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Preparation of Topical Cream for Athletes Foot and Foot Crack using Five Indian Medicinal Plants and their Evaluation

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Abstract: *About 15-20% people suffering from Athlete's foot. Athlete's foot is a fungal infection caused by dermatophyte. The fungus causing athlete's foot can be easily spread through direct contact with infected skin or contaminated surfaces. Open cracks in the feet can become entry points for bacteria which leads to further complications. This study aimed to investigate the antifungal and antibacterial activities of a topical cream formulated for the treatment of athlete's foot and foot cracks. The cream was prepared using ethanolic extracts from the whole plant of Centella asiatica, the root of Rubia cordifolia, and the leaves of Chromolaenaodorata, Azadirachta indica. The presence of secondary metabolites including alkaloids, flavonoids, tannins, glycosides, carbohydrates and saponins in these extracts was determined through phytochemical screening. The antifungal and Antibacterial activity of herbal formulation can be evaluated using the fungus Candida albicans and the bacterium Staphylococcus aureus species .The results of the study demonstrated that the topical formulation exhibited significant antifungal and antimicrobial activities at various concentration. Additionally, the cream effectively inhibited the fungi responsible for athlete's foot and reduced skin dryness, thereby preventing foot cracks.*

Keywords: Antifungal activity, Antimicrobial activity, Athlete's foot, Foot crack

I. INTRODUCTION

Fungal skin infections are a global health concern, affecting nearly a billion people worldwide. Their prevalence is influenced by various factors such as climate, socioeconomic conditions, and cultural practices.Two of the most common foot fungi infections are tinea pedis (Athlete's foot) and onychomycosis. These infections can affect the outer layers of the skin, hair, and nails on the feet. Athlete's foot is a cutaneous fungal infection caused by dermatophyte infection. It is characterised by itching, flaking and fissuring of the skin. It may manifest in three ways, the skin between the toes may appear macerated (white) and soggy, the soles of the feet may become dry and scaly, and the skin all over the foot may become red and vesicular eruptions may appear.[1]The most common symptom are cracked foot, flaking, peeling of skin between the toes or on the sides of the foot.

Cracked feet also known as heel fissures is a common foot problem characterized by yellowish colour of the skin on the heel of the foot, hard skin growth, hardening and cracks in the feet associated with pain, bleeding or itching.[2]. Skin conditions such as psoriasis and eczema may also cause cracked heels and feet. Obesity increases the chances of cracks by putting more pressure on fat pads under the heel thereby causing it to expand.In extreme cases, cracked heels can get infected and lead to cellulitis [3]

Azadirachta indica (Neem) is a perennial tree belonging to Meliaceae family. Neem has special importance in various traditional systems of medicine in India and is known to be the "Panacea for all diseases.". Scientific research substantiate that the neem has antifungal, antibacterial properties [4]. Due to its antifungal property neem leaves are potential in treatment of athlete's foot. *Rubia cordifolia* (Rubiaceae) is also known as, Manjishtha, Indian madder known to contain substantial amounts of anthraquinones, especially in the roots[5] which is responsible for antimicrobial

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and antifungal activity[6]. *Rubia cordifolia* is highly valuable plant in Ayurvedic system of medicine used for treatment of various skin diseases [7].

Chromolaena odorata is traditionally recognized for its broad spectrum of medicinal properties, particularly in the management of external conditions such as wounds, dermal infections, and inflammatory responses.Studies have demonstrated that the leaf extract has antioxidant, anti-inflammatory, analgesic, antimicrobialand many other medicinally significant properties.[8]Ethanolic extract of *Centella asiatica* plant shows significantly higher rate of antifungal activity against various fungal infections.[9]. A specific component of *Centellaasiatica,* asiaticoside, is reported to increase the tensile strength of skin and stimulate angiogenesis.[10]Aloe vera is incredibly hydrating and a great moisturiser. Additionally, it reduces skin moisture and increases collagen production, both of which will help heal cracked heels.[11]

II. MATERIALS AND METHODS

2.1 Collection of plant material

The leaves of *Azadirachta indica*were collected from Mala, Thrissur. The whole plant of *Centella asiatica*and the leaves of *Chromolaena odorata*and *Rubia cordifoli*a were gathered from Angamaly, Ernakulam, while the leaves of Aloe vera were obtained from Alappuzha. The authentication of all five medicinal plants was conducted at the Department of Botany, St. Thomas College, Thrissur, to ensure the accurate identification and verification of the plant species used in the study. This authentication process confirmed the botanical identity of the specimens, ensuring their suitability for further research or medicinal applications.

