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In Vitro Evaluation of Antidermatophytic Activity of Selected Medicinal Plant Extracts Against Microsporum Gypseum and Microsporum Canis

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ABSTRACT: Dermatophytes account for a significant proportion of widespread fungal infections affecting individuals worldwide. Dermatophytic infections present considerable challenges in therapeutic management due to their tendency for recurrence and increasing resistance to treatment. According to the World Health Organization, approximately 20–25% of the global population is affected by dermatophyte infections. The emergence of drug resistance among fungal pathogens to currently available antifungal agents has become another major concern. In this context, herbal plant extracts are considered promising alternatives for the treatment of fungal infections as they offer dermal compatibility and relatively fewer adverse effects. The World Health Organization also reports that medicinal plants represent an important source of therapeutic agents, with nearly 80% of the global population relying on traditional medicine, much of which involves the use of plant extracts or their active compounds. India possesses more than 18,000 species of angiosperms, of which around 2,500 are recognized as important sources of medicinal and aromatic compounds. In the present study, fourteen medicinal plants were screened based on ethnobotanical information and assessed for their antifungal activity against Microsporum gypseum and Microsporum canis. Five plants exhibiting the highest antifungal activity were further evaluated using the disc diffusion method, and antifungal activity was recorded after 5–7 days of incubation. Senegalia rugata showed maximum activity, with a zone of inhibition of 28 ± 0.3 mm at a concentration of 20 mg/mL against Microsporum gypseum and 32 ± 0.6 mm (20 mg/mL) against Microsporum canis. Senegalia rugata showed an MIC of 78 µg/mL against Microsporum gypseum and 156 µg/mL against Microsporum canis. Phytochemical characterization of Senegalia rugata was carried out by thin layer chromatography and column chromatography, and bioautography of the fractions obtained was performed to assess their antifungal activity. GC–MS analysis of fraction 8 of the ethanolic extract of Senegalia rugata identified 40 volatile constituents. Cyclopentane carboxylic acid ester, which showed the highest peak area, has been reported to exhibit antimicrobial and antioxidant properties. These findings highlight the potential antidermatophytic efficacy of the selected medicinal plants and suggest their possible application in the development of topical therapies for the management of dermatomycoses. Cytotoxicity studies indicated that the extract is moderately cytotoxic.

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1. INTRODUCTION

Dermatophytes are a group of keratinophilic fungi that utilize keratin as a nutrient source and are responsible for superficial infections of the skin, hair, and nails. The infections caused by these fungi are collectively known as dermatophytosis. Transmission may occur directly through animals (zoophilic), from infected individuals (anthropophilic), or from soil (geophilic organisms), as well as indirectly through contaminated fomites. Diagnosis is usually established through clinical examination and patient history, and confirmed by potassium hydroxide (KOH) microscopy. Additional diagnostic methods such as fungal culture, histopathological examination and Wood's lamp evaluation are also used to confirm infection [1].

In recent years, the incidence of dermatophytosis has increased significantly worldwide, posing a major public health concern, particularly among immunocompromised and elderly individuals [2]. Approximately 20–25% of the global population is affected by superficial dermatophytic infections. Clinical observations have revealed increasingly, atypical morphology and more severe manifestations of the disease across different age groups. Among elderly patients, onychomycosis and tinea pedis are particularly common [3]. Moreover, there has been a notable rise in recurrent, chronic, and relapsing cases. This trend has been attributed to several factors, including the misuse of topical corticosteroids, easy constituent availability of over-the-counter antifungal–steroid combination creams and irrational use of such formulations [4].

The development of antifungal therapies remains challenging because fungi are eukaryotic organisms and share several structural and biochemical similarities with their host cells [5]. Although dermatophyte infections are generally limited to keratinized tissues, they may become invasive and lead to severe systemic infections in immunocompromised individuals [6]. Major risk factors for invasive fungal infections include HIV infection in AIDS patients, cytotoxic chemotherapy in cancer patients, immunosuppressive therapies that impair host defence mechanisms, and the presence of indwelling medical devices such as catheters [7].

Dermatophytes possess several adaptive mechanisms that enable them to survive and proliferate within host tissues. Processes such as mitotic proliferation and the activation of genes responsible for oxidative stress responses allow these fungi to evade host immune defenses and establish chronic infections [8]. Approximately 30 species of filamentous fungi are capable of degrading keratin in keratinized tissues and thereby cause superficial infections of the skin, hair, and nails [9]. Transmission can occur through contact with infected individuals, animals, soil, or contaminated objects, and infected body sites may also act as reservoirs of infection. Dermatophyte infections are considered the most common fungal diseases affecting humans, with an estimated 20–25% of the global population affected by these superficial skin infections [9].

The clinical manifestations of dermatophytosis include onychomycosis (nail infection), tinea pedis (athlete's foot), tinea corporis (ringworm of the body), and tinea capitis (scalp ringworm) [10]. Anthropophilic dermatophyte species typically produce desquamated and pruritic lesions with minimal inflammation. The most commonly encountered species include *Trichophyton rubrum*, *T. interdigitale*, *T. tonsurans*, *T. violaceum*, *T. soudanense*, and *Microsporum audouinii*. In contrast, zoophilic species such as *T. mentagrophytes* and *Microsporum canis* generally produce more inflammatory lesions in humans. Geophilic species may also induce similar inflammatory responses [11]. Since the 1990s, terbinafine has been widely used as a first-line therapy for the treatment of dermatophytosis [12].

Over the past decade, an increasing number of dermatophytic infections with atypical clinical presentations and transmission patterns have been reported. These include invasive infections, extensive lesions involving unusual anatomical sites, difficult-to-treat infections that do not respond to standard first-line therapies, and sexually transmitted dermatophytosis caused by *T. mentagrophytes*. Collectively, such cases are often described as "complex dermatophytosis." Atypical clinical manifestations may lead to delayed or incorrect diagnosis, resulting in inappropriate use of antibiotics and topical corticosteroids that can mask or exacerbate the infection. Delayed treatment also contributes to persistent transmission of the disease. Furthermore, recent outbreaks involving zoophilic dermatophytes and their transmission to humans have raised additional concerns regarding public health [13,14].

Although terbinafine has long been the drug of choice for dermatophytosis, increasing resistance to conventional antifungal agents has recently been reported. In India, approximately 14.3% of *T. rubrum* isolates have demonstrated resistance to terbinafine [15]. This growing resistance highlights the urgent need to identify and develop novel antifungal agents. Natural products, particularly those derived from plants, represent an important source of bioactive compounds with potential

antifungal activity. Plant-derived compounds provide an environmentally sustainable and promising approach for the development of new antimycotic agents [16].

