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Antifungal Activity of *Rhizophora apiculata* Leaf Extract for the Management of Rice Sheath Blight

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ABSTRACT

The present study was undertaken to assess the antifungal activity of different solvent extract of *Rhizophora apiculata* against *R. solani* and evaluation of its antimicrobial compounds. Different solvent extracts of *R. apiculata* (15%) considerably inhibited the growth of test pathogen when compared to control. The highest percent inhibition (95.82%) over control recorded with methanol extract which accounted with minimum mycelial growth of 3.76mm followed by aqueous, ethanol and choloroform extract of *R. apiculata* (15%) accounting 93.37, 91.45 and 89.37 per cent inhibition in the decreasing order of merit respectively. The maximum mycelial growth (13.58 mm) was recorded with acetone extract which account for the lowest per cent inhibition (84.91%) over control. The standard chemical fungicide Hexaconazole 5% EC at 0.1% concentration showed 100 per cent inhibition of the test pathogen. Also, lyoniresinol-3α-O-β-arabinopyranoside was recorded highest peak area (69.757%) with retention time RT (8.013 min). My-3-O-ribose was recorded least peak area (2.144%) with retention time RT (13.042 min). Remaining compounds are elevated at various retention time under HPLC analysis.

***Key words*:** *R. solani, R. apiculata,* Solvent extract, HPLC analysis

Rice (*Oryza sativa* L.) is one of the most widely grown cereal crop in the world. Among various fungal diseases of rice, sheath blight is a very destructive disease in rice growing regions and causing substantial yield losses on rice cultivation [1]. Sheath blight caused by *Rhizoctonia solani* and yield loss was estimated upto 45% [2]. The highly effective method for controlling plant diseases is application of fungicide. However, another concern over fungicide use, fungicides are non- biodegradable and associated with hazard to human and natural ecosystems. Botanicals is the integral part and safer alternative method of plant disease management. Hence, being focused on the alternative methods for pathogen control which are eco-friendly and also enhance the crop yield. Botanicals are now emerging as safer and more compatible approach to control phytopathogens [3].

Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth [4]. Among the botanicals, mangroves are rich in various secondary metabolites and are widely used in the traditional medicine practices [5].

The antifungal activity of some mangrove species has been well documented against plant pathogens viz., *Avicennia marina* against *Alternaria citri*, *Avicennia marina* and *Rhizophora mucronata* against *A. alternata*, *Rhizophora apiculata* against *Macrophomina phaseolina* [6-7]. More than 200 bioactive compounds identified from mangroves with antibacterial and antifungal properties belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics [8-10].

cathertica), Meheadi (*Lawsonia alba*) and Duranta (*Duranta plumeiri*) [11]; *Pithecellobium dulce* [12]; *Z. jujuba* and *I. carnea* [13]; *Saraca asoca* [14]; *Alhagi camelorum* [15]; garlic [16]. Leaf extract of *R. apiculata* showed antifungal compounds against fungal pathogen *M. phaseolina* [17]. Meanwhile *R. apiculata* exhibited antifungal principles while against *Penicillium sp.* and *A. alternata* [18]. Methanol leaf extract of *R. apiculata* effectively inhibits the growth of *Xanthomonas oryzae pv. oryzae* and assume that the most prevailing components are methyl 4-O-methyld-arabinopyranoside (38.66%) and 1, 6, 10, 14Hexadecatetraen-3, 7,11,15-tetramethyl-(E,E) (30.24%) are quite reasonable for its antibacterial activity [19]. Hence, the present study was undertaken to assess the antifungal activity of *R. apiculata* against *A. solani* and evaluation of their chemical compounds (GC-MS).

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MATERIALS AND METHODS

Source of the pathogen and plant extract

The test pathogen *R. solani* (Rs. 5) and the mangrove species *R. apiculata* that have being used in this study, were selected based on their previous results in the study of [20], where they demonstrated potent pathogenicity and in vitro antifungal potential, respectively.

