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ANTIFUNGAL ACTIVITY OF *RHIZOPHORA APICULATA* AGAINST *ALTERNARIA SOLANI*

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Abstract

The present investigation was undertaken to assess the antifungal activity of different solvent extract of *Rhizophora apiculata* against A. solani and evaluation of its antimicrobial compounds. The experiment clearly revealed that all the solvent extracts of *R. apiculata* (15%) considerably inhibited the growth of test pathogenwhen compared to control. The minimum mycelial growth (3.65mm) was recorded with methanol extract which accounted for the highest percent inhibition (95.54%) over control. This was followed by aqueous extract, ethanol extract and chloroform extract of *R. apiculata* (15%) accounting 94.47, 92.28 and 89.94 percent inhibition. The standard chemical fungicide Mancozeb 75% WP at 0.25% conc. showed 100 percent inhibition of the test pathogen. Also, 13-Docosenamide, (Z)-, -13-Docosenamide, 9-Octadecenamide, (Z)-was recorded highest peak area with 36.86% with retention time (RT) 25.14 min. ç-Sitosterol, á-Sitosterol, Cholest-8(14)-en-3-ol, 4,4-dimethyl-, (3á,5à) was identified as most abundant compounds of *R. annamalayna* with 3.31 % peak area with highest retention time (RT) 33.33 min under GC-MS analysis.

Keywords: *Rhizophora apiculata*; *Alternaria solani*; antifungal activity; GC-MS.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetables in the world. Among the fungal diseases of tomato, early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout causing yield loss ranged between 72 to 80 percent (Joseph *et al.,* 2016) depending on the variety, severity and stage of infection. Generally, the fungicides are widely used for controlling the disease, but are not economically feasible under most conducive weather. Beside, *A. solani* has low sensitive with fungicides because of its production of dark brown to black pigment called melanin which enhanced survival and competitive abilities of the pathogen under certain environmental conditions (Bell and Wheeler, 1986). Hence, much attention is being focused on the alternative methods of pathogen control which are eco-friendly and also enhance crop yield.

Plant species have been known for their medicinal and antimicrobial properties called biologically active compounds/secondary metabolites.However, among the botanicals, mangroves are mainly distributed around seashores and mangrove swamps in coastal proximal and middle zones (Prabhakaran and Kavitha, 2012) are known to be a rich source of various secondary metabolites and are widely used in the traditional medicine practices. 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* (Mehdi *et al.,* 2000; Muthukumar *et al.,* 2014; Behbahani *et al.,* 2016; Rastegar and Mohsen Gozan, 2016). More than 200 bioactive compounds identified from mangroves with antibacterial and antifungal properties belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics (Bandaranayake, 2002; Bose and Bose, 2008; Chandrasekaran *et al.,* 2009; Vengadeshkumar, 2017).

Several workers have studied the efficacy of various plant extracts against *A. solani viz., Cinnmomum zeylanicum* (Bowers and Locke, 2004) *Vitexnegunda* (Panda *et al.,* 2009), *Psoralea corylifola* (Gidwani *et*

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al., 2010), *Boswellia serrate* (Raja *et al.*, 2011), *Calendula officinalis* (Bissa and Bora, 2011). Rasteger and Gozari, (2016) reported that *R. apiculata* exhibited antifungal principles while against *Penicillium* sp. and *A. alternata*. Likewise, the leaf extracts of *R. apiculata* showed antifungal compounds against fungal pathogen *M. phaseolina* (Muthukumar *et al.*, 2014). The extract of *Ceriops decandra* showed maximum inhibition against *Candida albicans* (Selvam and Kolanjinathan, 2014). Ethanolic extract of mangrove species *A. marina* was found to inhibit the radial growth of *M. phaseolina* under *in vitro* was reported (Mehdi *et al.*, 1999). Vengadeshkumar *et al.*, (2019) reported that 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-methyl-d-arabinopyranoside (38.66%) and 1, 6, 10, 14-Hexadecatetraen-3, 7,11,15-tetramethyl-(E,E) (30.24%) are quite reasonable for its antibacterial activity. 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).

