2SO_4 test	++	+	+	+	++
	FeCl3 test	+	+	+	+	+

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 732-740
DoI-10.004687/1000-9035.2025.100

Table 8: Phytochemical evaluation of Madhuca longifolia leaves extract

Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Water	Hydroalcoholic (1:1)
Carbohydrate	*Molisch's test*	+	+	++	+	+
	Fehling's test	++	-	+	+	++
Glycosides	*Legal's test*	+	+	+	+	+
	Borntrager's test	+	+	++	+	+
	Baljet test	++	+	+	+	+
Fixed oil and Fats	*Spot test*	+	+	-	+	++
	Saponification test	+	++	+	+	+
Proteins and Amino Acids	*Biuret test*	+	+	+	+	+
Saponins	*Foam test*	+	+	+	+	+
Phenolic Comp. and Tannins	*$FeCl_3$ test*	+	+	++	+	+
Steroids	*Libermann-bucchard test*	++	+	+	+	++
Alkaloids	*Dragendorff's test*	+	+	+	+	+
	Mayer's test	+	+	-	+	+
	Wagner's test	+	+	+	+	+
Terpines		+	+	+	+	+
Flavonoids	*Lead acetate test*	-	+	+	+	+++
	Con. H_2SO_4 test	+	++	+	+	++
	FeCl3 test	+	+	+	+	+++

3.5 Pharmacological evaluation viz. antifungal activity

The antifungal activity of dried leaves extracts were tested against human pathogenic fungi. The plants extract at different concentrations such as 25 mg/ml, 50 mg /ml and 100 mg /ml were taken for study against three fungal species *Aspergillus niger, Aspergillus ochraceus, Candida albicans* for the determination of zone of inhibition.

Table 9: The zone of inhibition of *Phyllanthus acidus* leaves extracts

Zone of inhibition (in mm)				
Leaves Extract	**Concentration (mg/ml)**	***Aspergillus niger***	***Aspergillus ochraceus***	***Candida albicans***
Chloroform	**25**	10.4	12.4	12.7
	50	14	13.3	13.1
	100	16.7	14.7	15.7
Ethyl acetate	**25**	11.5	10.5	11.1
	50	12	10.8	11.5
	100	12.3	11.7	11.7
Ethanol	**25**	11.4	12.4	12.7
	50	13	13.6	13.4
	100	14.7	15.7	15.7
Water	**25**	10.4	12.7	12.7
	50	12.3	15.3	13.1
	100	13.7	17.7	16.6
Hydro-alcoholic	**25**	**15.4**	**14.4**	**15.1**
	50	**17**	**16.3**	**17.1**
	100	**21.7**	**19.7**	**22.7**

Table 10: The zone of inhibition of *Cinnamomum tamala* leaves extracts

Zone of inhibition (in mm)				
Leaves Extract	**Concentration (mg/ml)**	***Aspergillus niger***	***Aspergillus ochraceus***	***Candida albicans***
Chloroform	**25**	10.7	12.7	13
	50	14.3	13.6	13.4
	100	17	15	16
Ethyl acetate	**25**	11.8	10.8	11.4
	50	12.3	11.1	11.8
	100	12.6	12	12
Ethanol	**25**	11.7	12.7	13
	50	13.3	13.9	13.7
	100	15	16	16
Water	**25**	10.7	13	13
	50	12.6	15.6	13.4
	100	14	18	16.9
Hydro-alcoholic	**25**	**15.7**	**14.7**	**15.4**
	50	**17.3**	**16.6**	**17.4**
	100	**22**	**20**	**23**

Table 11: The zone of inhibition of *Madhuca longifolia* leaves extracts

Zone of inhibition (in mm)				
Leaves Extract	**Concentration (mg/ml)**	***Aspergillus niger***	***Aspergillus ochraceus***	***Candida albicans***
Chloroform	**25**	7.2	9.4	10
	50	10.8	10.3	10.4
	100	13.5	11.7	13
Ethyl acetate	**25**	8.3	7.5	8.4
	50	8.8	7.8	8.8
	100	9.1	8.7	9
Ethanol	**25**	8.2	9.4	10
	50	9.8	10.6	10.7
	100	11.5	12.7	13
Water	**25**	7.2	9.7	10
	50	9.1	12.3	10.4
	100	10.5	14.7	13.9
Hydro-alcoholic	**25**	**12.2**	**11.4**	**12.4**
	50	**13.8**	**13.3**	**14.4**
	100	**18.5**	**16.7**	**20**

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 732-740
DoI-10.004687/1000-9035.2025.100

Table 12: Comparative study of different leaves extract and polyherbal extract on zone of inhibition

