Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Plant Pathology Journal

ISSN 1812-5387
DOI: 10.3923/ppj.2019.39.46

Research Article
Antibacterial Activity of *Rhizophora apiculata* Leaf Extract for the Management of Rice Bacterial Blight Disease

Vengadeshkumar Lakshmanan, Meera Thangaraj, Balabaskar Ponnusamy, Sanjaygandhi Santhirakasan, Rajamohan Kannan, Udhayakumar Regunathan and Sudhasha Selvaraj

Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, 608 002 Chidambaram, Tamil Nadu, India

Abstract

Background and Objectives: Bacterial blight of rice is a serious disease and causes yield loss up to 70% wherever rice is grown. So the current study was aimed to examine the *in vitro* and *in vivo* antibacterial activity of *Rhizophora apiculata* leaf extract for the management of bacterial leaf blight of paddy and identifies the bioactive compounds present in it through gas chromatography-mass spectrometry (GC-MS) analysis. **Materials and Methods:** The *in vitro* efficacy of *R. apiculata* was assessed following agar well method. A pot culture study was conducted to evaluate the bio efficacy and phytotoxicity effect of *R. apiculata* leaf extract for the management of leaf blight of paddy. The bio-active compounds present in *R. apiculata* leaf extract were identified by GC-MS analysis. **Results:** The study revealed that the methanol extract of *R. apiculata* at 15% concentration significantly inhibited the growth of Xoo under *in vitro,* reduced the percent disease index of rice bacterial blight disease and enhanced the biometrics of rice in pot trial without any phytotoxicity symptoms. The phytochemical analysis of *R. apiculata* by GC-MS revealed the 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. **Conclusion:** So it was concluded that the aqueous extract of *R. apiculata* at 15% concentration significantly inhibited the growth of Xoo under *in vitro,* reduced rice bacterial blight disease and enhanced the biometrics of rice in pot trial without any phytotoxicity symptoms.

Key words: Rice, leaf blight, *Rhizophora apiculata*, bacterial blight, biometrics of rice

Citation: Vengadeshkumar Lakshmanan, Meera Thangaraj, Balabaskar Ponnusamy, Sanjaygandhi Santhirakasan, Rajamohan Kannan, Udhayakumar Regunathan and Sudhasha Selvaraj, 2019. Antibacterial activity of *Rhizophora apiculata* leaf extract for the management of rice bacterial blight disease. Plant Pathol. J., 18: 39-46.

Corresponding Author: Vengadeshkumar Lakshmanan, Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, 608 002 Chidambaram, Tamil Nadu, India Tel: 91-8667806189

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice (*Oryzae sativa* L.) is principally grown in tropical and sub-tropical zones and would be the staple food for about 9.3 billion years around the world[1]. The bacterial leaf blight disease (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is the most important, oldest known and serious bacterial disease of rice in Asia and many rice growing regions of world. The BLB disturbs the emergence of panicles and grain filling which leads to the low production[2]. The yield loss due to this disease is estimated between 20-80% depending on the variety, severity and stage of infection[3]. Currently, the disease is being managed by application of chemical, antibiotics like streptocycline+copper oxychloride, bacterinashak, agrimycin 100 and kasugamycin[4]. Although, the use of antibiotics is common for the control of bacterial disease yet there is no true effective bactericide available for the proper management of the disease[5]. So an effective and economical chemical control is yet to be developed for BLB disease. This may be because of variability among the pathogen population and its sensitivity to the chemicals. Hence, much attention is being focused on the alternative methods of pathogen control which are eco-friendly and also enhance the crop yield. Botanicals provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth[6]. Among the botanicals, mangroves are known to be a rich source of various secondary metabolites and these higher plants are widely used in the traditional medicine practices. They are mainly distributed around seashores and mangrove swamps in coastal proximal and middle zones[7].

