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ANTIFUNGAL ACTIVITIES OF FOUR *STROBILANTHES* SPECIES FROM NORTHERN WESTERN GHATS OF INDIA

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ABSTRACT

Antifungal activities of leaf and stem of *Strobilanthes ixiocephalus, Strobilanthes ciliatus, Strobilanthes sessilis* var. ritchiei, *Strobilanthes integrifolius* were studied for their potential against three fungal species i.e. *Aspergillus niger, Penicillium* sp., *Saccharomyces cerevisiae*. The antifungal activities were evaluated using agar well diffusion method. The strong antifungal activity was observed in the ethanolic and methanolic extracts of all four species than water extract, while the chloroform extract showed complete inhibition of growth. Among the plant species studied, *Strobilanthes integrifolius* revealed with highest inhibitory activity towards all three fungal species, followed by *Strobilanthes ciliatus, Strobilanthes sessilis* var. ritchiei and *Strobilanthes ixiocephalus.* These findings provide scientific evidence to support traditional medicinal uses and have a great significance for the development of an antifungal drug from these *Strobilanthes* species.

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INTRODUCTION

Plants are the basis for the development of modern drugs and medicinal plants have been used for many years in daily life to treat diseases all over the world (Mythili and Ravindhran, 2012)[1]. The increase in prevalence of multiple drug resistance has showed the development of new synthetic antifungal drugs and the new drugs are necessary to be used as alternative sources (Muthu *et al*., 2012)[2]. The use of the leaves, flowers, stem, seed berries and roots of the plants are known to prevent, relieve and treat illness. They also play vital role as an antifungal agent (Deshpande *et al*., 2005)[3].

Strobilanthes is a genus of perennial flowering herbs and shrubs belongs to the family Acanthaceae. In Genus, *Strobilanthes* some species bloom annually, others are plietesials and shows diversified habits and gregarious nature (Venu, 2006)[4]. Most of the species are frost-tender and require protection in frost-prone areas and these species show an unusual flowering behaviour, varying from annual to 16-year blooming cycles. *Strobilanthes* species is known for its medicinal uses such as antiarthritic, anti-inflammatory, gout, jaundice and rheumatism (Preethi and Suseem, 2014)[5]. In the present study, antifungal activity of both leaf and stem of *S. ixiocephalus, S. ciliatus, S. sessilis* var. ritchiei, *S. integrifolius* were investigated against three fungal species viz. *Aspergillus niger, Penicillium* sp., *Saccharomyces cerevisiae*.

The aim of the present study was to investigate the antifungal activity of leaf and stem of *Strobilanthes ixiocephalus, Strobilanthes ciliatus, Strobilanthes sessilis* var. ritchiei, *Strobilanthes integrifolius* against *Aspergillus niger, Penicillium* sp., *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh healthy leaves and stem of four *Strobilanthes* species viz. *Strobilanthes ixiocephalus* and *Strobilanthes ciliatus* were collected from Bondla, Ponda, Goa and *Strobilanthes sessilis* var. ritchiei was collected from Kavalesad, Amboli, Maharashtra. *Strobilanthes intigrifolius* was collected from Nagargao, Sattari, Goa. The plant materials were washed thoroughly with tap water and shade dried leaves were grinded to powder and stored in an air tight container for use.

Plate 1. Habit of *Strobilanthes* species. A. *Strobilanthes ixiocephalus,* B. *Strobilanthes ciliatus,* C. *Strobilanthes sessilis* var. ritchiei, D. *Strobilanthes integrifolius.*

Preparation of Extract

Two grams of leaf and stem powder was extracted with 20ml of various organic solvents like water, methanol, ethanol and chloroform. Later, slurry was filtered through muslin cloth. The extracts were condensed to 1/10th volume by heating at 50-60°C in water bath and then it was filtered using Whatman No. 1 filter paper. The filtrate was centrifuged at 2500 rpm for 15min and the supernatant was collected in sterile bottles.

Evaluation of Antifungal Activity

Culture of fungal species

Pure culture of fungal species such as *Aspergillus niger, Penicillium* sp. and *Saccharomyces cerevisiae* were obtained from Goa University Fungus Culture Collection (GUFCC), Department of Botany, Goa University, Goa, India. Fungal species were sub cultured using Malt Extract medium. Media was poured in the pre- sterilised Petri plates and kept for solidification. Loop full of pure culture from the plate was taken and it was streaked on the plate to obtain fine isolated colonies. The Petri plates were incubated at 37°C for 1 week.

