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Research Article

EVALUATION OF ANTIBACTERIAL PROPERTIES OF AVICENNIA MARINA (FORSSK.) VIERH FLOWERS AND ITS ENDOPHYTE KUSHNERIA AVICENNIAEA, ALONG WITH GC-MS ANALYSIS OF SECONDARY METABOLITES

ASHA SELVA MALAR M, REENA A*

Department of Microbiology, Mohamed Sathak College of Arts and Science, Chennai, Tamil Nadu, India.
***Corresponding author: Reena A; Email: reena.denzil@yahoo.com**

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ABSTRACT

Objectives: This research aimed to evaluate the antibacterial effects of solvent extracts obtained from the flowers of *Avicennia marina* (Forssk.) Vierh and its endophyte, *Kushneria avicenniae*, and to analyze the bioactive compounds produced by the endophyte.

Methods: This research gathered flowers from the mangrove species *A. marina* (Forssk) Vierh in Manakudi village, located in the Ramanathapuram district of Tamil Nadu. The flowers underwent surface sterilization, followed by the isolation of an endophyte using a Starch casein agar medium. The antibacterial properties of various solvent extracts from the flower and its endophyte were evaluated against *Listeria monocytogens* ATCC 19115 and *Escherichia coli* ATCC 25922 using the well diffusion method. The isolated endophyte was identified through genotypic methods, and gas chromatography-mass spectrometry was employed to analyze the bioactive compounds produced by this endophyte.

Results: The different solvent extracts of the flower of the mangrove *A. marina* (Forssk) Vierh showed no inhibition zone for the pathogens, and the extract of the bacteria showed an inhibition zone of 11 mm for *E. coli* ATCC 25922 and 12 mm for *L. monocytogenes* ATCC 19115. The endophyte was identified as *K. avicenniae*. The gas chromatography- mass spectrometry analysis detected the presence of nearly 40 potential bioactive compounds.

Conclusion: This study emphasised the importance of exploring the mangrove ecosystem and its relatively understudied endophytes, capable of producing secondary metabolites with distinctive features and bioactivities.

Keywords: *Avicennia marina* (Forssk) Vierh, *Kushneria avicenniae*, Gas chromatography- mass spectrometry, Endophyte, Antibacterial activity.

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INTRODUCTION

Nature has been a reservoir of medicinal compounds for thousands of years and across various eras. Many contemporary drugs have been derived from natural sources, leveraging their applications in traditional medicine. Natural products remain a reliable source of pharmaceutical agents and potential drug candidates, with numerous fascinating molecules discovered from naturally occurring and genetically modified microorganisms [1]. Mangroves are tree-like plants specifically adapted to thrive in the wetlands of intertidal zones found in tropical and subtropical coastal areas, making them the second most vital marine ecosystem after coral reefs [2]. Mangrove ecosystems possess intricate nutritional environments and generate a variety of pharmaceutical compounds through microorganisms. These microbes can obtain nutrients through reverse processes, allowing them to grow and create new bioactive metabolites that influence the elements' geochemical cycles. In addition, they play a significant role in material cycling and energy flow within mangrove ecosystems [3]. Research has indicated that mangrove plants possess effective antibacterial, anticancer, anti-inflammatory, antiviral, and antidiabetic properties. The genus *Avicennia* is recognized globally as a significant plant with the potential to generate novel biopharmaceutical products. Prior studies have indicated that the mangrove species *Avicennia marina* possesses a high concentration of tannins in its wood. Additionally, the bark of *A. marina* is utilised in the leather and dyeing industries. This species is noted for its remarkable healing properties, which are attributed to its rich content of phytochemicals, including terpenoids, glucosides, and naphthalene [4]. The abundant presence of phenols and flavonoids in *A. marina* has been recognised for its bioactive compounds and antimicrobial characteristics [5]. Phyto derivatives in the plant play a crucial role in synthesising chemical compounds and contribute significantly to the plant's defence mechanisms, safeguarding it against biotic and abiotic stress factors. They enhance the plant's resilience and adaptability in challenging environmental conditions while supporting essential biological processes [5]. The abundant availability of these derivatives underscores their medicinal properties and potential role in health remedies. The shortcomings and limitations of existing synthetic drugs have steered attention toward exploring new plant-based materials and their associated chemical derivatives. This shift has also enabled the discovery of novel chemical compounds that enhance the efficacy of natural substances. Notably, approximately 95% of synthetic drugs are linked to significant hazardous side effects, which can severely undermine their intended efficacy. Due to these challenges, recent research has increasingly focused on discovering natural product drugs from diverse sources. Among these, the largely unexplored mangrove ecosystems have emerged as a vital resource for identifying potential drug candidates with minimal harmful effects against various pathogens [6].