More than 200 bioactive compounds identified from mangroves in different geographical population with antibacterial and antifungal properties belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics[8,9,10,11]. The anti bacterial activity of mangroves has been well documented against various human pathogenic bacteria *viz*., *Excoecaria agallocha* against *Staphylococcus aureus*, *Avicennia marina* against *Pseudomonas aeruginosa*, *Lumnitzera littorea* against *E. coli*[12-14]. But, there is no significant work reported against bacterial pathogens causing plant diseases. However, the antibacterial activity of various other plant extracts apart from the mangroves viz., *Allium sativum*, *Adathoda vasica*, *Curcuma longa* and *Andrographis serpyllifolia* (leaves) against Xoo has been well documented[15-18]. Towards this direction the preliminary screening study conducted with extracts of various plant species under *in vitro* conditions revealed the supremacy of marine mangrove extract of *R. apiculata* against Xoo. Subsequently, the present study was aimed at evaluating the *in vitro* and *in vivo* antibacterial activity of *R. apiculata* leaf extract for the management of bacterial leaf blight of paddy. Also studies were proposed to take up chemical characterization of *R. apiculata* extract using gas chromatograph interfaced with a mass spectrometer (GC-MS) analysis. This study may be the first report on an antibacterial nature of mangrove species against plant pathogenic bacterium.

MATERIALS AND METHODS

Location and time duration of study: The *in vitro* and pot culture experiments were carried out during July, 2017-May, 2018 in the Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.

Material used: In the present study the pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo) was isolated from infected leaf tissue of rice crop in Kathiramangalam village, Nagapattinam district, Tamilnadu, India and *R. apiculata* was selected on the basis of maximum antibacterial activity observed in the earlier study under *in vitro*[19].

Collection and authentication of *R. apiculata*: The fresh leaves of *R. Apiculata* were collected from Pichavaram Mangrove forest, Tamil Nadu and authenticated in the herbarium of C.A.S. in Marine Biology, Annamalai University, Parangipettai, India. The leaves were carefully examined and healthy leaves were washed, shade dried, coarsely powdered and stored in air tight bottles for further work.

Preparation of aqueous extract: The preparation of aqueous and solvent extract of *R. apiculata* was followed by the method suggested earlier by Bele *et al.*[20] and Chandrasekaran and Rajappan[21]. Fresh leaves of *R. apiculata* were collected, washed, ground by adding sterile water (1:1w/v) and filtered though muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 min and filter (44 µm) sterilized. The extract thus prepared served as the standard (100%).

Preparation of solvent extract: The shade dried leaves of *R. apiculata* was ground to fine powder (15 g dry weight) and extracted with 100 mL of ethanol, methanol and ethyl acetate each for 48 h using a soxhlet extractor and was filtered through Whatman No. 1 filter paper. The solvent was evaporated from crude extract by a rotary evaporator and the dry extract was stored at 4°C until further use[22].

Antibacterial activity of *R. apiculata*: The antibacterial activity of *R. apiculata* was assessed by Agar well method[23]. Both aqueous and solvent extracts of *R. apiculata* at 15% concentration (1 µL/cavity) were poured in cavities of 5mm dia. formed in Petri dish containing peptone sucrose agar medium seeded with Xoo (10^8 CFU mL^{-1}). The plates were incubated at 28±2°C for 72 h. The cavity filled with sterile distilled water served as control. Three replications were maintained for each treatment and the relative antibacterial potency was calculated by comparing the zone of inhibition. The activity index was calculated by using formula:

$$\text{Activity index (AI)} = \frac{\text{Inhibition zone of test sample}}{\text{Inhibition zone of the standard}}$$

Pot trial: *Rhizophora apiculata* leaf extract which showed significant reduction on the growth of Xoo was tested at different concentration for the management of BLB disease in pot trial. The BLB susceptible variety BPT 5204 grown in rectangular pots of size, 30×45 cm was used for the study. The upper most leaves of plants at the maximum tillering stage [30 DAT (days after transplanting)] were inoculated with Xoo by clipping method[24]. *Rhizophora apiculata* leaf extract as per treatment schedule was sprayed two days after inoculation of the pathogen and a second spray was given at fifteen days interval. The crop was maintained in a poly house with frequent spraying of water to provide adequate moisture and relative humidity to enable successful infection by the pathogen. The experiment was conducted in a randomized block design with three replications for each treatment and a suitable control. The antibiotic streptomycin at 100 ppm was used for comparison and the standard agronomic practices as recommended by the State Agricultural Department were followed. The observations on lesion length and percent disease incidence were assessed at 14 days after inoculation and recorded as centimeters (cm). Also, the reduction in the per cent disease index over control is worked out and recorded. The biometric parameters viz., plant height (cm), No. of productive tillers, panicle length (cm), number of grains per panicle and yield (g/pot) were assessed at harvest and recorded. The details of the treatments are given below.

