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ORIGINAL ARTICLE

Evaluation of antifungal potential of selected medicinal plants against human pathogenic fungi

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Context: Evaluation of medicinal plants used in traditional medicine lead to novel bioactive compounds with antifungal activity that could be exploited as therapeutic agents. **Aims:** The aim was to screen selected medicinal plants for antifungal activity against three important human pathogenic fungi and to identify the broad group of phytochemicals responsible for the activity. **Materials and Methods:** A total of 8 medicinal plants were screened for antifungal activity against three human pathogenic fungi. Aqueous and the solvent extracts of the plant materials were prepared by polarity based solvent extraction. Antifungal activity was tested by well and disc diffusion methods. Minimum inhibitory concentration (MIC) of the active extract was determined by micro-broth dilution technique. Phytochemical analysis of the active extract was done. **Statistical Analysis Used:** The results were statistically analysed by One-Way analysis of variance with *Post-hoc* Tukey's *B* test at $P < 0.05$ using the Software SPSS version 20 (IBM Corp. Armonk, NY Released 2011). **Results:** Significant antifungal activity was observed in the aqueous extracts of the fruits of *Terminalia chebula* (47.75 mm) against *Microsporum gypseum* and the mesocarp of *Persea americana* (40.5 mm) against *Microsporum canis*. *Candida albicans* was inhibited by the ethyl acetate (20 mm) and aqueous extracts (16 mm) of *T. chebula* fruits and aqueous extract of the seeds of *Syzygium jambos* (16 mm). The aqueous extract of mesocarp of *P. americana* showed lowest MIC value (312.5 µg/ml) against *M. canis* and *M. gypseum*. Phytochemical analysis of the active extracts revealed the presence of phenols, tannins, alkaloids and flavonoids. **Conclusions:** The study validates the use of the plants in the treatment of fungal infections and has provided important leads for the discovery of new plant-based antifungal agents.

Key words: Antifungal activity, dermatophytes, *Persea americana*, phytochemical analysis, syzygium jambos, *Terminalia chebula*

INTRODUCTION

Medicinal plants are a rich source of novel biologically active compounds with antimicrobial activity. They are believed to be an important source of new chemical substances with potential therapeutic effects. Medicinal herbs are used by 80% of the world population living in rural areas for their primary health-care.[1] Plant derived natural products are generally bioactive secondary metabolites with better potential to treat different diseases. Some examples of these compounds include terpenoids, alkaloids, phenols, unsaturated lactones, phenolic glycosides, saponins, sulphur compounds, cyanogenic glycosides and glucosinolates.[2] Biological and chemical screenings are complementary approaches for the detection and isolation of interesting new plant constituents.[3]

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Fungal diseases represent a critical problem to the health, and they are one of the main causes of morbidity and mortality worldwide. Human infections particularly those involving the skin and mucosal surfaces constitute a serious problem especially in tropical and subtropical developing countries.[4] The prominent groups of fungal pathogens of the skin are species of *Candida* and dermatophytes. Fungal infections are usually associated with a compromised host immune system. *Candida albicans* and related species can become pathogenic causing oral, vaginal and/or systemic candidiasis.[5] Around 75% of adult women have at least one episode of vulvo-vaginal candidiasis during their life, with the prevalence of *C. albicans* in 70–90% of the cases.[6] Dermatophytes have the ability to invade keratinised tissues of animals and humans and cause a disease called dermatophytosis, which is the most common human contagious fungal disease.[7] In the past decade, the incidence of dermatophytosis has risen dramatically. The humid weather, overcrowding and poor hygienic conditions are ideally suited for the growth of dermatophytes and these factors are more important in a tropical country like India.[8]

Due to the emergence of drug-resistant pathogens and the ineffectiveness of chemically derived anti-fungal agents in combating this emergency there is a need

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to discover new antifungal compounds derived from medicinal plants with diverse chemical structure and novel mechanism of action.[9] The first step in this direction is to evaluate the antifungal activity of medicinal plants indicated in the literature for the cure of fungal infections and to chemically screen the active extract to identify the secondary metabolites present in them.

