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Antifungal efficacy of leaf, flower and root of *Aerva lanata* (Linn.) against selected fungal pathogens

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Abstract
Background: The aim of this study is to determine the antifungal activity of different parts like leaf, flower and root extracts of *Aerva lanata* (L.).
Material and Methods: The antifungal activity of different solvents like acetone, aqueous, benzene and ethylacetate extracts of *A. lanata* against *Candida parapsilosis, Aspergillus flavus, Trichosporon asa*hii and *Mucor indicus* was studied by agar well diffusion method.
Results: Benzene extract of leaf of *A. lanata* showed maximum zone of inhibition against *Aspergillus flavus*. The benzene extract of flower showed antifungal activity against *Trichosporon asahii*. The acetone extract of root showed antifungal activity against *Candida parapsilosis, Aspergillus flavus, Trichosporon asahii* and *Mucor indicus.*
Conclusion: The best antifungal activity was observed in all solvent extracts of root of *A. lanata* against selected fungal species such *as Candida parapsilosis, Aspergillus flavus, Trichosporon asahii* and *Mucor indicus*, when compared with leaf and flower extracts. In future, there is in need of study to isolate and purify the active phytocompounds, which possess antifungal activity against above mentioned fungal species and it may be useful in the treatment of fungal diseases.
Key words: *Aerva lanata, antifungal activity, leaf, flower, root.*

Introduction
Ifectious diseases are caused by fungi, bacteria, viruses and parasites. These are the major threat to public health despite tremendous growth in human chemotherapeutic medicine. Their impact is particularly great in developing countries because of the unavailability of medicines and the emergence of widespread drug resistance (1). The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. The essential oils and phytocompounds of plant extracts such as alkaloids, flavonoids, phenolics, tannins and steroids have evoked interest as sources of natural products. The screening of antimicrobial activity of medicinal plants is an alternative remedies for the treatment of many infectious diseases (2).
The majority of the world population depends on traditional medicine for primary health care. Medicinal and aromatic plants which are widely used as medicine constitute a major source of natural organic compounds. Plants have limitless ability to synthesize aromatic substances and most of which are phenols or their oxygen derivatives.

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The phytocompounds have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (3).

Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (4). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions (5). More side effects were observed in patients using allopathic drugs for treating diseases. So the scientists focused their research on to find new antimicrobial agents from medicinal plants. Plant based drugs are cheap and very less side effects. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (6). Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts of different regions of the world (7).

Herbal plants have become increasingly popular and their use is widespread. Clear-cut proof of their efficacy on microorganisms inducing pathogenesis is yet to be explored. Various medicinal plants have been used for years in daily life to treat disease all over the world. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (8). Over 50% of all modern clinical drugs are of natural product origin (9) and natural products play an important role in drug development programs in the pharmaceutical industry (10). It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents (11).

Aerva lanata (L.) belongs to Amaranthaceae family is known as "Chaya" in Hindi and "Bhadram" in Sanskrit and "Palai" in Tamil (12). *A. lanata* has been used as diuretic, antihelmintic, antidiabetic, expectorant and hepatoprotective drugs (13). Antimicrobial and cytotoxicity activities (14), diuretic activity (15) urolithiasis (16) and anti-inflammatory activity (17) of *A. lanata* were reported. It has been reported that canthin-6-one and β-carboline alkaloids were isolated from leaves of *A. lanata* (18). The antidiabetic activity of *A. lanata* was reported (19, 20) and the studies have shown that diabetic patients are susceptible to infections (21). Diabetic patients are at great risk of bacterial and fungal infections and they have an increased susceptibility to develop skin and soft tissue infections. In this study, the antifungal activities of various solvent extracts of leaf, flower, and root of *A. lanata* were screened against selected fungal species such as *Candida parapsilosis, Aspergillus flavus, Trichosporon asahii* and *Mucor indicus.*

Methods and materials

Collection of plant material

The medicinal plant A. lanata was collected from Mayiladuthurai, Nagapattinam District, Tamilnadu, India during the month of November 2013 and authenticated by the Botanist Dr. S. John Britto, Director, Rabinet Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli-620 002, Tamilnadu, India.

Preparation of plant extracts

The root, flower and leaves were separated from the collected plant and cleaned. The separated parts were dried under shade and then ground well into powder. 30 g powder of root, flower and leaves were taken separately in different conical flasks and labeled. 500 ml of solvents like acetone, benzene and ethyl acetate were added in each conical flask separately shaken well and plugged with cotton and then kept at room temperature for 3 days. On the fourth day, the contents were shaken well and filtered through muslin cloth and then filtered again using Whatmann no. 1 filter paper. Then the filtrates were concentrated through hydro-distillation process. The extracts were dried until a constant weight of each was obtained (24). 30 g powder of root, flower and leaves were soaked separately in distilled water for 12 to 16 hours and boiled and then it was filtered through muslin cloth and then Whatmann no. 1 filter paper. The aqueous extracts were concentrated and made the final volume to one-fifth of the original volume (25). The extracts were stored in air tight containers at 4 ºC until the time of use.