Preparation of fungal suspension

Fungal suspensions were prepared by transferring one loop full of sub culture of respective fungal species to 5ml of sterile distilled water in a conical flask. The suspension was incubated at low temperature in refrigerator for 24 hours.

Agar Well Diffusion Method

Spreading of fungal Suspension

Malt Extract Agar media was poured on to sterile Petri plates and allowed for solidification. The plates with media were seeded with respective fungal suspension (200µl) using sterile swab and with the help of sterile glass spreader the suspension was spread on the plates uniformly under aseptic conditions. The suspensions on plates were dried for 1 hour.

Preparation of Wells

Wells were made with the help of sterile cork borer having a size of 7 mm diameter at four different places in a plate.

Determination of minimum inhibitory concentration (MIC)

The extracts of different concentrations such as 100, 200 and 300 µl were added individually to respective three different wells using micropipette. The antibiotic tetracycline hydroxide (300 µl) was added in fourth well as a positive control. Later the plates were kept for incubation in an incubator at 37° C for 1 week. After incubation period, antifungal activity was determined by measuring the zone of inhibition around each well and expressed in mm (Harkare *et al*., 2013)[6]. All the above procedures were carried out in a laminar air flow system under sterile aseptic condition.

RESULTS AND DISCUSSION

The antifungal activity of crude leaves and stem extracts of *S. ixiocephalus, S. ciliatus, S. sessilis* var. ritchiei, *S. integrifolius* were studied in different concentration (100µl, 200µl, 300µl). As positive control 300µl of tetracycline hydroxide was used. The leaves and stem extract of *S. ixiocephalus, S. ciliatus, S. sessilis* var. ritchiei, *S. integrifolius* was extracted using different solvent (water, methanol, ethanol and chloroform), their antifungal activities were studied against *Aspergillus niger*, *Penicillium* sp., *Saccharomyces cerevisiae.* The result revealed that among the extracts, ethanol extract showed high inhibitory effect followed by methanol extract at diffenent concentrations (100µl, 200µl, 300µl) against the three fungal species used. The water solvent extracts exhibited low activity on all species of fungi and chloroform extract showed complete inhibitory activity against all three fungi. Therefore, no inhibition zones were noted in chloroform extract. Among the different concentrations of extract evaluated, 300µl concentrations of extract exhibited excellent inhibitory effect, even more than the positive control antibiotic, tetracycline hydroxide against three fungal species *Aspergillus niger*, *Penicillium* sp., *Saccharomyces cerevisiae* (Tables 1-4).

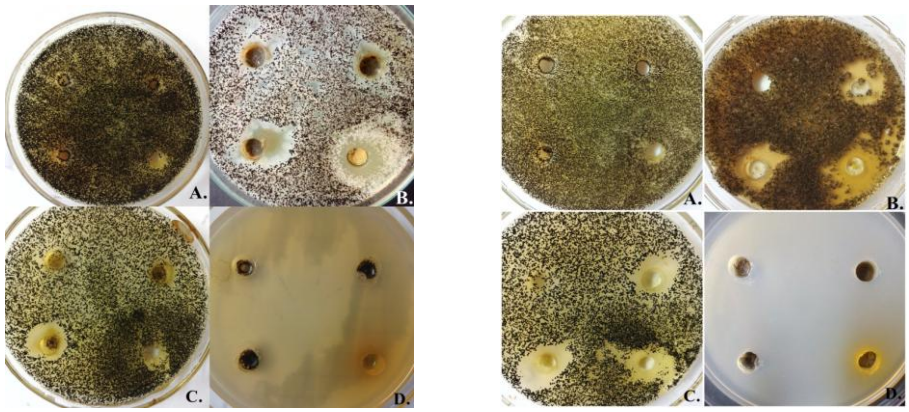
S. ixiocephalus, methanol extract of leaf showed higher activity against *penicillium* sp. (0, 3, 10mm) and ethanol extract against *Aspergillus niger* (5, 4, 10mm) and *Saccharomyces cerevisiae* (0, 2, 9mm) (Table 1). Ethanol extract of stem revealed high inhibitory effect against all three fungi (Fig. 1). The zone size measured was 2, 5, 7mm for *Aspergillus niger*; 3, 3, 8mm for *Penicillium* sp., and 1, 2, 8 mm for *Saccharomyces cerevisiae* (Table 1).

Table 1. Antifungal Activity of *Strobilanthes ixiocephalus*.