Materials and Methods

Source of the pathogen and plant extract

The test pathogen *A. solani* (AS_5) and the mangrove species *R. apiculata* and *R. annamalayana* that have being used in this study, were selected based on their previous results in the study of Mahalakshmi, (2019), where they demonstrated potent pathogenecity 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*., (2009) and then the extract was filtered through a Buchner funnel and using Whatmann 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

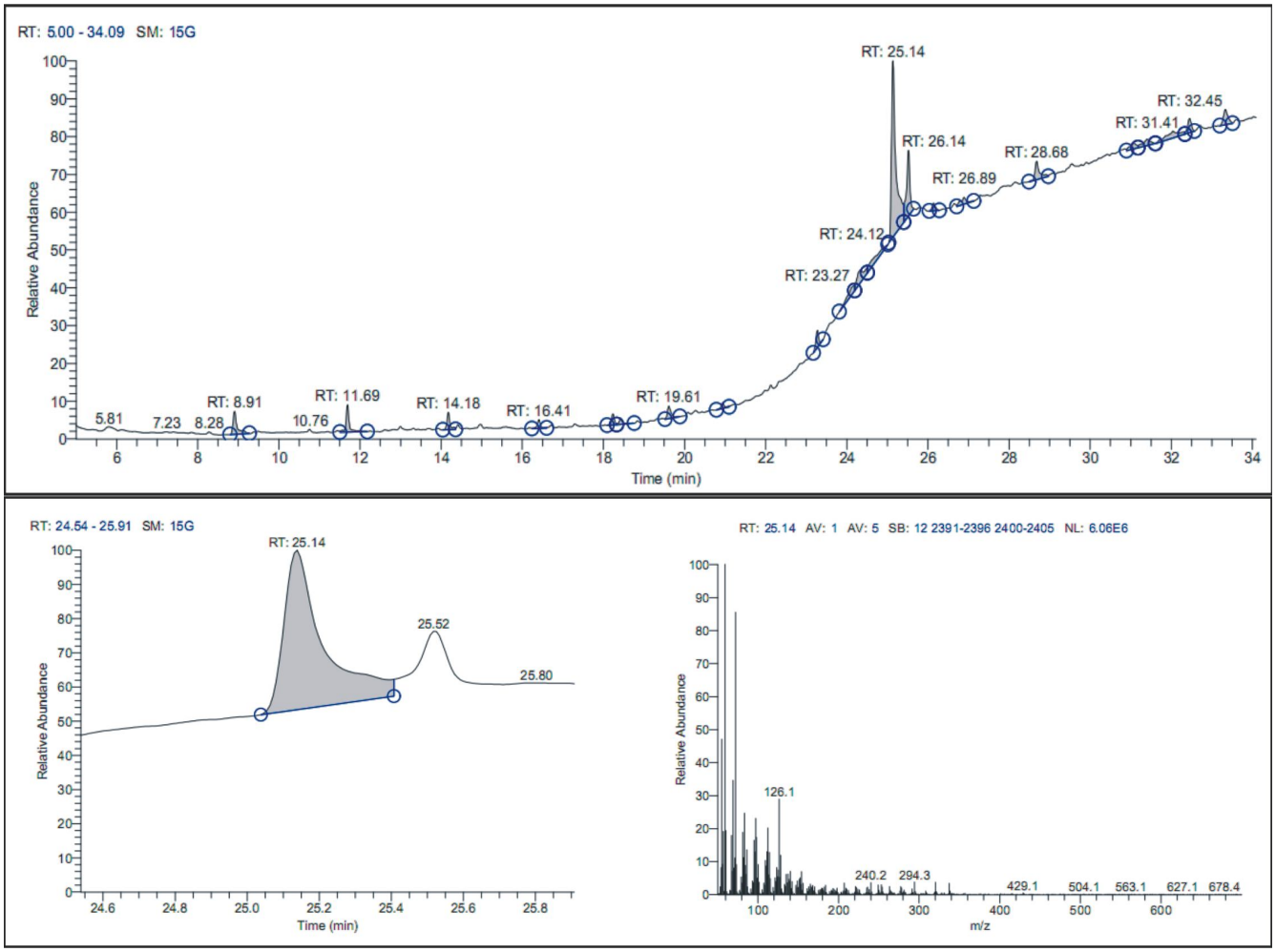

Fig. 1: GC-MS Chromatogram of *R. annamalayana*.