Fungal strains

The fungal strains *Candida parapsilosis, Aspergillus flavus, Trichosporon asahii* and *Mucor indicus* were used in this study and the fungal cultures were collected from Microbiology Laboratory, Doctors Diagnostic Centre, Tiruchirappalli, Tamilnadu, India and where the fungal strains were isolated from blood smear and urine cultures of urinary tract infected (UTIs) patients. The fungal cultures were maintained in Rose Bengal Agar slants at 4 ºC. For this study, the fungi were sub-cultured in broth and incubated at 37 ºC for 72 hours before use.

Preparation of medium

The composition of Rose Bengal Agar media is papaic digest of soya bean meal - 5 g; Dextrose - 10 g; Mono potassium phosphate - 1 g; Magnesium sulphate - 0.5 g; Rose Bengal - 0.05 g and Agar - 15 g. 31.55 g of Rose Bengal Agar in 1000 ml of distilled water and boiled to dissolve the medium completely then sterilized by

autoclaving at 15 lbs pressure (121 ºC) for 15 minutes, and then cooled to 45 ºC (pH 7.2±0.2). It is mixed throughly and poured into sterile petri plates.

Determination of antifungal activity by agar well diffusion method

Rose Bengal Agar (RBA) plates were swabbed using sterile cotton swabs with 8 hr old broth culture of the respective fungi such as Candida parapsilosis, Aspergillus flavus, Trichosporon asahii and Mucor indicus. A sterile cork borer was used to make wells. In this study, 0.6 g of dried extracts was dissolved in 2 ml of DMSO and used as sample. In this study the DMSO (inert organic solvent) and amphotericin B (10 µg, antifungal drug) were used as negative control and positive control, respectively. The plant samples were used at different concentrations like 25 µl (7.5 mg), 50 µl (15 mg), 75 µl (22.5 mg) and 100 µl (30 mg). The plant extract was added into each well using sterilized micropipette and allowed the extracts for diffusion at room temperature for 2 hrs. The plates were incubated at 28 ºC for 72 hrs. Diameter of the inhibition zones was measured (26).

Statistical Analysis

All the results were subjected to statistical analysis and the results are expressed as mean ± standard deviation of three replicates.

Results

In this study, the pharmacological evaluation of antifungal activities of four different solvent extracts of leaf, flower and root of *A. lanata* was carried out using agar well diffusion method and the results are represented in Tables 1, 2 and 3. It is an important to investigate scientifically plants that have been used in traditional medicines to determine potential sources of novel antimicrobial compounds.

Antifungal activity of different solvent extracts of leaf of *A. lanata* against selected fungal species such as *Candida parasilosis, Aspergillus flavus, Trichosporon asahii* and *Mucor indicus* was determined. Aqueous and ethyl acetate extracts of leaves showed minimum zone inhibition against Aspergillus flavus, but no antifungal activity against the other three selected microorganisms. Benzene extract of leaf showed maximum activity against *Aspergillus flavus,* at the concentration of 22.5 mg (16±0.35 mm) and 30 mg (18±0.43 mm). Acetone extract of leaves showed less activity against *Trichosporan asahii* when compared with standard drug Amphotericin B (Table1).

Table 2 represented the results of antifungal activities of different solvent extracts of flower of *A. lanata* against selected fungal species. Aqueous and acetone extracts of flower of *A. lanata* showed no zone of inhibition against *Candida parapsilosis, Aspergillus flavus, Trichosporon asahii* and *Mucor indicus.* Benzene and ethylacetate extracts of flower of *A. lanata* exhibit maximum activity against Trichosporon asahii as 18±0.4 mm and 15±0.45 mm, respectively at the concentration of 30 mg. Ethyl acetate extract of flower also exhibits better activity against Mucor indicus (14±0.63 mm).

Table 3 showed that the results of antifungal activity of different solvents extracts of root of *A. lanata*. All solvent extracts of root of *A. lanata* showed maximum inhibition against tested fungal species. The acetone extract showed antifungal activity against *Candida parasilosis* (20±0.48 mm), *Aspergillus flavus* (18±0.55 mm), *Trichosporon asahii* (18±0.58 mm), and *Mucor indicus* (24±0.60 mm). Whereas ethyl acetate extract showed higher degree of inhibition against selected fungal species, when compared to aqueous and benzene extracts of root.