Treatment schedule:

T1	:	Foliar spray of *R. apiculata* at 5% at 35 DAT
T2	:	Foliar spray *R. apiculata* at 10% at 35 DAT
T3	:	Foliar spray *R. apiculata* at 15% at 35 DAT
T4	:	T_1+second foliar spray at 50 DAT
T5	:	T_2+second foliar spray at 50 DAT
T6	:	T_3+second foliar spray at 50 DAT
T7	:	Streptomycin (100 ppm) as foliar spray at 35 and 50 DAT
T8	:	Control

Phytotoxicity effect of *R. apiculata*: To know the crop tolerance and extent of phytotoxicity, ten leaves were randomly selected from each pot and the total number of leaves showing phytotoxic symptoms such as Leaf injury on tips/surface, Wilting, Vein clearing, Necrosis, Epinasty, Hyponasty if any were counted on 1, 3, 5, 7 and 10 days after spraying and grading was done as per CIB guidelines adopting 0-10 scale.

Phytotoxicity rating scale:

Injury (%)	Rating	Injury (%)	Rating
0-10	1	11-20	2
21-30	3	31-40	4
41-50	5	51-60	6
61-70	7	71-80	8
81-90	9	91-100	10

Phytochemical studies

Qualitative analysis of *R. apiculata*

Test for alkaloids (Tannin and phlobatannins): About 5 mL of the methanol extract was added to 2 mL of HCl. To this acidic medium, 1 mL of Wagners reagent was added. A reddish precipitate brown produced immediately indicated the presence of alkaloids[25].

Test for glycosides: To as small amount of methanol extract, 1 mL of Fehling's solution was added and heated, orange precipitate indicated the presence of glycosides[25].

Test for flavonoids: To 1 mL of methanol extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid indicated the presence of flavonoids[13].

Test for saponins: The methanol extract was diluted with 20 mL of distilled water and it was agitated in a graduated cylinder for 15 min, the formation of 1cm layer of foam showed the presence of saponins[25].

Test for phenolic compounds (Steroids): Small amount of methanol extracts were taken separately in water and tested for the presence of phenolic compounds with diluted ferric chloride solution. Violet color indicated the presence of phenolic components[25].

Test for quinins (Terpenoids): To a small amount of methanol extract, concentrated sulphuric acid is added. Appearance of red color indicated the presence of quinines[25].

Identification of bioactive compounds (GC-MS analysis): For the identification of bioactive compounds the plant extract was dissolved in methanol and filtered with polymeric solid phase extraction (SPE) column and analyzed in GC-MS for different components. The GC-MS analysis was performed using a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) and employing the following conditions: Column Elite-1 and silica capillary column (30×0.25 mm ID×1 µm df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 mL min^{-1} and an injection volume of 0.5 µL was employed (split ratio of 10:1) with injector temperature at 250°C; ion-source temperature at 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C min^{-1}, to 200°C, then 5°C min^{-1} to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 sec and fragments from 45-450 Da. Total GC running time was 36 min.

Identification of components: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained and recorded.

Statistical analysis: The statistical analysis of the experimental results was performed employing the computer software package 'SPSS', by Duncan's Multiple Range Test (DMRT) and the values are expressed as mean[26].

RESULTS

Effect of solvent extracts of *R. apiculata* on the growth of Xoo (Agar Well Method): The methanol extract of *R. apiculata* at 15% concentration recorded the highest inhibition zone (14.44 mm) and activity index (0.80) against the growth of Xoo. The efficacy of ethyl acetate extract (0.77 activity index) and ethanol extract (0.73 activity index) of *R. apiculata* came next in the order of merit in reducing the bacterial growth. Also, the results obtained with the methanol extract of *R. apiculata* in reducing the bacterial growth was almost similar and comparable with the results obtained with aqueous extract (15%) of *R. apiculata* (Table 1). Hence, subsequent pot culture study was carried out with aqueous extract of *R. apiculata.*

Efficacy of *R. apiculata* on the management of rice BLB (Pot culture): The results revealed that among the treatments with *R. apiculata* leaf extract the treatment T_6 viz., foliar spray with *R. apiculata* extract at 15% at 35+50 DAT recorded maximum reduction of lesion length (3.21cm) and per cent disease index (23.24%). This was followed by the treatments with T_5 and T_4 in the decreasing order of merit. The least effect was recorded with foliar spray of *R. apiculata* extract at 5% at 35 DAT (T_1). However, the antibiotic treatment with Streptomycin (100 ppm) as foliar spray at 35 and 50 DAT proved its superiority and recorded only 15.36% BLB incidence (Table 2).