Endophytes

Endophytic bacteria are plant-associated microorganisms that reside within plant tissues without causing any harm to their host. Endophytes, protected by their host plants from environmental challenges and microbial competition, are widespread within plant tissues. They can benefit their host by synthesising natural products with potential medical, agricultural, and industrial applications [7].

Genus *Kushneria*

Kushneria (Kush.ne9ri.a.): A Latin feminine noun, *Kushneria*, named in honour of Dr. Donn J. Kushner, a Canadian microbiologist renowned for his groundbreaking research on halophilic microorganisms. The genus *Kushneria*, belonging to the family *Halomonadaceae* within the

Table 1: Antibacterial activity of the ethyl acetate extract of *Kushneria avicenniae*

S. No	Name of the pathogens	Zone of inhibition (mm)	Positive control chloramphenicol	Negative control DMSO
1	*Listeria monocytogenes* ATCC 19115	12±0.5	10±0.5	-
22	*Escherichia coli* ATCC 25922	11±0.5	11±0.5	-

DMSO: Dimethyl sulfoxide

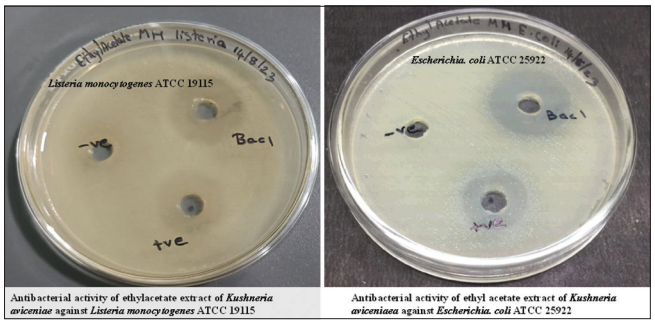

Fig. 1: The antibacterial activity of the ethyl acetate extract of the endophyte, *Kushneria avicenniae,* against the pathogens, *Escherichia coli* 25922 and *Listeria monocytogens* 19115

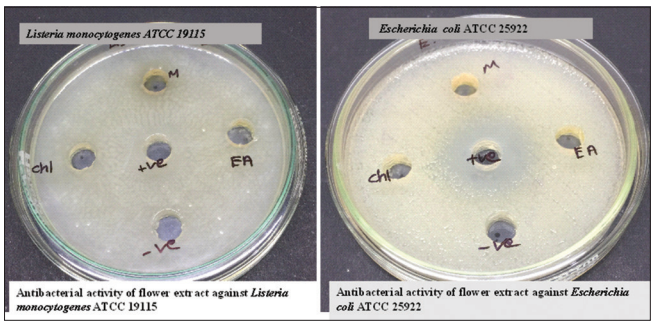

Fig. 2: The antibacterial activity of the methanol, chloroform, and ethyl acetate extracts of the flower of *Avicennia marina* (Forsk) Vierh against *Escherichia coli* 25922 and *Listeria monocytogens* 19115

order *Oceanospirillales*, was first introduced in 2009 to describe the novel species *Kushneria aurantia* [8]. Currently, the genus comprises ten recognised species: *K. aurantia*, *Kushneria phosphatelytica* [9], *Kushneria avicenniae* [10], *Kushneria endophytica* [11], *Kushneria phyllosphaerae* [11]. *Kushneria indalinina* [12], *Kushneria marisflavi* [13], *Kushneria pakistanensis* [14], *Kushneria konosiri* [15], and *Kushneria sinocarnis* [16]. The type strains of *Kushneria* are predominantly isolated from hypersaline environments, such as salt-cured leaves of *Avicennia germinans*, halophyte plants, solar salterns, salt mines, cured meats, seawater, salt-fermented seafood, and halophyte plants [9].

The salt-encrusted leaf surfaces of the black mangrove, *A. germinans*, close to the solar salterns of Cabo Rojo, Puerto Rico, were used to identify the unique species *Halomonas avicenniae* spp. nov. [10] In the Odiel wetlands of Huelva, Spain, *K. phyllosphaerae* spp. nov. and *K. endophytica* spp. nov. were identified as endophytes from the aerial portions of the halophyte plant *Arthrocnemum macrostachyum*. Trypticase soy agar supplemented with 2.5% NaCl was used to culture these strains, which were then incubated for 2 days at 30°C. [11].

In this research, the bacteria identified as part of the genus *Kushneria* exhibited similarities to *K. avicenniae*, which had previously been documented in *A. germinans*. However, this is the first instance of it being reported as a flower endophyte in *A. marina* (Forssk) Vierh.

