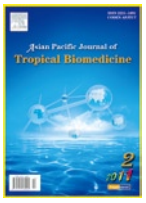
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading

Antifungal activity of traditional medicinal plants from Tamil Nadu, India

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ARTICLE INFO

Article history:
Received 16 August 2011
Received in revised form 5 September 2011
Accepted 29 September 2011
Available online 15 October 2011

Keywords:
Antifungal activity
Medicinal plants
Hexane extract
Ethyl acetate extract
Methanol extract
Minimum inhibitory concentration
Broth microdilution method
Phytochemical
Active principles
Fungi

ABSTRACT

Objective: To assess the antifungal activity of hexane, ethyl acetate and methanol extracts of 45 medicinal plants and to determine the minimum inhibitory concentration for each extract against human pathogenic fungi. **Methods:** A total of 45 medicinal plants were collected from different places of Tamil Nadu and identified. Hexane, ethyl acetate and methanol extracts of 45 medicinal plants were assessed for antifungal susceptibility using broth microdilution method. Two known antifungal agents were used as positive controls. **Results:** Most of the extracts inhibited more than four fungal strains. From the evaluation we found that ethyl acetate extracts inhibited large number of fungal growth. Hexane extracts also nearly showed the same level of inhibition against fungal growth. Methanol extracts showed the minimum antifungal activity. Among the 45 plants tested, broad spectrum antifungal activity was detected in *Albizzia procera* (*A. procera*), *Atalantia monophylla*, *Asclepias curassavica*, *Azima tetracantha*, *Cassia fistula* (*C. fistula*), *Cinnomomum verum*, *Costus speciosus* (*C. speciosus*), *Nymphaea stellata*, *Osbeckia chinensis*, *Piper argyrophyllum*, *Punica granatum*, *Tinospora cordifolia* and *Toddalia asiatica* (*T. asiatica*). Promising antifungal activity was seen in *A. procera*, *C. speciosus*, *C. fistula* and *T. asiatica*. **Conclusions:** It can be concluded that the plant species assayed possess antifungal properties. Further phytochemical research is needed to identify the active principles responsible for the antifungal effects of some of these medicinal plants.

1. Introduction

Traditional medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations[1]. The specific plants to be used and the methods of application for particular ailments were passed down through oral tradition. Plants with possible antimicrobial activity should be tested against some microbes to confirm the activity. The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world[2,3]. As a result, antifungal therapy is playing a greater role in health care and the screening of traditional plants in search of novel antifungals is now more frequently performed[4]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products[5].

Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide[6]. Human infections, particularly those involving the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries[7]. In humans, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in recent years. The treatment of mycoses has lagged behind bacterial chemotherapy and fewer antifungal than antibacterial substances are available. Therefore, a search for new antifungal drugs is extremely necessary[8].

During the past several years, there has been an increasing incidence of fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. This fact coupled with the resistance to antibiotics and with the toxicity

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Foundation Project: This work was financially supported by Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India (grant No. ERI/2008/EP-06).

during prolonged treatment with several antifungal drugs[9] has been the reason for an extended search for newer drugs to treat opportunistic fungal infections[10].

Pathogenic fungi, dermatophytes, have the ability to invade keratinized tissues of animals and humans and cause a disease, dermatophytosis, which is the commonest human contagious fungal disease[11,12]. *Trichophyton rubrum* (*T. rubrum*) is the most prevalent pathogenic fungus worldwide and it represents 80% of clinical isolates[13]. Due to the increasing development of drug resistance in human pathogens as well as the appearance of undesirable effect of certain antimicrobial agents, there is a need to search for new antifungal agent without toxicity and side effect.

Plant extracts or plant-derived compounds are likely to provide a valuable source of new medicinal agents[14,15]. Infectious diseases, particularly skin and mucosal infections, are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits. An important group of these skin pathogens are the fungi, among which dermatophytes and *Candida* spp are prominent[16,17]. Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information[18-25], and a few attempts were made on inhibitory activity against certain pathogenic bacteria and fungi.

In the present study, the antifungal activities of hexane, ethyl acetate and chloroform extracts of 45 medicinal plants were investigated against dermatophytes and opportunistic pathogens.

2. Materials and methods

2.1. Chemicals and media

Dimethyl sulphoxide (DMSO), Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB) were obtained from Himedia, Mumbai, India. The organic solvents *i.e.*, hexane, ethyl acetate and methanol were obtained from Rankem Company, India. The antifungal agent fluconazole was obtained from Himedia, Mumbai.

2.2. Collection of plants and identification

The selection of the species used in this study was mainly based on their ethnomedical evidence (literature) of use for conditions related to microbial infections. These include skin infections, healing of wounds, diarrhoea and fever, *etc*. Some plants without ethnomedical precedents but not previously studied were also included.

The plants were collected from different places of Tamil Nadu and authenticated by a plant taxonomist from the Department of Botany, Loyola College, Chennai. A voucher specimen (ERILC 1-45) is deposited at the herbarium of Entomology Research Institute, Loyola College, Chennai. The following 45 plants were selected for the present study.