Table 1: Effect of different solvent extracts of *R. apiculata* (15%) on the growth of Xoo (Agar Well Method)

Treatments	Inhibition zone (mm)	Activity index
***R. apiculata* (15%)**		
Aqueous extract	14.42[b]	0.80
Ethanol	13.15[d]	0.73
Ethyl acetate	13.88[c]	0.77
Methanol	14.44[b]	0.80
Streptomycin (100 ppm)	18.00[a]	-
Control	0.00	0.00

Values in the column followed by same letters not differ significantly by DMRT (p = 0.05)

Table 2: Efficacy of *R. apiculata* on the management of rice BLB (Pot culture)

Treatment No.	*R. apiculata*	Lesion length (cm)	PDI	Percent disease reduction
T_1	Foliar spray at 5% at 35 DAT	13.22[g]	49.11[g]	23.56
T_2	Foliar spray at 10% at 50 DAT	11.14[f]	46.51[f]	27.61
T_3	Foliar spray at 15% at 35 DAT	9.17[e]	43.57[e]	32.18
T_4	T_1+second foliar spray 50 DAT	7.20[d]	34.32[d]	46.58
T_5	T_2+second foliar spray 50 DAT	5.24[c]	25.46[c]	60.37
T_6	T_3+second foliar spray 50 DAT	3.21[b]	23.24[b]	63.82
T_7	Streptomycin (100 ppm) as foliar spray at 35 and 50 DAT	1.32[a]	15.36[a]	76.09
T_8	Control	12.32[h]	64.25[h]	0.00

Values in the column followed by same letters not differ significantly by DMRT (p = 0.05). DAT: Days after transplanting, PDI: Percent disease index

Table 3: Efficacy of *R. apiculata* on the biometrics of paddy variety BPT 5204 (Pot culture)

Treatment No.	*R. apiculata*	Plant height at harvest (cm) DAT	Number of productive tillers/hill	Panicle length (cm)	Grains/panicle	Yield (g/pot)
T_1	Foliar spray at 5% at 35 DAT	76.12[g]	7.52[g]	12.48[g]	70.51[g]	28.47[g]
T_2	Foliar spray at 10% at 50 DAT	78.24[f]	8.33[f]	12.83[f]	85.39[f]	29.39[f]
T_3	Foliar spray at 15% at 35 DAT	78.83[e]	9.42[e]	13.45[e]	89.47[e]	31.68[e]
T_4	T_1+second foliar spray 50 DAT	79.87[c]	12.23[c]	16.46[c]	95.35[c]	33.91[c]
T_5	T_2+second foliar spray 50 DAT	80.21[b]	12.27[b]	16.58[b]	97.21[b]	34.56[b]
T_6	T_3+second foliar spray 50 DAT	82.37[a]	13.21[a]	16.87[a]	100.23	35.32[a]
T_7	Streptomycin (100 ppm) as foliar spray at 35 and 50 DAT	79.37[d]	11.34[d]	15.92[d]	92.44[d]	33.15[d]
T_8	Control	74.32[h]	6.21[h]	10.32[h]	67.43[h]	25.76[h]

Values in the column followed by same letters not differ significantly by DMRT (p = 0.05). DAT: Days after transplanting

Table 4: Phytotoxicity effect of *R. apiculata*

Treatment No.	Plant extract	Phytotoxicity symptoms/days after application: Chlorosis 1	3	5	7	10	Necrosis 1	3	5	7	10	Scorching 1	3	5	7	10	Epinasty 1	3	5	7	10	Hyponasty 1	3	5	7	10
T_1	Foliar spray at 5% at 35 DAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T_2	Foliar spray at 10% at 50 DAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T_3	Foliar spray at 15% at 35 DAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T_4	T_1+second foliar spray 50 DAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T_5	T_2+second foliar spray 50 DAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T_6	T_3+second foliar spray 50 DAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T_7	Streptomycin (100 ppm) as foliar spray at 35 and 50 DAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

DAT: Days after transplanting

Table 5: Phytochemicals in methanol extract of *R. apiculata*

Phytochemicals screened	Status
Tannin	Present
Saponin	Present
Flavonoids	Present
Steroids	Present
Terpenoids	Present
Cardiac glycosides	Present
Phlobatannin	Absent