There is growing concern about the emergence of dermatophyte resistance to commonly used antifungal drugs such as azoles and allylamines, which has led to increasing therapeutic failures. Although resistance was initially associated mainly with *T. rubrum*, recent reports indicate that *T. interdigitale* has also developed resistance. Cases of terbinafine-resistant dermatophytes have been documented in several countries including Japan, multiple European nations, Iran, Mexico, and the United States. Mechanisms underlying antifungal resistance include alterations in drug targets, enhanced drug efflux, and biofilm formation [17].

Several factors contribute to the development of resistant dermatophyte strains, including poor treatment compliance, administration of sub-therapeutic drug doses, self-medication, and misuse of topical steroid formulations. Furthermore, the number of antifungal drug targets is limited, and many antifungal agents act on overlapping pathways. For instance, allylamines, azoles, morpholines, and thiocarbamates all interfere with ergosterol biosynthesis, although they act on different enzymes within this metabolic pathway. These limitations have encouraged research aimed at developing new antifungal compounds as well as improved formulations of existing drugs. Currently, dermatophytosis is treated using both topical and systemic antifungal agents such as tolnaftate, terbinafine hydrochloride, and imidazole derivatives including ketoconazole, miconazole nitrate, and clotrimazole [18]. Despite the availability of effective treatments, the prolonged duration of therapy required for fungal infections often results in poor patient compliance. Financial constraints may also lead patients to discontinue treatment prematurely, resulting in recurrence of infection [19,20].

Plants possess inherent defence mechanisms that enable them to combat microbial pathogens and therefore represent a rich source of antimicrobial compounds. Traditionally, numerous plants have been used by indigenous communities to treat fungal infections, particularly in regions such as Latin America, Asia, and Africa. Many of these medicinal plants have been investigated scientifically for their antifungal activity and are often selected based on their traditional medicinal use [21]. Growing global interest in traditional knowledge has led to increased documentation and scientific evaluation of ethnomedicinal practices. Ethnopharmacological studies frequently validate the traditional use of medicinal plants in the treatment of infectious diseases [22]. Owing to their abundance of bioactive phytochemicals and secondary metabolites, plants may serve as promising therapeutic agents for various ailments. It has been estimated that nearly 80% of the world's population relies on traditional medicine for primary healthcare, with a significant proportion involving the use of plant-based preparations [23]

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All reagents and chemicals used in this study were of analytical grade and procured from Hi-Media Pvt. Ltd., Mumbai, India. Glassware used for the experimental procedures was obtained from Borosil, India.

2.2 Collection and Authentication of Plant Materials

Fourteen angiosperm plant species were selected based on ethno botanical relevance, namely *Piper longum, Plectranthus amboinicus, Mangifera indica, Senna siamea, Senegalia rugata, Curcuma longa, Nerium oleander, ficus racemosa, Eucalyptus globulus, Calotropis gigantea, Trigonella foenum graecum, Azadirachta indica and Piper betle*

Plant materials were collected from the University campus and surrounding regions of Bangalore, Karnataka, India. The specimens were authenticated by taxonomists from the Central Council for Research in Ayurvedic Sciences (CCRAS), Bangalore, India.

Based on the preliminary screening results, five plants exhibiting maximum antidermatophytic activity were selected for further investigation. These included *Senegalia rugata*, *Senna siamea*, *Syzygium cumini*, *Mangifera indica*, and *Calotropis procera*.

2.3 Preparation of Plant Extracts

Fresh plant parts were collected, washed thoroughly, shade-dried, and powdered using an electric grinder. Sequential hot extraction was performed using a Soxhlet apparatus with solvents of varying polarity, namely hexane, ethyl acetate, and methanol/ethanol. Approximately 60 g of powdered plant material was extracted with 300 mL of each solvent for 72 h following the procedure described by Kalaivanan *et al.* (2013). The obtained extracts were concentrated using a rotary

evaporator and stored at 4 °C for further experimental studies. The plants, plant parts, solvents and percentage yields are given in Table 1.

Table 1: Plants used for extraction and percentage yield obtained from 60 g crude sample

S. No	Plant	Family	Common name	Part used	Solvent	Yield (%)
1	*Nerium oleander* Linn.	Apocynaceae	Oleander	Leaves	Ethanol	6.1
2	*Ficus racemosa* L.	Moraceae	Cluster fig	Leaves	Ethanol	1.0
3	*Senna siamea* Lam.	Fabaceae	Golden shower	Leaves	Ethanol	1.5
4	*Syzygium cumini* L.	Myrtaceae	Java plum	Leaves	Ethanol	1.0
5	*Senegalia rugata* (Lam.)	Fabaceae	Shikakai	Pods	Ethanol	1.5
6	*Mangifera indica* Linn.	Anacardiaceae	Mango	Leaves	Methanol	2.87
7	*Azadirachta indica* A. Juss	Meliaceae	Neem	Leaves	Methanol	4.9
8	*Calotropis gigantea* (L.)	Apocynaceae	–	Leaves	Methanol	3.9
9	*Piper betle* L.	Piperaceae	Betel	Leaves	Ethanol	3.5
10	*Trigonella foenum-graecum*	Fabaceae	Fenugreek	Seeds	Ethanol	0.5
11	*Eucalyptus globulus*	Myrtaceae	Eucalyptus	Leaves	Methanol	4.6
12	*Curcuma longa* L.	Zingiberaceae	Turmeric	Rhizome	Ethanol	2.4
13	*Piper longum* (L.)	Piperaceae	Pepper	Leaves	Methanol	1.2
14	*Coleus amboinicus*	Lamiaceae	Mexican mint	Leaves	Methanol	0.7

2.4 Fungal Strains and Culture Maintenance

Dermatophytic fungal strains *Microsporum gypseum* (MTCC 3270) and *Microsporum canis* (MTCC 2829) were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. The cultures were sub-cultured and maintained on Sabouraud Dextrose Agar (SDA) slants and plates (Hi-Media, India) and incubated at 30 °C. Master cultures were preserved in 10 % glycerol stocks at 4 °C.

2.5 Phytochemical Screening

Preliminary phytochemical screening of crude plant extracts was performed to identify major classes of secondary metabolites responsible for antifungal activity. The analysis included tests for flavonoids, phenolics, alkaloids, glycosides, anthocyanins, tannins, saponins, quinones, anthraquinones, and terpenoids following standard protocols [24].

2.6 Preparation of Fungal Inoculum

Fungal inoculum was prepared according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines with slight modifications. Five-day-old fungal cultures grown on SDA plates were flooded with sterile distilled water containing Tween-20 to obtain conidial suspensions. The suspensions were centrifuged at 6000 rpm for 15 min. The pellet was

resuspended in sterile distilled water and vortexed for 15 s. The turbidity was adjusted to a 0.5 McFarland standard using a spectrophotometer at 530 nm, corresponding to approximately 10^5 CFU/mL.