Acalypha fruticosa (*A. fruticosa*) Forsk, *Achyranthes bidentata* Blume, *Albizzia procera* (*A. procera*) (Roxb.) Benth, *Aristolochia tagala* (*A. tagala*) Cham, *Asclepias curassavica* (*A. curassavica*) L, *Atalantia monophylla* (*A. monophylla*) Correa, *Azima tetracantha* (*A. tetracantha*) Lam, *Bauhinia tomentosa* (*B. tomentosa*) L, *Biophytum sensitivum* (*B. sensitivum*) DC, *Caesalpinia pulcherrima* (*C. pulcherrima*) Swartz, *Cassia fistula* (*C. fistula*) L, *Cassia alata* (*C. alata*) Linn, *Cassia auriculata* (*C. auriculata*) Linn, *Casearia elliptica* (*C. elliptica*) Willd, *Cinnomomum verum* (*C. verum*) Presl, *Costus speciosus* (*C. speciosus*) Sm, *Couroupita guianensis* (*C. guianensis*) Aublet, *Croton sparsiflorus* (*C. sparsiflorus*) Morong, *Diospyros ebenum* (*D. ebenum*) Koenig, *Dodonaea angustifolia* (*D. angustifolia*), *Elephantopus scaber* (*E. scaber*) L, *Gomphrena celosioides* (*G. celosioides*) Baan, *Hydnocarpus alpina* (*H. alpina*) Wt, *Hyptis suaveolens* (*H. suaveolens*) Poit, *Ichnocarpus frutescens* (*I. frutescens*) (L.) R.Br, *Mundulea sericea* (*M. sericea*) Chevel, *Nymphaea stellata* (*N. stellata*) Willd, *Ocimum basilicum* (*O. basilicum*) Linn, *Osbeckia chinensis* (*O. chinensis*) Linn, *Olax scandens* (*O. scandens*) Roxb, *Ophiorrhiza mungos* (*O. mungos*) Linn, *Peltophorum pterocarpum* (*P. pterocarpum*) (DC.), *Pergularia daemia* (*P. daemia*)R.Br, *Pterolobium hexapetalum* Roth, *Plumbago zeylanica* (*P. zeylanica*) Linn, *Piper brachystachyum* (*P. brachystachyum*) Wall, *Piper argyrophyllum* (*P. argyrophyllum*) Miq, *Punica granatum* (*P. granatum*), *Syzygium cumini* (*S. cumini*) (DC.), *Syzygium lineare* (*S. lineare*) Wall, *Sphaeranthus indicus* (*S. indicus*) L, *Solanum xanthocarpum* (*S. xanthocarpum*) Sch.&wendl, *Tinospora Cordifolia* (*T. cordifolia*) Miers, *Toddalia asiatica* (*T. asiatica*) Lamk and *Zizyphus oenoplia* (*Z. oenoplia*) Mill.

2.3. Preparation of plant extracts

The plants were collected and shade dried at room temperature and ground in a manual mill. The powder was extracted with hexane for a period of 48 h. The extract was filtered through a Buchner funnel with Whatman No.1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator. The remaining residue of the plant material was extracted with ethyl acetate and methanol sequentially in a similar manner. The extracts were stored at 4 ℃ until further use.

2.4. Tested fungi

The following fungi were used for experiments: *Trichophyton rubrum* (*T. rubrum*) MTCC 296, *T. rubrum* 57/01, *Trichophyton mentagrophytes* (*T. mentagrophytes*) 66/01, *Trichophyton simii* (*T. simii*) 110/02, *Epidermophyton floccosum* (*E. floccosum*) 73/01, *Scopulariopsis* sp. 101/01 *Aspergillus niger* (*A. niger*) MTCC 1344, *Botrytis cinerea* (*B. cinerea*), *Curvularia lunata* (*C. lunata*) 46/01, *Magnaporthe grisea* (*M. grisea*) and *Candida albicans* (*C. albicans*) MTCC 227.

2.5. Preparation of fungal spore

The filamentous fungi were grown on SDA slants at 28 ℃ for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on SDB at 28 ℃ for 48 h.

2.6. Antifungal assays

The antifungal activity was performed according to the standard reference method[26]. The extracts were dissolved in 2% DMSO. The initial concentration of extract was 1 mg/mL. The initial test concentration was serially diluted two-fold. Each well was inoculated with 5 μL of suspension containing 104 spore/mL of fungi. The antifungal agent fluconazole was included in the assays as positive control; the plates were incubated for 24 h to 72 h at 27 ℃ for dermatophytes strains. MIC was defined as the lowest extract concentration showing no visible fungal growth after incubation time.

3. Results

The traditional uses of selected medicinal plants are described in Table 1. Results of antifungal activity are summarized in Table 2. Of the 45 plants tested, most of the plants showed varying degree of antifungal activity. Most of the extracts inhibited more than four fungal strains. From the evaluation we found that ethyl acetate extracts inhibited large number of fungi. Hexane extracts also nearly showed the same level of inhibition against fungi. Methanol extracts showed minimum antifungal activity. Some plants were moderately active. We have tested two types of fungal strains such as, *T. mentagrophytes*, *T. rubrum* 296, *T. rubrum* 57, *T. simii*, *E. floccosum*, *C. albicans* and *Scopulariopsis* sp which are dermatophyte and *A. niger*, *B. cinerea*, *M. grisea* and *C. lunata* which are opportunistic pathogen.

The dermatophytes cause skin eruptions which last for long time. The selected plants are widely used to treat skin diseases and diarrhoea. Broad spectrum of antifungal activity was detected among tested plants of *A. procera*, *A. monophylla*, *A. curassavica*, *A. tetracantha*, *C.*

Table 1
Traditional uses, medicinal properties and chemical constituents of selected medicinal plants.

