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A Study on the Preparation and Evaluation of an Herbal Antifungal Spray

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ABSTRACT
This study presents the formulation and evaluation of herbal antifungal spray developed using natural ingredients like karanja oil, neem oil, black pepper extract, camphor and other ingredient. Now a days the fungal infection is risen in humans. Along with its growth rate, the kind of fungal species also increased considerably. For this situation effective formulation is necessary to treat these fungal infections in a simple manner. The primary objective of my research project was to create a formulation for a herbal antifungal spray. Research into tropical antifungal formulations has demonstrated considerable effectiveness in the treatment of fungal infections. Compared to conventional dosage forms, these herbal drug delivery systems provide greater benefits and remarkable advantages. The spray formulation includes a combination of natural and synthetic ingredients such as Black pepper oil (Piper nigrum) exhibits potent antimicrobial properties, while neem oil (Azadirachta indica) and karanja oil (Pongamia pinnata) etc.

The pH of the final product was found to be in the skin-compatible range of under 4-6 , ensuring minimal irritation and suitability for topical use. UV-Visible spectroscopy was employed to analyze the stability and phytochemical profile of the formulation, confirming the presence of active constituents from the oils, with characteristic absorbance peaks in the 250–350 nm range. Antifungal activity was evaluated using the agar well diffusion method against Candida albicans and Aspergillus niger, where the herbal spray demonstrated significant zones of inhibition, comparable to standard antifungal agents. The spray pattern was assessed for uniformity and droplet dispersion, indicating consistent and even coverage over the application area, which is critical for effective therapeutic action.

Keywords: Fungal infection, Herbal spray, Karanja oil, Neem oil, Black pepper extract, Camphor.

I. INTRODUCTION

FUNGAL INFECTIONS

Fungal infections of the skin, hair, and nails are widespread dermatologic disorders that afflict people all over the world. Dermatophytosis is an infection caused by Dermatophytes which are keratinophilic fungi that primarily affect the keratinized layers of the skin, which include both human and animal hair and nails. A fungal infection can affect anyone, including individuals who are otherwise healthy. Fungi are abundant in the environment, and humans breathe in or come into touch with fungal spores on a daily basis without becoming ill. These fungi, however, are more likely to cause infection in individuals with weakened immune systems.

Serious fungal infections can be separated into two major categories: The opportunistic mycoses that include candidiasis, cryptococcosis, and invasive mold infections such as aspergillosis and zygomycosis, and the endemic mycoses, including histoplasmosis, blastomycosis, and coccidioidomycosis. The majority of fungi are widespread and can successfully reproduce in their native habitats without the aid of humans or other animals as substrates. Nonetheless, some species can infect people accidentally and cause systemic, subcutaneous, or superficial infections. The majority of fungi that cause systemic (or deep-seated) infection do so either by directly invading a wound site or the lung.

Superficial fungal infections occur in the outermost layers of the skin, nails, hair and mucous membranes. Superficial fungal infections include

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some of the most frequently observed skin diseases, affecting millions of people worldwide. Dermatophytes specifically, Trichophyton spp., Microsporum spp. and Epidermophyton spp. are responsible for most superficial fungal infections, although yeasts and some non-dermatophyte moulds can also be causative agents.

Tinea pedis:

Tinea pedis is the most common dermatophytosis and may affect up to 70% of adults worldwide. Also referred to as athlete's foot or ringworm of the foot, it involves the plantar surface and interdigital spaces of the foot and can include inflammatory and non-inflammatory lesions. Trichophyton rubrum accounts for most dermatophytic foot infections. Epidermophyton floccosum and Trichophyton mentagrophytes have also been reported to be causative agents.

Figure no.1

Tinea capitis:

It is a dermatophytic infection of the head and scalp, usually found in infants, children, and young adolescents. Tinea capitis can have a wide range of clinical presentations. Before 1960, most tinea capitis infections in the United States were caused by Microsporum audouinii. Today, the great majority of infections are caused by Trichophyton tonsurans. 1Initially, the condition may be asymptomatic and have a subtle appearance. As the inflammatory response to infection increases, inflammatory alopecia may develop.

Figure no.2

Tinea unguium or Onychomycosis:

Onychomycosis is fungal infection of the nail unit, including the nail plate and nail bed. There are four types of onychomycosis: proximal subungual, distal subungual, superficial white, and candidal. Dermatophytes usually cause the first three types, although Candida can be present in all types. The incidence of onychomycosis has been increasing worldwide, and at present it accounts for almost half of all nail disorders.