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

S. No.	Solvents	Growth (mm)	Percent inhibition over control
1.	Acetone	9.64^{g}	87.79
2.	Chloroform	8.43^{e}	89.94
3.	Ethanol	5.72^{d}	92.28
4.	Ethyl acetate	9.22^{f}	88.84
5.	Methanol	3.65^{c}	95.54
6.	Aqueous extract	4.42^{b}	94.47
7.	Mancozeb @ 75% WP	0.0^{a}	100.00
8.	Control	80.00^{h}	0.00
Values in the column followed by same letters not differ significantly by DMRT (P=0.05)			

evaporator. The dry extract was stored at 4°C until further use (Laith *et al*., 2012).

Poisoned food technique (Narenderkumar *et al*., 2017)

Potato dextrose agar medium mixed separately with different solvent extract of *R. apiculata* at 15% concentration poured into sterile Petri dishes and allowed to cool and solidify. Mycelial disc (9mm) of 15 days old culture of test pathogen placed at the centre of the petri dishes and incubated at 28±2°C for 10 days. The PDA medium with the same concentration of sterile distilled water alone served as control. Similarly a fungicide *viz.*, Mancozeb (0.25% conc.) as also tested against the pathogen for comparison for each treatment. The experiments was replicated thrice and the percent inhibition of mycelial growth if any was determined by the formula:

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

Where C = Diameter of *A. solani* in control,

T= Diameter of *A. solani* in treated.

GC-MS analysis

The GC-MS analysis was carried out using a Clarus 500 Perkin - Elmer of auto system XL Gas Chromatograph equipped and coupled to mass detector Turbo mass gold – Perkin Elmer Turbomass 5.1 spectrometer with Elite–1 (100% Dimethyl poly siloxane), 30×0.25 mm ID × 1µm of capillary column. The initial temperature of the instrument was set to 110°C and continuously maintained for 2 min. After end of 2 minutes, the oven temperature was increased 5°C for every 1 min. and finally reached up to 280°C and maintained for 9 min. The temperature of injection port was ensured as 250°C and flow of helium was 1ml/min. the ionized voltage was 70eV and the sample was injected in split mode as 10:1. The mass spectral scan range was set at 45-450 (m/z).

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 (Gomez and Gomez, 1976).

Results

Effect of different solvent extract of *R. apiculata* (15%) on the growth of *A. solani* (AS_5)

The data depicted in table 1, 2 revealed that all the solvent extracts of *R. apiculata* (15%) considerably

Table 2: Evaluation of chemical compounds in dried leaves of *R. annamalayana* (GC-MS analysis)

Compound Name	RT	Molecular Formula
3-Dodecene, (E)-	8.91	C12H24
1-Dodecene	8.91	C12H24
Cyclododecane	8.91	C12H24
Cyclotetradecane	11.69	C14H28
1-Tetradecene	11.69	C14H28
1-Hexadecanol	11.69	C16H34O
Hexadecen-1-ol, trans-9-	14.18	C16H32O
1-Hexadecanol	14.18	C16H34O
Cetene	14.18	C16H32
Hexadecen-1-ol, trans-9-	16.41	C16H32O
2-Hexadecanol	16.41	C16H34O
10-Heneicosene (c,t)	16.41	C21H42
Dibutyl phthalate	18.23	C16H22O4
Phthalic acid, butyl hept-3-yl ester	18.23	C19H28O4
Phthalic acid, butyl hex-3-yl ester	18.23	C18H26O4
10-Heneicosene (c,t)	18.43	C21H42

Table 2 Continue...