2.7 Antifungal Activity by Disc Diffusion Method

The antifungal activity of plant extracts was evaluated using the disc diffusion method following CLSI guidelines (CLSI, 2006). Extracts were prepared at a concentration of 20 mg/mL in 10 % dimethyl sulfoxide (DMSO). Sabouraud Dextrose Agar plates supplemented with chloramphenicol (50 µg/mL) were inoculated with fungal spore suspensions using sterile swabs.

Sterile 6 mm diameter discs were placed on the agar surface, and 10 µg of plant extract was dispensed onto each disc. Ketoconazole (25 µg/mL) served as the positive control, while 10 % DMSO served as the negative control. Plates were incubated at 28–30 °C for 72–96 h. Zones of inhibition (ZOI) were measured in millimeters, and all experiments were conducted in triplicates.

2.8 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of plant extracts against dermatophytes was determined using the broth microdilution method according to CLSI M38-A guidelines (CLSI, 2006). Extracts prepared at 50 mg/mL in 10 % DMSO were diluted in RPMI-1640 medium to obtain concentrations ranging from 3.9 to 1000 µg/mL.

Two hundred microliters of the diluted extract were added to the first well of a 96-well microtiter plate, followed by serial two-fold dilutions across subsequent wells. Each well received 100 µL of standardized fungal inoculum (~10^4–10^5 CFU/mL). Ketoconazole served as the reference antifungal drug, while 10 % DMSO served as the negative control. The plates were incubated at 30 °C for 72 h. The MIC was defined as the lowest concentration of extract that showed no visible fungal growth.

The plant extract exhibiting the highest antidermatophytic efficacy among the evaluated extracts was selected for further investigation. Subsequent to chromatographic separation and purification, GC–MS and molecular docking analyses were employed to describe the selected extract. The efficacy of the selected fraction against dermatophytes was then evaluated in an in vivo experimental model.

2.9 Thin layer chromatography

TLC plates coated with silica gel were utilised for the chromatographic analysis of *Senegalia rugata* extract. A suitable solvent was used to dissolve the sample and spotted onto the TLC plate using a thin capillary tube approximately 1 cm above the lower end of the plate.

The developing chamber was prepared by adding the standardized mobile phase, **ethanol : methanol (65:35:10, v/v/v)**, and allowing the chamber to equilibrate and become saturated with the solvent vapour. The spotted TLC plate was carefully placed in the developing chamber.

The solvent was allowed to migrate up the plate by capillary action. The plate was removed from the chamber when the solvent front had travelled approximately 5cm. The solvent front was marked immediately and the plates were allowed to dry at room temperature. The chromatogram was further visualised under short and long wavelength.

Under short- and long-wavelength UV light, separated bands were documented. The retention factor (Rf) of each separated component was calculated

Rf = Distance travelled by the compound / Distance travelled by the solvent front.

The solvent system was chosen based on resolution of the separate bands and clarity. The data obtained from TLC was further employed as a guide for the column chromatography

2.10 Column Chromatography

The selected fraction of *Senegalia rugata* extract was subjected to column chromatography using silica gel as the stationary phase. Silica gel was initially equilibrated with the starting mobile phase and carefully packed into a glass chromatography column to obtain a uniform stationary bed.

The sample was dissolved in a minimum volume of suitable solvent and carefully loaded onto the top of the silica-gel bed. Elution was performed using a solvent system of chloroform, methanol and water with increasing polarity. The composition of the mobile phase was optimized based on the TLC profile of the sample.

The elute was collected in separate fractions and each fraction was monitored by TLC using silica gel 60 F254 plates. Fractions exhibiting similar TLC profiles were pooled and concentrated under reduced pressure. The resulting sub-fractions were subsequently subjected to phytochemical screening and TLC-bio autography to identify fractions containing the antifungal-active constituents.

For fractions suspected to contain saponins, chloroform : methanol was used as an appropriate TLC solvent system, as reported previously for *Senegalia rugata* saponins.

Fraction from F1 to F12 were collected and further assessed for antidermatophytic activity by bio autography.

2.11 Bioautography

The selected fraction of *Senegalia rugata* was subjected to thin layer chromatography (TLC) on silica gel TLC plates as stationary phase. The specimen was observed on the plate and developed with chloroform: methanol (65:25:4, v/v/v) as the mobile phase. The TLC plate was then removed from the chamber and allowed to dry thoroughly before the development. Then, the fabricated plate was coated with liquefied Sabouraud dextrose agar (SDA)/potato dextrose agar (PDA) containing spores of *Microsporum canis*. The temperature was kept between 25 and 28°C to favor the growth of fungi. After incubation, fungal growth was assessed and clear zones of inhibition in the chromatogram were considered as indicative of antifungal-active compounds. Then the R_f values (retention factor) of the active bands were calculated based on the distance travelled by the molecule compared to the solvent front.

2.12 GC–MS

The compounds were identified by comparing The *Senegalia rugata* crude extract and a fractions obtained from column chromatography exhibiting strong antifungal activity assessed by bioautography. Crude extract of *Senegalia rugata* subjected to GC-MS analysis using a Shimadzu GCMS-QP2010 S gas chromatograph–mass spectrometer equipped with GC–MS Solutions software. The compounds were separated using a Rtx-5 capillary column. The crude extracts were appropriately diluted with the matching solvent (1:100, v/v) and then filtered to produce particle-free samples. A 1 µL aliquot of the diluted sample was injected into the GC injector in the split mode (split ratio 30:1). The temperature of the injector was set at 300°C. Helium was used as the carrier gas with a flow rate of 1.0 ml/ min. The column temperature was held constant at 60°C and then programmed to 300°C with a 10 minute hold. Mass spectral detection was carried out using an electron ionization (EI) source with an ionization energy of 70 eV. The mass spectrometer was operated in the full scan mode in the m/z range of 40-550 amu. The measured mass spectra were compared with the existing spectral library.

2.13 Scanning Electron Microscope (SEM)

Fungal mycelia from control and herbal-treated samples were harvested and fixed in 2.5% glutaraldehyde at 2–4 h at 4 °C. Fixed samples were washed three times in 0.1 M phosphate buffer and then post-fixed in 1% osmium tetroxide for 1–2 h. The specimens were then dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, 95% and 100% ethanol). Specimens were then dehydrated and subjected to critical point drying (CPD). The dried specimens were mounted on SEM stubs and coated with gold before examination. The prepared specimens were viewed under scanning electron microscope and the surface morphology of untreated and herbal treated *Microsporum canis* and *Microsporum gypseum* was compared to identify the structural alterations associated with treatment

2.14 MTT assay-based cytotoxicity assessment

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test was used to assess the cytotoxicity of the chosen plant extract/formulation using the human keratinocyte cell line HaCaT. The assay relies on metabolically active cells reducing MTT to insoluble formazan crystals with a purple hue. The amount of formazan produced, which was measured spectrophotometrically at 570 nm, is proportionate to the number of live cells that are metabolically active.

