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Antifungal activity of seeds of *Alangium salvifolium* (L.f.)Wangerin (Alangiaceae)

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Various extracts (Petroleum ether, Acetone, Chloroform, Methanol and water) of seeds of *Alangium salvifolium* (l.f) wangerin were investigated for their *in vitro* antifungal properties. The antimicrobial activity of the extract was tested against the pathogenic fungi, *Aspergillus flavus, Fusarium oxysporum*, *Cladosporium fulvum* and *Mucor racemosus.* Methanol was the best solvent to extract when compared to other extracts. The strongest inhibitory effect of the extracts was observed against *Aspergillus flavus* and weakest effect was against *Cladosporium fulvum.* Result clearly indicate that the seeds of *Alangium salvifolium* is a promising source of antimicrobial compounds.

Key words: *Alangium salvifolium,* Antifungal activity, Alangiaceae and mycotoxins.

In India, different parts of several medicinal plants or their extracts are used for the treatment of various diseases. Several antibiotics used for the treatment of human infections, which have limited antimicrobial spectrum. They could develop drug resistance in pathogens and lead to serious ill effect. Hence, plant derived antimicrobial properties have received considerable attention in recent years. Several plants have been indicated in folk and other traditional system of medicine as aseptic agents. Efforts are thus directed to identify the plant products which have broad spectrum of antimicrobial property with no ill effects. The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigation on plants as a source of new biomolecules for human disease management.

Fungi cause severe damage to store food commodities. Among different species of fungi *Aspergillus* sp., *Fusarium* sp. and *Pencillium* sp. are associated with heavy loss of grains, fruits, vegetables and other plant products during pickling, transit and storage rendering them unfit for human consumption by producing mycotoxins and affecting their nutritive value[1-3]. In recent time application of plant metabolite for plant disease management has become important viable component of Integrated Pest Management, as plant metabolites are eco-friendly.

A. salvifolium is a small deciduous tree or shrub, belongs to the family alangiaceae,which grows in the wild throughout the hotter parts of India the major phytochemical constituents of the plant are alangine A and B, alangicine, markindine, lamarckinine and emetine. The root of *A. salvifolium* has been used in the Indian system of medicine as an acrid, diuretic, astringent and

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antidote for several poisons. The fruits of the plant are useful in treating burning sensation and haemorrhages[4]. However, no scientific evidence is available regarding its antimicrobial activity. The present study was carried out to investigate the effect of the seed extract of *A. salvifolium* on pathogenic fungal strains.

MATERIAL AND METHODS

Collection and Authentification of plant material

The seeds for the proposed study were collected from Bolluvampatty village, of Coimbatore District, Tamilnadu, India. Flowering shoots of the plant were also collected for identification. The collected plant material was identified and their authenticity was confirmed by the voucher specimen at the Botanical Survey of India (BSI), Southern circle, Coimbatore, Tamilnadu. The voucher specimens (AS-1010-1021) were deposited in the Ethnobotany unit, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu.

Preparation of extracts

The collected seeds from *Alangium salvifolium* plant were thoroughly washed in tap water and shade dried for about 15-30 days and made into coarse powder with mixer grinder. The powder obtained was passed through 60 mesh sieve plate and then used for extraction. Powder is extracted successively with petroleum ether, acetone, chloroform and methanol in a Soxhlet extraction for 20 hr. the extract was evaporated to dryness at 37 ^{0}C. The aqueous extract was prepared by soaking 30g of powder in 200ml of distilled water. After 24 hrs. elapsed with interval stirring, the mixture were filtered using Whatman No.1 filter paper and the filtrate was left for dryness by evaporation using steam bath at 100^{0}C. The dried extracts were stored at 4^{0}C for further investigations.

Analysis of bioactive compounds

The various extracts were analyzed for alkaloids, tannins, phenols, steroids, glycosides, saponins, flavonoids and fixed oils were carried out by standard procedure [5,6]

Test pathogens

Fungal pathogens like *Aspergillus flavus, Fusarium oxysporum*, *Cladosporium fulvum* and *Mucor racemosus* were the pathogens selected for the study. The fungal species in potato dextrose agar slants and stored at 4^{0}C.