Fungal species	Concentration (µl)	Water		Methanol		Ethanol		Chloroform	
		Zone of inhibition in (mm)							
		L	S	L	S	L	S	L	S
Aspergillus niger	100µl	2	0	0	0	5	2	-	-
	200µl	3	0	3	6	4	5	-	-
	300µl	3	0	0	7	10	7	-	0
	+ve control	5	0	4	6	10	9	-	-
Penicillium sp.	100µl	0	3	0	2	2	3	0	-
	200µl	0	4	3	3	0	3	-	-
	300µl	0	10	10	5	7	8	-	-
	+ve control	0	0	3	2	3	4	0	-
Saccharomyces cerevisiae	100µl	0	0	0	0	0	1	-	0
	200µl	2	0	1	4	2	2	-	0
	300µl	0	0	6	7	9	8	-	-
	+ve control	2	0	0	0	0	0	-	-

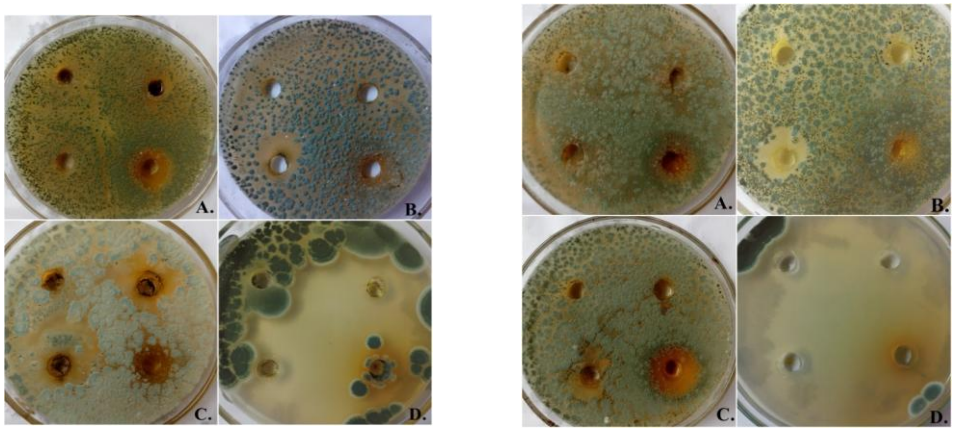
(-) indicates No Growth, (0) indicates No Zone of inhibition
L, leaf extract; S, stem extract

S. ciliatus, methanol extract of leaf exhibited higher antifungal activity against *Aspergillus niger* (3, 5, 8mm) and ethanol extracts against *Penicillium* sp. (2, 13, 17mm) and *Saccharomyces cerevisiae* (3, 6, 10mm) (Table 2). The ethanol extract of stem showed high inhibitory effect against all three fungi (Fig 1). The zone size measured was 0, 6, 9mm for *Aspergillus niger*; 1, 0, 8mm for *Penicillium* sp. and 1, 11, 15mm for *Saccharomyces cerevisiae* (Table 2).



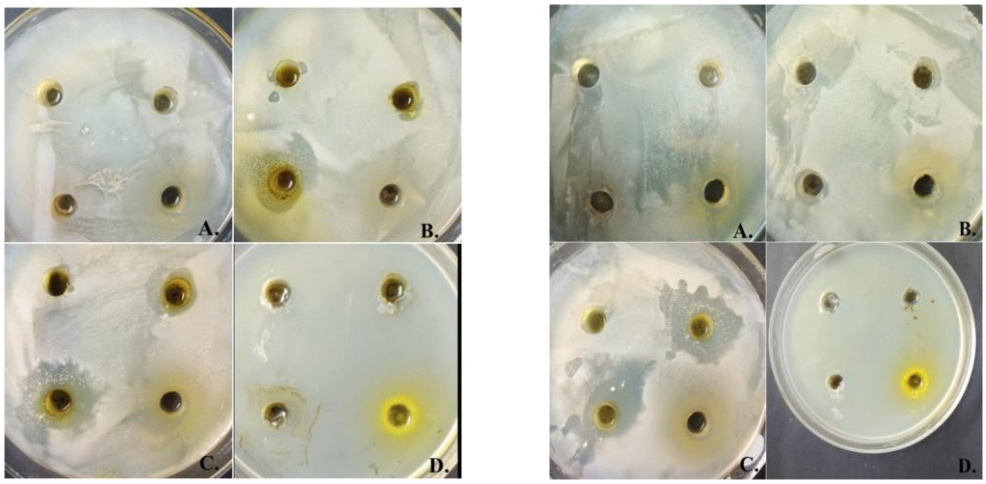
Leaf extract **Stem extract**

Plate 2. Antifungal activity of *Strobilanthes ciliatus* (Leaf & Stem). Images showing inhibition zone against *Aspergillus niger*. A. Water extract, B. Methanol extract, C. Ethanol extract, D. Chloroform extract.