Name of the plants	Local name	Part used	Medicinal uses	Chemicals constituents	References
A. fruticosa L (Euphorbiaceae)	Siru chinni	Leaves, root	Antioxidant, anti-inflammatory, skin diseases, dysentery	Alkaloid, Flavonoids	[27,28]
Achyranthes bidendata (Amaranthaceae)	Sivappu naayuruvi	Leaves, root, stem	Roots are dried and ground into a powder or used in decoctions, asthma, pyorrhea, Anti-HIV activity	–	[29,30]
A. procera (Roxb.) Benth (Leguminosae)	Konda vaghe	Leaves , bark	Leaves are used as insecticides, The leaves are also used to ulcer	–	–
A. tagala Cham. (Aristolochiaceae)	–	Leaves, root	The roots are used to skin diseases	–	[31]
A. curassavica L. (Asclepidaceae)	Pallai chedi	Leaves	The juice of *A. curassavica* is used against roundworm infestation, as an anthelmintic, haemostatic and in gonorrhoea	–	[32]
A. monophylla Correa. (Rutaceae)	Kattuelumicaapazham.	Leaves , bark	Leaves of *A. monophylla* are used to treat rheumatism, paralysis as antidysentric and antipyretic	–	[33,34]
A. tetracantha Lam Salvadoraceae	Changam	Leaves	Cough, asthma, ulcer, Diarrhea	Azimine, azcarpine, carpaine, friedelin	[35]
Bauhinia tomentosa L. (Caesalpiniaceae)	Kanchini, Thiruvatti	Leaves, root, seed	Antidysenteric and anthelmintic, dried leaves, buds and young flowers are prescribed in dysentery	–	–
B. sensitivum DC Oxzalidaceae	Tintaanaalee	Whole plant	Diabetes, asthma	Phenolic compounds	[36]
Caesalpinia pulcherima Swarts. (Caesalpiniaceae)	–	Leaves, bark, root	Diarrhoea and dysentery, rheumatism, skin infections and as anti-ulcerogenic	–	[37,38]
C. elliptica Willd. (Samydaceae)	Kadichai, Kutti	Leaves , root, bark	in traditional medicine against malaria	–	[39]
C. alata L (Caesalpiniaceae)	Seemai agathi	Leaves	Leprosy and liver disease, antihelminthic, antibacterial, laxative, diuretic, for treatment of snakebites and uterine disorders	Kaempferol, naringenin, guercetin	[40,41]
C. auriculata L (Caesalpiniaceae)	Aavaarai	Leaves	Root is used to treat skin diseases, Immunostimulant and Endocrine stimulant, Antiviral and anti spasmodic	Triterpenoid, glycosides	[42]
C. fistula L (Caesalpiniaceae)	Sarakonnai	Flower	Diarrhea and skin disorder, antimicrobial, antidiabetic, antioxidant	Anthraquinone, glycosides, fistulic acid, benzoic acid	[43]

Table 1, continued

C. verum Presl. (Lauraceae)	Lavangampattai	Leaves, bark	Antifungal, antibacterial	Cinnamaldehyde	[44]
C. speciosus Sm. (Zingiferaceae)	–	Rhizome, leaves	burningsensation, constipation, leprosy, worminfection, skindiseases, fever, asthma, bronchitis, inflammation and anaemia, anti-diabetes	Costunolide, eremanthin	[45-47]
C. guianensis Aubl. (Lecythidaceae)	Nagalingam	Leaves, bark	respiratory and other diseases	Gum, and malic, citric and tartaric acids	[48]
Diospyros ebenum Koenig (Ebinaceae)	Tumbi-Karunkaali	Stem bark	Cough, asthma, diabetes	Naphthaldehyde and dispyrin	[49]
D. angustifolia L. (Sabindaceae)	Virali	Leaves, wood	Decoction, purgative, fever and the young twigs are used as tonic, tuberculosis	–	[50]
Elephentopus scaber L (Astreaceae)	Aanai chuvadi	Leaves	Wound-healing, skin-breaking strength	Deoxyelephantopin, Isodeoxy-elephantopin	[51]
Gompherena celosioides Baan. (Amarantaceae)	Sivappu Kerai	Leaves	Antimicrobial	Phenolic compound	[52]
H. alpina Watt (Flacouriaceae)	Neervetti	Leaves	Anticancer	Cyclopantenoid, fatty acids, enoic acids	[53]
H. suaveolens Poit (Lamiaceae)	Perum thulasi	Leaves	Anthelamatic and stomachic, antibacterial, antifungal	Suaveolol and methyl suaveolate	[54]
I. frutescens R. Br. (Apocynaceae)	Illu-katte	Leaves, flowers	Skin diseases, diabetes, hepatoprotective	–	[55,56]
Lippia javanica (Verbinaceae)	–	Leaves	Asthma, bronchitis, cold and fever	Dihydroactinidiolide	[57]
N. stellata Willd. (Nymphaceae)	–	Leaves, rhizome, flowers	Diarrhea, diabetes	Steroid	[41,58]
O. basilicum L (Lamiaceae)	Thirunoothipachai	Leaves	Cough, diuretic and anthelamatic	Methyl havicol, eugenol, limonene	[59]
O. mungos L. (Rubiaceae)	Keerippundu, Keripuranan	Leaves, root	The roots are useful in the treatment of cancer. Decoction of roots, leaves and barks are used as stomachic. The leaves are used for dressing ulcers.	–	[60-62]
P. pterocarpum Backer. (Leguminosae)	Ivalvagai	flowers	The bark of *Peltophorum* spp. is used to treat dysentery and is externally used as lotion for eye troubles, muscular pains, sores and antimicrobial	Bergenin, flavanol glucoside, cyanomaclurin	[63-65]
P. daemia Forsk.) Chiov. (Asclepiadaceae)	Velipparuthi	Leaves	Antimicrobial, anthelmintic, laxative, anti-pyretic, expectorant and used in infantile diarrhoea	Cardenolides, alkaloids, triterpenes and saponins	[66,67]
P. argyrophyllum Miq. (Piperaceae)	–	Flowers, leaves	It is used to treat wound, purgative and anthelmintic	–	–
P. zeylanica	–	Bark	To treat for skin diseases, antifungal, antibacterial	plumbagin	[68]
P. granatum L (Punicaceae)	Maadhulai	Roots	Diarrhea and dysentery, antioxidant, antimalarial, antimicrobial	Alkaloids, pelletierine, isopelletierine	[69]
S. xanthocarpum Schrad-Wendl (Solanaceae)	Kanttankathiri	Leaves	Cough, asthma, fever and chest pain, antimicrobial	Solasonine, solocarpine, solocarpidin	[70]
S. indicus L Astreaceae	Karandai	Seeds	Diabetes, leprosy, fever and cough, antifungal	Cinnamaldehyde, geraniol, geranyl acetate	[71,72]
Syzygium leniare	–	Leaves	Skin diseases, antimicrobial, diabetes	–	[73]
Syzygium jambolanum L Myrtaceae	Naaval	Seeds	Dysentery, diabetes, ringworms, inflammations	Alkaloid, jambosine, glycoside	[74-76]
T. cordifolia Miers. (Rutaceae)	–	Stem	Anti-allergic, anti-diabetic, anti-hepatotoxic, anti-pyretic and anti-inflammatory, anticancer	–	[77]
T. asiatica Lamk Rutaceae	Kaattukari	Leaves	Diarrhea, malaria, fever, Antimicrobial	Alkaloids, skimmiamine, oxyavicine, flindersine	[78-81]
Z. oenoplia	–	Leaves, fruit	sore throat, for dysentery and inflammation of the uterus	–	[82]

