Original Article

Bioactivity-Guided Investigation of Some Medicinal Plants of Bangladesh In Search of Active Principles Against Common Dermatophyte Strains

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Abstract

Background: *Fungal infections, particularly dermatophytosis, represent a significant global health concern, including in Bangladesh. Although numerous antifungal agents are available, the management of these infections has become increasingly challenging due to the emergence of drug-resistant fungal strains, a higher incidence of adverse effects, and poor cost-effectiveness. Given the growing issues of antifungal resistance, toxicity, and treatment costs, there is an urgent need to explore and evaluate the antifungal potential of naturally occurring medicinal plants found in Bangladesh.*

Objective: *To find out newer antifungal principles from bioactive plants available in Bangladesh.*

Materials and Methods: *It was an experimental study. The study was conducted from December, 2024 to August 2025. The study was carried out by Pharmacology lab; the Department of Pharmacology and Therapeutics, Khwaja Yunus Ali Medical College and Hospital (KYAMCH), Enayetpur, Sirajgonj situated in Bangladesh. At first Phytochemical investigation was done. For this, the five plant materials (Leaves of Achyranthes aspera and Dalbergia sissoo and also leaves & seeds of Cassia obtusifolia, whole plants of Eclipta prostata and leaves & fruits of Morienga oleifera) were collected and indentified. Then all parts of the plants were dried and dried parts were fine ground, weighed and labeled and preserved. Extraction of all plant materials was dissolved with MeOH for 7 days under room temperature. After 7 days all dissolved material were filtered and then crude extract were collected. Collected crude extracts were subjected the process of fractionation. A total 10 fractions were found and these fractions were further evaluated for biological activities.*

Results: *From this study it has been observed that the methanolic extract of methanol fraction (100 µg/ml) and hexane fraction (100 µg/ml) of Dalbergia sisso that produced 20 mm and 10 mm area of inhibition respectively against common dermatophytes. Methanolic extract of Methanol fraction (100µg/ml) & Hexane fraction (100µg/ml) of Cassia obtusifolia produced maximum area of inhibition is 12 mm and 20 mm respectively.*

Conclusion: *Experiments described in this article demonstrate that Dalbergia sisso and C.obtusifolia extracts exhibit the antifungal potential. Further studies are needed for isolation of bioactive chemical compound and development of new anti-fungal principles from Dalbergia sisso and C.obtusifolia leaves.*

Keywords: *Antifungal principles, Dermatophytes, Bioactive medicinal plants, Fungal infections, Anti-fungal resistance.*

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Introduction

Fungal infections are caused by agents like yeasts and molds can affect skin, mucus membranes and in some cases, it can affect lungs and brain. Now a days fungal infections have created a worldwide epidemic. The prevalence and incidence of such infections are increasing day by day. In spite of existence numerous antifungal agents' fungal infections are becoming difficult to treat due to the higher potential of resistance against the antifungal agents. A large number of patients are suffering from various fungal infections for long duration with or without uses of different antifungal medicines. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world.[1]

The prevalence of fungal infections with other diseases including Covid-19 is coupled with life-threatening mycoses and mortality. Fungal infections can incorporate superficial, cutane

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ous, sub-cutaneous, mucosal and systemic infections with varying extent of severity. Organisms like *Candida spp.* are part of human normal flora which can cause opportunistic infections and life-threatening infections (invasive candidiasis) in immu no-compromised individuals such as HIV patients, cancer patients treated with chemotherapy, and immuno-suppressive drugs. In addition, opportunistic and systemic infections, fungal pathogens like *Candida, Aspergillus, Fusarium,* Mucorales and molds are capable of causing healthcare-associated infections (HAI) in patients with underlying diseases.[2]

In definite geographical areas, fungal pathogens cause prevalent life-threatening endemic mycoses like Blastomycosis, Coccidiodomycosis, Talaromycosis, Histoplasmosis Paracoccidiodomycosis and Sporotrichosis.[3]

Systemic fungal infections are seldom diagnosed lately with escalating mortality rates. Fungal disease awareness weeks is declared by CDC from September 19-23 in 2022 to highlight the importance of antifungal drugs reducing resistance and recognizing severe fungal disease early to provide lifesaving treatment.[4]

