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In vitro antifungal activity of four folklore medicinal plants used among tribal communities of Western Ghats, Coimbatore, Tamil Nadu.

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ABSTRACT

The present study was conceded to investigate the *in vitro* antifungal activity of four medicinal plants *Solanum trilobatum, Spathodea campanulata, Syzygium jambos* and *Tylophora indica* used in folklore treatments among tribal community in Western Ghats of Coimbatore district. The cold macerated crude aqueous and organic solvent leaf extracts were subjected to antifungal activity against *Aspergillus flavus* and *Aspergillus fumigatus* by agar plate dilution method. At an MIC of 500 µg/mL the methanolic leaf extracts of *S.jambos*, *T.indica* and *S.trilobatum* showed significant ($p<0.001$) fungal inhibition against *A.flavus* with IC_{50} values 94.56, 84.52 and 40.63 µg/mL respectively. Furthermore, the ethanolic extracts of *S.trilobatum*, *S.campanulata*, *S.jambos* and *T.indica* showed substantial ($p<0.01$) antifungal activity against *A.flavus* with IC_{50} values ranging between 102.34-146.79 µg/mL, whereas the methanolic extract of *S.campanulata* and chloroform extract of *S.jambos* influenced an inhibition against *A.flavus* with IC_{50} values at 147.92 and 111.08 µg/mL respectively. On the other hand the methanolic leaf extracts of *S.campanulata* exhibited exceptionally significant antifungal activity ($p<0.001$) against *A.fumigatus* with an IC_{50} value at 95.3 µg/mL. Comparable mycelia inhibition was also observed among methanolic extracts of *S.jambos* and *T.indica* against *A.fumigatus* with IC_{50} values predicted at131.52 and 114.51 µg/mL respectively. The alcoholic leaf extracts of the selected plants remained more active against tested fungal strains when compared to the chloroform and aqueous extracts, showing no pronounceable inhibition of two fungal strains used in the assay signifying not up to the score. Further investigations are mandatory to identify the antifungal compounds present in the chosen herbs.

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

INTRODUCTION

Dissemination of fungal spores emerges as a major problem in the spread of spore derived allergic pulmonary diseases. In temperate and tropical climates, *Aspergilli* are well recognized agents of allergic bronchopulmonary infections. Aspergillosis has been recorded in India, frequently in tropical areas disseminating with the development of fungus ball and pulmonary cavitation in the patients causing severe haemoptysis secondary to tuberculosis.[1] A type of aspergillosis called Farmer's Lung infection is a housing of mold spores in pulmonary cavities among paddy field agrarians involved in farming practices. Farm workers in Narasipuram, Semmedu, and areas near Velliangiri foothills (Poondi) of Western Ghats, Coimbatore are the chief cultivator's of grains and sugarcane. The husk, hay and other debris are the major growth substrates of *Aspergillus* species. Constant exposure of field workers during harvesting of crops may frequently prone to farmer's lung Aspergillosis infection. Treatment of such fungal infections should be considered with utmost significance, untreated may be chronic leading to morbidity and sometimes fatal consequence among infected individuals.

Fungal infections with multidrug resistances have posed a great threat in tropics and emerge with resistance to antifungal agents. Since the availability of antifungals are lesser compared to antibacterial agents, a search of alternative antifungal agents are mandatory with nil side effects. Phytotherapy is an arena practiced among the folklore traditional healers for various diseases and disorders. From time immemorial traditionally from generation to generation phytotherapy information's has been conceded as oral communication. Among secondary metabolites from herbal sources the plant polyphenolics, essential oils, terpenoids, saponins, alkaloids, 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 *Solanum trilobatum*, *Spathodea campanulata*, *Syzygium jambos* and *Tylophora indica* either in the form of extracts or decoctions for remedy. With the el-

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ementary information and empirical usages of selected plants among specific communities have made a rationale to review and evaluate the *in vitro* antifungal activity as a preliminary attempt for documentation.

MATERIALS AND METHODS

Collection of plant material

The plant samples were collected from different areas around Western Ghats, Coimbatore, Tamilnadu and authenticated by Dr. G.V.S. Murthy, Scientist 'F' and Head, Botanical Survey of India, 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 fresh disease free shade dried powdered leaves were defatted with petroleum ether and extracted with organic solvents viz. methanol, ethanol, chloroform, and distilled water by cold maceration method. After a week of socking, filtration was conducted with whatmann filter paper no.1, and concentrated via. rotary vacuum evaporator. The concentrated 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) gifted by Dr. M.Palaniswamy, Director, Faculty of Life Sciences, Karpagam University, Coimbatore was maintained in Sabouraud dextrose agar plates. The seven day old fungal colonies with spores were flooded with sterile double 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 of plant extract 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 final concentration of the drug in the petri-plates ranged from 1000 to 31 µg/mL from the 1st to 6th plate respectively. About 1 µL of standardized fungal spore suspension ($1x10^7$ spores/mL) was carefully liquidated using micropipettes at the center of each petri-plate amended with plant extract in different concentration and allowed to diffuse in the media. The antifungal agent Amphotericin B (1.5 µg/mL) and 2 mL of 2% DMSO was used as positive and negative assay controls respectively. The plates were incubated at 27 ±2°C for 7days and fungal growth (mm) in each plate was measured and averaged. The assay was carried out in triplicates to attain statistical significance and fungal growth inhibition percentage was calculated 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 AND DISCUSSION