2.2 Sample Preparation and extraction procedure

The leaves of *Azadirachta indica* were dried in a microwave oven at 100°C. After drying, the leaves were ground into a fine powder and stored in an airtight container in a cool, dry place, away from direct sunlight or bright light to prevent damage. The leaves of *Chromolaena odorata* and *Centella asiatica* were shade-dried and powdered. For *Aloe vera*, fresh pulp was collected, crushed, and the juice was extracted. The dried roots of *Rubia cordifolia* were crushed into powder.

For the extraction 20 g of the powdered material from each plant was packed into separate thimbles and extracted with 250 ml ethanol using a Soxhlet apparatus for 8 cycles at a temperature not exceeding 40°C. The ethanol extracts obtained were dried using an electric water bath, and the dried extracts were weighed separately.

2.3 Preparation of cream

To prepare the oil phase, weigh 5g of stearic acid, 1.25g of cetyl alcohol, and 1.75ml of paraffin oil into a clean china dish. Heat the mixture at 75˚C and dissolve the content with constant stirring.For the aqueous phase, weigh 2g each of *Azadirachta indica*, *Centella asiatica*, *Chromolaena odorata*, *Rubia cordifolia*, and aloe vera extract into a beaker. Add 0.675ml of triethanolamine, 0.09g of methyl paraben, 5ml of glycerin, and a sufficient amount of distilled water. Heat the mixture in a water bath at 75˚C.Once both phases reach 75˚C, gradually add the aqueous phase to the oil phase with continuous stirring to produce cream of appropriate texture. Allow the cream to cool at room temperature. Transfer the final mixture into an airtight container for storage.

2.4 Evaluation Methodologies

2.4.1 Preliminary Phytochemical Screening

The crude extracts were screened for the presence of phytochemicals like alkaloids, glycosides, carbohydrates, tannins, flavonoids and saponin by using the standard procedures[12]

Test for Alkaloids

Mayers Test: Take 2-3 ml of plant extract using a clean pipette in to a test tube.Add 2-3 drops of Mayer's reagent (potassium mercuric iodide) Gently shake the test tube to mix the content let it stand for couple of minutes and observe for any colour change in the solution.

Dragendroff'stest: Take 2 ml of extract and add 2-3 drops of Dragendroff's reagent (potassium bismuth iodide solution) and mix well. The presence of brown or reddish- brown colour precipitate indicate the presence of alkaloids.

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Test for carbohydrate

Benedict's test: Take 2 ml of plant extract and add 2ml of Benedict's reagent and boiled, a reddish- brown precipitate formed which indicated the presence of the carbohydrates.

Molisch's test: Take small amount of extract and mixed with 2ml of Molisch's reagent and shaken properly. After that, 2ml of concentrated Hydrochloric acid was poured carefully along the side of the test tube. Appearance of a violet ring at the junction of two liquid indicated the presence of carbohydrate. [13]

Test for saponin

Foam test: Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for flavonoids

Lead subacetate: To 2ml of filtrate add few drops of lead subacetate solution and observe the colour change.

Ferric chloride:To 2ml of filtrate add,add few drops of 10% ferric chloride solution were added and observe the colour change.

Test for glycosides

Borntragger's test: Boil powdered drug with dilute sulphuric acid. Filter immediately and mix the filtrate with organic solvents like benzene or chloroform. shake and separate the organic layer. To the layer add equal quantity of dilute ammonia.The layer becomes pink and finally turns to red.

Baljet Test: Take 2-3 ml of plant extract in a clean test tube add 1ml of baljet reagent.(picric acid sodium hydroxide reagent) Mix the contents gently and observe for the colour change.