Preparation of solvent extract of R. apiculata

The leaves of *R. apiculata* was washed with both tap and distilled water to remove any epiphytes present and other wastes and then dried under shade for 3 weeks. The dried plant material was ground to fine powder (15 gm dry weight) and then extracted with 100ml of each solvent (acetone, chloroform, ethanol, ethyl acetate and methanol) separately for 48 h., using a Soxhlet extractor according to the method of Bele *et al*. [21] and then the extract was filtered through a Buchner funnel and using Whatman No. 1 filter paper. This was repeated three times for the complete extraction of solvent. The solvent was evaporated from crude extract by a rotary evaporator. The dry extract was stored at 4°C until further use [22].

Poisoned food technique [23]

Potato dextrose agar PDA medium mixed separately with various species mangrove leaf extracts at different concentrations viz., 5, 10, 15 and 20% poured into sterile petri dishes, allowed to cool and solidify. Mycelial disc (9mm) of 15 days old culture of test pathogen placed at the center of the petri dishes and incubated at 28±2°C for 7 days. The PDA medium with the same concentration of sterile distilled water alone served as control. Similarly, a fungicide viz., Hexaconazole (0.1% conc.) as also tested against the pathogen for comparison for each treatment. The experiments was replicated thrice and the per cent inhibition of mycelial growth if any was determined by the formula:

$$PI = C - T / C \times 100$$

Where;
PI = Percent inhibition,
C = Diameter growth of *R. solani* in control
T = Diameter growth *R. solani* in treated

HPLC analysis

10 g of dried leaf powder of *R. apiculata* was added into a flask with 150 ml of methanol. The flask was placed onto a magnetic stirrer at 50°C for 40 min. After that, the solution was filtered with the help of filter paper. Furthermore, the filtered solution was concentrated using a rotary evaporator; then, the concentrate was collected and kept in a refrigerator at 4°C until the time of the experiment. Samples were analyzed by using Agilent 1200 high-performance liquid chromatography (HPLC) system, and the UV detector was set at 254 nm with a column size of 250 mm x 4.6 mm (5 µm) Hypersil Gold C18 (Thermo Electron Corporation). Furthermore, the combination of water, methanol and acetonitrile (45/40/15 v/v/v) has been used as a mobile phase. The injection volume was 10 µl, and the flow rate was set at 0.7 ml/min. Methanol has been used as a solvent for the preparation of sample and standard. For sample preparation, 10 mg of leaf extract of *R. apiculata* was dissolved in 10 ml of the solvent same as standard preparation.

Statistical analysis

The statistical analysis of the experimental results was performed employing the computer software package 'SPSS', by Duncan Multiple Range Test (DMRT) and the values are expressed as mean. The data on percent infection and disease severity was arcsine transformed before undergoing statistical analysis [24].

RESULTS AND DISCUSSION

Effect of different solvent extract of R. apiculata (15%) on the growth of R. solani

Different solvent extracts of *R. apiculata* (15%) considerably inhibited the growth of test pathogen when compared to control. The highest percent inhibition (95.82%) over control recorded with methanol extract which accounted with minimum mycelial growth of 3.76mm followed by aqueous, ethanol and choloroform extract of *R. apiculata* (15%) accounting 93.37, 91.45 and 89.37 per cent inhibition in the decreasing order of merit respectively. The maximum mycelial growth (13.58 mm) was recorded with acetone extract which account for the lowest per cent inhibition (84.91%) over control. The standard chemical fungicide Hexaconazole 5% EC at 0.1% concentration showed 100 per cent inhibition of the test pathogen (Table 1). Hence, the solvent extract (methanol extract) was used for HPLC analysis for identification of compounds and the aqueous extract of *R. apiculata* was used for further studies.

Table 1 Effect of different solvent extract of *R. apiculata* (15%) on the growth of *R. solani*

Solvent extract *R. apiculata*	Radial growth (mm)	Percent inhibition over control
Ethanol	7.69^{d}	91.45
Ethyl acetate	11.66^{f}	85.76
Choloroform	9.56^{e}	89.37
Acetone	13.58^{g}	84.91
Aqueous extract	5.96^{c}	93.37
Methanol	3.76^{b}	95.82
Hexaconazole 5% EC	0.0^{a}	100
Control	90.00^{h}	-

Values are mean of three replications
In column means followed by same letter(s) are not significantly different (P=0.05) by DMRT