Table 2 Continue...

2-Hexadecanol	18.43	C16H34O
9-Hexacosene	18.43	C26H52
Phytol	19.61	C20H40O
Isophytol, acetate	19.61	C22H42O2
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.61	C20H40O
Cyclononasiloxane, octadecamethyl-	20.94	C18H54O9Si9
Tetracosamethyl-cyclododecasiloxane	20.94	C24H72O12Si12
Cyclodecasiloxane, eicosamethyl-	20.94	C20H60O10Si10
1-Monolinoleoylglycerol trimethylsilyl ether	23.27	C27H54O4Si2
9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	23.27	C27H52O4Si2
Glycine,N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	23.27	C36H69NO6Si3
Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15hexadecamethyl	24.12	C16H50O7Si8
1Monolinoleoylglycerol trimethylsilyl ether	24.12	C27H54O4Si2
Glycine,N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	24.12	C36H69NO6Si3
Cyclodecasiloxane, eicosamethyl-	24.30	C20H60O10Si10
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15hexadecamethyl	24.30	C16H50O7Si8
Cyclononasiloxane, octadecamethyl-	24.30	C18H54O9Si9
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15hexadecamethyl	24.59	C16H50O7Si8
1Monolinoleoylglycerol trimethylsilyl ether	24.59	C27H54O4Si2
Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11dodecamethyl	24.59	C12H38O5Si6
13-Docosenamide, (Z)-	25.14	C22H43NO
-13-Docosenamide	25.14	C22H43NO
9-Octadecenamide, (Z)-	25.14	C18H35NO
Squalene	25.52	C30H50
6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)-	25.52	C30H51BrO
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	25.52	C30H52O
Tetrapentacontane, 1,54-dibromo-	26.14	C54H108Br2
17-Pentatriacontene	26.14	C35H70
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	26.14	C26H54
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15hexadecamethyl	26.89	C16H50O7Si8
1Monolinoleoylglycerol trimethylsilyl ether	26.89	C27H54O4Si2
N[(3à,5á,7à,12à)24oxo3,7,12tris[(trimethylsilyl)oxy]cholan24yl], methyl ester	26.89	C36H69NO6Si3
Tetratriacontane	28.68	C34H70
Hexatriacontane	28.68	C36H74
Heptadecane, 2,3-dimethyl-	28.68	C19H40
Prosta5,13dien1oic acid, 9,11,15tris[(trimethylsilyl)oxy], trimethylsilyl ester, (5Z,9à,11à,13E,15S)	31.07	C32H66O5Si4
Pregn4ene3,11,20trione, 6,17,21tris[(trimethylsilyl)oxy],3,20bis(Omethyloxime), (6á)	31.07	C32H58N2O6Si3
17(1,5Dimethylhexyl)10,13dimethyl3styrylhexadecahydrocyclopenta[a]phenanthren2one	31.07	C35H52O
Campesterol	31.41	C28H48O
.psi.,.psi.Carotene, 1,1',2,2'tetrahydro1,1'dimethoxy	31.41	C42H64O2
Lycoxanthin	31.41	C40H56O
28,33-Dinorgorgost-5-en-24-one, 3-hydroxy-, (3á)-	32.04	C28H44O2
Stigmasterol	32.04	C29H48O
Ent-3a,10-dihydroxy-13-iodomethyl-16-oxo-8,13-ep i-17,20-dinorgibberell-1-en-7, 19-dioic acid,19,10-lactone,7-methyl ester	32.04	C20H23IO6
Hexatriacontane	32.45	C36H74
Tetratriacontane	32.45	C34H70
Triacontane	32.45	C30H62
ç-Sitosterol	33.33	C29H50O
á-Sitosterol	33.33	C29H50O
Cholest-8(14)-en-3-ol, 4,4-dimethyl-, (3á,5à)-	33.33	C29H50O