In the present study, eight Indian medicinal plants were investigated for antifungal activity. Minimal inhibitory concentration assay and preliminary phytochemical analysis of the active extract were carried out to identify the class/group of compounds responsible for the antifungal activity.

MATERIALS AND METHODS

Test Plants

Healthy plants were selected, and the plant parts were collected from different areas of Mysore, extracted and further subjected to antifungal activity assay. The list of test plants, parts used, place of collection and medicinal uses are presented in Table 1. The voucher specimens of the test plants were deposited at the herbarium of the Department of Studies in Botany, University of Mysore.

Preparation of Plant Extracts

Aqueous extract

The fresh plant materials were washed thoroughly under tap water and then cut into small pieces and crushed in a mixer-grinder with distilled water in the ratio of 1:2. The macerate was filtered through a double layered muslin cloth and centrifuged at 8000 rpm for 15 min. The supernatant was sterilised by autoclaving at 120°C, 15 lbs pressure and stored at 4°C until further use.[18]

Solvent extract

The plant material was successively extracted with solvents of increasing order of polarity, starting with non-polar solvents like Petroleum ether and then moving up to polar solvents like methanol. Shade dried plant materials were crushed into a coarse powder in a mixer-grinder. About 35 g powder of each plant material was taken in a 500 ml conical flask. The solvent was added to it in the ratio 1:3 or 1:5 w/v. It was allowed to stand for 24 h and then filtered through Whatmann no. 1 filter paper. The filtrate was then dried to get the concentrated extract. It was stored at 4°C until further use.[19]

Test Fungi

Candia albicans (yeast) Microbial Type Culture Collection Centre (MTCC183), *Microsporum canis* (MTCC2820) and *Microsporum gypseum* (MTCC2830) (dermatophytes) were selected as test organisms for the present study. The test fungi were procured from Microbial Type Culture Collection Centre, Institute of Microbial Technology Chandigarh. The organisms were maintained on Sabourad's dextrose agar [Table 2].

Antifungal Activity Assay

The antifungal activity assay of aqueous extracts was done by agar-well diffusion method[20], and that of the solvent extracts was done by Disc diffusion method.[21] 24 h old culture of *C. albicans* and 10-day-old cultures of dermatophytes grown in Sabourad's dextrose broth (SDB) were taken as inoculum for the assays. Miconazole and nystatin were employed as a positive control. The concentration of the solvent extracts and the positive control was 10 mg/ml, and 50 µl was added to each disc. 100 µl of the aqueous extracts were added to the agar wells. Negative control was maintained by taking the respective solvents used for preparation of the extracts. Four replicates were maintained.

Determination of Minimal Inhibitory Concentration

The extracts possessing antifungal activity were chosen for determination of MIC. The MIC was determined by micro broth dilution technique, using 96 well plates.[20] 1-day-old culture of *C. albicans* and 10 days old culture of filamentous fungi were used for preparing the inoculum.

Table 1: Test plants, parts used, place of collection and medicinal uses

Plant name	Family	Part used	Place of collection	Medicinal uses
T. chebula Retz.	Combretaceae	Fruit	Retail shop, Devaraja Market, Mysore	Skin disorders like allergies, urticaria[10]
T. arjuna (Roxb. ex DC.) Wight and Arn	Combretaceae	Peel bark Bark, leaves	CSRTI* Garden, Mysore	Antiviral, anticancer, antimicrobial property[11]
T. tomentosa Wight and Arn	Combretaceae	Bark	CSRTI Garden, Mysore	Ulcers, wounds and burning sensation[12]
T. catappa L.	Combretaceae	Bark and leaves	Manasagangotri, Mysore	Anticancer and anti-HIV properties[13]
P. americana Mill.	Lauraceae	Fruit	Horticulture Market, Mysore	Skin infections, wound healing, inflammation and fungal infections[14]
P. longifolia (Sonn.) Thwaites.	Annonaceae	Bark and leaves	Manasagangotri, Mysore	Leucorrhoea, uterus ailments and mouth ulcers[15]
P. corylifolia Linn.	Fabaceae	Seed	Retail shop, Devaraja Market, Mysore	Leucoderma, psoriasis and leprosy[16]
S. jambos Alston. L.	Myrtaceae	Fruit and seed	Horticulture Market, Mysore	Diarrhoea, dysentery, diabetes and catarrh[17]