Leaf extract **Stem extract**

Plate 3. Antifungal activity of *Strobilanthes ciliatus* (Leaf & Stem). Images showing inhibition zone against *Penicillium* sp. A. Water extract, B. Methanol extract, C. Ethanol extract, D. Chloroform extract.



Leaf extract **Stem extract**

Plate 4. Antifungal activity of *Strobilanthes ciliatus* (Leaf & Stem). Images showing inhibition zone against *Saccharomyces cerevisiae*. A. Water extract, B. Methanol extract, C. Ethanol extract, D. Chloroform extract.

Table 2. Antifungal Activity of *Strobilanthes ciliatus*.

Fungal species	Concentration (µl)	Water		Methanol		Ethanol		Chloroform	
		Zone of inhibition in (mm)							
		L	S	L	S	L	S	L	S
Aspergillus niger	100µl	0	0	3	0	1	0	-	-
	200µl	0	0	5	6	2	6	-	-
	300µl	0	0	8	7	6	9	-	-
	+ve control	0	0	5	6	2	7	-	-
Penicillium sp.	100µl	4	0	0	3	2	1	0	-
	200µl	3	0	3	3	13	0	-	-
	300µl	2	0	8	8	17	8	-	-
	+ve control	4	0	3	4	5	6	0	-
Saccharomyces cerevisiae	100µl	0	0	5	0	3	1	-	-
	200µl	0	0	6	1	6	11	-	-
	300µl	1	0	8	2	10	15	-	-
	+ve control	2	3	0	3	2	2	-	-

(-) indicates No Growth, (0) indicates No Zone of inhibition
L, leaf extract; S, stem extract

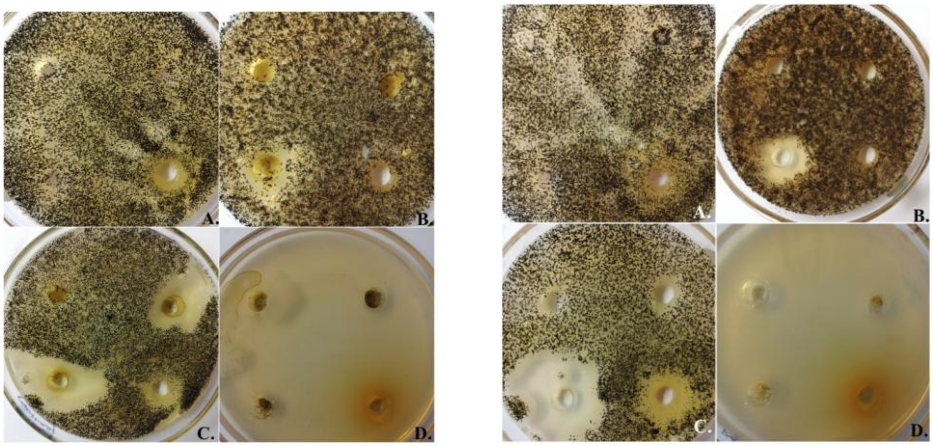
S. sessilis var. ritchiei leaf and stem extract was also extracted using different solvents. As compared to other solvents, ethanol extract showed higher activity for both leaf and stem (Fig. 1). The zone size measured was 0, 5, 11m) in leaves, 3, 5, 15mm in stem for *Aspergillus niger*; 0, 0, 4mm in leaf, 0, 7, 12mm in stem for *Penicillium* sp.; 1, 15, 10 mm in leaf, 3, 4, 10mm in stem for *Saccharomyces cerevisiae* (Table 3).

Table 3. Antifungal Activity of *Strobilanthes sessilis* var. ritchiei.

Fungal species	Concentration (µl)	Water		Methanol		Ethanol		Chloroform	
		Zone of inhibition in (mm)							
		L	S	L	S	L	S	L	S
Aspergillus niger	100µl	0	0	0	0	0	3	-	-
	200µl	0	0	0	0	5	5	-	0
	300µl	0	0	0	4	11	15	-	-
	+ve control	0	3	2	3	4	8	-	-
Penicillium sp.	100µl	0	2	0	5	0	0	0	0
	200µl	0	6	0	6	0	7	-	-
	300µl	0	0	2	6	4	12	-	-
	+ve control	0	0	0	4	0	6	0	0
Saccharomyces cerevisiae	100µl	0	0	0	0	1	3	-	-
	200µl	0	0	0	10	15	4	-	-
	300µl	5	0	0	0	10	10	-	0
	+ve control	2	3	0	2	2	2	-	-