fistula, *C. verum*, *C. speciosus*, *N. stellata*, *O. chinensis*, *P. argyrophyllum*, *P. granatum*, *T. cordifolia* and *T. asiatica*. However the remaining plant extracts such as *C. alata*, *C. auriculata*, *C. sparsiflorus*, *D. ebenum*, *E. scaber*, *I. frutescens* and *O. basilicum*, *O. mungos* inhibited a few tested fungi. *C. guianensis*, *D. angustifolia*, *G. celosioides*, *H. alpina*, *M. sericea*, *Pterolobum hexapetalum*, *P. daemia* and *S. xanthocarpum* did not show any activity against the tested fungi.

Hexane, ethyl acetate and methanol extracts of *A. procera* bark inhibited growth of *T. mentagrophytes*, *T. simii*, *T. rubrum* 296, 57 and *E. floccosum*. The MIC values ranged between 1.0 and 0.125 mg/mL. In addition the methanol extract inhibited growth of *C. lunata* at 0.125 mg/mL and *M. grisea* at 0.125 mg/mL. Ethyl acetate extract of *A. tagala* root inhibited the growth of *T. mentagrophytes*, *E. floccosum* at 0.62 mg/mL and also inhibited the growth of *T. simii* and *T. rubrum* (296).

Hexane extract of *A. curassavica* leaves inhibited the growth of *T. mentagrophytes* at 0.250 mg/mL, *T. simii* at 0.250 mg/mL, *T. rubrum* 57 at 0.5mg/mL, *E. floccosum* at 0.125 mg/mL and *M. grisea* 0.5mg/mL. Ethyl acetate extract also inhibited the growth of the above fungi, but methanol extract inhibited only one fungus *E. floccosum* at 0.125 mg/mL.

All the extracts of *A. monophylla* leaves inhibited the growth of *E. floccosum* at 0.125 mg/mL and methanol extract inhibited the growth of *T. mentagrophytes T. simii*, *T. rubrum* 57, *E. floccosum* and *C. lunata*. The MIC values ranged between 1.0 and 0.125 mg/mL.

The hexane extract of *A. tetracantha* leaves inhibited large number of tested fungi and also significant MIC values were observed. For example, *T. rubrum*, 296 (0.62 mg/mL), *T. rubrum* 57 (0.250 mg/mL), *T. mentagrophytes* (1.0 mg/mL), *T. simii* (0.62 mg/mL), *E. floccosum* (0.62 mg/mL), *A. niger* (0.62 mg/mL), *C. lunata* (0.62 mg/mL) and *C. albicans* (1.0 mg/mL) and *M. grisea* (0.250 μg/mL).

Hexane, ethyl acetate and methanol extracts of Bahunia tomentosa inhibited the growth of *T. mentagrophytes* and *E. floccosum* at 0.125 mg/mL and also methanol extract inhibited the growth of *T. simii* at 0.125 mg/mL. Hexane and ethyl acetate extract of *C. alata* (flower) and *C. auriculata* (flower) inhibited the growth of *T. mentagrophytes* and *E. floccosum*. Also ethyl acetate extract of *C. alata* inhibited the growth of *T. simii*, *T. rubrum* 296 and *T. rubrum* 57. Ethyl acetate extract of *C. fistula* flower inhibited the growth of *T. mentagrophytes*, *T. simii*, *T. rubrum* 296, *T. rubrum* 57, and *E. floccosum*. *C. lunata* and *Scopulariopsis* sp. Hexane and methanol extract of *C. fistula* flower showed some activity.

Hexane extract of *Cinnamomum verum* (*C. verum*) inhibited the growth of eight fungi, namely strains of *T. rubrum* 296 (1.0 mg/mL), *T. mentagrophytes* (0.125 mg/mL), *T. simii* (05 mg/mL), *E. floccosum* (0.125 mg/mL), *A. niger* (1.0 mg/mL), *B. cinerea* (1.0 mg/mL), *C. lunata* (1.0 mg/mL) and *M. grisea* (1.0 mg/mL). The ethyl acetate extract also nearly showed the same level of inhibition. Methanol extract did not show any inhibition against tested fungi.

All the solvent extracts of *N. stellata* flower inhibited the growth of *T. mentagrophytes*, *E. floccosum* and *T. simii*. Hexane extract of *O. scandens* inhibited the growth of six fungi, namely, *T. rubrum* 296, *T. rubrum* 57, *T. mentagrophytes*, *T. simii*, *E. floccosum*, *C. lunata* and *M. grisea*.

Hexane extract of *O. chinensis* leaves showed activity against tested fungi such as *T. rubrum*, 296 (1.0 mg/mL), *T. mentagrophytes* (0.62 mg/mL), *T. simii* (0.5 mg/mL), *E. floccosum* (0.62 mg/mL), *A. niger* (1.0 mg/mL), *C. lunata* (1.0 mg/mL) and *B. cinerea* (1.0 mg/mL). Ethyl acetate extract inhibited *T. mentagrophytes* (0.5 mg/mL), *T. simii* (0.5 mg/mL), *E. floccosum* (0.250 mg/mL) and *T.rubrum* 296 (0.5 mg/mL). Methanol extract also inhibited the above fungi moderately.