Efficacy of *R. apiculata* on the biometrics of paddy variety BPT 5204 (Pot culture): Among the treatments, the treatment (T_6) with *R. apiculata* at 15% at 35 and 50 DAT recorded maximum plant height (82.37 cm), productive tillers (13.21), length of panicle (16.87 cm), grains per panicle (100.23) and maximum yield of rice (35.32 g/pot). This was followed by the treatments with T_5 and T_4 in the decreasing order of merit. The antibiotic treatment T_7 (Streptomycin (100 ppm) as foliar spray at 35 and 50 DAT) recorded a plant height of 79.37cm, productive tillers (11.34), panicle length (15.92 cm), grains per panicle (92.44) and yield (33.15 g/pot). The untreated control recorded the minimum plant biometric values of rice (Table 3).

Phytotoxicity effect of *R. apiculata*: The results revealed that none of the treatments with *R. apiculata* exhibited any kind of phytotoxicity symptoms and also revealed a normal plant growth pattern (Table 4).

Bioactive compounds in methanol extract of *R. apiculata*

Qualitative and GC-MS analysis of *R. apiculata*: The GC-MS analysis revealed the presence of six groups of phytochemicals in the extract of *R. apiculata*. Tannin, saponin, flavonoids, Steroids, terpenoids and cardiac glycosides are the phytochemicals identified in the methanolic extract of *R. apiculata*. The phytochemical phlobatannin is found absent in the extract (Table 5).

Regarding the bioactive compounds, the GC-MS analysis revealed the presence of eight bio active compounds. The active principles were identified based on their retention time (RT), molecular formula, molecular weight (MW) and concentration (%). The most prevailing compounds are methyl 4-O-methyl-d-arabinopyranoside (12.70, $C_7H_{14}O_5$, 178 and 38.66%) and 1,6,10,14-Hexadecatetraen-3,7,11,15-tetramethyl-, (E,E) (32.77, $C_{20}H_{34}O$, 290 and 30.24%) with corresponding retention time, molecular formula, molecular weight and concentration respectively (Table 6). The corresponding chemical shift peaks of the spectrum are shown in Fig. 1.