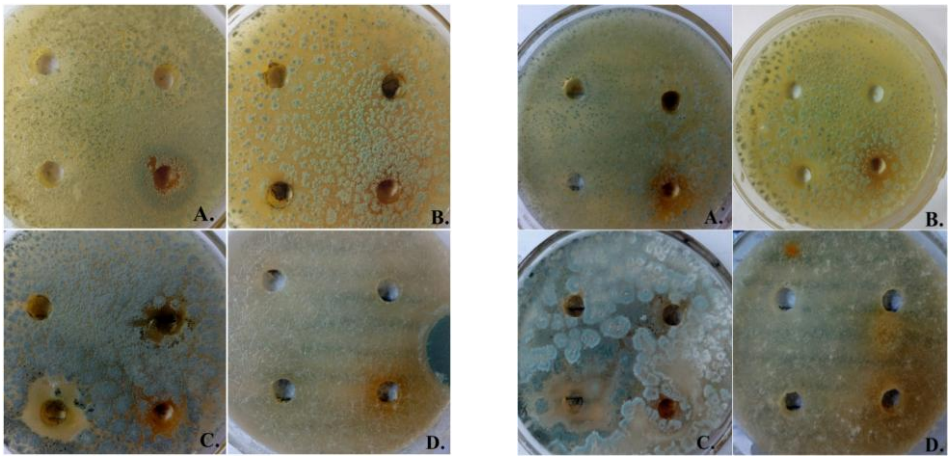
(-) indicates No Growth, (0) indicates No Zone of inhibition
L, leaf extract; S, stem extract

Similarly *S. integrifolius* ethanol extracts of leaf and stem showed higher antifungal activity against all three fungi (Fig.1). The inhibition zone size was measured for leaf (0,10,16mm) and stem (2, 3, 17mm) against *Aspergillus niger*; 0, 4, 8mm for leaf extract and stem extract 0, 3, 8mm against *Penicillium* sp.; 0, 5, 13mm for leaf and stem 1, 7, 14 against *Saccharomyces cerevisiae* (Table 4).



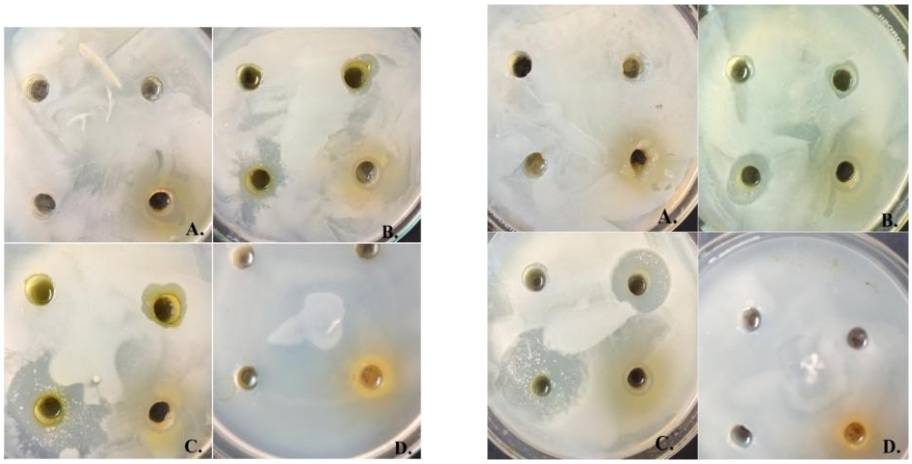
Leaf extract Stem extract

Plate 5. Antifungal activity of *Strobilanthes integrifolius* (Leaf & Stem). Images showing inhibition zone against *Aspergillus niger*. A. Water extract, B. Methanol extract, C. Ethanol extract, D. Chloroform extract.



Leaf extract Stem extract

Plate 6. Antifungal activity of *Strobilanthes integrifolius* (Leaf & Stem). Images showing inhibition zone against *Penicillium* sp. A. Water extract, B. Methanol extract, C. Ethanol extract, D. Chloroform extract.



Leaf extract Stem extract

Plate 7. Antifungal activity of *Strobilanthes integrifolius* (Leaf & Stem). Images showing inhibition zone against *Saccharomyces cerevisiae.* A. Water extract, B. Methanol extract, C. Ethanol extract, D. Chloroform extract.