METHODS

Sample collection

The flowers of *A. marina* (Forssk.) Vierh were gathered from Manakudi village in the Ramanathapuram district of Tamil Nadu, positioned between latitude 9.65955 and longitude 78.950214.

Taxonomic identification

The gathered plant specimens were dispatched to the Department of Pharmacognosy at SCRI, located in Arumbakkam, Chennai—6, and were taxonomically identified as *A. marina* (Forssk.) Vierh (Requisition no. 535.07062301).

Surface sterilization

The gathered flowers were rinsed under flowing water to eliminate dust and dirt, followed by a wash with distilled water. A 70% ethanol solution was used to surface sterilize the flowers for 1 min. Then, they were treated with sodium hypochlorite for 5 min and again with 70% ethanol for 30 s. The flowers were then rinsed 3 times with sterile distilled water. Finally, the cleaned flowers were placed on sterile filter paper to dry.

Isolation of endophytes

Following surface sterilization, the flowers were chopped into small fragments, placed onto the starch casein agar (SCA) medium enriched with 0.5% NaCl, and incubated at 37°C for 7 days. The resulting cultured colonies were then streaked onto fresh sterile SCA medium for further culturing and incubated again. The isolated pure colonies were subsequently transferred into 500 mL SCA broth to facilitate fermentation for secondary metabolite production.

Molecular identification

The bacterial DNA was extracted following the manufacturer's guidelines using the NucleoSpin® Tissue Kit (Macherey – Nagel). The quality of the isolated DNA was assessed through agarose gel electrophoresis. Polymerase chain reaction (PCR) amplification of the 16S rRNA gene was carried out in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The primers employed were (CAGGCCTAACACATGCAAGTC - 16S-RS-F Forward and GGGCGGWGTGTACAAGGC - 16S-RS-R Reverse). Sequencing of the reaction was performed in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) utilizing the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA), following the instructions provided by the manufacturer. The ABI 3500 DNA Analyzer (Applied Biosystems) was used to sequence the PCR product, employing the Sanger sequencing method. Sequence Scanner Software v1 (Applied Biosystems) reviewed the sequences' quality. Sequence alignment and necessary adjustments of the resulting sequences were made using Geneious Pro v5.1 [17].

Antibacterial activity of the endophyte using agar well diffusion method

The bacterial strains cultured overnight were swabbed to Muller Hinton agar plates using sterile cotton swabs. Sterile borers were used to create wells with a diameter of approximately 8 mm. Approximately 50 µL of the endophyte extract was introduced into the well. Chloramphenicol and dimethyl sulfoxide served as the negative and positive controls, respectively. The inoculated Muller-Hinton agar plates were then incubated for 24 h at 37°C. All experiments were performed in triplicate, and observations were made after 24 h of incubation [18].

Antibacterial activity of the flower extracts of *A. marina* (Forssk.) Vierh the well diffusion method

The antibacterial properties of flower extracts in methanol, chloroform, and ethyl acetate were evaluated against the pathogens

Table 2: GC-MS analysis of the secondary metabolites of the endophyte, *Kushneria avicenniae*