The results of antifungal activity of medicinal plant extracts against *A.flavus* and *A.fumigatus* are summarized in Table 1 and Table 2 respectively**.** Complete growth inhibition was noticed in positive control plates with Amphotericin B (1.5 µg/mL) and ample growth was recorded in negative control plates. Of the 4 plants tested, the leaf extracts showed varying degree of antifungal activity against the tested fungal strains. The most significant 100% inhibition against *A. flavus* with MICs at 500 µg/mL were found to be STMLE, SJMLE and TIMLE with their IC_{50} values ranging 40 -95 µg/mL. The inhibitory level 85-100% at MICs $\geq$1000 µg/mL 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 at $\geq$1000 µg/mL. On the other hand the SCMLE, SCELE, SJMLE and TIMLE were 100% actively fungistatic against *A. fumigatus* at MIC 500 µg/mL 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 80-95% fungal inhibition by STMLE, STELE and TIELE were recorded at $\geq$ 1000 µg/mL 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*.

Terrestrial plants synthesize a broad range of secondary metabolites such as alkaloids, flavonoids and polyphenols. The natural polyphenols phytoanticipin and phytoalexin called as preformed antibiotic compounds from plants attribute to the defensive mechanism against wide range of bacterial and fungal pathogens. Literature reviews indicates that phytoalexin phenolic acids are toxic against *Aspergillus* species.[2] In addition to plant phenolic acids, the flavonoids have also been proved to be fungicidal against fungal pathogens. The varying degree of antifungal activity by selected plant extracts may be due to the presence of preformed antifungal metabolites such as polyphenols, flavonoids, essential oils, terpenoids, saponins, alkaloids, peptides and proteins that may be associated with the plant growth.[3] So for no written documents are found among the tribes of Western Ghats using the selected plants in different ailments. Further investigations are required to isolate the specific antifungal compounds present in all the four selected plants.

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	500	40.635***
STELE	23.95	41.32	54.49	73.65	86.23	95.21	>1000	103.52**
STCLE	2.395	5.988	7.784	20.96	32.34	55.09	>1000	888.3^{ns}
SCALE	0	0	0	0	0	0	ND	ND
SCMLE	20.96	26.95	46.71	64.67	71.86	100	1000	147.926**
SCELE	0.599	37.72	56.89	75.45	95.21	100	1000	102.34**
SCCLE	1.198	5.988	40.72	56.89	66.47	71.86	>1000	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	500	94.56***
SJELE	7.186	17.37	45.51	71.26	82.63	100	1000	146.79**
SJCLE	4.192	29.94	55.69	69.46	83.23	88.02	>1000	111.08**
TIALE	0	0	3.593	4.79	5.389	9.581	>1000	ND
TIMLE	19.16	40.12	67.66	83.83	100	100	500	84.52***
TIELE	9.581	23.35	59.88	70.66	81.44	100	1000	107.95**
TICLE	5.389	7.186	19.16	40.72	68.26	80.24	>1000	334.17^{ns}
Amphotericin B	100						$\leq$100	$\leq$100
Control	0							0

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- not significant .

Table 2. Percentage antifungal 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	ND	ND
STMLE	2.26	6.77	18.8	54.14	72.18	81.2	>1000	235.38^{ns}
STELE	1.5	26.3	42.86	66.92	81.2	94	>1000	162.09*
STCLE	0	0	2.256	1.504	13.53	43.6	>1000	$>1000^{ns}$
SCALE	0	0	0	0	0	0	ND	ND
SCMLE	0	36.1	62.41	88.72	100	100	500	95.3***
SCELE	0	33.1	52.63	73.68	100	100	500	116.52**
SCCLE	0	0	0	0	19.55	36.1	>1000	$>1000^{ns}$
SJALE	0	0	0	0	0	9.92	ND	$>1000^{ns}$
SJMLE	5.26	25.6	48.12	84.21	100	100	500	131.52**
SJELE	0.75	2.26	31.58	63.91	78.2	100	1000	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	500	114.51**
TIELE	6.77	26.3	48.12	63.16	76.69	82.7	>1000	140.62^{ns}
TICLE	0	0	0	0	0	0	ND	ND
Amphotericin B	100						$\leq$100	$\leq$100
Control	0						ND	ND

Values in the parenthesis represent 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- not significant .

CONCLUSION

In the present study the alcoholic extracts were effective against *A.flavus* and *A.fumigatus* which may be due to the presence of natural defensive compounds present 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[7-9] and practice of selected medicinal plants by tribes of Western Ghats in Coimbatore.

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CONFLICT OF INTEREST

We all authors declare that, we have no conflict of interest.

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