Table 4. Antifungal Activity of *Strobilanthes integrifolius.*

Fungal species	Concentration (µl)	Water		Methanol		Ethanol		Chloroform	
		Zone of inhibition in (mm)							
		L	S	L	S	L	S	L	S
Aspergillus niger	100µl	0	0	0	0	0	2	-	-
	200µl	0	0	0	0	10	3	-	-
	300µl	0	0	4	8	16	17	-	-
	+ve control	3	0	2	2	5	6	-	-
Penicillium sp.	100µl	0	0	0	3	0	0	-	-
	200µl	0	0	0	4	4	3	-	-
	300µl	0	0	5	5	8	8	-	-
	+ve control	0	4	0	4	5	0	-	-
Saccharomyces cerevisiae	100µl	0	0	0	1	0	1	-	-
	200µl	0	0	4	3	5	7	-	-
	300µl	0	0	8	6	13	14	-	-
	+ve control	0	0	3	2	2	1	-	-

(-) indicates No Growth, (0) indicates No Zone of inhibition
L, leaf extract; S, stem extract

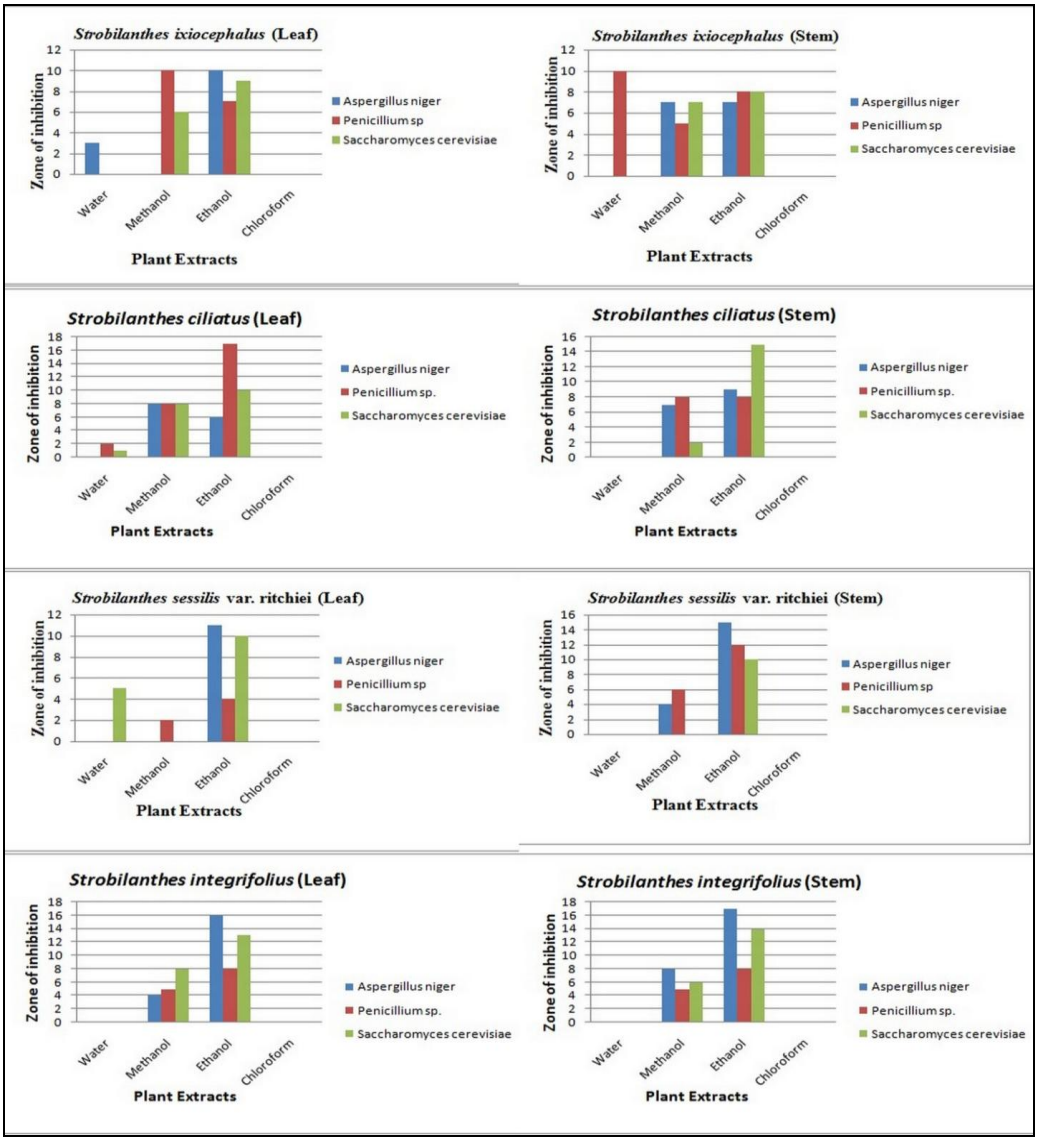


Fig 1. Comparison of different solvent extractions at 300µl concentration of leaf and stem of *S. ixiocephalus, S. ciliatus, S. sessilis* var. ritchiei, *S. integrifolius* against antifungal activity.