Zone of inhibition (in mm)				
Leaves Extract	**Concentration (mg/ml)**	**Aspergillus niger**	**Aspergillus ochraceus**	**Candida albicans**
Phyllanthus acidus Hydro-alcoholic (1:1) Extract	**25**	15.4	14.4	15.1
	50	17	16.3	17.1
	100	21.7	19.7	22.7
Cinnamomum tamala Hydro-alcoholic (1:1) Extract	**25**	15.7	14.7	15.4
	50	17.3	16.6	17.4
	100	22	20	23
Madhuca longifolia Hydro-alcoholic (1:1) Extract	**25**	12.2	11.4	12.4
	50	13.8	13.3	14.4
	100	18.5	16.7	20
Blend of Hydroalcoholic extract of Phyllanthus acidus, Cinnamomum tamala and Madhuca longifolia (1:1:1)	**25**	**18.2**	**21.4**	**22.4**
	50	**23.8**	**23.3**	**24.2**
	100	**28.5**	**26.7**	**30.5**

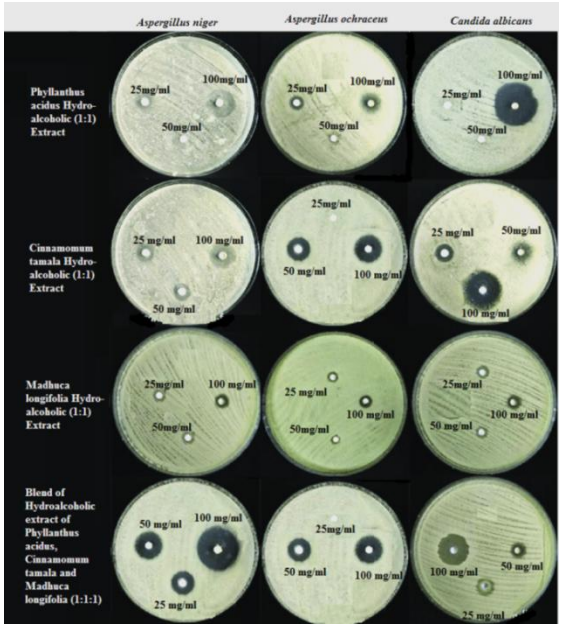

Figure 1: Zone of inhibition

Table 13: Minimum inhibitory concentration of selected extract

Minimum Inhibitory Concentration MIC (%w/v)				
Sample	**Concentration (mg/ml)**	**Aspergillus niger**	**Aspergillus ochraceus**	**Candida albicans**
Polyherbal Hydro-alcoholic	**0.1**	N	N	N
	0.19	N	N	N
	0.39	N	N	N
	0.78	Y	Y	Y
	1.56	Y	Y	Y
	3.13	**Y**	**Y**	Y
	6.25	**Y**	**Y**	Y
	12.5	**Y**	**Y**	**Y**
	25	**Y**	**Y**	**Y**

3.6 Formulation and development of herbal cream:

20 g oil-in-water (O/W) cream will be formulated. The oil phase (Part A) consists of stearic acid, cetyl alcohol, liquid paraffin, and other oil-soluble ingredients, heated to 75°C. The aqueous phase (Part B) is made by dissolving polyherbal leaves extract in propylene glycol, adding it to water, and mixing in glycerin, methylparaben, and triethanolamine. Both phases are heated to 75°C and then combined with continuous stirring until emulsification occurs. Different concentration of 2.5, 5, 7.5 and 10% w/w cream will be prepared using this method.[120]

Table 14: Formulation of herbal cream

Ingredients	**Formulation %w/w**			
	F1	**F2**	**F3**	**F4**
Polyherbal Extract	1	2	3	4
Stearic acid	2.2	2.2	2.2	2.2
Cetyl alcohol	0.8	0.8	0.8	0.8
Liquid paraffin	0.8	0.8	0.8	0.8
Triethanolamine	0.3	0.3	0.3	0.3
Glycerin	1	1	1	1
Methylparaben	0.2	0.2	0.2	0.2
Water	QS 20gms	QS 20gms	QS 20gms	QS 20gms

3.7 Evaluation of herbal cream

Table 15: Evaluation of polyherbal cream

Parameters	**Formulation %w/w**			
	F1	**F2**	**F3**	**F4**
Colour	Dark Brown	Dark Brown	Dark Brown	Dark Brown
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Appearance	Smooth	Smooth	Smooth	Smooth
pH	6.9	6.8	6.9	6.8
Viscosity	2090±4.52	2105±4.66	2085±4.34	2150±4.82
Spreadability	6.15±0.16	6.22±0.18	6.22±0.14	6.28±0.20

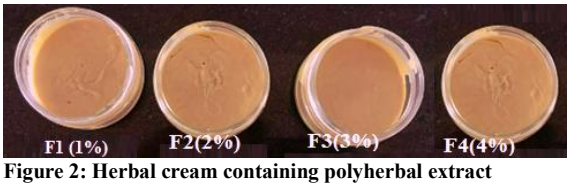

Figure 2: Herbal cream containing polyherbal extract

1. Organoleptic Properties

All four formulations displayed dark brown color, characteristic odor, and a smooth appearance

2. pH

The pH values ranged from 6.8 to 6.9, which is

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within the ideal range for topical applications, aligning with the skin's natural pH (typically 4.5–6.5).