Ascomycota and Basidiomycota are the key contributor of all fungal pathogens and infections in humans. Ascomycota organisms are recognized for causing oropharyngeal, otolaryngeal, dermatological, ophthalmic, neuronal, genitourinary, cardiac, pulmonary and systemic infections. Basidiomycota such as *Cryptococcus* and *Malassezia* are well-known fungus for invasive meningitis and superficial skin infections.[5]

Fungal pathogens principally use direct contact and/or inhalation route for spread. Dermatophytic fungi belong to the genera of *Microsporum, Trichophyton* and *Epidermophyton, Malassezia spp.* and *Sporothrix* infect the damaged skin by direct contact. Other are transmitted by inhalation of spores/ conidia that causes pulmonary infections. *Blastomyces dermatitidis* (Blastomycosis), *Paracoccidioides brasiliensis* and *P. lutzii (Paracoccidioidomycosis), Pneumocystis jirovecii* (Pneumocystis pnuemonia), *Histoplasma capsulatum* (Histoplasmosis), *A. flavus* (Aspergillosis) and *Aspergillus fumigates, Coccidioides immitis* and *C. neoformans, C. posadasii* (Coccidioidomycosis) and *C. gattii* (Cryptococcosis) are mostly transmitted through inhalation. While, *Talaromyces marneffei* (talaromycosis) uses both direct contact and inhalation route.[6]

Now, five common classes of antifungal drugs such as azoles, polyenes, echinocandins, allylamines and pyrimidine analogs are accessible for superficial and systemic antifungal therapies.[7] Azole class of drugs includes imidazoles (miconazole and ketoconazole) and triazoles (fluconazole and voriconazole) comprise the most successful backbone in building large number of antifungal compounds for clinical use. These agents are useful against *Candida spp.* and other fungal pathogens and suitable due to flexibility of administration through different routes.[8]

Allylamine include terbinafine and naftifine are commonly suggested for controlling superficial dermatophytoses.[9] Drugs such as pyrimidine analogue (5-fluorocytosine, 5-FC) are active against *Candida spp.* and *Cryptococcus sp* Antifungal drug resistance is an emerging clinical problem in antifungal therapy.[10]

Numerous host factors, disease factors and environmental factors exacerbate the development of resistance. Fungal resistance are two types: microbial resistance or in vitro resistance and clinical resistance or in vivo resistance.[11,12] Microbial resistance refers to non-susceptibility of fungus to an antifungal agent. It is done by in vitro susceptibility testing in which there is minimum inhibitory concentration of the drug exceeds the susceptibility breakpoint of that organism. Microbiological resistance is of two types: primary (intrinsic) and secondary (acquired)[13] where the fungi are resistance to a drug before exposure it is known as primary microbial resistance. Certain fungal species are intrinsically resistant such as *Candida krusei* to fluconazole and *Criptococcus neoformans* to echinocandins and nonalbicans candida to 5-flucytosine (5 FC).[12,13]

Secondary microbial resistance develops in response to exposure to a microbial agent, usually develops among previously susceptible strains and it is dependent on altered gene expression.[12] For example, terbinafine resistance in T. rubrum, fluconazole resistance among C. albicans. A fungal infection is referred as clinical resistance despite the administered antifungal agent with in vitro activity against that organism. This resistance cannot be always predicted.[12]

Mutations in target genes, adaptive phenotypic plasticity, chromosomal aneuploidy, sexual reproduction and horizontal gene transfer are the powerful driving forces for appearance of antifungal resistance.[11]

According to zanna et al *R. crispus, B. dalzieli, L. hastate, O. gratissimum, C. alata, B. nivosa Andm. oleifera* were assessed for their antifungal activities against *Candida albicans, Trichophyton mentragophyton* and *Trichophyton rubrum*. Methanolic extracts from above all medicinal plants showed that antifungal properties having variability. Methanolic leaf extracts of *B. dalzielii, O. gratissimum* and root extracts of *C. alata* exhibited most potent and dose dependent antifungal activity. Leaf extracts of *B. dalzielii* exhibited no activity against *C. albicans* but having antifungal activity against *Trichophyton rubrum* and *Trichophyton mentragophyton*.[14]