*CSRTI – Central Sericulture Research and Training Institute; *T. arjuna – Terminalia arjuna; T. tomentosa – Terminalia tomentosa; T. catappa – Terminalia catappa; P. americana – Persea americana; P. longifolia – Polyalthia longifolia; P. corylifolia – Psoralea corylifolia; S. jambos – Syzygium jambos; T. chebula – Terminalia chebula*

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Table 2: Antifungal activity of selected medicinal plants against test fungi

Plant name	Plant part used	Extracts	Diameter of the zone of inhibition (mm)* against test fungi		
			Candida albicans	*Microsporum canis*	*Microsporum gypseum*
T. arjuna	Bark	Aqueous	0±0.00[a]	28.75±1.1[e]	28.5±1.55[e]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Methanol	0±0.00[a]	0±0.00[a]	15.5±0.28[b,c]
	Leaves	Aqueous	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Methanol	0±0.00[a]	0±0.00[a]	0±0.00[a]
	Peel bark	Aqueous	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Methanol	0±0.00[a]	0±0.00[a]	0±0.00[a]
T. catappa	Bark	Aqueous	0±0.00[a]	15.0±0.57[b]	0±0.00[a]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Methanol	0±0.00[a]	0±0.00[a]	16.5±0.5[b,c]
	Leaves	Aqueous	0±0.00[a]	25.25±1.25[d]	33.25±1.37[f]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Methanol	0±0.00[a]	0±0.00[a]	30.0±0.0[e,f]
T. tomentosa	Bark	Aqueous	0±0.00[a]	28.25±0.62[e]	23.25±1.1[d]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Methanol	0±0.00[a]	0±0.00[a]	16.5±0.5[b,c]
T. chebula	Fruit	Aqueous	16.0±1.15[c]	36.5±1.7[f]	47.75±1.31[g]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	20.0±0.0[d]	19.5±0.5[c]	19.0±0.57[c]
		Methanol	0±0.00[a]	0±0.00[a]	30.0±0.0[e,f]
P. longifolia	Bark	Aqueous	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	0±0.00[a]	0±0.00[a]	17.0±0.57[b,c]
		Methanol	0±0.00[a]	0±0.00[a]	0±0.00[a]
	Leaves	Aqueous	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	0±0.00[a]	13.75±0.75[b]	17.0±0.57[b,c]
		Methanol	0±0.00[a]	0±0.00[a]	0±0.00[a]
P. corylifolia	Seeds	Aqueous	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Petroleum ether	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Chloroform	0±0.00[a]	0±0.00[a]	0±0.00[a]
		Ethyl acetate	0±0.00[a]	15.5±0.28[b]	0±0.00[a]
		Methanol	0±0.00[a]	0±0.00[a]	0±0.00[a]
P. americana	Seed	Aqueous	0±0.00[a]	0±0.00[a]	0±0.00[a]
	Fruit (ectocarp)	Aqueous	0±0.00[a]	0±0.00[a]	0±0.00[a]
	Fruit (mesocarp)	Aqueous	0±0.00[a]	40.5±0.50[g]	31.25±0.47[e,f]

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Table 2: Contd...