2.14.1 Maintaining and cultivating cells

The National Center for Cell Science (NCCS) in Pune, India, provided the HaCaT cells. The cells were kept in Dulbecco's Modified Eagle Medium (DMEM) with high glucose (HiMedia, India), supplemented with 1% antibiotic-antimycotic solution and 10% fetal bovine serum (FBS; HiMedia). After reaching the proper confluency, cells were periodically subcultured in a humidified incubator at 37°C with 5% CO_2.

2.14.2 MTT test

In a 12-well culture plate, HaCaT cells were planted at a density of 50,000 cells per well and given a full day to adhere and stabilize. The cells were exposed to the test samples at the necessary concentrations after incubation. The negative control was untreated cells kept in culture media. The positive control was doxorubicin (1 μM). To account for background absorbance, a medium-only control devoid of cells was included.

The test samples were incubated with the cells for 48 hours at 37°C in a humidified environment with 5% CO_2. The culture medium was taken out after treatment, and MTT solution was added to reach a final concentration of 0.5 mg/mL. To enable the development of formazan crystals, the plates were shielded from light and incubated for three hours at 37°C.

Following incubation, the formazan crystals were dissolved by adding dimethyl sulfoxide (DMSO) after the MTT-containing liquid was carefully removed. To ensure that the crystals completely dissolved, the plates were gently shaken. A microplate reader was used to detect absorbance at 570 nm.

Using the following formula, cell viability was represented as a percentage in relation to the untreated control:

Cell viability (%) = (Absorbance of treated cells / Absorbance of untreated control cells) × 100 Equation 1

When applicable, the concentration–response curve was used to calculate the concentration of the test sample that produced a 50% reduction in cell viability (IC_{50}).

2.15 Statistical Analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation. Differences among treatments were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc test using SPSS version 20. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Antidermatophytic activity of the plant extracts

Among the fourteen medicinal plant extracts screened for antidermatophytic activity, the crude ethanolic pod extract of *Senegalia rugata* exhibited the highest inhibitory activity against both *Microsporum canis* and *Microsporum gypseum*. The extract produced zones of inhibition of 32 ± 0.6 mm against *M. canis* and 28 ± 0.3 mm against *M. gypseum* (Figures 1 and 2), indicating strong antifungal activity.

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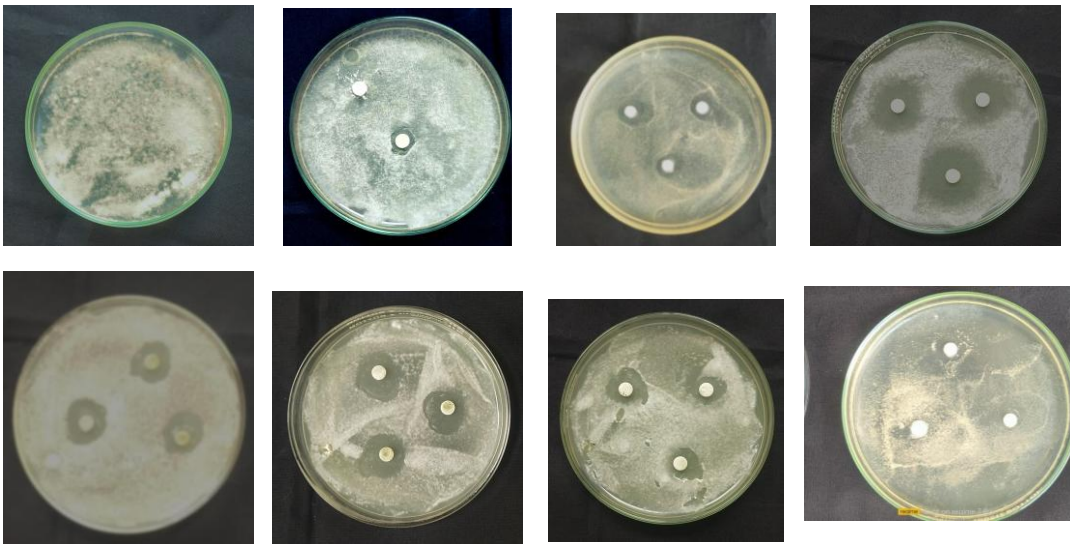


Figure 1: Antifungal activity of plant extracts against *Microsporum canis*. 1) Organism control, 2) Positive control, 3) *Senegalia rugata*, 4) *Mangifera indica*, 5) *Syzygium cumini*, 6) *Senna siamea*. Concentrations: a) 10 mg/mL, b) 20 mg/mL.

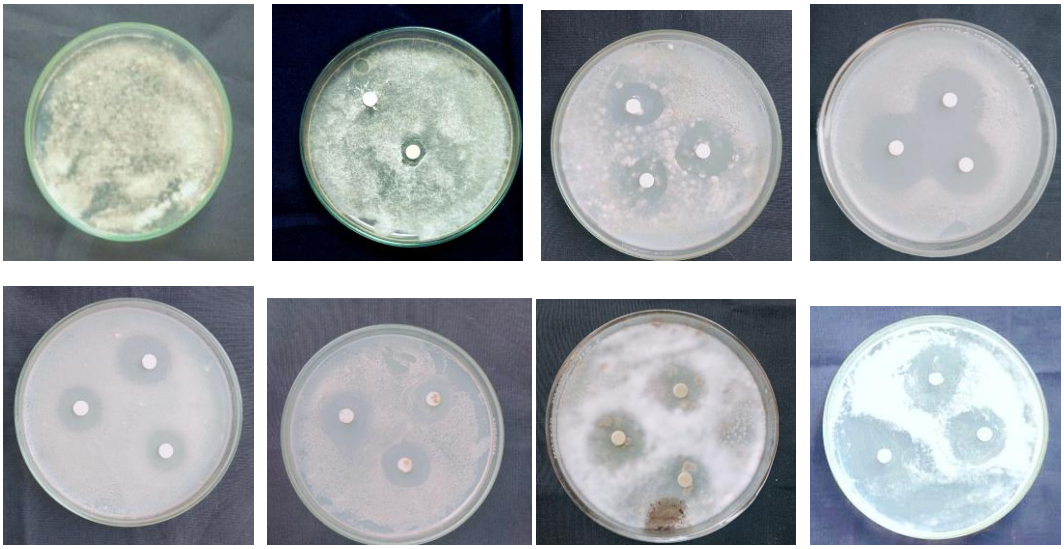


Figure 2: Antifungal activity of plant extracts against *Microsporum gypseum*. 1) Organism control, 2) Positive control, 3) *Senegalia rugata*, 5) *Syzygium cumini*, 6) *Cassia fistula*. Concentrations: a) 10 mg/mL, b) 20 mg/mL.