S. No.	Name of the compound	Molecular formula	Molecular weight	Probability %	Class of compounds
1	Dodecyl acrylate	$C_{15}H_{28}O_2$	240.38 g/moL	10.59	Ester
2	2-Propenoic acid, pentadecyl ester	$C_{18}H_{34}O_2$	282.5 g/moL	8.95	Ester
3	2-Propenoic acid, tridecyl ester	$C_{16}H_{30}O_2$	254.41 g/moL	7.56	Ester
4	1,4-diazabicyclo[4.3.0]nonan-2,5-dione, 3-methyl	$C_8H_{12}N_2O_2$	168.19 g/moL	95.84	A pyrrole derivative
5	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione, N-acetyl-	$C_{10}H_{14}N_2O_3$	210.23 g/moL	1.61	Piperazine
6	Uric acid	$C_{13}H_{19}NO_4S$	285.36 g/moL	0.34	
7	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	154.17 g/moL	76.82	Organonitrogen compound
8	Glycyl-L-proline	$C_7H_{12}N_2O_3$	172.18 g/moL	17.82	Dipeptide
9	1-Oxaspiro (4,5) decan-2-one	$C_9H_{14}O_2$	154.21 g/moL	0.75	Spirolactone
10	Cyclo (L-prolyl-L-valine)	$C_{10}H_{16}N_2O_2$	196.25 g/moL	94.17	Diketopiperazines
11	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210.27 g/moL	2.41	Diketopiperazine
12	5-Azacytosine, N, N, N'-trimethyl-	$C_3H_4N_4O$	112.09 g/moL	1.31	Cytosine analog
13	(2S,6R)-2,6-Dibutyl-4-methylpiperidine	$C_{14}H_{29}N$	211.39 g/moL	0.59	Piperidine
14	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34 g/moL	11.24	Plasticiser
15	Phthalic acid, butyl hex-3-yl ester	$C_{19}H_{24}O_4$	320.4 g/moL	10.36	Plasticiser
16	Phthalic acid, butyl 2-pentyl ester	$C_{17}H_{24}O_4$	292.4 g/moL	6.49	Plasticiser
17	2,5-Piperazinedione, 3,6-bis (2-methylpropyl)-	$C_{12}H_{22}N_2O_2$	226.3153	63.67	Diketopiperazine
18	l-Leucyl-d-leucine	$C_{12}H_{24}N_2O_3$	244.33 g/moL	11.93	Peptides
19	2-Butylamine, N-nonyl-	$C_4H_{11}N$	73.14 g/moL	6.87	Secondary aliphatic amine
20	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	$C_{14}H_{16}N_2O_2$	244.29 g/moL	87.91	Diketopiperazine
21	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-,	$C_{33}H_{37}N_5O_5$	583.7 g/moL	11.42	KETONE
22	Ergotamine	$C_{33}H_{35}N_5O_5$	581.7 g/moL	0.45	Alkaloid
23	Tetracosane	$C_{24}H_{50}$	338.7 g/moL	14.68	Alkanes
24	Pentacosane	$C_{25}H_{52}$	352.7 g/moL	10.07	Alkanes
25	Eicosane, 10-methyl-	$C_{21}H_{44}$	296.6 g/moL	8.89	Alkanes
26	Triacontane	$C_{30}H_{62}$	422.8 g/moL	10.16	Alkanes
27	Phthalic acid, di (2-propylpentyl) ester	$C_{24}H_{38}O_4$	390.6 g/moL	29.83	Ester
28	Phthalic acid, di (oct-3-yl) ester	$C_{24}H_{38}O_4$	390.6 g/moL	8.61	Ester
29	1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.6 g/moL	7.27	Ester
30	Tetratriacontane	$C_{34}H_{70}$	478.9 g/moL	9.09	Alkanes
31	Cyclo-(l-leucyl-l-phenylalanyl)	$C_{15}H_{20}N_2O_2$	260.329 g/moL	23.3	Diketopiperazines
32	Pyrimidine-2 (1H)-thione, 4,4,6-trimethyl-1-(1-phenylethyl)-	$C_{15}H_{20}N_2S$	260.399 g/moL	14.12	
33	2-Acetylamino-3-phenylpropionic acid, 1-carbamoylethyl ester	$C_{14}H_{18}N_2O_4$	278.3 g/moL	8.56	
34	Nonacosane	$C_{29}H_{60}$	408.8 g/moL	11.4	Alkanes
35	Octacosane	$C_{28}H_{58}$	394.8 g/moL	30.91	Alkanes
36	Hexatriacontane	$C_{36}H_{74}$	507 g/moL	6.7	Alkanes
37	Tritetracontane	$C_{43}H_{88}$	605.2 g/moL	11.41	Alkanes
38	Betulinaldehyde	$C_{30}H_{48}O_2$	440.7 g/moL	22.79	Triterpenoid
39	Lupeol	$C_{30}H_{50}O$	426.7 g/moL	21.02	Triterpenoid
40	Cedran-diol, (8S,14)	$C_{15}H_{26}O_2$	238.3657	5.73	Alcohol
41	Octadecane, 1,1'-[1,3-propanediylbis (oxy)]bis-	$C_{39}H_{80}O_2$	581.1 g/moL	13.23	Lipid
42	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7 g/moL	9.07	Alkane
43	Stearic acid, 3-(octadecyloxy) propyl ester	$C_{39}H_{78}O_3$	595 g/moL	4.95	Lipid
44	Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester	$C_{20}H_{28}O_3$	316.4 g/moL	15.68	Ester

GC-MS: Gas chromatography- mass spectrometry

Listeria monocytogenes ATCC 19115 and *Escherichia coli* ATCC 25922 using the agar well diffusion technique. Approximately 100 µL of an overnight culture of the pathogenic strains was spread onto Muller-Hinton Agar medium. Wells measuring about 8mm in diameter were created, and 50 µL of each extract was introduced into the wells, with the plates being incubated at 37°C for 24 h. Following incubation, the diameter of the clear zones surrounding the wells was measured and recorded.