Plant name	Plant part used	Extracts	Diameter of the zone of inhibition (mm)* against test fungi		
			Candida albicans	*Microsporum canis*	*Microsporum gypseum*
S. jambos	Seed	Aqueous	16.0±0.0^{c}	28.75±0.47^{e}	30.25±1.31^{e,f}
		Petroleum ether	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}
		Chloroform	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}
		Ethyl acetate	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}
		Methanol	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}
Miconazole			10.75±0.25^{b}	13.5±0.28^{b}	14.25±0.25^{b,c}
Nystatin			21.5±0.28^{e}	16.5±0.28^{b}	22.5±0.28^{d}

*The values are mean±SE of 4 replicates. Values with different superscript are significantly different from each other at $P<0.05$ (one-way ANOVA with Tukey's B analysis) (SPSS ver. 20). SE – Standard of error; ANOVA – Analysis of variance; *T. catappa – Terminalia catappa; T. arjuna – Terminalia arjuna; T. tomentosa – Terminalia tomentosa; T. chebula – Terminalia chebula; P. longifolia – Polyalthia longifolia; P. corylifolia – Psoralea corylifolia; P. americana – Persea americana; S. jambos – Syzygium jambos*

The test inoculum was prepared by diluting the culture with 1% peptone water to the turbidity level of 0.5 McFarland (1×10^8 cells/ml). The extract was tested at the concentrations ranging from 10,000 µg/ml to 9.8 µg/ml. The total volume per well was 200 µl (100 µL SDB + 100 µl inoculums). Three replicates were maintained. Miconazole and nystatin were taken as a positive control. 1 mg of each Miconazole and nystatin were dissolved in 1 ml of methanol and water respectively and were serially diluted to concentrations ranging from 1000 µg/ml to 0.98 µg/ml. Negative control was also maintained. The plates were incubated at 37°C for one day in case of *C. albicans*, two days for filamentous fungi. MIC was determined visually for dermatophytes. MTT [3-(4, 5-Dimethyl thiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide] was used (0.1 mg/ml) for detection of growth with *C. albicans*. MTT changes colour from yellow to violet in the presence of live cells. The MIC was defined as the lowest concentration of the extract that inhibits the growth of the test organism.

Preliminary Phytochemical Analysis of the Bioactive Plant Extracts

The active extracts were subjected to preliminary phytochemical analysis by following the methods of Harborne[22] and Sofowora.[23]

Test for Phenols

Ferric chloride test

About 2 ml of the extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour.

Test for Tannins

Braymer's test

About 2 ml of the extract was treated with 10% alcoholic ferric chloride solution and observed for formations of blue or greenish colour indicating the presence of tannins.

Test for Alkaloids

Dragendroff's test

Two ml of the extract was taken, and 2 ml of Dragendroff's reagent was added to it. The formation of orange, red precipitate was taken as a positive for alkaloids.

Mayer's test

Two ml of the extract was taken, and 2 ml of Mayer's reagent was added to it. A dull white precipitate confirmed the presence of alkaloids.

Test for Flavonoids

Shinoda's test

Two ml of the extract was taken, and 2–3 pieces of magnesium turnings and 1–2 drops of concentrated hydrochloric acid were added to it. Formation of red colour shows the presence of flavonoids.

Test for Terpenoids

Liebermann-Burchard test

Two ml of the extract was taken, and 2 ml of chloroform, 1 ml of acetic anhydride and 3–5 drops of concentrated sulphuric acid were added and observed for the formation of dark green colour.

Test for Steroids

Sulphuric acid test

Two ml of the extract was treated with ethanol and 2–3 drops of concentrated sulphuric acid. Formation of violet/blue or green colour indicated the presence of steroids.

Test for Saponins

Foam test

Two ml of the extract was shaken with water and observed for the formation of persistent foam.

Test for Glycosides

Salkowski's test

Two ml of the extract was dissolved in 2 ml of chloroform; 2 ml of concentrated sulphuric acid was added carefully and shaken gently. A reddish brown colour indicated the presence of a steroidal ring (that is a glycone portion of glycoside).

Keller-Kiliani test

Two ml of the extract was dissolved in 2 ml of glacial acetic acid containing 1–3 drop of 5% ferric chloride solution. The mixture was then poured into a test tube containing 1 ml of

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concentrated sulphuric acid. A brown ring at the interphase indicated the presence of a de-oxy sugar, characteristic of glycosides.