Against *M. gypseum*, crude leaf extracts of *Cassia fistula*, *Calotropis gigantea* and *Syzygium cumini* at 20 mg/mL demonstrated considerable antifungal activity, producing zones of inhibition of 26 ± 3.6 mm, 24 ± 3.4 mm and 25 ± 0.6 mm, respectively (Table 2). The MIC values ranging from 156–625 µg/mL further confirmed their inhibitory activity at relatively low concentrations.

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Table 2: Activity of plant extracts against *Microsporum gypseum* (zone of inhibition in mm)

Sl. No	Plant extract	10 mg/mL	20 mg/mL
1	*Senna siamea*	25 ± 3.5	26 ± 3.6
2	*Calotropis gigantea*	23 ± 0.6	24 ± 3.4
3	*Senegalia rugata*	20 ± 0.9	28 ± 0.3
4	*Syzygium cumini*	23 ± 0.6	25 ± 0.6
5	Ketoconazole (25 µg/mL)	20 ± 1.5	–

Similarly, against *M. canis*, extracts of *Calotropis gigantea*, *Syzygium cumini* and *Mangifera indica* exhibited zones of inhibition of 29 ± 0.8 mm, 22 ± 2.9 mm and 17 ± 0.8 mm, respectively (Table 3). The MIC values for these extracts ranged between 78–312.5 µg/mL (Table 4), indicating strong antifungal activity.

Table 3: Activity of plant extracts against *Microsporum canis* (zone of inhibition in mm)

Sl. No	Plant extract	10 mg/mL	20 mg/mL
1	*Syzygium cumini*	19 ± 0.8	22 ± 2.9
2	*Mangifera indica*	15 ± 0.5	17 ± 0.8
3	*Senegalia rugata*	25 ± 1.7	32 ± 0.6
4	*Calotropis gigantea*	25 ± 0.8	29 ± 0.8
5	Ketoconazole (25 µg/mL)	28 ± 1.5	–

Table 4: Minimum inhibitory concentration (MIC) of plant extracts against dermatophytes

Sl. No	Plant name	MIC (*M. gypseum*)	MIC (*M. canis*)
1	*Calotropis gigantea*	312 µg/mL	78 µg/mL
2	*Senna siamea*	156.5 µg/mL	78 µg/mL
3	*Senegalia rugata*	78 µg/mL	156.5 µg/mL
4	*Syzygium cumini*	312 µg/mL	312 µg/mL
5	Ketoconazole	–	–

Overall statistical comparison ($p < 0.05$) demonstrated that *Senegalia rugata* exhibited the most potent antidermatophytic activity, followed by *Calotropis gigantea*, *Syzygium cumini*, *Senna siamea* and *Mangifera indica* in decreasing order of antifungal potential.

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3.2 Thin layer chromatography and bioautography

Thin layer chromatography followed by bioautography clearly indicated that fraction 8 and fraction 10 showed antidermatophytic activity (Figures 3–7). The R_f values of fractions 5–10 are given in Table 5.

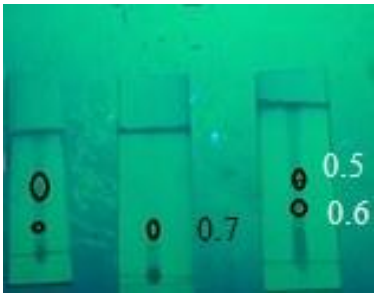


Figure 3: Thin layer chromatography. A – fraction 8, F – fraction 9, G – fraction 10.

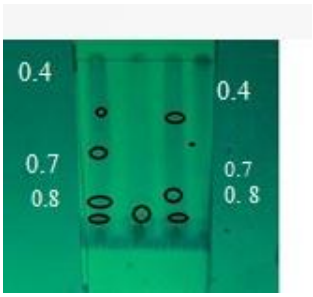
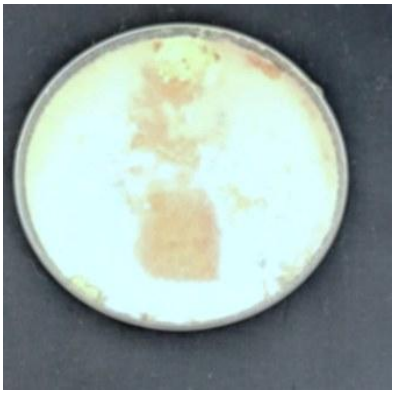


Figure 4: Thin layer chromatography. E – fraction 8, F – fraction 9, G – fraction 10.



Fraction 8



Fraction 10

Figure 5: Bioautography of fraction 8 and fraction 10.

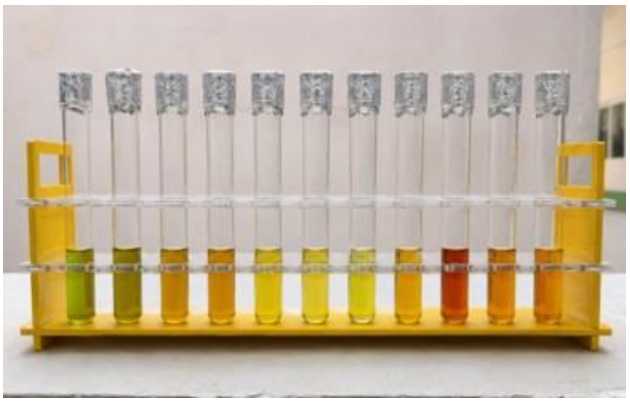


Figure 6: Tubes showing the different fractions obtained by column chromatography.



Figure 7: Packed column used for chromatography.

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Table 5: R_f values of fractions 5, 6, 7, 8, 9 and 10

Fraction	R_f
5	0.5, 0.8
6	0.7
7	0.5, 0.6
8	0.4, 0.7, 0.8
9	0.7, 0.8
10	0.4, 0.7, 0.8

3.3 GC–MS analysis

GC–MS analysis of fraction 8 of the ethanolic extract of *Senegalia rugata* exhibited 40 volatile constituents. The extract was rich in organic acids, esters and fatty acid derivatives. The major constituents were cyclopentane, ethyl 2-oxocyclopentane-1-carboxylate (30.76%) and 3-hydroxy-2,2-dimethyl-, diethyl ester (20.58%) (Figures 8 and 9).