Test for tannins

Gelatin test: take small quantity of plant extract, aqueous solution of gelatin and sodium chloride are added. a white buff coloured precipitate is formed.

Ferric chloride: Take aliquot amount of plant extract add few drops of freshly prepared ferric chloride solution then mix the solution gently by tapping the test tube and observe the colour change.

2.4.2 Evaluation for Antifungal and Antibacterial activity

Inoculum preparation: Transfer a loopful of fungal and bacterial culture from working stock slants to 5ml of Broth and incubated at 37ºC till getting a visible turbidity equivalent to 0.5 MacFarland unit. 25 ml of respective agar(Antifungal – MGYPA, for bacteria – MHA) medium were added to sterile petriplates and allow to dry for 5 minutes.Then the cultures were inoculated on plates by swabbing on the surface of the media.Using sterile Cork Borer of 8mm diameter prepare wells on the swabbed agar plates. 100µl sample was added to the well using micropipette.Kept the plates in the biosafety cabinet till the diffusion of sample occurs and after that incubate the plates. After incubation, using a ruler measure the diameter (mm) of the zone of inhibition. Record the results.

III. RESULT AND DISSCUSSION

3.1 Phytochemical Screening

The preliminary phytochemical investigation of different extracts revealed the presence of alkaloids, glycosides, saponin, tannins, flavonoids, carbohydrates. Detailed result is represented in table 1

TABLE 1: REPRESENTS THE PHYTOCHEMICAL CONSTITUENTS IN EXTRACT

SI NO	EXPERIMENT	Ethanolic Extract			Aqueous Extract	
		Neem Leaves	Communist Pacha Leaves	Kodangal Plant	Manjistha Root	Aloe Vera Gel
1	Alkaloid test Dragendroffs test				×	
	Mayers test				×	
2	Carbohydrate test Molisch test					
	Benedicts test					

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3	Flavonoids test Ferric chloride test				×	×
	Lead subacetate test				×	×
4	Glycosides test Borntraggers test	×	×	×	×	
	Baljet test	×	×	×	×	
5	Tannins test Gelatin test	×		×	×	×
	Ferric chloride test	×		×	×	×
6	Saponin test Foam test	×	×	×	×	

3.2 ANTI FUNGAL ACTIVITIES

Fungicidal activity of formulation was determined on Candida albicans and the zone of inhibition was 22mm-13mm. As the formulation concentration increased the zone of inhibition also increased which is represented in table 2

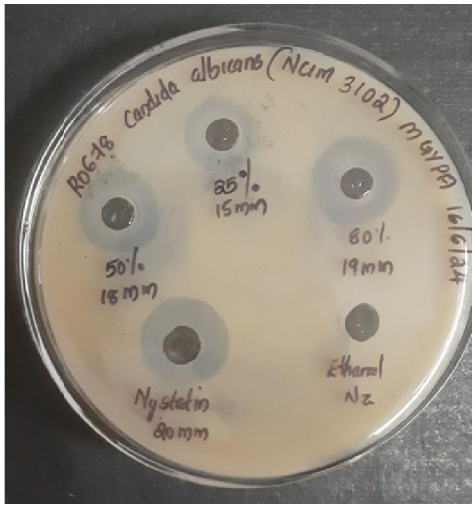

TABLE 2: REPRESENTS ANTIFUNGAL ACTIVITIES

SI.NO	Test organism	Test result-(Zone of inhibition in mm)				Test method
		80%	50 %	25 %	Diluent control	
1	*CandidaAlbicans* NCIM 3102	22 mm	13mm	No Zone	9mm	CKL/MB/ MOA-044

3.3 ANTI BACTERIAL ACTIVITIES

Antibacterial activity of formulation was determined on*Staphylococcus aureus* and the Zone of inhibition was 15mm-19mm. As the formulation concentration increased the zone of inhibition also increased which is represented in table 2

TABLE 3: REPRESENTS ANTIFUNGAL ACTIVITIES

SI. NO.	Test Organism	Test result–(Zone of inhibition in mm)				Test method
		80 %	50 %	25 %	Diluent control	
1	*Staphylococcus aureus* NCIM 2127	17mm	15mm	12mm	No Zone	CKL/MB/ MOA-044

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