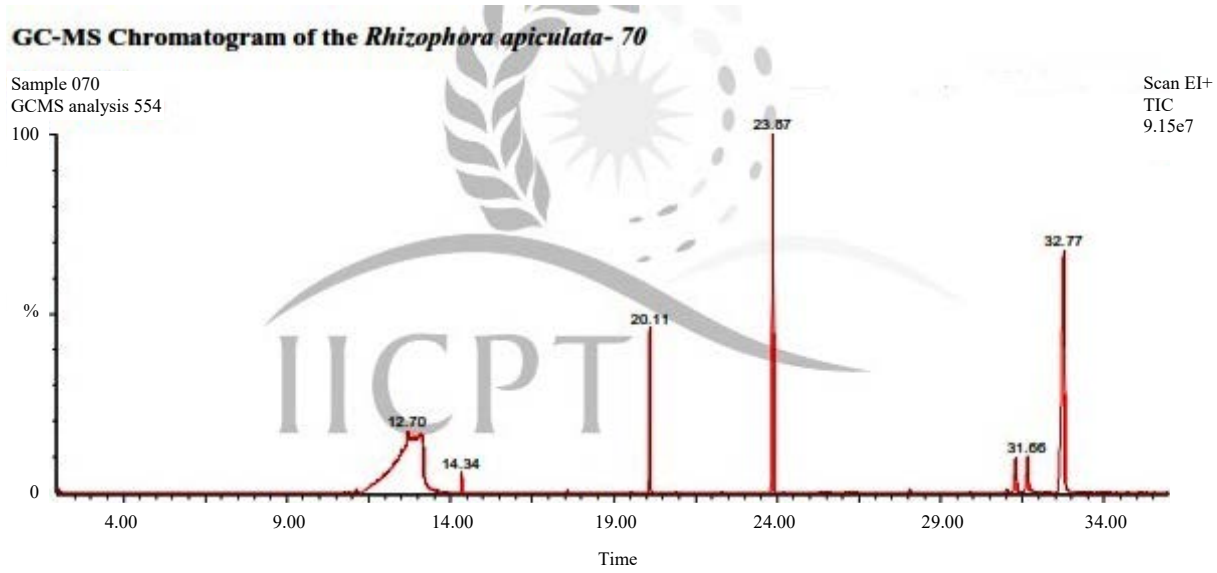

Fig.1: GC-MS analysis of *R. apiculata*

Table 6: Bioactive compounds in methanol extract of *R. apiculata*

Name of the components	Molecular formula	RT	MW	Peak area (%)
Methyl 4-O-methyl-d-arabinopyranoside	$C_7H_{14}O_5$	12.70	178	38.66
Phytol	$C_{20}H_{40}O$	14.34	296	0.67
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	20.11	390	6.99
Squalene	$C_{30}H_{50}$	23.87	410	17.42
Vitamin E	$C_{29}H_{50}O_2$	28.05	430	0.14
2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol	$C_{17}H_{30}O_3$	31.29	282	2.82
Urs-12-en-24-oic acid, 3-oxo-methyl ester, (+)-	$C_{31}H_{48}O_3$	31.66	468	3.06
1,6,10,14-Hexadecatetraen-3,7,11,15-tetramethyl-, (E,E)-	$C_{20}H_{34}O$	32.77	290	30.24

Parameters tested are not covered under the scope of NABL accreditation. RT: Retention time, MW: Molecular weight

DISCUSSION

Mangrove plants are reported to possess diverse medicinal properties and novel biologically active compounds which are active against human and plant pathogens[27-29]. The present study also clearly revealed that the methanol extract of *R. apiculata* exhibited highest antibacterial activity against Xoo under *in vitro* conditions. It is quite reasonable to assume that the antibacterial activity of *R. apiculata* could be largely due to the presence of most prevailing bioactive compounds like methyl 4-O-methyl-d-arabinopyranoside and 1, 6, 10, 14-Hexadecatetraen-3, 7,11,15-tetramethyl-(E,E). Presence of similar bioactive compounds such as Tannin, flavonoids, saponins, phenols, terpenoids, sterols etc. have been reported in the extracts of mangrove plants which are attributed for the antibacterial properties of the respective mangroves studied by Hong *et al.*[30], Poompozhil and Kumarasamy[31] and Surya and Hari[32].

Similar to the present study, the efficacy of methanol extract of mangroves like *Avicennia*, *R. apiculata*, *Aviceniia* sp. and *B. gymnorrhiza* against the growth of human pathogenic bacterium viz., *S. aureus* and *Pseudomonas aeruginosa* have been reported by Saravanan and Radhakrishnan[33] and Seepana *et al.*[34]. Also *n*-hexane extract of mangrove *L. littorea* inhibited the growth of *S. aureus* and *E. coli*[14]. Likewise, the ethanol extract of *R. apiculata* exhibited antibacterial activity against *S. aureus* and *C. albicans*[35]. These earlier reports corroborates with the present findings.

In the pot culture study the leaf extract of *R. apiculata* at 15% concentration as foliar spray at 35 and 50 DAT recorded significant reduction in the BLB incidence and enhanced biometrics of rice when compared to other treatments. As observed in the present study, the leaf extract of *D. metal* was found most effective in reducing rice BLB severity in glass house condition[36]. Besides, the efficacy of plant extracts viz., *Allium sativum*, *Datura metal*, *Adathoda vasica*, *Curcuma longa* and *Lentana camera* foliar spray for the control of BLB of rice have been reported by Bandaranayake[15], Bose and Bose[16], Chandrasekaran *et al.*[17], Kumar[37] and Kagale *et al.*[38]. These earlier reports are in line to the present observations. However, the efficacy of *R. apiculata* extract needs to be tested under field conditions and workout Benefit cost ratio comparing with streptomycin sulphate.

CONCLUSION

The study revealed that the methanol extract of *R. apiculata* at 15% concentration significantly inhibited the growth of Xoo under *in vitro* and the aqueous extract of *R. apiculata* at 15% concentration reduced the percent disease index of rice bacterial blight disease and enhanced the biometrics of rice in pot trial without any phytotoxicity symptoms. The GC-MS analysis of *R. apiculata* revealed the 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.

SIGNIFICANCE STATEMENT

This study discovered the potential antibacterial nature of *R. apiculata* that can be beneficial for biological management of bacterial blight disease of rice. Thus the present study had opened the possibility of further exploration of the *R. apiculata* extract for formulation and field level testing. Also, this study will help the researchers to uncover the critical areas on the use of botanicals/phytochemicals in crop disease management without any deleterious effect on the environment.

