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Original Article

A Study of Folklore Antifungal Medicinal Herbs among the Tribal Groups of Western Ghats, Coimbatore, Tamil Nadu

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ABSTRACT

Background: The present study has been carried out to explore antifungal property of folklore medicinal herbs *Solanum trilobatum, Spathodea campanulata, Syzygium jambos* and *Tylophora indica* prevalent among the tribal communal in the Western Ghats of Coimbatore district. **Materials and Methods:** The aqueous and organic leaf extracts obtained by cold maceration method was confirmed for its antifungal activity against *Aspergillus flavus* and *Aspergillus fumigatus* by agar plate dilution method. **Results:** The methanolic extracts of *S. trilobatum, S. jambos* and *T. indica* exhibited substantial ($p<0.001$) fungal growth inhibition against *A. flavus* with IC_{50} values 40.63, 94.56 and 84.52 µg/mL respectively. The ethanolic extracts of *S. trilobatum, S. campanulata, S. jambos* and *T. indica* also revealed ($p<0.01$) inhibitory activity against *A. flavus* with IC_{50} values ranging between 102.34-146.79 µg/mL, whereas the *S. campanulata* methanolic and chloroform fractions of *S. jambos* influenced mycelial inhibition against *A. flavus* with a IC_{50} values at 147.92 and 111.08 µg/mL respectively. Furthermore, the methanolic extracts of *S. campanulata* exposed highly significant antifungal activity against *A. fumigatus*, ($p<0.001$) with IC_{50} value at 95.3 µg/mL. Analogous antifungal significances were also observed among methanolic extracts of *S. jambos* and *T. indica* against *A. fumigatus* with IC_{50} values at 131.52 and 114.51 µg/mL respectively. The other extracts were not up to the pronounceable level of fungal growth control against the two fungal strains used in the assay. **Conclusion:** The alcoholic leaf extracts of selected plants were more active against tested fungal strains when compared to the chloroform and aqueous extracts. Further surveys and findings have to be fixed to recognize the antifungal metabolites present in the chosen herbs.

Keywords: Antifungal, Aspergillosis, Bronchopulmonary, Folklore, Mycosis.

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INTRODUCTION

Fungal spore dissemination in the environment poses a great problem leading to the spore derived allergic pulmonary diseases. *Aspergilli* allergic bronchopulmonary infections are common in moderate and tropical climates that has been recorded in India with the progress of pulmonary cavitation and fungus ball in patients causing frightful haemoptysis incidental to tuberculosis.[1] Fungal aspergillosis called Farmer's Lung infection, a type of hypersensitivity pneumonitis due to the housing of mold spores in the hay or saw in pulmonary cavities of paddy field agrarians involved in farming practices. The moist husk, hay and other grain debris are the major growth substrates of *Aspergillus* species and frequent exposer of these field workers during harvesting of crops are frequently prone to farmer's lung Aspergillosis infection. Treatment of such fungal infections should be considered with utmost consequence, untreated may result in chronic condition with morbidity and sometimes fatal consequences.

Multidrug resistance among fungal strains have constituted an inordinate obstacle in tropics, besides the availability of fungal drugs are inferior compared to anti-bacterial agents. Still search and research needs to come out with anti-fungals with zero side effects. Herbalism is a pitch run through the traditional healers with folklore knowledge for the various ailments. From time immemorial, traditionally phytotherapy information's has been conceded as oral communication from generation to generation. Among secondary metabolites from herbal sources plant polyphenolics, terpenoids, alkaloids, oils, saponins, peptides and proteins were evidenced to be natural antifungal compounds used in various ailments.[2,3] The Malasars, Irulas and Konars are the native people near poondi in Western Ghats of Coimbatore district, traditionally treat the fungal infections with herbal sources such as *S. trilobatum, S. campanulata, S. jambos* and *T. indica* either in a form of extracts or decoctions for remedy. With the elementary information and empirical usages of selected plants among specific communities have made a rationale to

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review and evaluate the *in vitro* antifungal activity as a preliminary attempt for documentation.