Study of zhang et al reported that most of the steriod saponins *tribulus terrestris* have antifungal activities against fluconazole resistant fungi. For this purpose, they conducted a series of experiment to investigate the activities of the eight steriod saponins like TTS-8, TTS-9, TTS-10, TTS-11, TTS-12, TTS-13, TTS-14, TTS-15. These steroid saponins were isolated from *tribulus terrestris*, the study showed that TTS-14 have activities but TTS-8, TTS-9, TTS-10, TTS-11, TTS-13 were inactive against *C. albicans, C. neoformans, C. glabrata and C. krusei.* In addition, TTS-12 and TTS-15 had significant antifungal properties against *C. albicans, C. glabrata, C. parapsilosis, C. krusei C. tropicalis and C. forman*.[15]

The methanolic leaf extracts of *Carica papaya* showed antifun

gal activity against *Aspergillus niger, Fusarium sp.* and *Cladosporium sp.*. Also, ethanolic extracts of *Crica papaya* showed pronounced antifungal activity against *Aspergillus niger*, *Cladosporium sp.* and *Aspergillus flavus*.[16]

Hussain et al showed that *Eclipta alba* had antifungal activity against four different strains like *Aspergillus niger, Fusarium solani, Aspergillus fumigates* and *Aspergillus flavus*.[17]

Devi et al showed that *Dalbergia sissoo* Roxb. stem had maximum antifungal activity in chloroform fraction and moderate antifungal activity in ethyl acetate and benzene fraction. And also, methanol fraction of *D. sissoo* showed antifungal activity against *Rhizoctonia solani* fungi.[18]

The aqueous leaf extracts of *Senna tora* on Downy Mildew of cabbage is effective and had antifungal activity against *Perenospora parasitica*.[19] The methanolic leaf extracts of *Achyranthes aspera* showed antifungal activity against *Microsporum canis*.[20]

The ethanolic extracts of *Calotropis giantea* latex possessed potent fungicidal activity.[21] Khamis et al showed that the acetone extracts of *Swietenia mahagoni* leaves had a partial antifungal activity against *Rhizoctonia solani* and potent fungicidal activity against *Fusarium equiseti*.[22]

Fungal resistance is a global emerging condition to combat the situation; it is urgent to evaluate about antifungal activity of several medicinal plants. In this study we will assess the antifungal activity in in *Dalbergia sissoo, Achyranthes aspera, Morienga olifera, Casia obtusifolia, Eclipta prostata.*

Materials and Methods

It was an experimental study. The study was conducted from December, 2024 to August 2025. The study was carried out by Pharmacology lab; the Department of Pharmacology and Therapeutics, Khwaja Yunus Ali Medical College and Hospital (KYAMC), Enayetpur, Sirajgonj situated in Bangladesh.

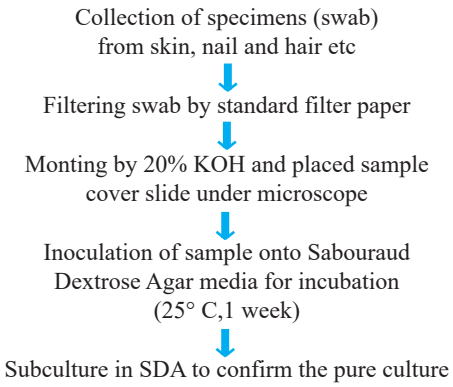
Table I: List of medicinal plants with their biological activities and constituents

Scientific name	Local name	Popular use	Parts used	Important chemical compound
Achyranthes aspera	Apang	Anti - bacterial & anti - fungal activity	Whole plant	An alkaloid Achyranthine; ecdysterone, inokosterone, henriacontane etc.
Dalbergia sissoo	Sishu	Antifungal activity	Chloroform and ethyl acetate fraction of leaves and bark	Leaves contain isoflavone, sissotrin. Stem bark contain dalberginone, dalbergin, methyldalbergin, 4 - phenylchromene, dalbergichromene
Morienga oleifera	Sajna gachh	Anti - fungal activity	Leaves, bark and fruits	Leaves contain a glycoside, bark contain moringine, moringinine etc.
Casia obtusifolia	Chakunda	Leaves and seeds are used in eczema and other skin diseases	Leaves and seeds	Leaves contain sennosides, D - mannitol, myricyl alcohol and beta sitosterol, emodin, flavonol glycoside. Seeds contain anthraquinone, chrysophanic acid, rhein, emodin, gluo -obtusifolin etc.
Eclipta prostrata	Keshraj	Plants are used in skin diseases	Whole plant	Plant contain an alkaloid ecliptine. Leaves also contain alpha -terthienylmethanol, beta amyrin, wedelolactone, demethyl wedelolactone, triterpane etc