Antimicrobial screening (Disc diffusion method[7])

The agar diffusion method was used to evaluate the antifugal effect of the seed extracts. Inoculums of each of the microbial strain was suspended in 2 ml of respective broth solution and incubated overnight at 30ºC. To screen for antimicrobial activity, sterile agar plates were used according to the disc diffusion assay. The contents of the media (15 ml) were poured into a sterile clean and dry Petri plates, then allowed the media to settle down. A bent glass rod (L - rod) was used for spreading diluted cultures on the plate then the sample extract impregnated sterile discs (6 mm in diameter) were kept on the seeded agar plates. Plates of culture were incubated 30ºC in the dark. Zones of inhibition were examined after 24-30 hr and they were measured using transparent ruler in millimeters. Assays were run in triplicates and the mean values were tabulated. The minimum inhibitory concentration (MIC) of methanol was also determined by turbidity method.

RESULTS AND DISCUSSIONS

The various extracts of seeds of *A. salvifolium* had antimicrobial effects against all fungi studied. Activity against test microorganisms showed in table 1. In general methanolic extract exhibited stronger activity followed by acetone, petroleum ether, chloroform and water.

Antifungal activity

Solvent extracts

The greatest zone of inhibition was observed in methanol extract against the fungi *A. flavus*, (16.7mm) and lowest inhibition zone was observed in petroleum ether against the fungi *C. fulvum* (6.3mm) (Table 1). Rest of the test pathogens exhibited moderate activity. The MIC results are shown in Table 2. Antifungal agents with low activity against an organism have high MIC value while highly active antifungal agents have low MIC value.

Aqueous extract

The aqueous extract has shown moderate activity on all the pathogens studied likely *Cladosporium fulvum*(9mm,) *Mucor racemosus*

Table 1. Antifungal activity of *A. salvifolium* seeds

Extracts (100mg/ml)	Zone of inhibition (mm)			
	A. flavus	*F. oxysporum*	*C. fulvum*	*M. racemosus*
Petroleum ether	10.3	12.5	6.3	8.3
Chloroform	10.2	9.3	9.3	5.3
Acetone	13.3	16.2	12.3	14.3
Methanol	16.7	15.0	10.2	14.3
Aqueous	9.1	8.0	9.0	8.8
Amphotericin	19.1	15.2	10.4	16.5

Table 2. MIC results of methanolic extract

Extract	Pathogens	MIC value in mg/ml
Methanol	*A. flavus*	2.4
	F. oxysporum	3.5
	C. fulvum	4.6
	M. racemosus	3.8

MIC-Minimum inhibitory Concentration

(8.8mm), *Aspergillus flavus* (9.1mm) and *Fusarium oxysporum* (8mm).

Phytochemical analysis

Phytochemical analysis of methanol extract revealed the presence of fixed oils and fats, gums and mucilages, alkaloids, saponins, glycosides, flavonoids, terpenoids, phenols, tannins and steroids (Table 3).

In the present study on the antimicrobial activity of *A. salvifolium* against plant pathogenic

Table 3. Bioactive compounds of *A. salvifolium* seeds

Bioactive compound	Petroleum ether	Chloroform	Acetone	Methanol
Fixed oils and fats	++	+	-	-
Gums and mucilages	+	-	-	-
Alkaloids	-	+	++	+++
Saponins	-	-	-	+++
glycosides	-	+	-	-
Flavonoids	-	-	++	+++
Terpenoids	++	++	-	-
Phenols	+	+	++	+++
tannins	-	++	+++	+++
steroids	++	++	-	-

The number of + indicates the intensity of reaction compound present and – indicates the absence of compound.

fungi, seed extracts proved a promising source of antimicrobial compounds. Variations in the antifungal effectiveness of different extracts against different organisms were most likely due to differences in the nature of the inhibitory materials they contained.[8] results from our phytochemical analysis (Table 3) suggest that the presence of biologically active compounds like carbohydrate, glycosides, proteins, aminoacids, alkaloids, phenol and tannins in the plant extracts could be related to the antifungal activity[9,10]. The mechanism (s) of action of the constituents of *A. salvifolium* might be due to the inhibition of fungal cell wall, protein and amino acid, sphingolipid biosynthesis and electron transport chain [11,12]

The higher plants may play an important role in controlling many plant diseases. Plants produce natural chemicals that are possible sources of non-phytoxic, systemic and readily biodegradable alternative pesticides[13]. This is the first paper reporting the antifungal activity of seeds of *A. salvifolium* crude extract. The finding suggest

that *A. salvifolium* seeds are potential source of compounds that are effective against many fungi (Table 2). Further studies on *A. salvifolium* seeds are recommended to identify the antifungal compounds.

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