Figure no.3

Tinea versicolor:

Tinea versicolor is common, primarily in young and middle-aged adults. The condition is caused by the lipophilic yeasts Pityrosporum orbiculare and Pityrosporum ovale. Tinea versicolor is typically found in regions of the body that have sebaceous glands, such as the upper trunk, neck, and arms. The characteristic finding is skin depigmentation, but lesions can range from red to hypopigmented to hyperpigmented. Hyperpigmentation is often found in dark skinned patients .

Figure no.4

Subcutaneous Fungal Infections although subcutaneous mycoses can disseminate, they are usually limited to the dermis and subcutaneous tissues. Sporotrichosis, for example, is caused by the dimorphic fungus Sporothrix schenckii and is the most prevalent subcutaneous infection in parts of Latin America.

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Chromoblastomycosis:

It is a chronic cutaneous or subcutaneous fungal infection caused by members of the Dematiaceae family found in wood, vegetable debris and soil; these include Fonsecaea pedrosoi, Cladosporium carrionii, F. compacta, Phialophora verrucosa and Rhinocladiella aquaspersa.

Figure no.5

Chronic mucocutaneous candidiasis:

It is a rare syndrome consisting of chronic infection of mucous membranes (usually by C. albicans), which may extend to the skin and nails. The condition is associated with impaired cell-mediated responses to Candida, although the underlying defect remains poorly understood.

Figure no.6

Opportunistic systemic fungal infections occur primarily when some aspect of the normal host defence is compromised.

Dermatophytes typically cause superficial infections of keratinized tissues like nails and hair, whereas Candida spp. is more frequently responsible for infections of mucous membranes. Subcutaneous infections are typically brought on by traumatic inoculation and are caused by a range of pathogens. Systemic fungal infections are the most dangerous and deadly.

PONGAMIA PINNATA

Description:

Pongamia pinnata Linn Pierre is a medium-sized glabrous tree known in Hindi as Karanja, English as Indian Beech, and Tamil as Pongam. Pongamia pinnata is used by the majority of Tamil Nadu physicians practising the Indian systems of traditional medicine Ayurveda and Siddha to address a variety of diseases. Pongamia has historically been used as a folk medicinal plant, especially in the Ayurveda and Siddha systems of Indian medicine. Traditionally, the leaves, seeds, and complete plant have been used to cure a variety of diseases. It has anti-ulcer, anti- diarrheal, anti-plasmodial, anti-inflammatory, anti-viral, anti-bacterial, and anti-lice properties.8 All parts of the plant have been used as crude medicine to treat tumours, piles, skin diseases, itches, abscesses, painful rheumatic joint wounds, ulcers, diarrhoea. It can be composted or used as livestock fodder, green manure, lumber, and fish poison. It has also been acknowledged as having applications in environmental and agricultural management. The seed powder of the plant is given as an expectorant in the treatment of bronchitis. An infusion of Pongamia leaves is used to relieve rheumatism. In the treatment of dyspepsia, the Pongamia seed oil is given as stomachic and cholagogue. By the process of trans-esterification, the seed oil of Pongamia pinata can be converted to biodiesel. According to reports, the seeds typically contain 28–34% oil, with a large concentration of polyunsaturated fatty acids.

Synonyms:

Pongamia pinnata linn Pierre is also known by various synonyms such as

- Millettia pinnata (L.) Panigrahi
- Pongamia glabra vent
- Derris indica (Lam)

Taxonomical classification:

- Kingdom: Plantae
- Order: Fabales
- Family: Fabaceae
- Genus: Pongamia (Millettia)
- Species: Pinnata [11]

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Karanja Flower
Figure no.7

Karanja Seed
Figure no.8

Table no.1: Medicinal value of different parts of the plant

Plant Part	Medicinal Value
Flower	Used to treat bleeding haemorrhoids, or piles
Fruit	Treatment of abdominal tumours, female genital system diseases, ulcers, and haemorrhoids.
Stem	Extracts are used to cure fever and relax the nervous system.
Seed	Extracts are used for scar tissue tumours, high blood pressure, and anaemia. Powder lowers the temperature and the treatment of bronchitis and whooping cough.
Bark	Relieves coughs and colds reduce spleen inflammation, and mental disorders Useful for the treatment of bleeding piles
Oil (extracted from seed)	Used as an astringent and to eliminate parasitic worms. The treatment of whooping cough, liver discomfort, chronic fever, ulcers, and leprosy. Relieves joint and muscular pain and arthritis. It is also used as an antifungal and antimicrobial agent.
Leaf	Whole leaves are used as a laxative and digestive aid, as well as to treat inflammation and sores. Leaf liquid can be used to cure leprosy, gonorrhoea, constipation, flatulence, coughs, and colds. Leaf infusions and preparations ease rheumatism and itching, respectively.
Root	Used as an oral hygiene toothbrush, to kill parasitic worms, and to cure vaginal and skin diseases. Juice is used to clean ulcers and heal exposed wounds. Juice, when mixed with coconut milk and lime water, can be used to cure gonorrhea [11].