3. Viscosity

The viscosity values for the formulations varied slightly:

Table 16: Viscosity and Spreadability

Formulation	Viscosity	Spreadability
F1	2090±4.52 cP	6.15±0.16 g·cm/sec
F2	2105±4.66 cP	6.22±0.18 g·cm/sec
F3	2085±4.34 cP	6.22±0.14 g·cm/sec
F4	2150±4.82 cP	6.28±0.20 g·cm/sec

Spreadability is a critical attribute that affects the user experience and the efficacy of topical formulations. The values recorded were:

3.8 *In-vitro* antifungal study of cream

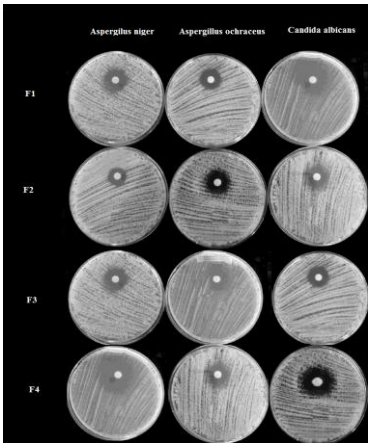

Figure 3: In-vitro antifungal activity of Polyherbal creams

Table 17: Zone of inhibition of Aspergillus niger, Aspergillus ochraceus and Candida albicans

Formulation	Zone of inhibition		
	Aspergillus niger	**Aspergillus ochraceus**	**Candida albicans**
F1	15.6	14.4	15.1
F2	17.2	16.3	17.1
F3	21.1	19.7	21.7
F4	21.7	22.2	22.8

3.9 Stability studies

Table 18: Stability Studies of polyherbal cream (F4)

Parameters		Colour	Odor	A ppearance	pH	Viscosity	Spreadability
Long term	**3 Month**	Dark Brown	Characteristic	Smooth	6.9	2090±4.52	6.08±0.16
	6 month	Dark Brown	Characteristic	Smooth	6.8	2054±4.64	5.95±0.14
Acceler-ated	**3 Month**	Dark Brown	Characteristic	Smooth	6.9	2090±4.46	6.15±0.14
	6 month	Dark Brown	Characteristic	Smooth	6.9	2076±4.38	6.15±0.18

4. CONCLUSION:

Phyllanthus acidus, *Cinnamomum tamala*, and *Madhuca longifolia* were selected for this research based on a thorough literature review. The leaves of these plants were collected in the month of July and authenticated by Professor S. R. Kshirsagar, Department of Botany, Dr. P. R. Ghogrey Science College, Dhule.

Phytochemical extractions were carried out using different solvents, followed by determination of extractive values and ash content. All extracts were subjected to preliminary phytochemical screening. In-vitro antifungal activity was evaluated, revealing that the hydroalcoholic extracts of all three plants demonstrated significant potency against *Aspergillus niger*, *Aspergillus ochraceus*, and *Candida albicans*. The Minimum Inhibitory Concentration (MIC) of the polyherbal hydroalcoholic formulation was assessed across a concentration range of 0.1 to 25 mg/mL. No inhibition was observed at lower concentrations (0.1–0.39 mg/mL). However, at concentrations of 0.78 mg/mL and above, the extract exhibited significant inhibitory activity, effectively suppressing the growth of all three fungal pathogens. This indicates strong antifungal potential of the formulation, with an MIC of approximately 0.78 mg/mL for the tested organisms.

A polyherbal cream was subsequently formulated using the hydroalcoholic extract at a concentration of 1, 2, 3 and 4%. The cream demonstrated desirable physicochemical characteristics, including smooth texture, appropriate viscosity, good spreadability, and a pH close to that of normal skin—making it suitable for topical application. The dark brown color and characteristic herbal odor reflect its natural composition, which may appeal to consumers seeking organic skincare alternatives. While preliminary results are promising, further studies are required to assess the formulation's stability, efficacy, and user acceptability—particularly in terms of scent and long-term effectiveness. Future work will include comprehensive in-vitro and in-vivo evaluations to further validate the antifungal potential of this polyherbal cream.

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