ACKNOWLEDGMENT

Sincere thanks to Directorate of Research, Annamalai University, Tamilnadu, India.

REFERENCES

1. Sheehy, J.E. and P.L. Mitchell, 2013. Designing rice for the 21st century: The three laws of maximum yield. Discussion Paper Series No. 48, International Rice Research Institute, Los Banos, Philippines, pp: 1-19.
2. Naqvi, S.A.H., R. Perveen, U.D. Umer, O. Malik, A.U. Rehman, S. Wazeer and T. Majid, 2014. Determination of antibacterial activity of various broad spectrum antibiotics against *Xanthomonas oryzae* pv. *oryzae*, a cause of bacterial leaf blight of rice. Int. J. Microbiol. Mycol., 2: 12-19.
3. Shivalingaiah, S. Umesha and M.K. Sateesh, 2012. Detection of *Xanthomonas oryzae* pv. *oryzae* in rice seeds by molecular techniques. Asian Australas. J. Plant Sci. Biotechnol., 6: 44-47.
4. Balanagouda, P., G.B. Jagadeesh, C. Karegowda, N. Seema and R.M. Revathi, 2017. Management of bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* under field condition. J. Pharmacog. Phytochem., 6: 244-246.
5. Lee, K.S., S. Rasabandith, E.R. Angeles and G.S. Khush, 2003. Inheritance of resistance to bacterial blight in 21 cultivars of rice. Phytopathology, 93: 147-152.
6. Zhang, J.D., Z. Xu, Y.B. Cao, H.S. Chen and L. Yan *et al*., 2006. Antifungal activities and action mechanisms of compounds from *Tribulus terrestris* L. J. Ethnopharmacol., 103: 76-84.
7. Bala, A., B. Rai, S. Priya and R. Kumar, 2017. *In vitro* evaluation of various fungicides and plant extracts against *Xanthomonas oryazae* pv. *oryzae* isolated from rice (*Oryza sativa* L.). Int. J. Current Microbiol. Applied Sci., 6: 421-427.
8. Govindappa, M., S. Umesh and S. Lokesh, 2011. *Adathoda vasica* leaf extract induces resistance in rice against bacterial leaf blight disease (*Xanthomonas oryzae* pv. Oryzae). Int. J. Plant Physiol. Biochem., 3: 6-14.
9. Jabeen, R., M. Ashraf, I. Ahmad and T. Iftikhar, 2011. Purification and bioassays of bioactive fraction from *Curcuma longa* against *Xanthomonas oryzae* pv. *oryzae* causing BLB disease in rice. Pak. J. Bot., 43: 1335-1342.
10. Jamuna, A., A. Ravishankar, V. Rai and V.S. Pradeepa, 2011. Evaluation of the antimicrobial activity of three medicinal plants against rice bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*). Malasian J. Microbiol., 7: 14-18.
11. Prabhakaran, J. and D. Kavitha, 2012. Ethnomedicinal importance of Mangrove species of Pitchavaram. Int. J. Res. Pharm. Biomed. Sci., 3: 611-614.
12. Prakash, M. and T. Sivakumar, 2013.. A study on antibacterial activity of mangrove plant *Excoecaria agallocha* L. Int. J. Curr. Microbiol. Applied Sci., 2: 260-262.
13. Ravikumar, S., M.S. Ali, A. Ramu and M. Ferosekhan, 2011. Antibacterial activity of chosen mangrove plants against bacterial specified pathogens. World Applied Sci. J., 14: 1198-1202.
14. Saad, S., M. Tahar, D. Susanti, H. Qaralleh and N.A.B. Abdul Rahim, 2011. Antimicrobial activity of mangrove plant (*Lumnitzera littorea*). Asian Pac. J. Trop. Med., 4: 523-525.
15. Bandaranayake, W.M., 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecol. Manage., 10: 421-452.
16. Bose, S. and A. Bose, 2008. Antimicrobial activity of *Acanthus ilicifolius* (L.). Indian J. Pharmaceut. Sci., 70: 821-823.
17. Chandrasekaran, M., K. Kannathasan, V. Venkatesalu and K. Prabhakar, 2009. Antibacterial activity of some salt marsh halophytes and mangrove plants against methicillin resistant *Staphylococcus aureus*. World J. Microbiol. Biotechnol., 25: 155-160.
18. Jun, W., Q. Xiao, J. Xu, M.Y. Li, J.Y. Pana and M.H. Yang, 2008. Natural products from true mangrove flora: Source, chemistry and bioactivities. Nat. Prod. Rep., 25: 955-981.
19. Kumar, L.V. and P.B. Baskar, 2013. *In vitro* antibacterial activity of plant extracts against *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight in rice. Int. J. Plant Protect., 6: 111-117.
20. Bele, A.A., V.M. Jadhav, S.R. Nikam and V.J. Kadam, 2009. Antibacterial potential of herbal formulation. Res. J. Microbiol., 4: 164-167.

