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Antifungal Activity of Medicinal Plants Collected from Servarayan Hills, Eastern Ghats, against Dermatophytic Fungi

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A B S T R A C T

Antimicrobial chemotherapy is ever growing research around the globe due to emerging new and drug resistant microorganisms. In the present study, different concentrations ethanolic leaf extracts of six different plant species, *Aristolochia indica* (Aristolochiaceae), *Coleus strobilifer* (Lamiaceae), *Lepidagathis cristata* (Acanthaceae), *Rhinacanthus nasutus* (Acanthaceae), *Spatholobus parviflorus* (Fabaceae), and *Tarenna asiatica* (Rubiacee), collected from various localities in Servarayan hill ranges, Salem district, south India were tested against the dermatophytic fungal pathogens, *Trichophyton mentegrophytes* and *Candida albicans* using disc diffusion technique. Fluconazole (10 µg) was used as standard antibiotic for comparison which showed 2.3 and 2.5 cm zone of inhibition respectively against *Trichophyton mentegrophytes* and *Candida albicans* respectively. At 1000 ppm concentration, the leaf extracts of *Lepidagathis cristata* showed a maximum of 2.1 cm zone of inhibition against both *Trichophyton mentegrophytes* and *Candida albicans* followed by *Coleus strobilifer* and *Tarenna asiatica*. The results reveal that the ethanolic leaf extracts of *Lepidagathis cristata* could be further investigated for the phytochemical component responsible for antifungal activity.

Introduction

The medicinal herbs have long been used as natural antimicrobial agents where the useful parts range from root, bark, stem, leaves, fruits, flowers, tubers, etc. (Khan *et al.*, 2013). In search of various medicinal ailments, researchers are seriously searching for the medicinal plants used by the traditional healers. Several medicinal plants have been screened for their antimicrobial activity across the countries to identify potential medicinal plants with reference to the development of drug resistance among microorganisms (Nascimento *et al.*, 2000; Vigneshwari *et al.*, 2014). Dermatomycosis or ringworm infection by fungi is a serious problem in humans and animals. *Trichophyton mentegrophytes*, a

dermal fungus known to be prevalent above the rate of 26% in Asia which has been frighteningly increased in the last few years (Gnat *et al.,* 2020). Candidiasis, is a fungal skin and membrane associated disease mostly develop by *Candida albicans* infection almost 70-80% of the cases (Aaron, 2020). These fungi are considered as emerging source of skin diseases in humans that developed resistance to drugs (Fattahi *et al.,* 2021; Martinez-Rossi *et al.,* 2018), requiring the development of antifungal drugs urgently.

The Servarayan hills of southern Eastern Ghats, Salem district, South India is known to harbor enormous potential medicinal plants used by local residents for curing several diseases in human beings as well as in cattle (Rekha *et al.,* 2020; Udayan *et al.,* 2006; Usha *et al.,* 2016). Parthiban and co-workers reported that a total of forty eight medicinal plants of included in forty five genera and twenty nine families practiced by the local populace of Yercaud, a part of Servarayan Hills (Parthipan *et al.,* 2011). About 21 different ethno-veterinary medicinal plants of 16 families have been explored to cure various diseases in cattle (Usha *et al.,* 2016). Recently, Rekha *et al.,* (2020) reported forty various ethnomedicinal plant species belonging to thirty eight genera and twenty five families used by Malayali tribes residing at Yercaud hills, Tamil Nadu. Ethanolic extracts of six different plants namely, *Cryptolepis buchanani*, *Gymnema sylvestre*, *Hemidesmus indicus*, *Secamone emetica*, *Leptadenia reticulata* and *Wattakaka volubilis* belonging to the family Asclepiadaceae collected from Servarayan hills have been tested for antimicrobial activity (Nagaraj *et al.,* 2018). The results also revealed that the ethanolic extract of *Leptadenia reticulata* showed moderate antimicrobial activity. However, the knowledge on the antifungal efficacy of several medicinal plants against dermatophytes is very limited. Hence, in the present study, six different medicinal plants that are largely available with known medicinal properties in Servarayan Hills of south India have been selected for the present study. In this study, the leaves of the selected medicinal plants have been subjected to ethanolic extraction and the crude extracts thus obtained were screened for two species of dermatophytic fungi, *Trichophyton mentagrophytes*, and *Candida albicans*.