Pharmacological activity

Anti-bacterial & Anti-fungal:

The seeds were extracted with N-Hexane in stages. The extracts were tested for antimicrobial activity against E. coli using the well diffusion technique. Seed extracts demonstrated an effective zone of inhibition.

The seed oil had considerable antifungal activity against the fungi tested. The highest level of suppression against Aspergillus niger and Candida albicans. Pure oil (100%) inhibited all studied fungi the most, with 40-45% of extracted oil inhibiting the least. It had high antifungal activity against yeast but poor antifungal activity against moulds.

AZADIRACHTA INDICA

Description:

Azadirachta indica, also known as Neem, is an evergreen tree that has been used in Ayurvedic medicine for thousands of years. It is grown in nations such as Pakistan, Nepal, India, and Iran, where it has been used for a variety of therapeutic purposes. The bark, flowers, leaves, twigs, fruits, roots, seeds, sap, and gum of the tree are all used as a source of numerous therapeutic substances in traditional native medicine. Neem extracts have antiviral, antifungal, anti-insecticidal, antibacterial, antiallergic, anti-helminthic, and anti-inflammatory qualities.

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Neem seed is a part of the neem tree that contains a high quantity of oil. Neem oil is extensively used as an insecticide, lubricant, and medication for a variety of diseases including diabetes and tuberculosis. Neem oil is usually light to dark brown in colour. It has a bitter taste and an offensive odour that is comparable to the odours of garlic and peanuts combined. It is primarily made up of triglycerides and is high in azadirachtin, which acts as an insect repellent, antifeedant, anti-fungal, and anti-viral. Phytochemicals such as quercetin and azadirachtin, as well as liminoids such as nimbin, nimbinin, and nimbidin, have been isolated from various sections of the plant. Furthermore, the leaves contain a variety of compounds including nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, nimbiol, different amino acids, and others.

Taxonomical Classification:
Kingdom:Plantae
Division:Magnoliophyta
Order: Sapindales
Family: Meliaceae
Genus: Azadirachta
Species: A. Indica

Neem Leaves
Figure no.9

Neem seeds
Figure no.10

Table no.2: Medicinal value of different parts of the plant

Plant part	Medicinal value
Fruit	Anti-inflammatory
Leaf	Wound healing, Hepatoprotective
Flower	Antifertility
Seed	Antimicrobial
Bark	Antimicrobial
Root bark	Antidiabetic
Stick	Reduction in plaque and gingival, Treatment of dental caries [14]

Pharmacological activity:
Anti-bacterial & Anti-fungal:

It inhibits the activity of bacteria such as Salmonella typhi, Staphylococcus aureus, Streptococcus mutants, M. tuberculosis, Vibrio cholerae, M. pyogenes and Klebsiella pneumonia.

Neem leaves aqueous, ethanolic and ethyl acetate concentrations (5, 10, 15 and 20%) from these extracts inhibited human pathogens (Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Candida albicans and Microsporum gypseum). The 20% ethyl acetate which is strongest above another extract, by the analysis of HPLC and NMR. Ethyl acetate contains nimonol which is the active component.

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PIPER NIGRUM.

Description

Black pepper, also known as the "King of Spices" or "Black Gold," is the most widely used spice in food items around the world. Piper nigrum L. is derived from a dried berry known as peppercorn, the flavour intensity of which differs based on its origin. It is a member of the Piperaceae family. The pungency of black pepper is due to its nonvolatile alkaloid component, piperine (1-peperoyl piperidine). Pepper is most frequently used in curry recipes, as masalas, and is also included in Ayurvedic and other traditional medicinal systems' prescriptions. Pepper is also used in folk medicine as an aphrodisiac, carminative, stomachic, antiseptic diuretic, and for the treatment of cough, rheumatoid arthritis, neuropathy, melanoderma, and leprosy due to the presence of volatile compounds, tannins, phenols, and other unknown substances. Black pepper contains few phytochemicals such as amides, piperidine, pyrrolidines and saffrole. The major components of black pepper essential oil are caryophyllene, limonene and camphene.