Pathogenic bacterial strains used in the study

This research utilized the pathogenic bacterial strains *L. monocytogenes* ATCC 19115 and *E. coli* ATCC 25922, obtained from the Microbial Type Culture Collection and Gene Bank in Chandigarh. Pure colonies of these bacterial pathogens were introduced into Muller Hinton agar broth and standardized to 0.5 McFarland turbidity standards.

Gas chromatography- mass spectrometry (GC-MS)

The crude extract of strain MMSASO1 was subjected to GC-MS analysis. This analysis was conducted using the Agilent 8890 GC gas chromatograph in conjunction with the Agilent 5977 MSD module type: GC/MS. A 30 m × 250 µm × 0.25 µm HP–5 ms capillary column was utilized for separation. The ethyl acetate extract of the sample was injected with a flow rate of 1 mL/min. Helium was the carrier gas at a 1 mL/min flow rate. The samples were analyzed at an oven

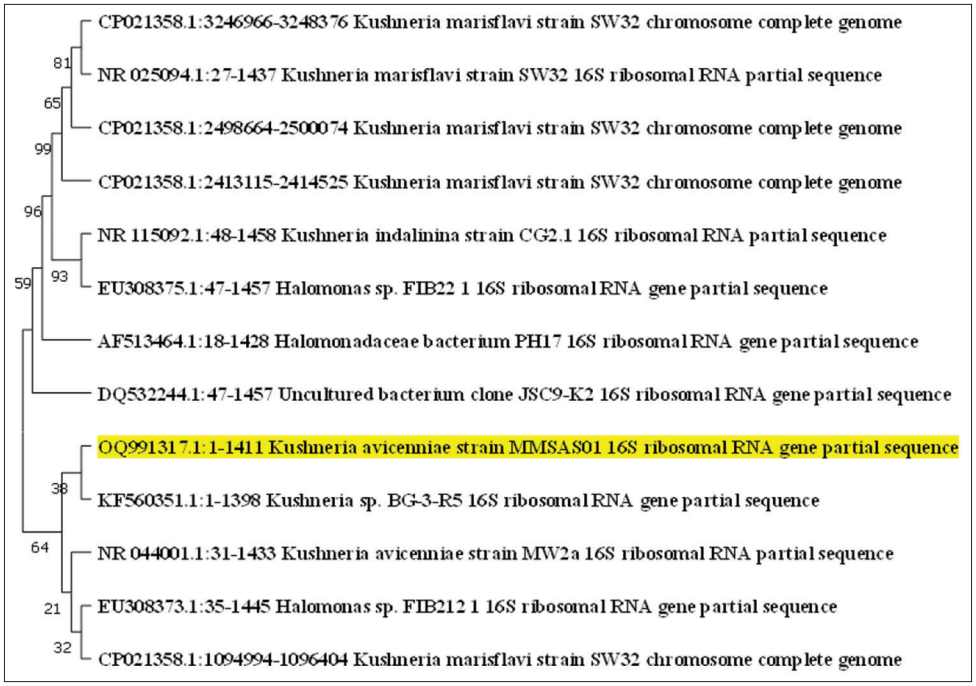

Fig. 3: Asm 1 *Kushneria avicenniae* Strain Mmsas 01 16s ribosomal rna gene, partial sequence

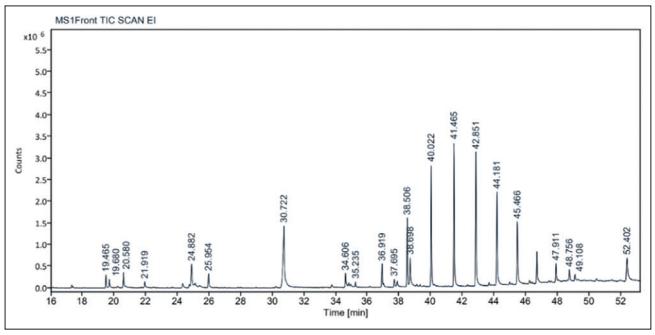

Fig. 4: Chromatogram of gas chromatography- mass spectrometry analysis of secondary metabolites

temperature of 10°C/min, ranging from 50 to 250°C. Impact ionization was performed using electrons at 70 ev. Data acquisition was executed in mass spectrometry scan mode. The components were identified by comparing their retention times and mass spectra with those in the National Institute of Standards and Technology library 7 mass spectrum database provided by the instrument's software.