Materials and Methods

Collection of medicinal plants

Based on the availability and medicinal knowledge, six species of medicinal plants, *Aristolochia indica* L., *Coleus strobilifer* (Roxb.) A.J.Paton, *Lepidagathis cristata* Willd., *Rhinacanthus nasutus* (L.) Kurz, *Spatholobus parviflorus* (DC.) Kuntze and *Tarenna asiatica* (L.) Kuntze ex K.Schum. were selected and the leaves of each plant were collected from different localities in Servarayan Hills, Salem district, Tamil Nadu, India, for the present study (Table 1; Fig. 1). Initially, the identification of plants and their current botanical names were confirmed with eflora of India Google group database (https://sites.google.com/site/efloraofindia/home). Various parts of the plants were packed in plastic covers and transported to the laboratory for further processing. The dust adhering to the outer surface of plant parts were removed and kept for air drying under shade for one week. Then the plant materials were individually powdered and kept in air tight containers until extraction.

Preparation crude extract

The powdered leaf materials were separately subjected to cold extraction using ethanol as solvent according to the method described by Kalpana and Prakash (2015). After 48 h immersion of powdered leaf materials with

intermittent stirring, the extract was filtered through Whatman No. 1 filter paper followed by centrifugation to remove plant powder, if any. The crude extracts thus obtained were condensed by rotary vacuum evaporator and the final greasy crude extracts were collected and stored under refrigeration prior to antifungal studies. A known quantity of each crude extract was made into different concentrations, 62.5, 125, 250, 500, and 1000 ppm using distilled water with few drops of Triton-X100, and finally used for studying antifungal activity.

Antifungal activity of crude extracts

For testing antifungal activity, two different dermatophytic fungi namely, *Trichophyton mentagrophytes*, and *Candida albicans* maintained in PDA slants, originally procured from Institute of Microbial Technology (IMTECH) were activated using PDA broth. The Petri plates of 100 mm diameter with PDA media were swabbed with broth culture of test fungus in separate plates with sterile swab. Filter paper discs impregnated with different concentrations of plant extracts were placed in environmental safety cabinet, in triplicates for each extract. Fluconazole (10 µg) was used as standard antibiotic along with control (without extract, 0 ppm). The plates were then incubated at 37ºC for 3-5 days and the zone of inhibition (ZI) was recorded.

Results and Discussion

Though there have been many new and novel drugs developed against fungal diseases, due to the lack of reasons pertaining to low impact and production, in addition to the development of drug resistance by pathogenic fungi diverted the researchers towards the medicinal plants with active phytochemical ingredients. The utilization of medicinal plants traditionally used in folk medicine certainly forms an alternative remedy for fungal infections, owing to the multifunctional phytochemical active ingredients with less toxic substances, economical, easily reachable and low side effects (Monteiro and Santos, 2019). In the present study, the ethanolic extracts of leaves of six different medicinal plant species collected from Servarayan hills, South India were tested against two species of dermatophytes. The results showed that the highest concentration of ethanolic leaf extracts of medicinal plants, *Aristolochia indica*, *Coleus strobilifer*, *Lepidagathis cristata*, *Rhinacanthus nasutus*, *Spatholobus parviflorus* and *Tarenna asiatica* at 1000 ppm were with 1.1, 1.8, 2.1, 1.2, 1.3 and 1.3 cm zone of inhibition against the dermatophytic fungi, *Trichophyton mentegrophytes* (Fig. 2). Overall, the inhibition of fungal organisms used in the study was dependent on the concentration gradient of the extracts used. Against, *Trichophyton mentegrophytes*, the lowest test concentration of 62.5 ppm showed minimal zone formation (0.9 cm zone) where it was 125 ppm for *Lepidagathis cristata* (0.9 cm), 500 ppm for *Aristolochia indica* (0.9 cm zone) and 1000 ppm for *Rhinacanthus nasutus* (1.2 cm zone). A maximum inhibition zone of 2.1 cm was recorded in the ethanolic leaf extract of *Lapidagathis cristata* against *Candida albicans* and 1000 ppm concentration followed by 1.8 cm for *Tarenna asiatica*>1.5 cm for *Coleus strobilifer*> 1.4 cm for *Aristolochia indica*> 1.1 cm for *Rhinacanthus nasutus*> 1.0 cm for *Spatholobus parviflorus* (Fig. 3).