The different parts (leaves, stem bark, seeds etc) of five investigating plants (enlisted herewith) were be collected from local market of Sadar upazila & Belkuchi upazilla of Sirajgang District and Netrokona Sadar of Netrakona District in the Month of April-May, 2023 and taxonomically identified by Prof. Dr. Mahbubur Rahman, Professor Scientific, Department of Botany, University of Rajshahi, Rajshahi, where a Voucher Specimens were collected (vide memo no. were 75, 30, 127, 63, 16, 91, 49, 07, 89 and 59 respectively). Then all parts (leaves, bark, seeds, roots etc.) of the plants were dried under sunlight and preserved it for investigation. All dried parts (leaves, bark, seeds, roots etc) of five plants were sent for fine Grinding, weighting and labeling and preserved all in air tight separate bottles. Extraction of all plant materials (leaves of *Achyranthes aspera*, of *Dalbergia sissoo*, of *Casia obtusifolia*, of *Eclipta prostrata* and of *Morienga oleifera*) is dissolved with MeOH for 7 days under room temperature. After 7 days, all dissolved material were filtered and crude extract of leaves of Achyranthes aspera, of Dalbergia sissoo, of Cassia obtusifolia, of Eclipta prostrata and of Morienga oleifera were collected. Then weighting all crude extracts were done and preserved in air tight bottles with code name. All preserved crude extracts have undergone the process of fractionation with different solvent system (from lower to higher polarity). After fractionation of the crude extracts, total 10 fractions were collected.

Methanol fraction of whole plant of *Eclipta prostrata* (100 mg), Hexane fraction of whole plant of *Eclipta prostrata* (600 mg), Methanol fraction of leaves of *Achyranthes aspera* (2370 mg), Hexane fraction of leaves of *Achyranthes aspera* (1160 mg), Hexane fraction of Leaves & seeds of *Cassia obtusifolia* (180 mg), Methanol fraction of Leaves & seeds of *Cassia obtusifolia* (93 mg), Hexane fraction of leaves & fruits of *Morienga oleifera* (1700 mg), Methanol fraction of leaves & fruits of *Morienga oleifera* (2300 mg), Hexane fraction of leaves of *Dalbergia sissoo* (640 mg), Methanol fraction of leaves of *Dalbergia sissoo* (985 mg). Collection of specimens (skin scraping, nail clipping, hair cutting) and tested dermatophytes were collected from the Out Patient Department (OPD) of KYAMC&H and stored in the Laboratory Services Department of KYAMC&H for culture, sub-culture and antifungal sensitivity test.

Figure 1: Flow chart of showing biological activity



Sterilize SDA medium was prepared by autoclaving according to the manufacturer's instructions. Pour the molten sterile SDA into sterile Petri dishes (approximately 15-20 mL for 90 mm plates) and allowed it to solidify on a level surface. Dip a sterile cotton swab into the standardized fungal suspension. Remove excess liquid by pressing the swab against the inside wall of the tube. Evenly swab the entire surface of the SDA agar plate in three different directions (swabbing the entire surface each time) to create a confluent lawn of fungal growth. Allow the inoculum to dry for 5-10 minutes.

Impregnate sterile blank discs with a known volume (e.g., 100 µL) of each plant extract concentration. Allow the discs to dry completely under lamilar air flow to ensure the solvent evaporates and the extract is absorbed. Using sterile forceps, carefully place the impregnated discs onto the surface of the inoculated SDA agar plates. Ensure the discs are evenly spaced and not too close to the edges of the plate typically 9 discs per plate. Place a disc with the solvent alone (negative control). Place a disc with the standard antifungal drug (positive control).