Test for protein and amino acids

Ninhydrin test

Two ml of the extract was treated with 2–5 drops of freshly prepared 0.2% ninhydrin reagent and heated on a water bath. Violet colour was formed in the extract containing amino acids.

Test for carbohydrates

Fehling's test

Two ml of the extract was taken, and an equal volume of Fehling's solution A and Fehling's solution B were added to it. The solution was heated on the water bath. Presence of carbohydrate was detected by the formation of brick red precipitate.

Statistical analysis

The results of the antifungal activity assay were subjected to statistical analysis using the software Statistical Package for Social Sciences (SPSS) version 20. One-way analysis of variance (ANOVA) with Tukey's B (*post-hoc*) test was applied at the significance level of $P < 0.05$.

RESULTS

Antifungal Activity Assay

The results of antifungal activity assay are presented in Table 2. Aqueous extract of the bark of *Terminalia arjuna* showed highest zone of inhibition of *M. canis* (28.75 mm), followed by *M. gypseum* (28.5 mm). Aqueous extract of the leaves of *T. catappa* was active against *M. canis* (25.25 mm) and *M. gypseum* (33.25 mm), while the methanol extract was found to be active against *M. gypseum* (30 mm). *T. tomentosa* showed activity with aqueous extract of its bark against *M. canis* (28.25 mm) and *M. gypseum* (23.25 mm).

The aqueous and ethyl acetate extract of the fruit of *Terminalia chebula* were found to be active against all the test fungi. The aqueous extract recorded a zone of inhibition of 16 mm against *C. albicans*, 36.5 mm against *M. canis* and 47.75 mm against *M. gypseum*. With the ethyl acetate extract, the zone of inhibition was 20 mm against *C. albicans*, 19.5 mm against *M. canis* and 19 mm against *M. gypseum*. The methanol extract was active only against *M. gypseum* (30 mm).

Aqueous extract of the mesocarp of *Persea americana* showed significant activity against *M. canis* (40.5 mm) and *M. gypseum* (31.25 mm). While did not show any activity against *C. albicans*.

Seeds of *Syzygium jambos* were also found to be possessing significant antifungal activity against *M. canis* (28.75 mm) and *M. gypseum* (30.25 mm). The aqueous extract showed activity against *C. albicans* (16 mm).

The two standard fungicides used in the present study as a positive control include miconazole and nystatin. Miconazole displayed an inhibition zone of 10.75 mm against *C. albicans*, 14.25 mm against *M. gypseum* and 13.5 mm against *M. canis*. Nystatin showed inhibition zone of 22.5 mm against *M. gypseum*, 21.5 mm against *C. albicans* and 16.5 mm against *M. canis*. The results of antifungal activity assay were subjected to statistical analysis by One-way ANOVA with *post-hoc* Tukey's *B* test. The ANOVA end table data revealed that the treatments were better than the positive control. The values which significantly differ from each other at $P < 0.05$ have been indicated in the table.

Determination of Minimum Inhibitory Concentration

Lowest MIC values were observed in the aqueous extract of the mesocarp of *P. americana* against *M. canis* (312.5 µg/ml) and *M. gypseum* (312.5 µg/ml). Ethyl acetate extract of the fruit of *T. chebula* was active against *C. albicans* (625 µg/ml).

Minimum inhibitory concentration value of 1250 µg/ml was observed against *M. canis* in the ethyl acetate extract of the fruits of *T. chebula* and in the aqueous extract of the bark of *T. arjuna* against *M. gypseum*. Nystatin recorded an MIC value of 15.625 µg/ml against *C. albicans* 62.5 µg/ml against *M. canis* and 31.25 µg/ml against *M. gypseum*. The MIC value of Miconazole was 15.625 µg/ml against *C. albicans* 0.243 µg/ml against *M.canis* and 0.30 µg/ml against *M. gypseum* [Table 3].