The medicinal plants are the vital source of cure for several illnesses in humans caused by pathogenic microorganisms. For case in a point, *Amaranthus spinosus* extracts are reported as a good antifungal source against *Candida albicans*, *Saccharomyces cerevisia*, *Aspergillus niger*, *Fusarium oxysporium* and *Aspergillus flavus* (Jadhav and Biradar, 2016).

Table.1 Plants and the parts used for the preparation of extract.

Botanical name	Common name	Family
***Aristolochia indica* L.**	Indian birthwort	Aristolochiaceae
***Coleus strobilifer* (Roxb.) A.J.Paton**	Thick leaved lavender	Lamiaceae
***Lepidagathis cristata* Willd.**	Crested Lepidagathis	Acanthaceae
***Rhinacanthus nasutus* (L.) Kurz,**	Snake jasmine	Acanthaceae
***Spatholobus parviflorus* (DC.) Kuntze**	Palas-climber	Fabaceae
***Tarenna asiatica* (L.) Kuntze ex K.Schum.**	Asiatic Tarenna	Rubiaceae

Fig.1 Plants selected for the present study. (A) *Aristolochia indica*, (B) *Coleus strobilifer*, (C) *Lepidagathis cristata*, (D) *Rhinacanthus nasutus*, (E) *Spatholobus parviflorus* and (F) *Tarenna asiatica*.

Fig.2 Antifungal activities of ethanolic leaf extracts of selected medicinal plants against *Trichophyton metagrophytes*. Std – Standard antibiotic, Fluconazole (10 µg). Values expressed are mean ± standard deviation (error bars).