Recent research indicates that black pepper extracts can prevent food spoilage and pathogens. We earlier investigated the inhibitory effects and minimal inhibition concentration (MIC) of black pepper chloroform extract (BPCE) against Escherichia coli and Staphylococcus aureus and investigated the optimum extraction process for black pepper using chloroform. Black pepper ethanolic extract had a wide antimicrobial spectrum and a significant antibacterial effect against both gram-positive and gram-negative bacteria.

Taxonomical Classification :
Kingdom: Plantae
Division: Angiosperms
Order: Piperales
Family: Piperaceae
Genus: Piper
Species: Piper nigrum

Black pepper plant
Figure no.11

Black pepper Seeds
Figure no.12

Pharmacological activity:

Anti-bacterial & Anti-fungal:

Antibacterial efficacy against S. aureus and B. subtilis, as well as E. coli.

Antifungal action of black pepper extract against Candida albicans was observed. The observed antifungal activity could be attributed to piperine, a volatile oil found in pepper that has been shown to have antimicrobial activity.

CINNAMOMUM CAMPHORA

Description:

Karpura is a small, glabrous, broad-leaved tree that can grow up to 40 m tall and has a 3m circumference. The plant's bark is golden brown in color, with a rough surface and vertical fissures. The botanical name is taken from Greek. It comes from the term kinnamomon, which means "spices." Camphor is produced by steam distillation, purification, and sublimation of tree timber, twigs, and bark. Camphor has numerous pharmaceutical uses, including topical analgesic, antiseptic, antispasmodic, antipruritic, anti-inflammatory, anti-infective, rubefacient, contraceptive, moderate expectorant, nasal decongestant, cough suppressant, and so on.

This plant contains a variety of active chemical components, such as 1% essential oil in the leaves and 3% in the wood. The plant's essential oil includes cineol, pinene, thymol, menthol, 10-15% 2- bornanon, terpineol, and no safrole. The brown version, on the other hand, contains 80% safrole and some terpenoids. Its yellow version contains safrole as well as other components such as esquiterpenes and sesquiterpene alcohol.

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Taxonomical Classification:
Kingdom - Plantae
Division -Tracheophyta
Order - Laurales
Family - Lauraceae
Genus - Cinnamomum
Species - Camphor

Figure no.13: Bhimseni kapur

MATERIALS & METHODS:

A. Pongamia pinnata

Seed Material:

The seeds are chosen based on their state, with damaged seeds being discarded. Seeds in excellent condition were cleaned, deshelled, and dried for 30 min at a high temperature of 100-105°C. The seeds were then extracted for oil.

Extraction of oil:

The shade dried seeds were powdered to appropriate size and about 200 g dried powder of seeds was extracted with 600 ml of Petroleum ether (40-60^{0}C) at temperature 40-60^{0}C with a soxhlet apparatus for 7 hours .Then the extract was filtered and concentrated in a water bath till an oily extract was obtained .

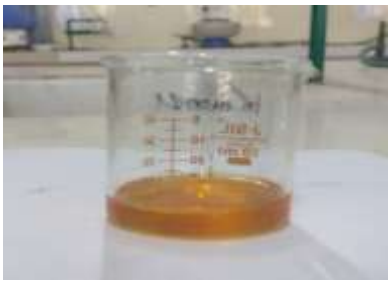
B. Azadirachta indica

Collection of leaves:

The neem leaves were dried in a dry oven. Weighed neem flour was measured using an analytical balance. Dichloromethane (DCM), N-hexane, Methanol, Toluene, and Ethanol were used to separate oil from neem powdered.

Extraction of oil:

100 g of neem powder was put in the soxhlet chamber in a thimble. The distillation process began after 500 ml of chosen solvents were put in a round bottom flask and assembled for soxhlet extractor. After the extraction process was finished, the solvent and extractor were placed in a water bath to evaporate the solvent .



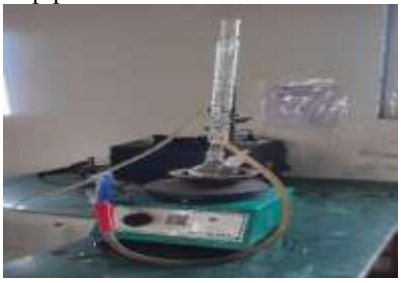
C. Piper nigrum

Collection of seeds:

The black pepper was purchased at a neighbourhood market. All chemicals were acquired from the college lab. The pepper seeds were then finely ground in a mechanical a mixer.