The study revealed that ethanol extract followed by methanol extract of leaf as well as stem of all four species showed more antifungal activity against three fungi used (Fig. 1). Further, observed that chloroform extract exhibited complete inhibition of growth against three fungi in all plant species. Therefore, inhibition zones were measured as 'minus (-)' (Table 1-4). Water extract as compared to other three extracts displayed no or less inhibitory effects against all three fungi tested with a relatively smaller zone of inhibition area ranging from 0-3mm (Fig. 1).

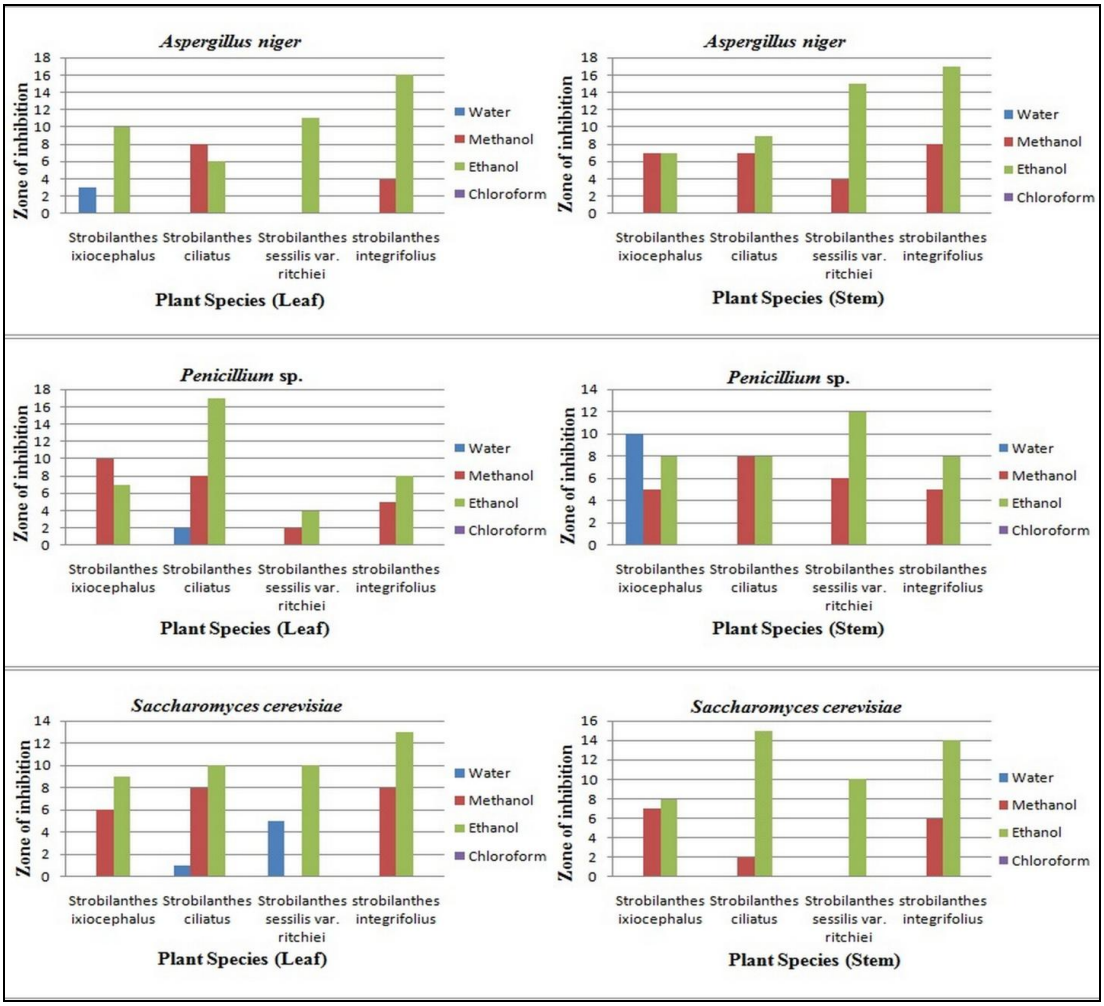


Fig 2: Antifungal activity of crude leaf and stem extracts against *Aspergillus niger*, *Pennicillium* sp., *Saccharomyces cerevisiae* in comparison to different plant species viz., *S. ixiocephalus*, *S. ciliatus*, *S. sessilis* var. ritchiei, *S. integrifolius* with different solvent extract at 300µl concentration.