Hexane and ethyl acetate extracts of *P. argyrophyllum* showed good activity against the tested fungi. The lowest inhibition was observed against *E. floccosum* and *T. simii* at 0.125 mg/mL respectively. Hexane and ethyl acetate extracts of *T. cordifolia* inhibited six fungi namely, *T. mentagrophytes*, *E. floccosum*, *T. simii*, *T. rubrum* 296, *T. rubrum* 57 and *M. grisea*. Methanol extract showed activity against four fungi, namely, *E. floccosum*, *T. rubrum* 296, *T. rubrum* 57 and *M. grisea*.

Ethyl acetate extract of *T. asiatica* leaves showed activity against 8 tested fungi namely, *T. rubrum* 296 (0.62 mg/mL), *T. rubrum* 57 (0.125 mg/mL), *T. mentagrophytes* (0.5 mg/mL), *T. simii* (0.125 mg/mL), *E. floccosum* (0.5 mg/mL), *C. lunata* (1.0 mg/mL) *M. grisea* (1.0mg/mL) and *Scopulariopsis* sp (0.5 mg/mL). However, the remaining plant extracts inhibited growth of one or two fungi only.

4. Discussion

In the present study *T. mentagrophytes*, *E. floccosum*, *T. rubrum* 296 and *T. rubrum* 57/01 were found to be the most sensitive fungal strains. The basis of varying degree of sensitivity of test organisms of fungi may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the crude extract. *T. mentagrophytes*, *T. simii* and *T. rubrum* are pathogenic fungi; the dermatophytes have the ability to invade keratinized tissues of animals and humans and cause a disease. *T. rubrum* is the main agent isolated in superficial mycosis, corresponding to almost 60% of all clinical cases in Brazil[11].

Methanol extract of *A. procera* showed antifungal activity against seven fungal strains and hexane ethyl acetate extracts showed activity against only five fungi. All the extracts inhibited growth of *T. mentagrophytes*, *E. floccosum*, *T. simii*, *T. rubrum* 296 and *T. rubrum* 57. No reports are available for this plant.

Methanol extract of *A. monophylla* leaves inhibited the growth of *T. mentagrophytes*, *E. floccosum*, *T. simii* and *C. lunata*. Ethyl acetate extract showed activity against four

Table 2
Antifungal activity of collected plant extracts against fungi (MIC) (mg/mL).

Sl No.	Plants	Part used	Extracts	*T. m*	*E. f*	*T. s*	*C. l*	*A. n*	*B. c*	*T. r* 296	*M. g* sp	*T. r* 57	*Scro*	*C. a*
1	*A. fruticosa*	Leaf	He	0.125	1.000	–	–	–	–	–	1.000	–	–	–
			Ea	–	–	–	–	–	–	1.000	0.250	–	–	–
			Me	–	–	–	–	–	–	1.000	–	–	–	–
2	*Achyranthes bidendata*	Leaf	He	–	0.500	–	–	–	–	–	1.000	–	–	–
			Ea	–	–	–	–	–	–	–	–	0.500	–	–
			Me	–	0.500	–	–	–	–	–	–	1.000	–	–
3	*A. procera*	Bark	He	0.500	0.125	0.250	–	–	–	0.250	–	1.000	–	–
			Ea	0.500	0.125	1.000	–	–	–	1.000	–	0.125	–	–
			Me	0.125	0.250	0.125	0.125	–	–	0.125	0.125	0.125	–	–
4	*A. tagala*	Root	He	–	0.500	–	–	–	–	–	0.250	–	–	–
			Ea	0.620	0.620	0.500	–	–	–	0.500	–	–	–	–
			Me	–	0.250	–	–	–	–	–	–	–	–	–
5	*A. curassavica*	Leaf	He	0.250	0.125	0.250	–	–	–	–	0.500	0.500	–	–
			Ea	0.250	0.25	0.500	1.000	–	–	–	–	0.500	1.000	–
			Me	–	0.125	–	–	–	–	–	–	–	–	–
6	*A. monophylla*	Leaf	He	–	0.125	–	–	–	–	–	–	1.000	–	–
			Ea	0.500	0.125	1.000	–	–	–	–	–	1.000	–	–
			Me	0.500	0.125	1.000	1.000	–	–	–	–	0.125	–	–
7	*A. tetracantha*	Leaf	He	1.000	0.62	0.62	0.620	0.620	–	0.620	0.250	0.250	–	–
			Ea	–	1.000	–	–	–	1.000	–	–	–	–	–
			Me	–	0.125	–	1.000	–	–	0.500	–	0.250	–	–
8	*Bauhinia tomentosa*	Seed	He	0.125	0.125	–	–	–	–	–	–	1.000	0.125	
			Ea	0.500	0.125	0.500	–	–	–	–	–	–	–	–
			Me	0.125	0.125	0.125	–	–	–	–	–	–	–	–
9	*B. sensitivum*	Leaf	He	–	–	–	–	–	–	–	0.125	–	–	–
			Ea	1.000	–	–	–	–	–	–	1.000	–	–	–
			Me	–	–	–	–	–	–	1.000	0.500	–	–	–
10	*Caesalpinia pulcherrima*	Flower	He	1.000	0.250	–	–	–	–	–	–	0.125	–	–
			Ea	–	0.500	–	–	–	–	–	–	0.250	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
11	*C. elliptica*	bark	He	–	0.125	–	–	–	–	0.500	0.500	0.250	–	–
			Ea	1.000	0.500	–	–	–	–	0.500	1.000	1.000	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
12	*C. alata*	Flower	He	0.500	0.250	–	–	–	–	1.000	–	–	–	–
			Ea	1.000	0.500	1.000				0.500	–	0.500	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
13	*C. auriculata*	Flower	He	1.000	0.125	–	–	–	–	–	–	0.500	–	–
			Ea	0.500	1.000	–	–	–	–	–	–	1.000	–	–
			Me	1.000	–	–	–	–	–	–	–	1.000	–	–
14	*C. fistula*	Flower	He	0.250	0.500	–	–	–	–	1.000	–	–	–	–
			Ea	0.250	0.500	1.000	1.000	–	–	1.000	–	0.500	0.500	–
			Me	–	1.000	–	–	–	–	–	–	1.000	–	–
15	*C. verum*	Leaf	He	0.125	0.125	0.500	1.000	1.000	1.000	1.000	1.000	–	–	–
			Ea	0.250	0.250	0.500	1.000	1.000	1.000	1.000	1.000	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
16	*C. speciosus*	Tuber	He	0.620	0.620	0.620	0.500	1.000	1.000	0.620	0.125	0.125	1.000	–
			Ea	0.625	0.620	0.620	0.250		0.500	0.125	0.125	0.125	–	–
			Me	–	0.125	–	–	–	–	0.500	–	0.125	0.500	–
17	*C. guianensis*	leaf	He	–	–	–	–	–	–	–	–	–	–	–
			Ea	–	–	–	–	–	–	–	–	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
18	*Crotons sparsiflorus*	Leaf	He	0.500	1.000	–	–	–	–	–	–	0.250	–	–
			Ea	1.000	0.500	1.000	–	–	–	0.500	–	0.500	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
19	*Diospyros ebenum*	Bark	He	–	–	–	–	–	–	1.000	1.000	–	–	–
			Ea	–	–	–	–	–	–	1.000	1.000	–	–	–
			Me	–	–	–	–	–	–	0.250	0.125	–	–	–