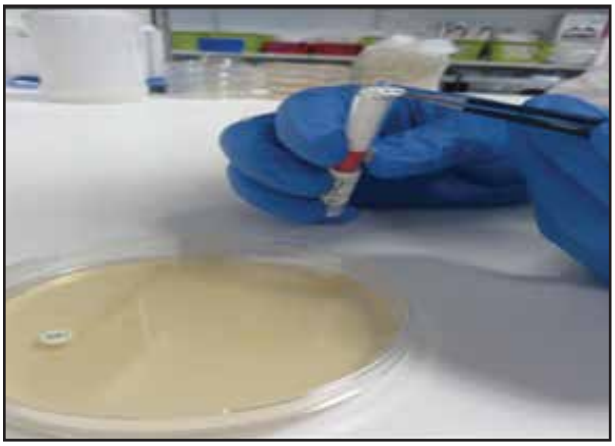


Figure 2: Procedure of blank disc impregnated with plant extract into inoculated SDA agar plates.

Figure 3: Blank disc impregnated with plant extract into inoculated SDA agar plates.

Incubate the plates at the optimal temperature for the test fungus (e.g. 28-37°C) for 24-72 hours. Observe for zones of inhibition regularly.

Interpretation showed that no zone of inhibition: The extract has no antifungal activity at the tested concentration. Zone of inhibition present means the extract exhibits antifungal activity. The larger the diameter of the zone, the greater the antifungal activity. Compare the zones of inhibition of the plant extracts with those of the positive control and negative control. The negative control should show no zone of inhibition.[23]

Results

The present study tested the antifungal activity of crude and their respective fractions from medicinal plants belonging to 5 plant families against dermatophytes. These medicinal plants were chosen based on folklore usage (Table I) suggestive of antifungal as well as antibacterial activity.

Sensitivity of drug sample can be explained on the basis of the following scale which was developed by Arora D.S et al (1997).

Table II: Relationship between zone of inhibition and drug sensitivity.

Sl.no	Zone of inhibition (mm)	Drug sensitivity
1	No inhibition/below 6	Insensitive
2	6-9	Less sensitive
3	9-12	Moderate sensitive
4	Above 12	Highly sensitive

It is clear that antifungal component within the extract could successfully inhibit fungal growth.

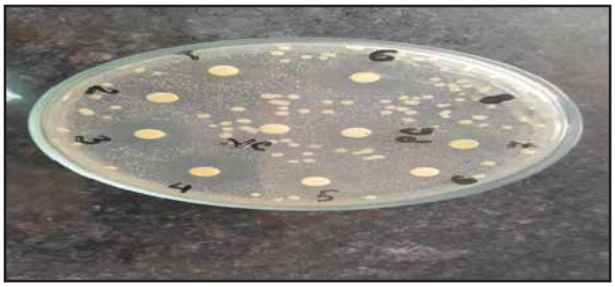


Figure 4: Fraction of plant extract shows zone of inhibition (1,2, 3, 4, 5, 6, 7, 8, 9 numbers, PC, NC are described in Figure 5)