inhibited the growth of test pathogenwhen compared to control. The minimum mycelial growth (3.65mm) was recorded with methanol extract which accounted for the highest percent inhibition (95.54%) over control. This was followed by aqueous extract, ethanol extract and chloroform extract of *R. apiculata* (15%) accounting 94.47, 92.28 and 89.94 percent inhibition in the decreasing order of merit respectively. The standard chemical fungicide Mancozeb 75% WP at 0.25% conc. showed 100 percent inhibition of the test pathogen. The results obtained with the solvent extracts (methanol extract) was almost similar with the results obtained with aqueous extract at 20 percent in reducing fungal growth (Table 1). However, the solvent extract (methanol extract) was used for GC-MS analysis for identification of the compounds.

Evaluation of chemical compounds in dried leaves of *R. annamalayana* (GC-MS analysis)

The analysis of the dried leaf of *R. annamalayana* identified many number of compounds listed in table 2. Of these, 10-Heneicosene (c,t), 2-Hexadecanol, 9-Hexacosene was recorded least peak area 1.13% with retention time (RT) 18.43min. The most abundant compounds of *R. annamalayna* was 13-Docosenamide, (Z)-, -13-Docosenamide, 9-Octadecenamide, (Z)-was recorded highest peak area with 36.86% with retention time (RT) 25.14 min. ç-Sitosterol, á-Sitosterol, Cholest-8(14)-en-3-ol, 4,4-dimethyl-, (3á,5à) was recorded with 3.31 % peak area with highest retention time (RT) 33.33 min. Remaining compounds are elevated at various retention time (Fig. 1 and Table 2).

Discussion

The presence of various secondary metabolites such as alkaloids, quaternary alkaloids, coumarins, flavanoids, steroids, terpenoids, phenols *etc*. have been reported in the various plants extracts (Aswal *et al*., 1984; Abraham *et al*., 1987; Chopra *et al*., 1992) which are attributed for the antifungal properties of the plants studied. Accordingly, in the present study, the GC-MS analysis revealed that *R. annamalayana* possess the most prevailing components are 13-Docosenamide, (Z)-, -13-Docosenamide, 9-Octadecenamide, (Z)-. It is quite reasonable to assume that the antifungal activity of *R. annamalayana* could be due to the presence of these compounds. In a similar line of GC-MS analysis to find out the bioactive compounds present in plant products, Yeole *et al.,* (2014) reported cinnamaldehyde and eugenol as the bio active compound of *C. zeylanicum* which is active against *A. solani* causing early blight of tomato.

Rukhsana *et al.,* (2011) reported curcumin as the bio active compound of *Curcuma longa* which is active against *X. oryzae*.pv. *oryzae* causing BLB of paddy. Nidhi *et al.,* (2013) reported that phthalazine as the most abundant phyto compound present in rhizome of *Barleria prionitis* exhibiting antibacterial activity. These earlier reports are in line and corroborates with the present study. Meera, (2014) reported that Eucalyptol as the bioactive compound of Eucalyptus globules which is active against rice sheath rot. Also, Vengadeshkumar *et al.,* (2019) reported that the antibacterial activity of *Rhizophora sp.* against rice BLB might be due to the presence of compounds *viz*., Methyl 4-O-methyl-d-arabinopyranoside and 1, 6,10, 14-Hexadecatetraen- 3,7,11,15-tetramethyl, (E,E). It has been reported that the antimicrobial activity of *R. apiculata* may be due to the presence of compounds like tannin (Lim *et al*., 2006), gallic acid (Lim *et al.,* 2011). Besides, Pyroligneous acid (by product from *R. apiculata*) has been reported to possess antibacterial (Chalermsan and Peerapan, 2009), antioxidant activities (Loo *et al*., 2007; Loo *et al*., 2008) and anticandidus activity (Ibrahim *et al.,* 2013), besides exhibited strong antifungal activity against several plant pathogenic fungi (Oramahi and Yoshimura, 2013).

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