In the present study revealed that the ethanol extract of *S. integrifolius* and *S. sessilis* var. ritchiei was found to be more effective against *Aspergillus niger* (Fig. 2). *S. ciliatus* and *S. integrifolius* showed high potential against *Penicillium* sp. and *Saccharomyces cerevisiae*. Whereas *S. sessilis* var. ritchiei and also *S. ciliatus* showed more or equal inhibitory activity or slightly greater against *Saccharomyces cerevisiae* and *S. ixiocephalus* showed less or equal antifungal activity against all three fungi (Fig. 2).

Among the plant species evaluated, ethanol extract of *S. integrifolius* showed highest inhibitory effect against *Aspergillus niger*, *Saccharomyces cerevisiae*. Similarly, ethanol extract of S. *ciliatus* was found to be more effective against *Penicillium* sp. (Fig. 6). When methanol extract of *S. integrifolius* and *S.ciliatus* compared with each other, they showed more or less equal zone of inhibition or slightly greater against some fungi. *S. sessilis* var. ritchiei showed equal effect against all three fungi studied. The *S. ixiocephalus* showed slightly lower inhibitory activity as compared to other three plant species (Fig. 2).

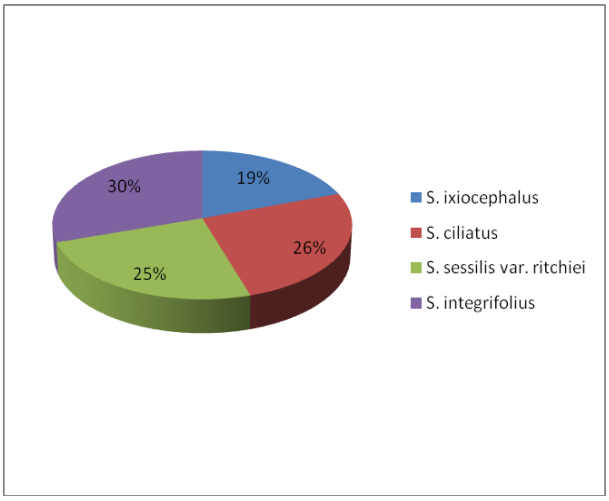


Fig. 3. Overall inhibitory activity of ethanol extract of both leaves and stem of all four species against all three fungi used at 300µl.

From the above study, it is found that ethanol followed by methanol extract was found to be more effective against three fungi used. The antifungal activity at the 300µl concentration was found excellent when compared with positive control antibiotic tetracycline hydroxide. The order of species inhibiting fungus is as follows: *S. integrifolius* > *S. ciliatus* > *S. sessilis* var. ritchiei > *S. ixiocephalus* (Fig. 3).

In the present study, *S. integrifolius. S. ciliatus, S. sessilis* var. ritchiei and *S. ixiocephalus* were evaluated for anti-fungal activities. Among the plant species, strong antifungal activity was noted in *S. integrifolius* followed by *S. ciliatus*. Antifungal activity may be due to the presence of lupeol in the plant species (Venkatachalapathi and Subban, 2013)[7]. Kavitha *et al*., (2015)[8] noted the presence of flavanoids, phenolic compounds, tannins, steroids, glycosides and triterpenoids in *strobilanthes ciliatus*. Shende *et al*., (2015)[9] found the presence of flavonoids, steroids and glycosides in *strobilanthes sessilis*. Rupali and Suresh, (2012)[10] carried out the phytochemical studies which revealed the presence of alkaloids, steroids, flavanoids, glycosides, carbohydrates in *Strobilanthes ixiocephalus*. The tri-terpenoids present in *S. callosus* showed the antimicrobial activities (Singh *et al*., 2002)[11]. These phytochemical compounds play a significant role in contributing this activity.

CONCLUSION

The antifungal study of leaf and stem of *S. integrifolius, S. ciliatus, S. sessilis* var. ritchiei, *S. ixiocephalus* demonstrated that the plants have considerable efficacy against various fungi viz, *Aspergillus niger*, *Penicillium* sp, *Saccharomyces cerevisiae*. The ethanol and methanol extract showed significant inhibitory activity against all three fungal species tested. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antifungal drug from *S. integrifolius* and *S. ciliatus*. This work has a great importance in pharmaceutical industries for preparing plant based antifungal drugs.

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