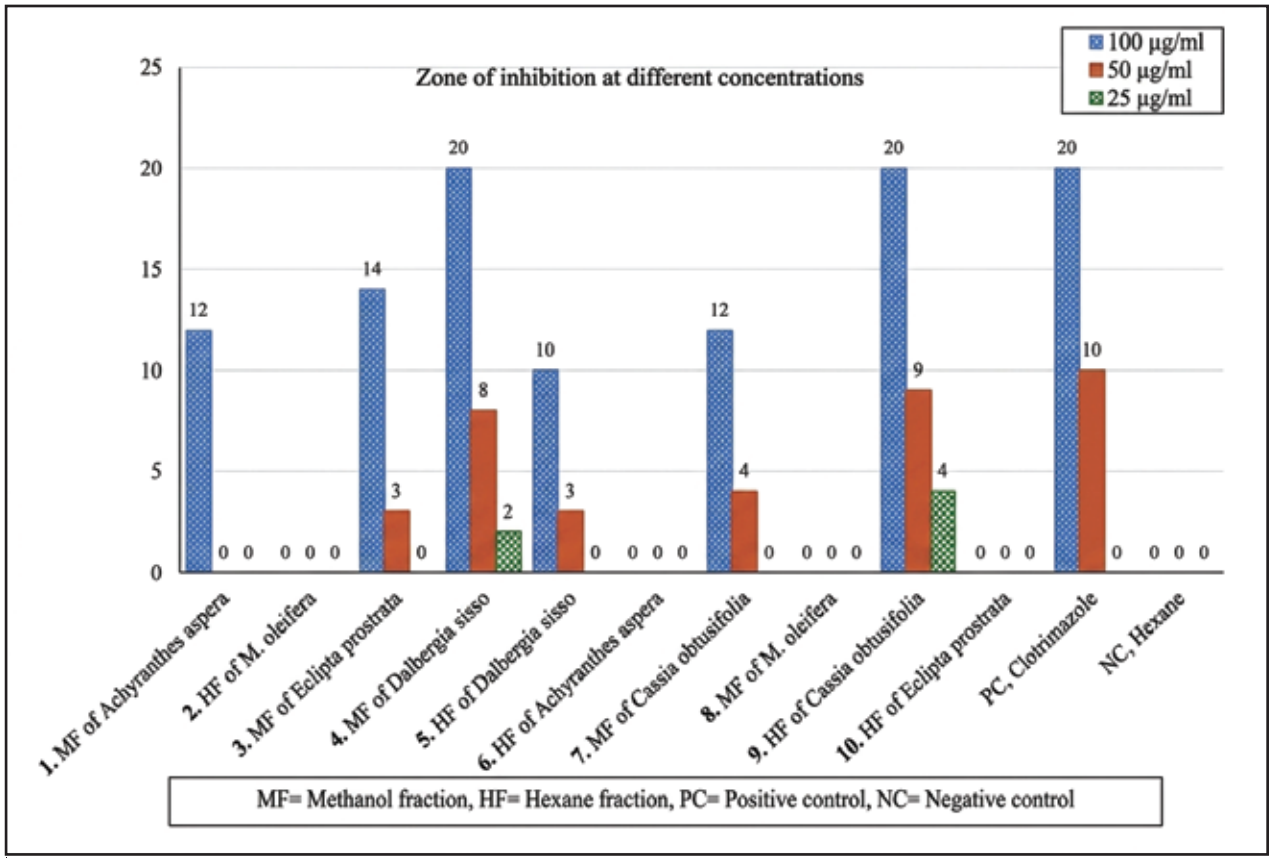


Figure 5: Zone of inhibition at different concentrations for each plant fraction.

Figure 5 shows that methanol extract of *Dalbergia sissoo* (100 µg/ml) and hexane fraction of *Cassia obtusifolia* (100 µg/ml) were found promising antifungal activity (zone of inhibition of each was 20 mm). Also methanol extract of *Dalbergia sissoo* (50 µg/ml) and hexane fraction of *Cassia obtusifolia* (50 µg/ml) were found mild and moderate antifungal activity because their zone of inhibition were 8 mm and 9 mm respectively. Two extracts (hexane fraction of *Eclipta prostrata* and methanol fraction of *M. olifera*) were discarded due to suspected contamination.

Discussion

The present study tested the antifungal activity of crude and their respective fractions from medicinal plants belonging to 5 plant families against dermatophytes. These medicinal plants were chosen based on folklore usage (Table 1) suggestive of antifungal as well as antibacterial activity. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made a significant contribution towards human health. Phytomedicine can be applied for the treated of diseases as is done in case of unani and ayurvedic technique of medicines or it can be the base for the improvement of a drug. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made a significant contribution towards human health.[24] Sequential isolation of alkaloids from plant resource is largely host on the type of solvent used in the isolation process. The present healers apply initially water as the solvent.[25] The increasing social and economic implication caused by pathogenic fungus means there is constantly striving to develop new antibacterial agents. The command for more natural antifungal drug has driven researchers to analyze the inhibitory drugs such as isolates from plants.[26] Various publications have documented the antimicrobial activity of plants extracts.[27,28] Thus, plants extracts are promising natural antifungal agents with pivotal roles in pharmaceutical industries for regulating the pathogenic fungus. From this study it has been observed that the methanolic extract of methanol fraction of *Dalbergia sisso* produced maximum area of inhibition (20 mm) and hexane fraction of *Cassia obtusifolia* produced largest area of inhibition (20 mm) and methanol fraction of *Eclipta prostrata* (14 mm). The MIC is the lowest concentration of the antimicrobial agent that shows no visible growth (the well remains clear).[29]