MATERIALS AND METHODS

Plant materials

The fresh disease-free plant leaves were collected around Poondi hills in Western Ghats, and authenticated at BSI, Southern Regional Centre, Coimbatore. The voucher specimens *Solanum trilobatum* L. (No.1269), *Spathodea campanulata* P. Beauv. (No.1371), *Syzygium jambos* L. Alston (No.1408) and *Tylophora indica* (Burm.f.) Merr (No.1194) were deposited in the Department of Microbiology, RVS College of Arts and Science, Sulur for future references.

Preparation of leaf extracts

The collected leaf samples were shade dried and powdered followed by defatting with petroleum ether, extracted with solvents viz. methanol, ethanol, chloroform, and distilled water by cold maceration method. Further, filtration was achieved with 90 mm Filter Paper Grade 1, concentrated in a rotary vacuum evaporator. The obtained crude extracts were stored at 4°C for further analysis.

Fungal spore preparation

The fungal strains *Aspergillus flavus* (MTCC 8834) and *Aspergillus fumigatus* (MTCC 2550) were maintained in Sabouraud dextrose agar plates. Seven day old fungal plates with spores were submerged with sterilized distilled water and disturbed with glass beads. The spore suspension from the plates obtained by filtration through Whatman no.1 filter paper was stored in sterile containers at 4°C till use.

In vitro antifungal assay by plate dilution method

The *in vitro* antifungal activity was performed according to the method described[4,5] with a minor modification in dilution and instead of test tubes petri plates were used. The actual drug concentration from the 1st to 6th in the petri plates ranged from 1000-31 µg/mL respectively. Using micropipettes around 1 µL of fungal spore suspension ($1x10^7$ spores/mL) was liquidated at the midpoint of each petri plates with plant extract in various concentration and the plates with fungal suspension was allowed to diffuse in the media. Antifungal Amphotericin B (1.5 µg/mL) and 2 mL of 2% DMSO were used as positive and negative assay controls. All the plates were incubated at 27 ±2°C for 7days. The growth of fungal colonies in each plate was measured (in mm) and averaged. The entire test was carried out in triplicates to calculate the statistical significance and fungal growth/mycelia inhibition was calculated in percentage with reference to the negative control by applying a formula described.[6]

$$\text{Fungal Growth inhibition (\%)} = \frac{\text{Growth in negative control (mm) - Growth in treatment (mm)}}{\text{Growth in negative control (mm)}} \times 100$$

The Minimum Inhibitory Concentration (MIC) of the drug inhibiting visible fungal growth after incubation time was recorded and the IC_{50} (half maximal inhibitory concentration) was determined using Graph Pad Prism software.

Statistical analysis

Statistical evaluations employed ANOVA one way by comparing control with test samples applying Dunnett's multiple comparison test, the values with $p<0.05$ was considered as statistically significant.

RESULTS

Antifungal potential of selected plant extracts against *A. flavus* and *A. fumigatus* are summarized in Tables 1 and 2 respectively. Complete fungal mycelia inhibition was observed in positive control with Amphotericin B (1.5 µg/mL) and ample growth in negative control plates. Of the four plants with their organic solvent leaf extracts used in the assay, varying degree of antifungal potential was exhibited determining their role in folklore application. The 100% inhibitory extracts against *A. flavus* were found to be STMLE, SJMLE and TIMLE with their IC_{50} values ranging 40-95 µg/mL. The inhibitory level 85-100% was exhibited by STELE, SCMLE, SCELE, SJELE, SJCLE and TIELE with their IC_{50} values ranging 102-148 µg/mL. The sensible mycotic inhibition (55-84%) was exhibited by STCLE, SCCLE and TICLE. On the other hand, the SCMLE, SCELE, SJMLE and TIMLE were actively (100%) fungistatic against *A. fumigatus* with IC_{50} values ranging from 95-132 µg/mL. The ethanolic extracts of *S. jambos* (SJELE) showed 100% fungal inhibition at 1000 µg/mL (IC_{50} at 196.25 µg/mL). A determined fungal inhibition (80-95%) of STMLE, STELE and TIELE were recorded with IC_{50} values ranging 140-236 µg/mL. The chloroform and aqueous leaf extracts of all the four plants did not show pronounceable antifungal activity against *A. fumigatus*.