HPLC analysis of Rhizophora apiculata

The analysis of the dried leaf powder of *Rhizophora apiculata* identified the compounds of lyoniresinol-3α-O-β-arabinopyranoside, Afzelechin-3-O-L-rhamno-pyranoside, My -3-O-(600-O-galloyl)-hexose, My-3-O-ribose and Q-3-O-rhamnose (quercitrin). Among these compounds,

lyoniresinol-3α-O-β-arabinopyranoside was recorded highest peak area (69.757 percent) with retention time RT (8.013 min). My-3-O-ribose was recorded least peak area (2.144 percent) with retention time RT (13.042 min). Remaining compounds are elevated at various retention time (Table 2, Fig 1).

Table 2 HPLC analysis of *Rhizophora apiculata*

Peak	Retention time	Area	Height	Area (%)	Height (%)	Compound name
1.	8.013	12811051	307991	69.757	70.324	Lyoniresinol-3α-O-β-arabinopyranoside
2.	10.530	3169695	68042	17.259	15.536	Afzelechin-3-O-L-rhamno-pyranoside
3.	12.213	1416029	33931	7.710	7.747	My-3-O-(600-O-galloyl)-hexose
4.	13.042	393755	16341	2.144	3.731	My-3-O-ribose
5.	22.984	574851	11657	3.130	2.662	Q-3-O-rhamnose (quercitrin)
	Total	18365380	437963	100.000	100.000	-