Discussion

Fungal infections are representing a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (27). Human infections, particularly those involving in the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries (28). In recent years, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in humans.

In recent years, the medicinal plants researches have attracted a lot of attentions globally. A large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides and phenolic compounds etc., which have been found in vitro antimicrobial properties (30). Similarly, the antifungal properties of petroleum

ether, chloroform and methanol extracts of Hybanthus enneaspermus were reported against Aspergillus flavus, *Aspergillus fumigatus*, *Candida albicans* and *Candida tropicalis* by well diffusion method (31). Further, the antifungal activity of methanol extract of leaf of Euphorbia hirta against C. albicans was reported (32). The antifungal activity of different solvent extracts of leaf and flower of Withania somnifera was reported (33). The antifungal activity of methanol and water extracts of Aerva lanata showed inhibition zones against selected fungal species (34).

Mathur et al. (35) reported that hydro-alcohol extract of *Valeriana jatamansi, Coleus barbatus, Berberis aristata, Asparagus racemosus, Andrographis paniculata, Achyranthes aspera, Tinospora cordifolia* and *Plantago depressa* showed antifungal activity against Aspergillus niger and Candida albicans. Similarly, Sule et al. (36) evaluated that the antifungal activity of *Senna alata* (L.) and in that study the crude leaf extract exhibited antifungal activity against *Microsporum canis, Trichophyton jirrucosum, Trichophyton mentagrophytes* and *Epidermophyton jlorrcosum.* Abera et al. (37) reported that the antifungal potential of aqueous and ethanol extracts of eight different plant species against Colletotrichum kahawae. Bohra and Purohit (38) reported that the antifungal activities of aqueous extracts of Azadirachta indica against Aspergillus flavus (39). Similarly, Taskeen-Un-Nisa et al. (40) reported that the antimycotic activity of onion (*Allium cepa* L.), garlic (*Allium sativum L*.) and mint (*Mentha arvensis* L.) extracts against some selected pathogenic fungi.

The results of the present study showed that the antifungal activity of leaf, flower and root extracts of *A. lanata*. Similarly, Manohari and Prasanna (41) reported that the antifungal activity of aqueous and ethanolic extract of leaf of *A. lanata* against *Aspergillus niger* and *Trichoderma viride*. The ethyl acetate and methanol extracts of whole plant of A. lanata showed antifungal activities against *Aspergillus fumigatus, Aspergillus niger, Candida albicans, Hensinela californica* and *Rhizopus oligosporum* (42). Amutha Kuppusamy (43) was also screened the antimicrobial activity of methanolic extract of *A. lanata* against Candida tropicalis. Earlier report showed that the ethyl acetate and methanol extracts of *A. lanata* have antimicrobial properties (42). Flavonoids, triterpenes and tannins are present in *A. lanata* and it may be responsible for antimicrobial properties (44). The methanol extract of *A. lanata* showed that the presence of alkaloids, tannins, saponins, flavonoids, carbohydrates, glycosides, phenols, steroids, phlobatannins, cardiac glycosides, proteins and resins (45). Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms (46). Coumarins are produced in carrots in response to fungal infection, which could be attributed to its antimicrobial activity (47). Antimicrobial property of saponins is due to its ability to cause leakage of proteins and certain enzymes from the cell (48). In the present study, the maximum level of antifungal activity was observed in root extracts of *A. lanata* against *Candida parapsilosis, Aspergillus flavus, Trichosporon asahii* and *Mucor indicus*. Similarly, the root extract of *Hypochaeris radicata* showed maximum antimicrobial activity when compared to other parts of plant (49). This may be due to the presence of flavonoids and phenolic compounds in the roots of plant (50). Flavonoids and phenolic compounds may have the capacity to rupture the cytoplasmic membrane of the fungal cells and damage the intracellular compounds (51) or they may interact with lipid bilayer or inhibit the protein and nucleic acid synthesis in fungal cell (52).

Conclusion

In this study, the antifungal activities of different solvent extracts of leaf, flower and root of *A. lanata* were confirmed against selected fungal species such as *Candida parapsilosis, Aspergillus flavus, Trichosporon asahii* and *Mucor indicus.* The root extracts of *A. lanata* were showed maximum activities against selected fungal species. So the root extracts were showed broad spectrum antifungal activity when compared to flower and leaf extracts against *Aspergillus flavus, Trichosporon asahii* and *Mucor indicus*. Antimicrobial properties of herbs are due to the presence of secondary metabolites like alkaloids, flavanoids, tannins, terpenoids, phenols, saponins and steroids. So, the further study is needed to find out the phytocompounds, which are responsible for antifungal activities.

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