RESULTS

Antibacterial activity of *K. avicenniae*

The ethyl acetate extract derived from *K. avicenniae* demonstrated antibacterial properties against *L. monocytogenes* ATCC 19115 and *E. coli* ATCC 25922. It produced an inhibition zone measuring approximately 11 mm for *E. coli* ATCC 25922 and 12 mm for *L. monocytogenes* ATCC 19115 (Table 1 and Fig. 1).

Antibacterial activity of flower extract

The ethyl acetate, chloroform, and methanol extract of *A. marina* Vierh (Forssk) flower showed no antibacterial activity against *L. monocytogenes* ATCC 19115 and *E. coli* ATCC 25922 Fig. 2.

Statistical analysis

Antibacterial assay was performed in triplicate, and the zone of inhibition was expressed as the mean average with standard deviation. All antimicrobial activity data were analysed using one-way analysis of variance followed by Tukey's honest significant difference *post hoc* comparison test ($p<0.05$).

Molecular identification

The identification and characterisation of selected isolates were done at the molecular level by amplifying the 16s rRNA gene using suitable primers. The obtained sequences were submitted to GenBank with accession numbers of SUB13362052, Contig_asmm01 OQ991317, and identified as *K. avicenniae* Fig. 3.

Phylogenetic analysis

Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbour-Joining method [19]. The bootstrap consensus tree inferred from 1000 replicates [20] represents the evolutionary history of the taxa analysed [20]. Branches corresponding to partitions reproduced in <50% of bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches [20]. The evolutionary distances were computed using the Maximum Composite Likelihood method [21] and are in the units of the number of base substitutions per site. This analysis involved 13 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1487 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [22].

GC-MS analysis of K. avicenniae

GC-MS analysed a complex mixture of bioactive compounds in the ethyl acetate extract of the bacteria K. avicenniae. About 44 different compounds were detected, and their structure was studied (Table 2). Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester has the highest retention time. About 20% of the compounds belong to the alkane class of compounds, and 18% belong to the ester class of compounds. 1,4-diazabicyclo [4.3.0] nonan-2,5-dione, 3-methyl is a pyrrole derivative with a highest probability percentage of 95.84. The detected compounds belong to diverse classes of compounds, such as esters, alkanes, triterpenoids, diketopiperazines, Spiro lactone, peptides,