Extraction of oil

In a round bottom beaker, 50g of ground pepper powder was refluxed with 300 ml of dichloromethane for 45 to 60 min. Water was permitted to run through the condenser to condense dichloromethane vapours. The beaker was then cooled and filtered through a Buchner funnel. After the extraction process was completed, the extract was put in a water bath to evaporate the solvent, and crude piperine oil was obtained.



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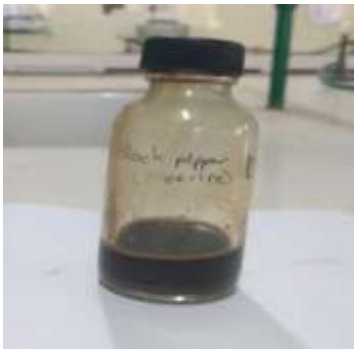

D. Camphor

Camphor was bought directly from market. Camphor was crushed with the help of a pestle to fine particles and later was added to the mixture of oil used in formulation.

FORMULATION:

Antifungal spray preparation:

An antifungal spray is sprayed onto infected tissue to treat fungal diseases. Only apply this medicine to your skin. Shake the container thoroughly before applying the spray form. The dosage and duration of treatment are determined by the sort of infection being treated.

Table no.3: Formulation Table

Sr. No.	Ingredients	Category	Amt. taken
1	Neem oil	Antifungal agent	3.5ml
2	Karanja oil	Antifungal agent	3.5ml
3	Camphor	Cooling effect, antiseptic	1.5gm
4	Black pepper extract	Antioxidant	1.5ml
5	Ethanol	Co-surfactant	2.5ml
6	Polyethylene glycol	Co-surfactant, Plasticizer	2.5ml
7	Tween 80	Surfactant	50ml
8	Water	solvent	qs.

Procedure:

- Preformulation studies was done to prepare the formula.
- Each ingredient was accurately weighed.
- With mild heating, all of the ingredients listed in the table are added in order.
- Continuous stirring can assist in achieving a homogeneous solution.
- Transfer the solution to a magnetic stirrer at 1200 rpm for 30min.
- It was then placed in an ultrasonic bath at 25^{o}C for 15 min

Ideal characteristics of Antifungal spray

- It should be harmless to skin.
- It should be convenient and easy to apply.

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- It should be stable on storage.
- It should form a satisfactory film on the skin.

EVALUATION PARAMETERS:

A. OIL & EXTRACT:

Chemical identification test:
Black pepper extract

Tests for Alkaloids:

a) Dragendorff test.: When 1 mL of Dragendorff's reagent was added to 2 mL of extract, an orange red precipitate was formed, showing the presence of alkaloids.
b) Mayer test: A few droplets of Mayer's reagent were mixed into 1 mL of extract. The existence of alkaloids was indicated by the formation of a yellowish or white precipitate.
(c)Wagner's test: A few droplets of Wagner's reagent were added to two millilitres of extract. The existence of alkaloids was indicated by the formation of a brown precipitate.

B. FORMULATION:

Physical appearance / visual inspection:
Colour Transparent
Odour Characteristic
Homogeneity No particulate matter present Clarity Translucent

pH: The pH was calculated using a digital pH meter.

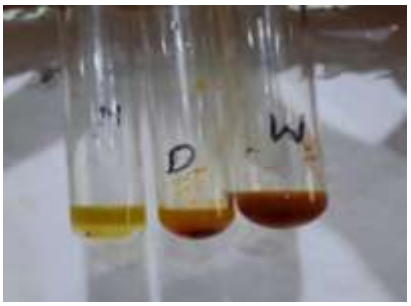
UV Spectrophotometry:

0.1ml of prepared formulation was diluted with ethanol as a solvent in a 50ml volumetric flask. The sample is poured into a cuvette and the checked at the range of 200-400nm using a Shimadzu UV Spectrophotometer.

Spray Pattern:
This method involves the use of a dye talc mixture treated paper onto which the spray pattern can be checked.

II. RESULT & DISCUSSIONS:

- The extract passed the test for the chemical identification test of alkaloids.