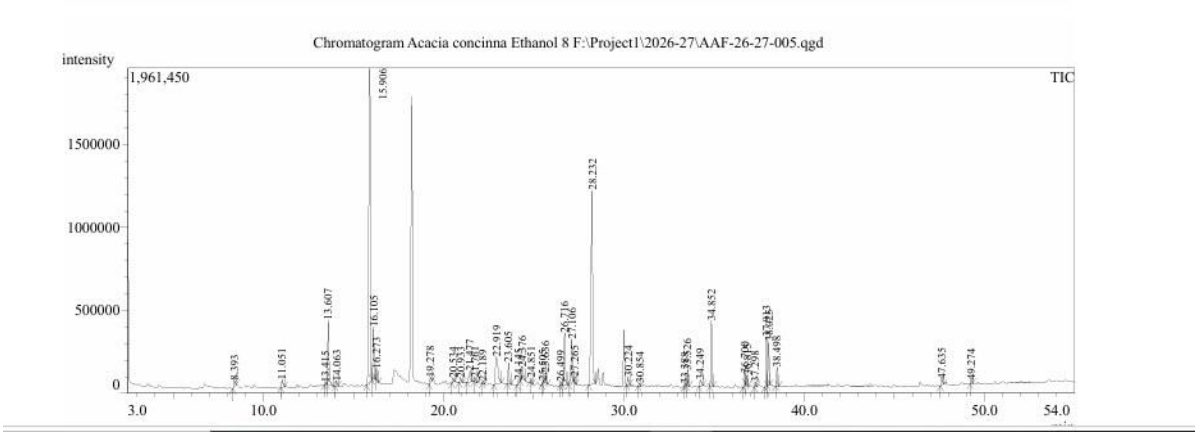


Figure 8: Chromatogram of fraction 8 obtained from column chromatography of the ethanolic extract of *Senegalia rugata*.

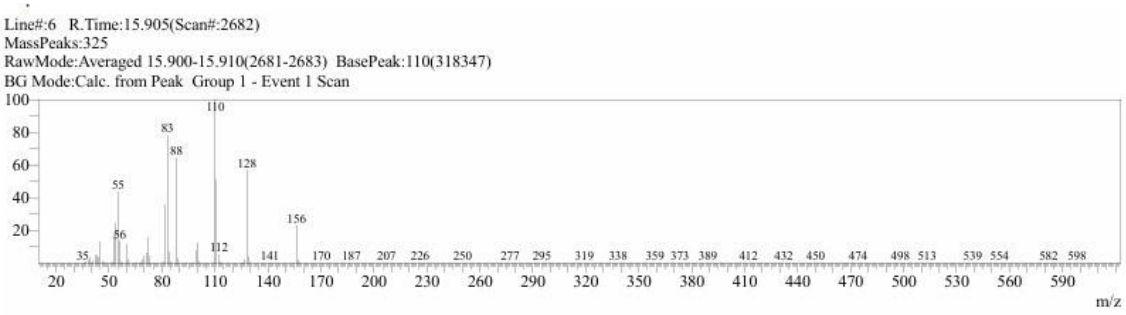


Figure 9: Spectra of cyclopentane, ethyl 2-oxocyclopentane-1-carboxylate.

In fraction 10, 3-ethoxy-3,7-dimethylocta-1,6-diene was identified as the major constituent of the ethanolic extract of *Senegalia rugata*, with a peak area of 18.08% at a retention time of 24.421 min (Figures 10 and 11). This terpenoid-related compound may contribute to the extract's antimicrobial and antifungal properties.

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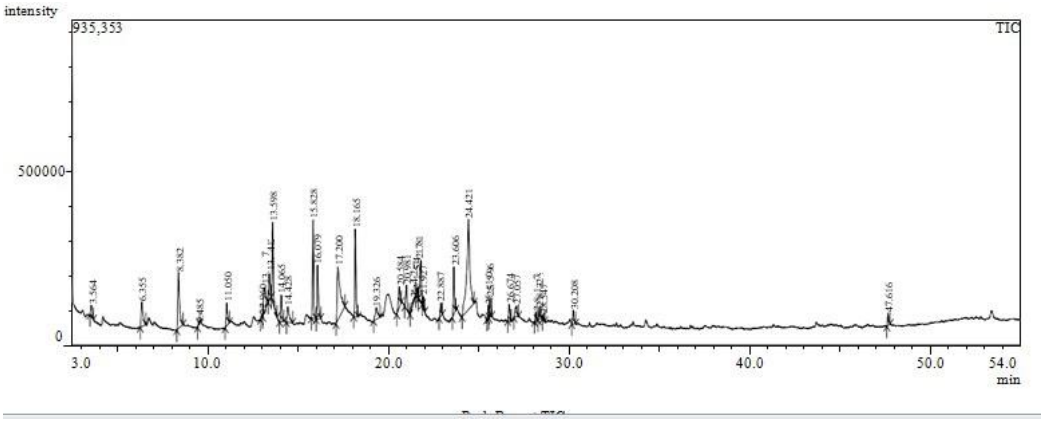


Figure 10: Chromatogram of fraction 8 obtained from column chromatography of the ethanolic extract of *Senegalia rugata*.

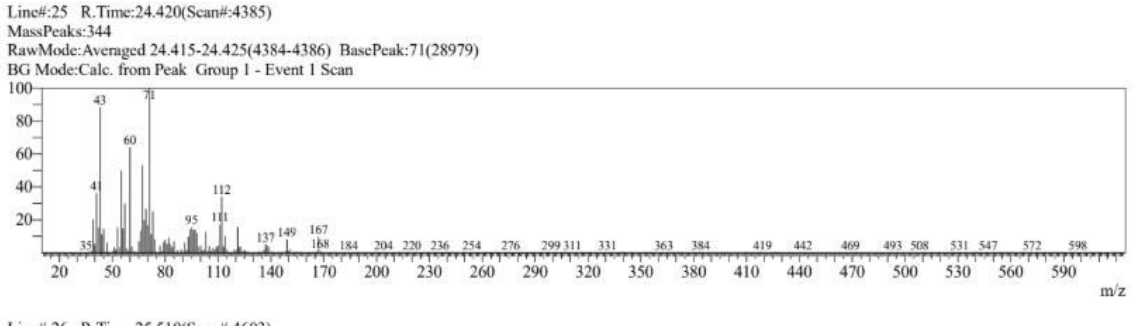


Figure 11: Spectra of 3-ethoxy-3,7-dimethylocta-1,6-diene.

3.4 Cytotoxicity (MTT assay on HaCaT cells)

The MTT assay demonstrated dose-dependent cytotoxicity of the *Senegalia rugata* extract against HaCaT cells. Growth inhibition increased from 7.28% (31.25 µg/mL) to 94.24% (500 µg/mL). Ketoconazole (1 µM) showed 97.26% inhibition, validating the assay. The IC_{50} was 54.95 µg/mL, indicating moderate cytotoxicity (Figures 12 and 13).