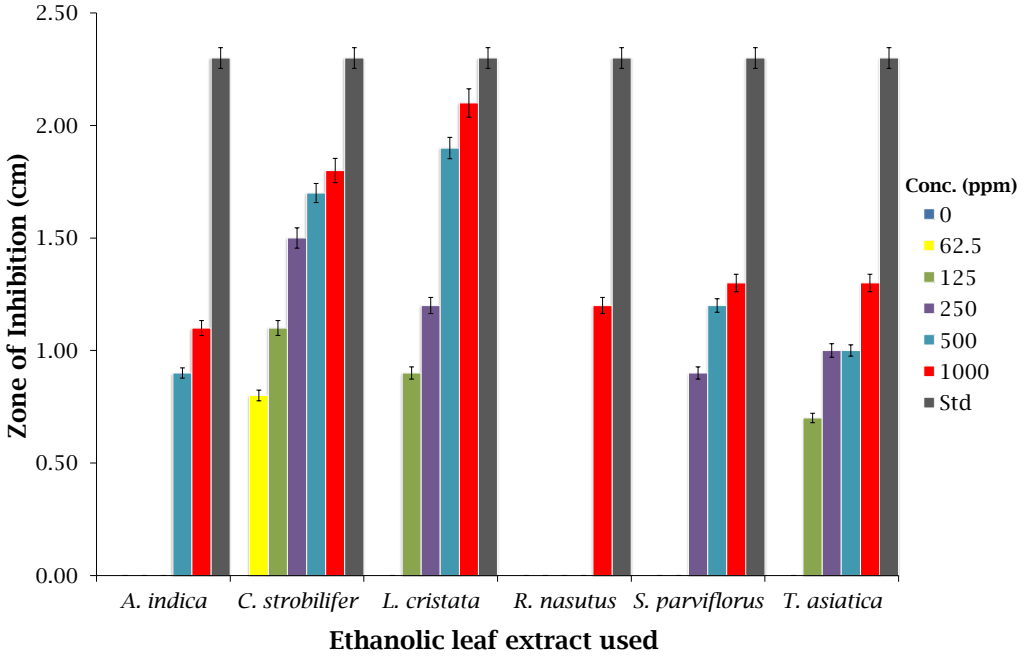
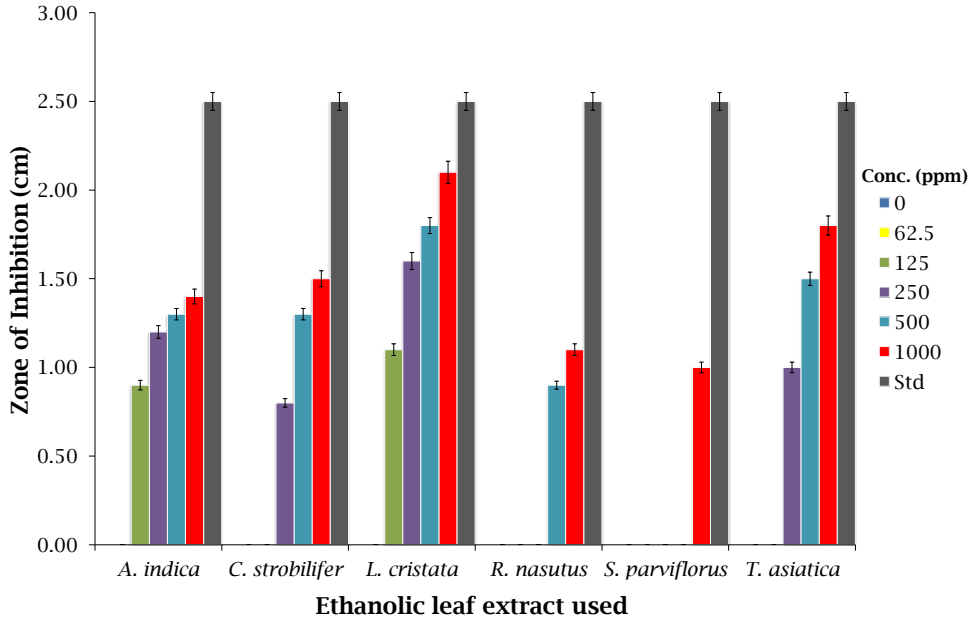


Fig.3 Antifungal activities of ethanolic leaf extracts of selected medicinal plants against *Candida albicans*. Std – Standard antibiotic, Fluconazole (10 µg). Values expressed are mean ± standard deviation (error bars).



The reports reveal that curcumin (200 mg/disc) has retained the highest antifungal activity on *Aspergillus flavus* and *Aspergillus fumigatus* in comparison to synthetic derivatives used as antifungal agents (Gitika *et al.*, 2019). The ethyl acetate extract of *Piper*

nigrum showed excellent antimicrobial activity against dandruff causing fungi (Gitika *et al.,* 2019).

The extract of the medicinal plant, *Coleus* exhibited antibacterial activity against several fungal organisms with a minimal inhibitory concentration range of 0.146-15.63 mg/ml (Tarh and Iroegbu, 2017).

A study conducted on antifungal activity of *Syzygium jambolanum, Cassia siamea, Odina wodier*, *Momordica charantia, Melia azedarach* against a large number *Candida* strains revealed the antifungal activity at 100 mg/ml for *Syzygium jambolanum* and *Cassia siamea* (Prabhakar *et al.,* 2008). The main mechanism of antifungal activity exerted by medicinal plants is due to the presence of a wide range of phytochemical compounds.

The effective plant species reported in the present study requires further investigation on the phytochemical compounds present in the extract to elucidate the exact active principle.

The findings of the current study clearly indicate that the antimicrobial activity of medicinal plants is variable depending on the concentration and the test microorganism.

Considering the antifungal activity of ethanolic leaf extracts tested, *Lepidagathis cristata* showed promising results that could be used for developing antifungal drug after further investigations with the specific phytochemical compound responsible through various advanced studies.

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