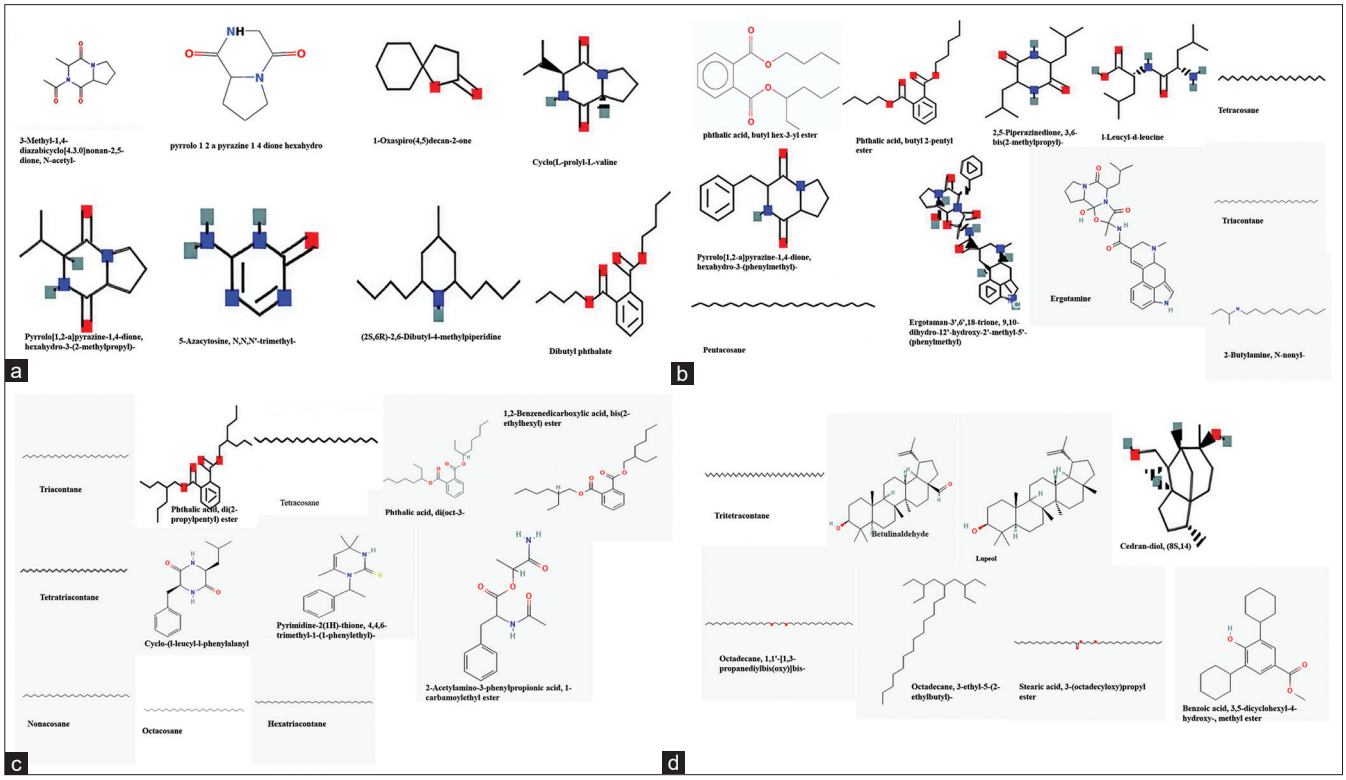

Fig. 5: (a-d) Chemical structure of the secondary metabolites of *Kushneria avicenniae*

Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester, which is an ester with a high retention time of 52.402 min (Fig. 4).

DISCUSSION

Marine habitats are attractive sources of bioactive compounds due to their complex class of compounds and extreme environments. Endophytes that inhabit the mangrove habitat produce unique metabolites due to the unique and extreme environmental conditions. The compounds produced exhibit various activities.

Antibacterial activity

The methanol, chloroform, ethyl acetate extract of the *A. marina* Vierh (Forssk) showed no zone of inhibition against the pathogens *L. monocytogenes* ATCC 19115 and *E. coli* ATCC 25922 Earlier studies have shown that the ethanol extract of flower of *A. marina* showed no antibacterial against *E. coli* (ATCC 25922) and multidrug resistant *Klebsiella* spp. and showed antibacterial action against *Staphylococcus aureus* (ATCC 29213) and multidrug-resistant *Acinetobacter* spp. and *Pseudomonas* spp. [23].

GC-MS analysis

The GC-MS analysis conducted in the current study revealed the presence of various classes of compounds with biological activities (Fig. 5).

Dodecyl acrylate [24,25], 2-Propenoic acid pentadecyl ester [26], 2-Propenoic acid tridecyl ester, Stearic acid 3-(octadecyl)propyl ester, 1,2-Benzenedicarboxylic acid bis(2-ethylhexyl) ester, Phthalic acid di(oct-3-yl) ester, Phthalic acid di(2-propylpentyl) ester, 2-Acetylamino-3-phenylpropionic acid 1-carbamoylethyl ester, and Benzoic acid 3,5-dicyclohexyl-4-hydroxy- methyl ester, which are part of the ester compound class, exhibit antimicrobial, insecticidal, antifungal, antioxidant, anticancer, anti-inflammatory, and larvicidal properties. They demonstrate extreme antifungal and antioxidant activities, as well as notable anticancer effects.

3-Methyl-1,4-diazabicyclo [4.3.0] nonan-2,5-dione has been identified in an endophytic organism known for combating bacterial blight in pomegranate, demonstrating antifungal and antibacterial properties [27]. N-acetyl-3-methyl-1,4-diazabicyclo [4.3.0] nonan-2,5-dione, a piperazine compound, has also exhibited antifungal activity [28].

Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- is an organonitrogen compound reported to possess antifungal, algicidal, and antioxidant activity [29]. The dipeptide glycyl-L-proline has attracted significant interest because of its neuroprotective effects and potential use in treating neurodegenerative diseases [30]. The compound, identified in the bacterium *Streptomyces albus* DR 57 from the Pichavaram Mangrove National Forest in Killai, Tamil Nadu, has shown potential as a promising agent for promoting wound healing, attributed to its involvement in collagen synthesis [31].

The dipeptide glycyl-L-proline has been identified as a promising agent for promoting wound healing due to its involvement in collagen synthesis [32]. In addition, it has attracted considerable attention for its neuroprotective properties and potential therapeutic applications, particularly in addressing neurodegenerative diseases [33]. The compound has also been detected in the bacterium *S. albus* DR 57, which was isolated from the Pichavaram Mangrove National Forest in Killai, Tamil Nadu [34].

1-Oxaspiro (4,5) decan-2-one is a spironolactone with a retention time of 20.578 min found in natural products exhibiting antifungal properties [35]. Cyclo(L-prolyl-L-valine) belongs to a cyclopeptide class that inhibits the fungus *Aspergillus fumigatus* and suppresses aflatoxin production [36].

Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro, a diketopiperazine compound produced by *Bacillus tequilensis* MSI45, acts as an antibiotic that inhibits the growth of *Staphylococcus* infections [37]. In addition, 2,5-piperazinedione, 3,6-bis(2-methylpropyl), another

diketopiperazine, has been identified in *Halobacillus* species [38]. Furthermore, pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl), demonstrates potential activity hindering biofilm formation of two *Pseudomonas aeruginosa* strains [39]. 5-Azacytosine, N,N,N'-trimethyl, has been documented to exhibit antimicrobial, antioxidant, and nematicidal properties [40]. Dibutyl phthalate, phthalic acid butyl hex-3-yl ester, and phthalic acid butyl 2-pentyl ester (retention time: 25.954 min) have demonstrated anticancer properties [41]. In addition, these compounds are reported to exhibit allelopathic, antimicrobial, insecticidal, and various other biological activities [42]. Ergot alkaloids, classified as secondary metabolites, are nitrogenous organic compounds produced by fungi. Globally, *Claviceps purpurea* is known to produce six common ergot alkaloids [43].

Tetracosane, pentacosane, eicosane-10-methyl, triacontane, nonacosane, octacosane, hexatriacontane, tritetracontane, and octadecane-3-ethyl-5-(2-ethylbutyl)- belong to the class of alkanes. These compounds have demonstrated various biological activities, including cytotoxic effects [44], antimicrobial and phototoxic properties [45,46], and high antioxidant potential [47]. They also exhibit antibacterial, antiviral, and antioxidant activities [48] and are utilised for anti-HIV, antioxidant, antibacterial, antimicrobial, cytotoxic, and antimalarial treatments [49]. Furthermore, they have shown wound healing effects in patients with diabetes mellitus [50], antioxidant and antimicrobial properties [51], antimycobacterial activity isolated from the hexane extract of mangrove plants [52], and antimicrobial and antifungal activities [53]. Phthalic acid di(2-propylpentyl) ester exhibits antimicrobial activity [54], while phthalic acid di(oct-3-yl) ester demonstrates anti-inflammatory properties [55]. Additionally, 1,2-benzenedicarboxylic acid bis(2-ethylhexyl) ester has been identified as a potent bioactive secondary metabolite, naturally synthesised by bacterial, fungal, and algal species [56,57].

Pyrimidine-2(1H)-thione,4,4,6-trimethyl-1-(1-phenylethyl)-reported in the *Halomonas* spp. known to possess antimicrobial properties [58]. Betulinaldehyde, a triterpenoid derived from birch bark, displays a variety of biological activities, such as antitumor, anti-inflammatory, and antimicrobial properties, positioning it as a promising candidate for therapeutic applications [59]. Similarly, lupeol, a pentacyclic triterpenoid found in numerous plants, demonstrates a broad spectrum of biological effects, including anti-inflammatory, antioxidant, anticancer, and antimicrobial actions, highlighting its potential as a therapeutic agent [60].

The essential compounds found with the help of GC-MS analysis exhibited different levels of antibacterial activity against the tested microorganisms. The tested microorganisms were susceptible to the essential oils of medicinal plants, likely stemming from their inherent properties, particularly regarding how permeable their cell surfaces are to these oils. With the rise of antibiotic-resistant pathogens, plants are increasingly viewed as a promising alternative to fight the spread of multidrug-resistant microorganisms [61].

CONCLUSION

Mangrove ecosystems represent an extraordinary reservoir of novel secondary metabolite products, characterised by unique structural and chemical properties typically absent in terrestrial bioactive metabolites. The challenging environmental conditions, such as intertidal fluctuations, drive microbes, plants, and organisms to generate highly effective bioactive metabolites. Among these, one notable feature of the genus *Kushneria* is its remarkable ability to thrive in high salt concentrations, with particular species capable of growing in up to 25% NaCl, far exceeding the salt content of seawater. This adaptability makes *Kushneria* a compelling research subject, particularly its potential applications in the bioremediation of saline soils and the development of salt-tolerant crops.

Furthermore, GS-MS analysis has identified the compounds responsible for these bioactivities, paving the way for novel compounds with significant environmental and pharmaceutical relevance. These metabolites hold promise as alternative natural drugs, exhibiting various biological activities, including antimicrobial, antibiofilm, and antioxidant effects, while ensuring minimal adverse side effects.

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AUTHOR'S CONTRIBUTION STATEMENT

Dr A. Reena designed the study, analyzed the data, and reviewed the manuscript. Ms. M. Asha Selva Malar performed the experiment, interpreted the result, and drafted the manuscripts.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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