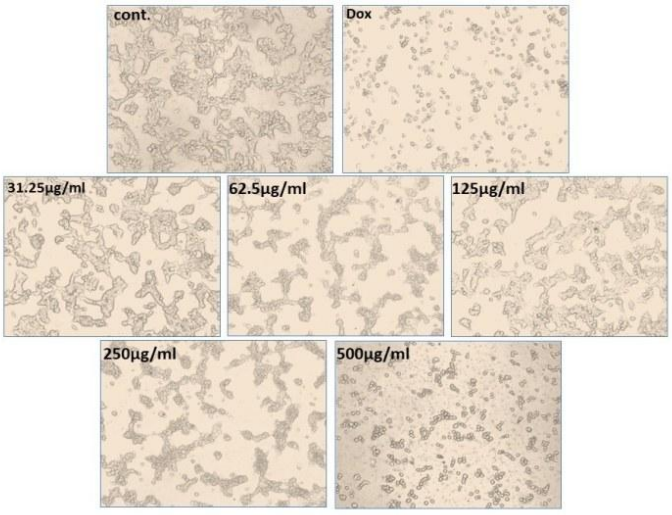


Figure 12: HaCaT cell morphology at different extract concentrations.

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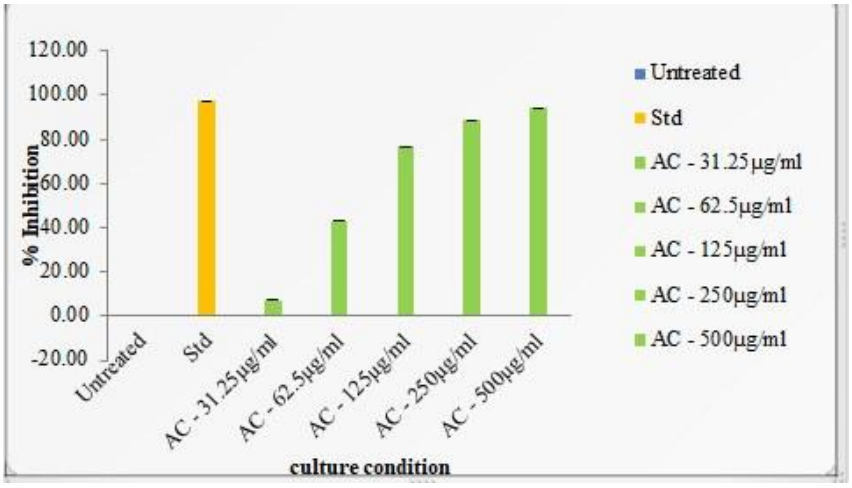
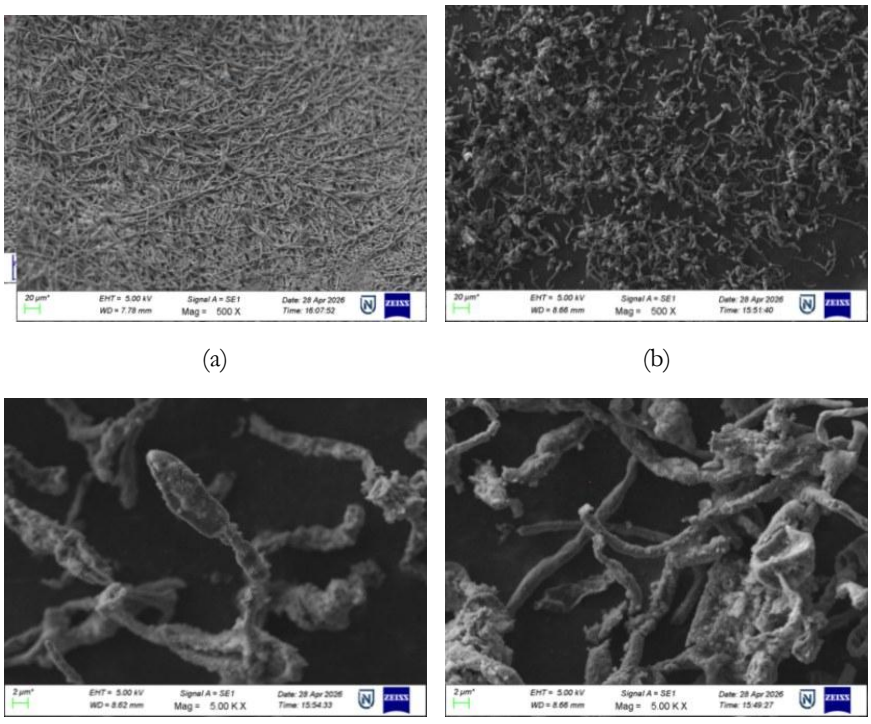


Figure 13: % cell inhibition values of *Senegalia rugata* against HaCaT cells after the incubation period of 48 h, along with controls.

3.5 Scanning electron microscopy (SEM) analysis

Scanning electron micrographs of control and *Senegalia rugata*-treated *Microsporum canis* and *Microsporum gypseum* are shown in Figures 14 and 15.

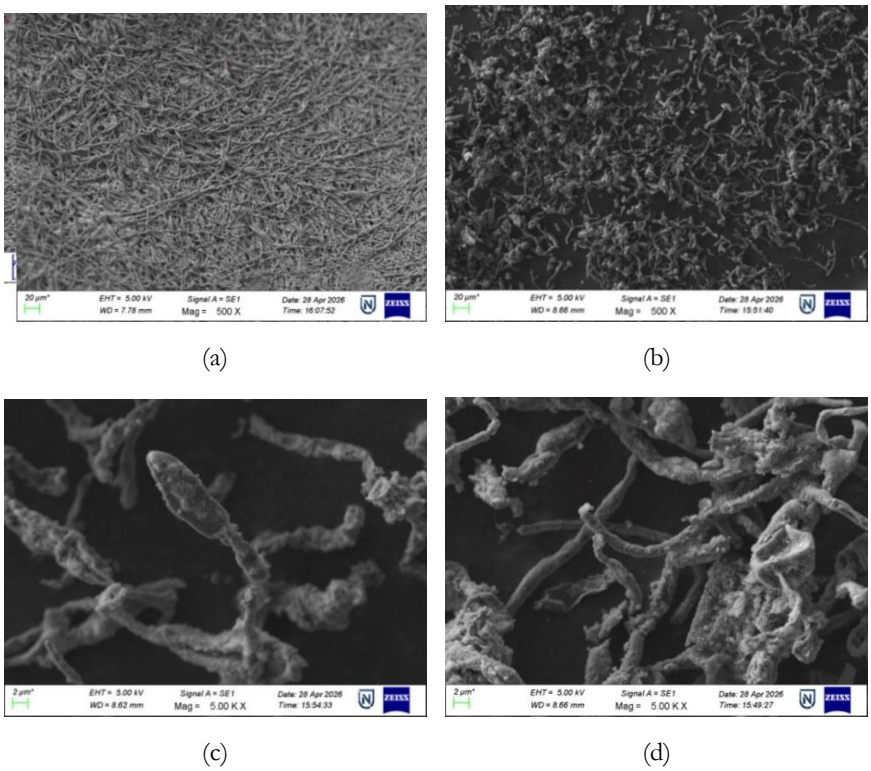


(a) (b)

(c) (d)

Figure 14: Scanning electron micrographs of *Microsporum canis*. (a) Control hyphae without treatment show clear, unbroken hyphae; (b) treated hyphae of *M. canis* are shrivelled with abnormal shapes; (c) hyphae showing spores of *M. canis*; (d) treated sample shows absence of spores.