21. Chandrasekaran, A. and K. Rajappan, 2002. Effect of plant extracts, antagonists and chemicals (individual and combined) on foliar anthracnose and pod blight of soybean. J. Mycol. Plant Pathol., 32: 25-27.
22. Laith, A.A., M. Najiah, S.M. Zain, A.W. Effendy, T.M. Sifzizul, M. Nadirah and M. Habsah, 2012. Antimicrobial activities of selected mangrove plants on fish pathogenic bacteria. J. Anim. Vet. Adv., 11: 234-240.
23. Manav, M. and B.S. Thind, 2002. Management of bacterial blight of rice with bioagents. Plant Dis. Res., 17: 21-28.
24. Kauffman, H.E., A.P.K. Reddy, S.P.Y. Hsieh and S.D. Merca, 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis. Rep., 57: 537-541.
25. Harborne, J.B., 1984. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd Edn., Chapman Hall, London, UK., ISBN: 0-412-25550-2, pp: 100-101.
26. Gomez, K.A. and K.A. Gomez, 1976. Statistical Procedures for Agricultural Research with Emphasis of Rice. International Rice Research Institute, Los Banos, Philliphines, Pages: 294.
27. Agoramoorthy, G., M. Chandrasekaran, V. Venkatesalu and M.J. Hsu, 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz. J. Microbiol., 38: 739-742.
28. Bandaranayake, W.M., 1998. Traditional and medicinal uses of mangroves. Mangroves Salt Marshes, 2: 133-148.
29. Ma, X., Q. Wei, S. Zhang, L. Shi and Z. Zhao, 2011. Isolation and bioactivities of organic acids and phenols from walnut shell pyroligneous acid. J. Anal. Applied Pyrol., 91: 338-343.
30. Hong, L.S., D. Ibrahim, J. Kassim and S. Sulaiman, 2011. Gallic acid: An anticandidal compound in hydrolysable tannin extracted from the barks of *Rhizophora apiculata* Blume. J. Applied Pharmaceut. Sci., 1: 75-79.
31. Poompozhil, S. and D. Kumarasamy, 2014. Studies on phytochemical constituents of some selected mangroves. J. Acad. Ind. Res., 2: 590-592.
32. Surya, S. and N. Hari, 2017. Studies on preliminary phytochemical analysis of some true mangrove species in Kerala. Int. J. Res. Pharm. Pharmaceut. Sci., 2: 15-17.
33. Saravanan, D. and M. Radhakrishnan, 2016. Antimicrobial activity of mangrove leaves against drug resistant pathogens. Int. J. PharmTech Res., 9: 141-146.
34. Seepana, R., K. Perumal, N.M. Kada, R. Chatragadda, M. Raju and V. Annamalai, 2016. Evaluation of antimicrobial properties from the mangrove *Rhizophora apiculata* and *Bruguiera gymnorrhiza* of Burmanallah Coast, South Andaman, India. J. Coastal Life Med., 4: 475-478.
35. Pimpliskar, M.R., R.N. Jadhav and B.L. Jadhav, 2012. Evaluation of antimicrobial principles of *Rhizophora* species along Mumbai Coast. J. Adv. Scient. Res., 3: 30-33.
36. Meena, C., J. Gopalakrishnan and P. Dureja, 2013. Antibacterial withametelin B from *Datura metel* against *Xanthomonas oryzae* pv. *oryzae*. Biopestic. Int., 9: 31-37.
37. Kumar, A., 2006. Pit canker disease of Siris (*Albizzia procera*)-a new report from Himachal Pradesh. Indian Phytopathol., 59: 124-125.
38. Kagale, S., T. Marimuthu, B. Thayumanavan, R. Nandakumar and R. Samiyappan, 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Physiol. Mol. Plant Pathol., 65: 91-100.