Table 2, continued

20	*D. angustifolia*	Leaf	He	–	–	–	–	–	–	–	–	–	–	–
			Ea	–	–	–	–	–	–	–	–	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
21	*E. scaber*	Root	He	–	–	–	–	–	–	–	–	–	–	–
			Ea	0.500	1.000	0.500	–	–	–	–	–	–	–	–
			Me	0.125	0.125	0.500	–	–	–	–	–	–	–	–
22	*G. celosioides*	Leaf	He	–	–	1.000	–	0.500	–	1.000	–	–	–	–
			Ea	–	–	–	–	–	–	–	–	–	–	–
			Me	–	–	1.000	–	–	–	–	–	1.000	–	–
23	*H. suaveolens*	Leaf	He	1.000	0.125	0.500	–	–	–	1.000	0.500	0.250	–	–
			Ea	0.500	0.250	1.000	–	–	–	–	0.500	–	–	–
			Me	–	–	–	–	–	–	–	–	0.500	–	–
24	*H. alpina*	Leaf	He	–	–	–	–	–	–	–	–	–	–	–
			Ea	–	–	–	–	–	–	–	–	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
25	*I. frutescens*	Leaf	He	–	–	–	–	–	–	–	–	–	–	–
			Ea	0.500	0.125	–	–		0.125	–	–	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
26	*M. sericea*		He	–	–	–	–	–	–	–	–	–	–	–
			Ea	–	–	–	–	–	–	–	–	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
27	*N. stellata*	Flower	He	0.500	0.125	0.500	–	–	–	–	–	–	–	–
			Ea	0.500	0.125	0.500	1.0	–	–	–	–	–	1.000	–
			Me	0.125	0.125	0.125	–	–	–	–	–	–	–	–
28	*O. basilicum*	Leaf	He	–	0.250	–	–	–	–	0.500	–	–	–	–
			Ea	–	0.500	–	–	–	–	1.000	–	–	–	–
			Me	0.500	0.250	1.000	–	–	–	0.250	0.500	–	–	–
29	*O. scandens*	Leaf	He	0.250	0.500	0.500	1.0			0.500	0.500	0.500		
			Ea	1.000	1.000	–	–	–	–	–	–	0.500	–	–
			Me	1.000	0.250	–	–	–	–	–	–	0.250	–	–
30	*O. mungos*	Leaf	He	–	–	–	–	–	–	0.500	–	0.500	–	–
			Ea	–	0.500	0.500	1.0	–	–	–	0.500	0.250	–	–
			Me	–	0.500	–	–	–	–	–	–	0.500	–	–
31	*O. chinensis*	Leaf	He	0.620	0.620	0.500	1.0	1.000	1.000	1.000	1.000	–	–	–
			Ea	0.500	0.250	0.500	–	–	–	0.500	–	–	–	–
			Me	1.000	1.000	0.500	–	–	–	1.000	–	–	–	–
32	*P. pterocarpum*	flower	He	1.000	1.000	–	–	–	–	0.500	1.000	1.000	–	–
			Ea	1.000	1.000	–	–	–	–	–	–	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
33	*P. argyrophyllum*	Leaf	He	0.250	0.125	0.125	0.5		1.000	0.500	0.500	–	–	–
			Ea	0.250	0.125	0.125	0.5	1.000	0.500	0.500	0.500	1.000	–	–
			Me	0.500	1.000	0.500		–	–	0.500	1.000	–	–	–
34	*P. brachystachyum*	Stem	He	1.000	0.250	–	–	–	–	0.500	–	0.125	–	–
			Ea	1.000	0.250	–	–	–	–	0.500	–	0.125	–	–
			Me	–	–	1.000	–	–	–	–	–	1.000	–	–
35	*Plumbago. zeylanica*	Bark	He	0.500	0.500	–	–	–	–	–	–	–	–	–
			Ea	1.000	0.500	–	–	–	–	0.500	1.000	–	–	–
			Me	0.620	0.620	0.250				1.000	1.000	1.000	1.000	
36	*Pterocarpum hexapetalum*	Leaf	He	–	–	–	–	–	–	–	–	–	–	–
			Ea	–	1.000	–	–	–	–	1.000	–	1.000	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
37	*P. granatum*	Root	He	0.500	0.125	–	–	–	–	0.500	0.250	0.125	–	–
			Ea	–	0.500	1.000	–	–	–	0.620	1.000	0.620	–	–
			Me	0.500	0.620	–	–	–	–	–	–	0.500	–	–
38	*P. daemia*	Leaf	He	–	–	–	–	–	–	–	–	–	–	–
			Ea	1.000	0.500	–	–	–	–	–	–	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–