- pH of normal skin is 4 - 6, the formulation has a pH of 5.34.
- UV spectrophotometry of prepared formulation

Peak Pick

No.	P/V	Wavelength nm.	Abs.	Description
1		305.40	1.379	
2		243.40	4.000	
3		232.40	4.000	
4		224.80	4.000	
5		289.00	1.307	
6		235.20	3.460	
7		228.80	3.365	

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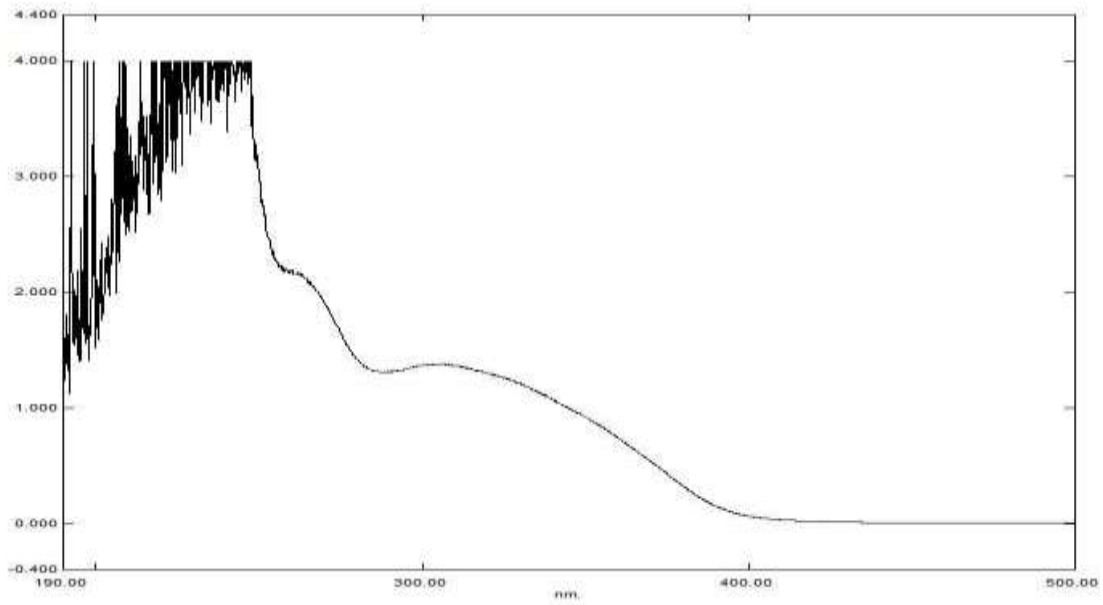

Spray pattern

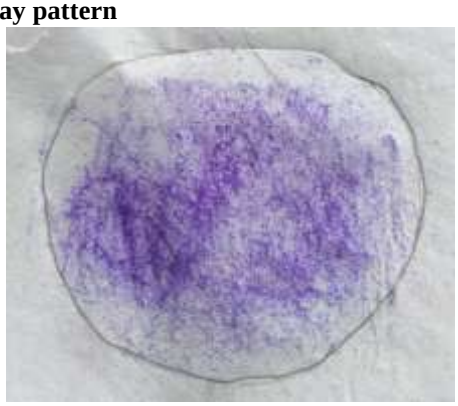

Figure no.18

The objective of this study was to prepare and evaluate an antifungal spray. The UV spectrophotometry results show that the oil was present in the prepared formulation. The formulation has a satisfactory inhibition of fungal species.

III. CONCLUSION:

The present antifungal spray formulation has been developed through a herbal approach, utilizing naturally derived ingredients and standard, easily accessible laboratory equipment. This low-complexity preparation method underscores the feasibility of producing the spray in both research and small-scale manufacturing settings without requiring advanced instrumentation.

The formulation demonstrates significant antifungal activity against common pathogenic fungi, as confirmed by laboratory assessments such

- The observed pattern was a full cone.

as antifungal bioassays and UV-visible spectroscopy. This efficacy is primarily attributed to the synergistic interactions of bioactive phytochemicals present in the selected plant-based ingredients—namely black pepper oil (Piper nigrum), neem oil (Azadirachta indica), karanja oil (Pongamia pinnata), and camphor (Cinnamomum camphora). These components are known for their well-documented antimicrobial and antifungal properties.

Importantly, the spray is devoid of synthetic preservatives, harsh solvents, or any toxic additives, rendering it non-toxic and non-irritant upon dermal application. This safety profile was verified through preliminary dermatological evaluations, suggesting suitability for use on sensitive skin without adverse effects.

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