PDA Multi 1: 1/254nm 4 nm; PDA Multi 2: 2/280nm 4 nm

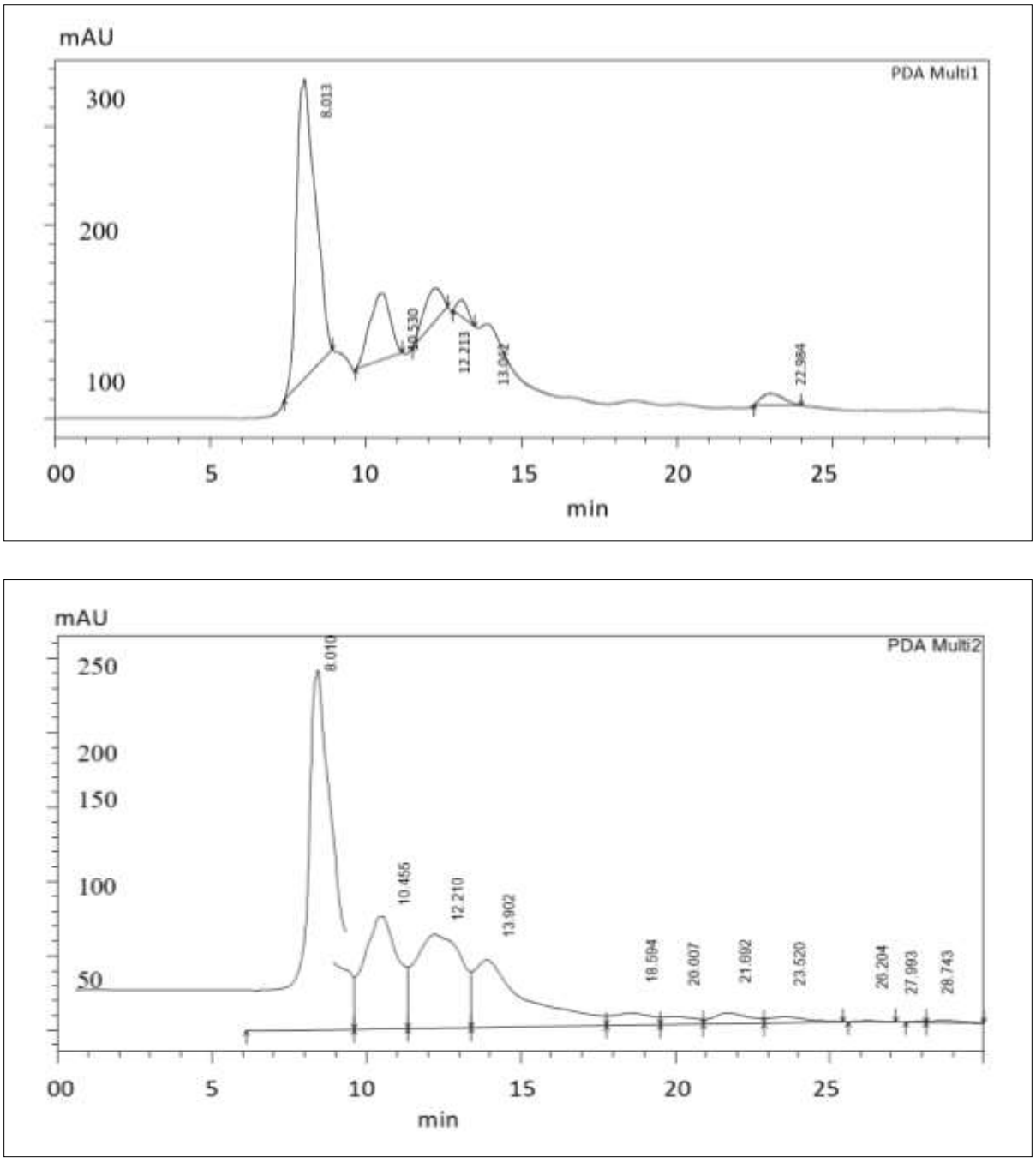

Fig 1 HPLC chromatogram of *Rhizophora apiculata*

The plant extracts have various secondary metabolites compounds such as alkaloids, quaternary alkaloids, coumarins, flavonoids, steroids, terpenoids, phenols etc., have been reported by several workers [25-27], these secondary metabolites possess antifungal properties. In present study, the HPLC analysis revealed that *Rhizophora apiculata* possess the most prevailing components are lyoniresinol-3α-O-β-arabinopyranoside and afzelechin-3-O-L-rhamno-pyranoside. It is quite reasonable to assume that the antifungal activity of *Rhizophora apiculata* could be due to the presence of these compounds.

Antimicrobial activity of *Rhizophora apiculata* may be due to presence of tannin, gallic acid also responsible for antimicrobial activity of *Rhizophora apiculata* [28]. Byproduct *Rhizophora apiculata*, Pyroligneous acid possess antibacterial and also have antioxidant activities [29-30]. Pyroligneous acid also possess anticandidus activity [31]. Besides, *Rhizophora apiculata* have antifungal activity against most of plant pathogenic fungi [32]. Presence of methyl 4-O-methyl-d-arabinopyranoside and 1,6,10,14-Hexadecatetraen-3,7,11,15-tetramethyl-, (E, E) as the most prevailing compounds in *Rhizophora apiculata* could be attribute for their antibacterial activity in respect of inhibits the growth *Xanthomonas oryzae* pv. *oryzae* [33]. "13-Docosenamide, (Z)-, -13-Docosenamide, 9-Octadecenamide, (Z)- and ç-Sitosterol, á-Sitosterol, Cholest-8(14)- en-3-ol, 4,4-dimethyl-, (3á,5à) was identified as most abundant antimicrobial compounds of *Rhizophora annamalayna* against *Alternaria solani* under GC-MS analysis [34].

Several secondary metabolites of *Acacia saligna* that possess antifungal and antibacterial and antioxidant properties and these compounds active against *F. culmorum, P. chrysogenum, R. solani, A. tumefaciens, E. cloacae, E. amylovora, and P. carotovorum subsp. Carotovorum* [35]. Antifungal and antibacterial activities of *Musa paradisiaca* and the compounds active against *Agrobacterium tumefaciens, Dickeya solani, Erwinia amylovora, Pseudomonas cichorii, Serratia pylmuthica, Fusarium culmorum* and *Rhizoctonia solani* [36].

CONCLUSION

It could be concluded that the maximum mycelial growth was recorded with acetone extract which account for the lowest per cent inhibition and standard chemical fungicide Hexaconazole 5% EC at 0.1% concentration showed 100 per cent inhibition of the test pathogen. Lyoniresinol-3α-O-β-arabinopyranoside was recorded highest peak area with retention time RT (8.013 min). My-3-O-ribose was recorded least peak area with retention time RT (13.042 min).

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