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(a) (b)

(c) (d)

Figure 15: Scanning electron micrographs of *Microsporum gypseum*. (a) Control hyphae without treatment show clear, unbroken hyphae; (b) treated hyphae of *M. gypseum* are shrivelled with abnormal shapes; (c) hyphae showing spores of *M. gypseum*; (d) treated sample shows absence of spores.

4. DISCUSSION

Dermatophytosis is one of the most common superficial fungal infections in humans globally. The rising incidence of chronic and recurrent dermatophytic infections along with the development of decreased susceptibility to currently used antifungal medicines has driven the need for the discovery of new antifungal drugs from natural sources. Medicinal herbs have traditionally been utilized for management of certain skin problems and are therefore a possible source of bioactive substances with antidermatophytic effect.

Fourteen medicinal plants traditionally used in ethnomedicine were examined for antidermatophytic action against *Microsporum gypseum* and *Microsporum canis* in the present investigation. The ethanolic extract of *Senegalia rugata* showed the strongest antifungal activity among the studied plants. The extract at 20 mg/mL showed inhibitory zones of 32 ± 0.6 mm against *M. canis* and 28 ± 0.3 mm against *M. gypseum*. The inhibition obtained shows that there are ingredients in ethanolic extract that are able to suppress growth of both dermatophytes. The difference in inhibition between the two species may be due to variation in their susceptibility to the phytochemical ingredients present in the extract.

The minimum inhibitory concentration (MIC) of the *S. rugata* extract was found to be 78 µg/mL against *M. gypseum* and 156.78 µg/mL against *M. canis*. These results further confirm the inhibitory effect seen in the original screening and suggest that the extract was active at relatively low concentrations. MIC values of the two species are different, which means the dermatophytes are susceptible to the bioactive compounds present in the extract differently. Such variances may be due to changes in fungal cell-wall composition, membrane properties or other physiological features affecting their susceptibility to plant-derived chemicals.

Preliminary phytochemical analysis of the plant extract showed the presence of numerous types of secondary metabolites like terpenoids, tannins, glycosides and other phytoconstituents. This chemicals have been previously linked to antibacterial and antifungal properties. In example, plant-derived saponins and terpenoids are found to interact with fungal cell membranes, while phenolic chemicals and flavonoids may interfere with cellular enzymes and other metabolic processes. Therefore, the

presence of these phytochemical groups in *S. rugata* may contribute individually or synergistically to the antidermatophytic activity shown in the present investigation. However, the present phytochemical screening does not indicate which single constituent is responsible for the observed action.

In an effort to further examine the compounds related to antifungal activity, the ethanolic extract of *S. rugata* was fractionated and the fractions obtained were analyzed by thin-layer chromatography and bioautography. Of the tested fractions, fraction 8 had antidermatophytic efficacy against *M. canis* in the bioautography experiment. The existence of a zone of inhibition related to fraction 8 suggests that one or more ingredients present in this fraction are responsible for the antifungal activity of the parent extract. Thus, bioautography was a valuable method for linking chromatographic separation to biological activity and for the identification of fractions requiring further chemical research.

The GC–MS analysis of fraction 8 revealed many chemical compounds, of which Cyclopentane Ethyl 2-oxocyclopentane-1-carboxylate gave a strong peak under the analytical conditions applied. This is evidenced by the high peak area which suggests that this molecule was one of the key detectable ingredients of fraction 8 in the GC-MS study. Cyclopentanecarboxylic acid ester related chemical class compounds have been studied for diverse biological characteristics. However, a large peak in GC-MS cannot confirm the presence of Cyclopentane Ethyl 2-oxocyclopentane-1-carboxylate as a cause of the antidermatophytic activity found. To verify the individual compound's contribution to the antifungal impact it would have to be isolated and tested.

The present study findings are also in agreement with earlier studies that have reported biological activity linked with *S. rugata* and its phytochemical constituents. Wasefa Begum et al. (2025) studied the saponins of *S. rugata* and described their biological features concerning fungal systems. Surface-active properties of plant saponins are well known and their antibacterial activity has been explored against a range of microbes. The results presented herein warrant future exploration of *S. rugata* as a source of bioactive phytochemicals. However, changes in extraction procedures, fungus species, concentrations and experimental circumstances should be considered while comparing the results of different studies.

Cytotoxicity testing on HaCaT cells provided some first information about the effect of the *S. rugata* extract on a human keratinocyte cell line. The cytotoxic reaction suggests a measurable effect of the extract on the viability of HaCaT cells under the experimental circumstances used. Thus, the cytotoxicity results should be evaluated jointly with the antifungal activity to determine the potential of the extract for topical applications. Further investigations in appropriate dosage ranges and utilizing other normal human cell types would be important to establish selectivity of the antifungal action.

To summarize, the present investigation revealed the promising antidermatophytic activity of *S. rugata* against *M. gypseum* and *M. canis*. The combination of primary screening, measurement of MIC, chromatographic fractionation and bioautography demonstrate that the antifungal activity can be connected to certain fractions of the plant extract, especially fraction 8. GC–MS analysis also provided preliminary information on the chemical makeup of the active fraction. However, further purification, structural characterization and individual testing of the bioactivity of the discovered compounds are needed to determine the ingredients responsible for the observed antidermatophytic effect. Further in vivo investigations and detailed toxicity evaluation would also be required to confirm the safety and therapeutic potential of preparations from *S. rugata*.

5. CONCLUSION

Overall, the strong zones of inhibition, sub-mg/mL, MIC, GCMS result especially for *Senegalia rugata* —support the hypothesis that these traditionally used plants contain antifungal constituents active against *Microsporum* spp. The results are concordant with recent studies on *Senegalia rugata*, *Cassia* spp., and *Calotropis procera* and justify bioassay-guided isolation, mechanistic studies, toxicity screening, and standardized potency testing as the next steps toward possible therapeutic development.

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AUTHOR CONTRIBUTIONS

: Mrinalini Devi U.G.: Conceptualization, Investigation, Formal analysis, Writing – original draft. Sharmila T.: Supervision, Methodology, Writing – review & editing. Sushma H.N.: Investigation, Data curation]

CONFLICT OF INTEREST

The authors declare no conflict of interest

ETHICS STATEMENT

Not applicable; the study used reference fungal strains and a commercially obtained human cell line, and involved no human participants or animals

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author on reasonable request.

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