DISCUSSION

The secondary metabolites such as polyphenols, alkaloids, and flavonoids were frequently reported from terrestrial plants. Certain naturally derived polyphenolic compounds such as phytoanticipin and phytoalexin are called as plants preformed antibiotic compounds featured with defensive mechanism against an extensive range of bacterial and fungal pathogens. Literature reviews reveals that the plant phytoalexin phenolic acids are highly toxic against *Aspergillus* species.[2] In addition, the plant flavonoids are also established with imposing fungicidal

property against fungal species. With an overview, the selected plant extracts with varying degree of antifungal mechanism may be due to the existence of preformed anti-fungal metabolites such as flavonoids, polyphenols, terpenoids, essential oils, alkaloids, saponins, peptides and proteins that might be allied with the plant growth.[3] On the other hand very few references were available for antifungal activity of selected plants in the present study.

Similar studies with Anti-inflammatory and antimicrobial properties of aqueous and organic solvent extracts of *S. trilobatum* exists with positive results.[9] Correspondingly, antifungal mechanism of *S. trilobatum* against *Aspergillus brasiliensis*–MTCC1344 and *Aspergillus fumigatus*–MTCC 343 was demonstrated to be good.[10] Reports with *Solanum trilobatum* cation sensitive heat-stable antifungal protein from the leaves with a molecular mass of 31 KDa showing stability for a wide range of pH between 5 and 8.5 has been proved to delay the hyphal extension making the fungi static.[11] Several phytochemical studies on *Spathodea campanulata* revealed the presence of spathodol, phenolic acids, caffeic acid, tannins, saponins, flavonoids, polyphenols and glycosides.[7,8,12] The *S. campanulata* extracts has been reported for its antimalarial activity against chloroquine sensitive and chloroquine resistant *P. falciparum* strain.[13] and hence the plant extract was proved for its parasite control. Fresh extracts of *S. campanulatum* P. Beauv was confirmed for its fungal and bacterial inhibition, and further the assay report discloses that storage of plant extracts has reduced the antifungal activity, whereas anti-bacterial property was largely unaffected.[14] The stem bark as a topical application paste is used for wound healing.[15] In Ghana the different parts of plant extracts were used for the treatment of several ailments such as dyspepsia, peptic ulcer; stomach ulcer, arthritis, fracture, tooth and stomach ache.[16] Few studies reports that anti-fungal property of *S. campanulata* aqueous cream base has lesser efficacy than that of commercially available miconazole anti-fungal cream.[14] The *Syzygium jambolanum* seed extracts has proved to be effective against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans, Bacillus subtilis* and *Staphylococcus aureus*.[17] Antifungal efficacy of two Phenanthroindolizidine alkaloids, Tylophorinidine Hydrochloride (TdnH) and Tylophorinine Hydrochloride (TnnH), isolated from the plant *Tylophora indica* was found to be effective.[18]

Reports pertaining to the antifungal potential of *T. indica* inhibiting *Aspergillus niger*, *A. fumigatus*, and *Trichoderma viride* has been well evidenced.[19] Potential inhibition of *T. indica* extracts against *A. niger*, and *Fusarium* species has also been reported formerly. The fungicidal significance of alcoholic extracts against *A. niger*, *A. flavus*, *A. fumigatus*, and *Penicillium* species proves the folklore property of the herb.[20] In another study, the leaf callus aqueous extract against *Candida parapsilosis* was well proved, while parent plant extract was found to be inactive in

Table 1: Percentage antifungal activity, MIC and IC_{50} of four medicinal plants against *Aspergillus flavus*.

Conc. µg/mL	31	62	125	250	500	1000	MIC µg/mL	IC_{50} µg/mL
STALE	0	0	0	0	0	22.16	≤1000	ND
STMLE	43.11	65.27	76.05	92.22	100	100	≤31	40.635***
STELE	23.95	41.32	54.49	73.65	86.23	95.21	≤31	103.52**
STCLE	2.395	5.988	7.784	20.96	32.34	55.09	31	888.3 ^{ns}
SCALE	0	0	0	0	0	0	ND	ND
SCMLE	20.96	26.95	46.71	64.67	71.86	100	≤31	147.926**
SCELE	0.599	37.72	56.89	75.45	95.21	100	≤31	102.34**
SCCLE	1.198	5.988	40.72	56.89	66.47	71.86	≤31	196.82 ^{ns}
SJALE	0	0	0	1.198	1.796	2.994	ND	ND
SJMLE	24.55	40.72	58.68	87.43	100	100	≤31	94.56***
SJELE	7.186	17.37	45.51	71.26	82.63	100	≤31	146.79**
SJCLE	4.192	29.94	55.69	69.46	83.23	88.02	≤31	111.08**
TIALE	0	0	3.593	4.79	5.389	9.581	≤125	ND
TIMLE	19.16	40.12	67.66	83.83	100	100	≤31	84.52***
TIELE	9.581	23.35	59.88	70.66	81.44	100	≤31	107.95**
TICLE	5.389	7.186	19.16	40.72	68.26	80.24	≤31	334.17 ^{ns}
Amphotericin B	100						≤100	≤100
Control	0							0