Table 2, continued

39	*S. xanthocarpum*	Leaf	He	–	–	–	–	–	–	–	–	–	–	–
			Ea	–	–	–	–	–	–	–	–	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
40	*S. indicus*	Flower	He	–	–	–	–	–	–	–	–	–	–	–
			Ea	–	–	–	–	–	–	–	–	–	–	–
			Me	–	–	–	–	–	–	–	–	–	–	–
41	*S. cumini*		He	0.250	0.250	1.000	–	–	–	1.000	1.000	–	–	–
			Ea	0.250	0.250	1.000	–	–	–	–	–	0.500	–	–
			Me	1.000	–	–	–	–	–	–	–	1.000	–	–
42	*S. lineare*	Leaf	He	–	–	–	–	–	–	–	–	1.000	1.000	–
			Ea	–	–	–	–	–	–	–	0.250	0.500	–	–
			Me	–	–	–	–	–	–	–	0.500	1.000	–	–
43	*T. cordifolia*	Stem	He	0.250	1.000	0.250	–	–	–	0.125	0.125	0.125	–	–
			Ea	0.500	0.125	0.500	–	–	–	0.250	0.500	0.125	–	–
			Me		0.125	–	–	–	–	0.125	0.125	0.125	–	–
44	*T. asiatica*		He	–	0.620	–	–	–	–	0.125	–	0.62	0.125	–
			Ea	0.500	0.500	0.125	1.000	–	–	0.620	1.000	0.125	0.500	–
			Me	–	–	–	–	–	–	1.000	–	1.000	–	–
45	*Z. oenoplia*	Leaf	He	–	–	–	–	–	–	–	1.000	0.125	–	–
			Ea	–	1.000	–	1.000	–	–	–	–	1.000	–	–
			Me	–	0.500	–	–	–	–	1.000	–	–	–	–

He – Hexane; Ea – Ethyl acetate; Me – Methanol.
T. r – *Trichophyton rubrum*; *T. m* – *T. mentagrophytes*; *T. s* – *T. simii*; *E. f* – *E. floccosum*; *Scro* – *Scopulariopsis* sp.; *A. n* – *A. niger*; *B. c* – *Botrytis cinerea*; *C. l* – *C. lunata*; *M. g* – *M. grisea*; *C. a* – *C. albicans* MTCC 227.

fungi. Hexane extract did not show much activity. All the extracts inhibited the growth of *T. mentagrophytes* at 0.125 mg/mL. The volatile oil from the leaves possessed inhibitory activity against the fungi *Aspergillus oryzae*, *A. nidulans*, *A. fumigatus*, *Penicillium aculeatum* and *Phomopsis destructum*[55]. Sharma *et al*[83] reported that the essential oil from the leaves of *A. monophylla* showed 100 percent inhibition of the mycelial growth of some of the saprophytic plant and human pathogenic fungi.

Ethyl acetate extract of *A. curassavica* leaves inhibited *T. mentagrophytes*, *T. simii*, *T. rubrum* 57, *C. lunata*, *E. floccosum* and *Scopulariopsis* sp. Hexane extract also showed some activity against the above fungi. Hexane and methanol extracts of leaf and stem of *A. curassavica* of inhibited the growth of *T. mentagrophytes* at 2 mg/mL[84]. Studies on latex of *Asclepia curassavica* have also identified terpenes and cardenolides, which are presumed to be responsible for the growth inhibition of *C. albicans*[85].

Hexane extract of *A. tetracantha* leaves inhibited large number of tested fungi. The lowest MIC was observed against *E. floccosum*, *T. simii*, *C. lunata* and *T. rubrum* 296 at 0.62 mg/mL concentration. Ethyl acetate and methanol extracts did not inhibit many fungi. *A. tetracantha* methanol extract of fruit exhibited broad spectrum antibacterial, antifungal activity against tested pathogen[86].

C. fistula is ornamental plant; it has been widely used to treat various ailments including ringworm and other fungal skin diseases[87]. We have found good antibacterial, antifungal activity in *C. fistula* flower[43]. Significant antibacterial activity has been reported that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents[88]. The methanol extract of leaves of *C. fistula* showed 100% antifungal activity at 10 mg/mL concentration against *Trichophyton rubrum*, *Microsporum gypseum* and *Penicillium marneffei*[89].

C. alata has been used traditionally to treat fungal infections in South East Asia[90]. Ibrahim and Osman[91] reported that ethanolic extract of *C. alata* leaves showed antifungal activity at 500 mg/mL concentration against fungi *Trichophyton* sp, *Microsporum* sp, *Aspergillus* sp and *Penicillium* sp, but not yeasts (*C. albicans* and *Cryptococcus neoformans*). We found significant antifungal activity in flower than the leaves. Khan *et al*[92] reported that methanol extracts of *C. alata* leaves, flowers, barks and roots at 4 mg/mL concentration inhibited many types of bacteria including *E. coli* and *S. aureus*, but not molds (*C. albicans*, *A. niger* and *Trichophyton mentagrophytes*).

C. verum exhibited antifungal activity against maximum number of fungi; hexane and ethyl acetate extract only showed activity. Methanol extract did not show activity against tested fungi. Several biological activities such as peripheral vasodilatory, antitumor, antifungal, antioxidant, cytotoxic, antiviral and antimutagenic activities have been attributed to cinnamaldehyde[93-96]. With its antifungal and antibacterial actions, *C. verum* helps to control the virulent actions of many microorganisms including the one which causes Botulism and *S. aureus*. It acts strongly against the fungi that produce aflatoxin, a potent poison and carcinogen[44]. Cinnamic aldehyde has been identified as an active fungitoxic constituent of cinnamon (Cinnamomum zeylanicum) bark oil. The fungitoxic properties of the vapours of the oil/active constituent against fungi involved in respiratory tract mycoses, *i.e.*, *A. niger*, *A. fumigatus*, *A. nidulans*, *A. flavus*, *C. albicans*, *C. tropicalis*, *C.*

pseudotropicalis, and Histoplasma capsulatum, were determined in vitro as minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC)[97].