Preliminary Phytochemical Analysis of Bioactive Plant Extracts

The preliminary phytochemical analysis of the extract possessing antifungal activity revealed that these extracts contained secondary metabolites known for their antifungal activity.

Phenols and tannins were found to be the predominant group of compounds present in these extracts. The aqueous extract of mesocarp of *P. americana* was found to be comprised of alkaloids and carbohydrates. The aqueous and ethyl acetate extract of *T. chebula* fruits contained phenols, tannins and carbohydrates. However, the aqueous extract showed the presence of flavonoids. The aqueous extract of *S. jambos* seeds showed the presence of phenols, tannins and carbohydrates. The results are presented in Table 4.

DISCUSSION

In the present study, eight medicinal plants were evaluated for antifungal activity against *Candida albicans*, *Microsporum*

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Table 3: MIC of active plant extracts against test fungi

Plant extract/positive control	MIC µg/mL*		
	Candida albicans	*Microsporum canis*	*Microsporum gypseum*
T. arjuna bark (aqueous)	-	2500	1250
T. arjuna bark (methanol)	-	-	1250
T. tomentosa bark (aqueous)	-	2500	2500
T. tomentosa bark (methanol)	-	-	1250
T. catappa leaves (aqueous)	-	2500	2500
T. catappa leaves (methanol)	-	-	1250
T. catappa bark (methanol)	-	-	2500
T. chebula fruit (aqueous)	-	5000	2500
T. chebula fruit (ethyl acetate)	625	1250	2500
T. chebula fruit (methanol)	-	-	2500
P. americana mesocarp (aqueous)	-	312.5	312.5
P. corylifolia seed (ethyl acetate)	-	2500	-
P. longifolia leaves (ethyl acetate)	-	2500	2500
P. longifolia bark (ethyl acetate)	-	-	10,000
S. jambos seed (aqueous)	-	2500	5000
Miconazole	15.625	0.243	0.30
Nystatin	15.625	31.25	31.25

- – Represents not tested. *The values are the concordant value of triplicates. *T. arjuna – Terminalia arjuna; T. tomentosa – Terminalia tomentosa; T. catappa – Terminalia catappa*; MIC – Minimal inhibitory concentration; *T. chebula – Terminalia chebula; P. americana – Persea americana; P. corylifolia – Psoralea corylifolia; P. longifolia – Polyalthia longifolia; S. jambos – Syzygium jambos*

Table 4: Phytochemical constituents of antifungal active extracts

Plant extracts	Phenols	Tannins	Alkaloids	Flavonoids	Terpenoids	Steroids	Glycosides	Proteins	Carbohydrates
T. arjuna bark (aqueous)	+	+	–	–	–	–	–	–	–
T. arjuna bark (methanol)	+	+	–	–	–	–	–	–	–
T. tomentosa bark (aqueous)	+	+	–	–	–	–	–	–	–
T. tomentosa bark (methanol)	+	+	–	–	–	–	–	–	–
T. catappa leaf (aqueous)	+	+	–	+	–	–	–	–	–
T. catappa leaf (methanol)	+	+	+	+	–	–	–	–	–
T. catappa bark (methanol)	+	+	+	+	–	–	–	–	–
T. chebula fruit (aqueous)	+	+	–	+	–	–	–	–	+
T. chebula fruit (ethyl acetate)	+	+	–	–	–	–	–	–	+
T. chebula fruit (methanol)	+	+	–	–	–	–	–	+	–
P. americana mesocarp (aqueous)	–	–	+	–	–	–	–	–	+
P. corylifolia seeds (ethyl acetate)	–	+	–	–	+	+	–	–	–
P.longifolia leaf (ethyl acetate)	–	+	–	–	+	+	–	–	–
S. jambos seeds (aqueous)	+	+	–	–	–	–	–	–	+

– – The absence of phytochemical; + – The presence of phytochemical. *T. arjuna – Terminalia arjuna; T. tomentosa – Terminalia tomentosa; T. catappa – Terminalia catappa; T. chebula – Terminalia chebula; P. americana – Persea americana; P. corylifolia – Psoralea corylifolia; P. longifolia – Polyalthia longifolia; S. jambos – Syzygium jambos*

canis and *M. gypseum*. Significant antifungal activity was displayed by the extracts of *T. chebula, P. americana* and *S. jambos*. Aqueous and methanol extracts of leaves of *Terminalia catappa* and the bark of *Terminalia arjuna* and *Terminalia tomentosa* also showed good activity.