The crude methanolic extract of methanol fraction (100µg/ml) & hexane fraction (100µg/ml) of *Dalbergia sisso* produced maximum area of inhibition 20 mm and 10 mm respectively. The MIC values were obtained from the methanolic extract of methanol fraction (100 µg/ml) and hexane fraction (100 µg/ml) of *Dalbergia sisso* that produced 20 mm and 10 mm area of inhibition against common dermatophytes. Also the MIC values were obtained from the methanolic extract of methanol fraction (50 µg/ml) and hexane fraction (50 µg/ml) of *Dalbergia sisso* that produced 8 mm (less sensitive) and 3 mm area of inhibition (this is insensitive)30 against common dermatophytes. Another similar study on antifungal potential on *Dalbergia sissoo* revealed that methanolic extract of *Dalbargia sisso* has higher potential than water extract in the same volume. The extract has more potential against *Microsporum canis* and *Epidermophyton floccosum*.[30]

The crude methanolic extract of methanol fraction (100µg/ml) & hexane fraction (100µg/ml) of *Achyranthes aspera* produced zone of inhibition 12 mm and no inhibition respectively. So, the MIC values obtained from the methanolic extract of methanol fraction (100µg/ml) of *Achyranthes aspera* that produced 12 mm. Another similar study of *Achyranthes aspera* showed that methanolic extract of *Achyranthes aspera* produced minimum inhibitory concentration values against *T. rubrum* (LM-09, LM-13) & *Microsporum canis*.[30]

The crude methanolic extract of methanol fraction (100µg/ml) & hexane fraction (100µg/ml) of *Achyranthes aspera* produced zone of inhibition 12 mm and no inhibition respectively. So, the MIC values obtained from the methanolic extract of methanol fraction (100µg/ml) of *Achyranthes aspera* that produced 12 mm (highly sensitive). Another similar study of *Achyranthes aspera* showed that methanolic extract of *Achyranthes aspera* produced minimum inhibitory concentration values against *T. rubrum* (LM-09, LM-13) & *Microsporum canis*.[31]

The crude methanolic extract of Methanol fraction (100µg/ml) & Hexane fraction (100µg/ml) of *Cassia obtusifolia* produced maximum area of inhibition is 12 mm and 20 mm respectively. The MIC values were obtained from the methanolic extract of methanol fraction (100µg/ml) and hexane fraction (100µg/ml) of *Cassia obtusifolia* that produced 12 mm and 20 mm area of inhibition that is highly sensitive. The MIC values were obtained from hexane fraction (50µg/ml) of *Cassia obtusifolia* that produced 9 mm area of zone of inhibition (moderate sensitive). Another study (Jain et al; 2012) revealed that methanol extraction of *Cassia obtusifolia* had antifungal potential against dermatophytes (Trichophyton and Epidermophyton). However, another evidence showed that methanol extraction of *Cassia obtusifolia* was able to minute inhibition of the growth of dermatophytes than petroleum ether extract but it is effective on maximum types of dermatophytes.[32]

The crude methanolic extract of methanol fraction (100µg/ml) of *Eclipta prostrata* produced maximum area of inhibition 14 mm. The MIC values were obtained from the methanolic extract of methanol fraction (100µg/) of *Eclipta prostrata* 14 mm area of inhibition against common dermatophytes. Another study evaluated *Eclipta prostata* for in vitro antifungal activity. Petroleum ether extract of *Eclipta prostrata* produced antifungal potential against dermatophyte species like *T. rubrum* and Microsporum species.[33]

The crude methanolic extract of hexane fraction (100µg/ml) of *Morienga oleifera, Achyranthes aspera* & *Eclipta prostrata* produced no zone of inhibition. There is no MIC values were obtained from the methanolic extract of hexane fraction of *Morienga oleifera, Achyranthes aspera* & *Eclipta prostrata.*

So, it may be possible that the production of new antifungal drug from *Dalbergia sisso*, *C. obtusifolia* which may be recommended for treatment option of common dermatophytes. The findings of this research suggest that the extracts of *Dalbergia sisso, C.obtusifolia* can be a source of natural antifungal agents with novel applications in pharmaceutical companies to control fungal infection causing severe illness in humans.

Conclusion

Further studies are needed for isolation and structure elucidation of compounds of *Dalbergia sisso, C.obtusifolia* that showed antifungal activity by using TLC, PTLC, UV spectrometer, column chromatography, HNMR, CNMR and finally HPLC with mass spectrometry. Physical & Chemical properties of these compounds will be determined. All bioactive compound will be undergone in vivo study among dermatophyte affected patients as Phase 1 clinical trials of new drug development.

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