Hexane and ethyl acetate extracts of *C. speciosus* tuber showed significant antifungal activity. The lowest MIC was observed against *T. mentagrophytes*, *E. floccosum* and *T. simii* (0.62 mg/mL). The methanol extract did not show any activity. No reports are available for this plant.

Hexane, ethyl acetate and methanol extracts of *N. stellata* flower inhibited growth of three dermatophytes species such as *T. mentagrophytes*, *T. simii* and *E. floccosum*. There are no reports on antimicrobial activity for this species.

Hexane extract of *O. chinensis* leaf showed some activity against tested fungi. The lowest MIC values were seen against *T. mentagrophytes* and *E. floccosum* at 0.62 mg/mL. All the extracts inhibited growth of *T. mentagrophytes*, *E. floccosum* and *T. simii*. There are no reports on antifungal activities for this plant. It is generally accepted that the medicinal use of *Osbeckia* plant extract increases the recovery rate of the damaged liver[98].

Hexane and ethyl acetate extracts of *P. argyrophylum* showed activity against more than seven fungi. Methanol extract showed activity against only five fungi. Twenty-three compounds, a novel neolignan, nine known neolignans and 13 known alkaloids, were isolated from methanol extract of stems of *P. argyrophyllum*[99].

Methanol extract of *P. zeylanica* bark inhibited the growth of *T. mentagrophytes*, *T. simii*, *T. rubrum* (57 & 296), *E. floccosum*, *M. grisea* and *Scopulariopsis* sp. Hexane and chloroform extracts of *P. zeylanica* contained naphthoquinone derivatives including plumbagin[100]. Alcoholic crude extract of *P. zeylanica* has been shown to possess greater antimicrobial activity than aqueous or hexane extracts[22].

Hexane and ethyl acetate extract of *P. granatum* root inhibited the growth of dermatophytes *E. floccosum* and *T. rubrum* 296 & 57. The lowest minimum inhibitory concentration was observed against *T. rubrum* 296 & 57 at 0.62 mg/mL. Prashanth *et al*[101] reported that different extracts of *P. granatum* fruit rind showed activity against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *B. subtilis* and *S. typhi*. Fruit extracts of *P. granatum* exhibited antimicrobial activity[102].

Hexane and ethyl acetate extracts of *T. cordifolia* stem inhibited growth of six fungi. Methanol extract inhibited only four fungi. This could be due to the insufficient amount of active compound present in the extract. All the extracts inhibited the growth of *T. rubrum* 296 & 57 and *M. grisea* at 0.125 mg/mL. *T. cordifolia* has been shown to possess anti-allergic, anti-diabetic, anti-hepatotoxic, anti-pyretic and anti-inflammatory properties[77]. This activity can be attributed to tinocordifolin[103]; sesquiterpene glucoside, tinocordifolioside[104]; cordifoliside D and cordifoliside[105]; tinosponone and tinocordioside, clerodane[106]; cordioside[107].

Ethyl acetate extract of *T. asiatica* showed significant antifungal activity against most of the tested fungi. The lowest MIC was observed against *T. rubrum*, 296 (0.62 mg/mL). Hexane and methanol extract did not inhibit many tested fungi. Antibacterial and antifungal activity of essential oil from this plant has been reported[108-110].

Garcia *et al*[84] used similar type of fungal pathogen for antifungal activity of eighteen plant extracts from nine traditional Mexican medicinal plants to test against two dermatophytes fungal species (*T. mentagrophytes* and *T. rubrum*), one non-dermatophyte (*A. niger*), and one yeast (*C. albicans*). The strongest effect was manifested by the hexane extracts from *Eupatorium aschenbornianum* and *Sedum oxypetalum*, as well as the methanol extracts from *Lysiloma acapulcensis* and *Annona cherimolia*. Similarly, Jeevan Ram *et al*[111] reported ethnopharmacological and antimicrobial properties of certain medicinal plants used by Adivasi tribes of the Eastern Ghats of Andhra Pradesh, and ethanol extracts of 23 crude extracts used for various skin diseases were assayed for antimicrobial activity against four bacterial and one fungal pathogen.

We found most of the methanol extracts did not show significant antifungal activity; similarly Portillo *et al*[7] reported the antifungal activity of aqueous, dichloromethane and methanol extracts from 14 Paraguayan plants used in traditional medicine for the treatment of skin diseases assayed *in vitro* by the agar disc diffusion method against 11 fungal strains comprising several filamentous fungi and yeasts. Among them, the dichloromethane extracts of *Acanthospermum australe*, *Calycophyllum multiflorum*, *Geophila repens* and *Tabebuia aellanedae*, as well as the aqueous and methanol extracts of the latter showed good activity.

Muschietti *et al*[112] assayed methanol extracts from 11 traditionally used Argentine medicinal plants *In vitro* for antifungal activity against yeasts, hialohyphomycetes as well as dermatophytes with the micro broth dilution method. The strongest effect was presented by *Eupatorium buniifolium* and *Terminalia triora*, *T. mentagrophytes* and *T. rubrum* being the most susceptible species with MICs ranging from 100 to 250 μg/mL.

Results of the present work indicate that the plant species assayed possess antifungal properties. This explains the use of these plants in folk medicine for the treatment of various diseases whose symptoms might involve fungal infections and underline the importance of the ethnobotanical approach for the selection of plants in the discovery of new bioactive compounds. We found maximum antifungal activity in ethyl acetate extract of *C. fistula* (flower), *T. asiatica* (leaves) and hexane extract of *A. tetracantha* (leaves). Further phytochemical research is needed to identify the active principles responsible for the antifungal effects of some of these medicinal plants.

Acknowledgements

We thank Dr. S Ignacimuthu, Director, Entomology

Research Institute, Loyola College, and Chennai for the collection of the plant and financial support.

Conflict of interest statement

We declare that we have no conflict of interest.

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