Different parts of *P. americana* have been scientifically evaluated for various pharmacological property such as wound healing activity, anticancer activity, anti-obesity activity and antimicrobial activity. An antifungal compound has been isolated from idioblast cells of the fruit of *P. americana* which is active against *Colletrotrichum gloeosporioides*. Acetone extract of the seed of *P. americana* revealed antifungal effect on *Aspergillus glaucus* and *Penicillium notatum*.[24]

None of the earlier workers have evaluated the antifungal potential of *P. americana* against human pathogenic fungi in general and dermatophytes, in particular. The present study is the first investigation on the antifungal activity of the extract of mesocarp of *P. americana*. Mesocarp is the fleshy edible portion of the fruit and hence it could be safely assumed that the compound responsible for the bioactivity is not toxic. Phytochemical analysis of the extract revealed the presence of alkaloids, which could be the bioactive compounds. MIC value of the extract is as low as 312.5 µg/ml, indicating high

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efficacy of the antifungal active compound. Thus, the results of the present investigation suggest that *P. americana* is an important plant for further investigation by *in vivo* systems to develop effective antifungal chemotherapeutic agent.

The previous reports on the antifungal activity of the different part of *T. chebula* including its fruits have primarily focused on activity against *C. albicans*.[25,26] The present study has found that the aqueous, ethyl acetate and methanol extract of *T. chebula* fruit possess antifungal activity with zones of inhibition ranging between 16 mm and 47.75 mm. This activity is better than the activity reported earlier. The MIC of ethyl acetate extract of the *T. chebula* fruit against *C. albicans* (625 µg/ml) appears promising. Phenols and tannins were found to be the predominant groups of secondary metabolites present in the active extracts. Flavonoids were found only in the aqueous extract. Our study is in agreement with the previous report[25] on the activity of the fruit extract against *C. albicans*. This is the first report of antifungal activity of fruits of *T. chebula* against *M. canis* and *M. gypseum*.

The fruit of *S. jambos* is edible, and its seeds are used in medicinal preparations.[17] The antibacterial activity of its different parts such as bark, leaves and seed has been reported earlier.[27-30] The ethyl acetate extract of the bark of *S. jambos* is reported to be active against *Microsprum audouinii*, *Trichophyton mentagrophyte*, and *T. soudanense* and two compounds, namely Betulinic acid, and Friedelolactone have been identified as the active compounds.[31] The antifungal activity of the seeds of *S. jambos* against human pathogenic fungi has not been reported. Hence, this is the first report of the aqueous extract of the seeds against the test dermatophytes.

Earlier report[32] on the antimicrobial activity of *T. catappa* suggest that the activity is due to alkaloids, steroids and tannins, while in the present study steroids were absent, but flavonoids were present.

Report suggests that the methanol extract of the bark of *T. arjuna* was active against the species of *Trichophyton* and *Microsporum*.[33] In the present study also no activity was observed against *C. albicans* whereas significant activity was recorded by the extracts of *T. arjuna* against *M. canis* and *M. gypseum*.

Among the test fungi, *M. gypseum* was found to be the most susceptible and was inhibited by 14 extracts. It was, followed by *M. canis* which was inhibited by ten extracts. *C. albicans* was the least susceptible as only three extracts inhibited the growth. Results indicate antifungal potential of the test plants and have validated the use of these plants in the treatment of fungal infections in the traditional medicine. Further analysis with chromatography and spectroscopic techniques are needed to identify the bioactive compound present in these plant extracts. This would pave the way for identification of chemical molecules responsible for the activity and exploitation of the same for treating fungal infections.

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