Note: Values in the parenthesis represents the percentage inhibition of fungal growth. MIC-Minimum inhibitory concentration; ND-Not detected; Inhibitory concentration (IC_{50}) with *, **, *** represents the values are significantly different at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, by Dunnett's multiple comparison test with control (DMSO); ns-no significant difference between the values.

Table 2: Percentage anti-fungal activity, MIC and IC_{50} of four medicinal plants against *Aspergillus fumigatus*.

Conc. µg/mL	31	62	125	250	500	1000	MIC µg/mL	IC_{50} µg/mL
STALE	0	0	0	0	0	1.5	≤1000	ND
STMLE	2.26	6.77	18.8	54.14	72.18	81.2	≤31	235.38 ^{ns}
STELE	1.5	26.3	42.86	66.92	81.2	94	≤31	162.09*
STCLE	0	0	2.256	1.504	13.53	43.6	≤125	>1000 ^{ns}
SCALE	0	0	0	0	0	0	ND	ND
SCMLE	0	36.1	62.41	88.72	100	100	≤62	95.3***
SCELE	0	33.1	52.63	73.68	100	100	≤62	116.52**
SCCLE	0	0	0	0	19.55	36.1	≤500	>1000^{ns}
SJALE	0	0	0	0	0	9.92	ND	>1000 ^{ns}
SJMLE	5.26	25.6	48.12	84.21	100	100	≤31	131.52**
SJELE	0.75	2.26	31.58	63.91	78.2	100	≤31	196.25*
SJCLE	0	0	0	0	0	0	ND	ND
TIALE	0	0	0	0	0	0	ND	ND
TIMLE	12.8	25.6	54.89	79.7	100	100	≤31	114.51**
TIELE	6.77	26.3	48.12	63.16	76.69	82.7	≤31	140.62 ^{ns}
TICLE	0	0	0	0	0	0	ND	ND
Amphotericin B	100						≤100	≤100
Control	0						ND	ND

Note: Values in the parenthesis represents the percentage inhibition of fungal growth. MIC-Minimum inhibitory concentration; ND-Not detected; Inhibitory concentration (IC_{50}) with *, **, *** represents the values are significantly different at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, by Dunnett's multiple comparison test with control (DMSO); ns-no significant difference between the values.

its anti-fungal potential.[21] The *in vitro* raised *T. indica* extract was explored comparing with the parent plant extract, where the alcoholic callus fractions outclassed the parent impeding *C. albicans* and *C. krusei*, as well as hampering *A. flavus*, *A. niger*, *A. fumigatus*, and *Penicillium* species.[22] The methanolic extracts of tissue cultured and wild *T. indica* showed sensible mycelia growth inhibition of *A. fumigatus* and *Verticillum lecanii*.[22] Even though the selected plants has been widely used for diverse ailments among the malasar tribes, no written documents was evidenced for its tribal usages in folklore among the community. Many preliminary approaches on antifungal assays in the selected herbs could prompt for further surveys to isolate the specific antifungal metabolites in all the four selected plants.

CONCLUSION

In the present study the alcoholic extracts were effective against *A. flavus* and *A. fumigatus* which may be due to the existence of natural shielding metabolites in the plant. The trial also confirms that the increased polarity of solvents may contribute to the release of antifungal metabolites tagged in the selected medicinal plants. The test facts support the assertions of traditional medicines using *S. trilobatum, S. campanulata, S. jambos* and *T. indica* leaf extracts to treat infectious diseases. Our *in vitro* study supports the previous studies and practice of selected medicinal plants by tribes of Western Ghats in Coimbatore. There is, therefore, the need for further studies into the stability of the extracts in antifungal assays.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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