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Research Article

A STUDY INVESTIGATING THE PRESENCE OF BIOACTIVE COMPOUNDS BY THIN-LAYER CHROMATOGRAPHY IN *AVICENNIA MARINA* (FORSSK.) VIERH AND THE ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS USING VARIOUS SOLVENTS

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ABSTRACT

Objective: The present study aims to find out the presence of potential bioactive compounds in the mangrove plant *Avicennia marina* (Forssk.) Vierh, through thin-layer chromatography (TLC), observes the antibacterial activity of various solvent extracts of the plant parts against the pathogens *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 19115.

Methods: TLC of the methanol, ethyl acetate, chloroform solvent extracts of stem, root, and leaf of mangrove plant *A. marina* (Forssk.) Vierh was studied with the mobile phase hexane: ethyl acetate in the ratio of 1;1, 3;7, and 7;3. The spots were identified at ultraviolet 240 nm. Antibacterial activity of the solvent extracts of different plant parts was done against *L. monocytogenes* ATCC 19115 and *E. coli* ATCC 25922 by well diffusion method.

Results: The thin-layer chromatographic technique revealed the presence of the potential bioactive compound in the *A. marina* (Forssk.) Vierh. The solvent extracts of different plant parts showed the zone of inhibition for the antibacterial effect ranges between 1 mm and 9 mm. The methanol root extract showed a maximum inhibitory effect against *L. monocytogenes* ATCC 19115. The methanol stem and leaf extract showed maximum inhibition against *E. coli* ATCC 25922.

Conclusion: The present study emphasizes the importance of exploring the mangrove plant *A. marina* (Forssk.) Vierh is a potent source of bioactive compounds.

Keywords: *Avicennia marina* (Forssk.) Vierh, Thin layer chromatography, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 19115, Antibacterial activity.

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INTRODUCTION

Microorganisms are found everywhere. Humans face ongoing threats from pathogenic microbes, as they can thrive within their hosts and cause infections. These organisms can be found in the food we eat, the soil, and the atmosphere. Antimicrobial agents play a vital role in preventing infections in both healthy and immunocompromised individuals. However, these pathogens eventually develop resistance to all synthetic medications. The prevalence of antimicrobial resistance has surged significantly over the last few decades, resulting in higher death rates, increased medication costs, and challenges in treating infections [1]. The World Health Organization has forecasted that mortality attributed to antimicrobial resistance may potentially escalate to 10 million by 2050 [2]. Urgent action is necessary to address the increasing prevalence of resistance to antibiotics and to seek new antimicrobial agents that can provide effective, cost-efficient treatment with prolonged antimicrobial activity.

Numerous biological elements typically utilized as antimicrobial agents are abundantly available in plants. Chemical antibiotics are ineffective as harmful bacteria develop resistance to commercial antibiotics, posing a threat to society. Scientists have been investigating medicinal plants to identify new antimicrobial substances. In developing countries, locals commonly use native herbal plants for treating diseases and infections. Following the recognition of the importance of mangroves, researchers redirected their attention from terrestrial medicinal plants to marine environments in search of novel bioactive compounds.

When it comes to production and tertiary yield, the mangrove ecosystem ranked second in importance. In addition to producing organic carbon and protecting the coastal shoreline from natural disasters, mangroves contribute significantly to the global carbon cycle [3]. They are the most varied and abundant type of wetland ecosystem. They rely on the muddy substrate to thrive that varies in texture and depth. Mangroves grow best in the coastal intertidal zones of tropical and subtropical regions. Because of its capacity to adjust to changing environmental conditions, including salinity, temperature, nutrients, and tidal currents, the mangrove ecosystem is thought to be extremely productive [4]. Because of the extreme environmental stress, the mangrove flora developed an adaptation mechanism that results in the production of secondary metabolites, which are important phytochemicals with medical applications.

This research attempts to look into the antibacterial action of the methanolic, ethyl acetate, and chloroform extracts of leaves, roots, and bark of *Avicennia marina* (Forssk.) Vierh against *Listeria monocytogenes* 19115 and *Escherichia coli* 25922 and the thin-layer chromatography (TLC) of the chloroform, methanol, and ethyl acetate extracts of leaves, bark, and root of *A. marina* (Forssk.) Vierh is performed to study the presence of bioactive compounds further.

Description of the plant

In terms of the biodiversity of mangroves, India ranks third in the world. India holds the title for having the most floristic variety among mangroves worldwide [5]. *A. marina* (Forssk.) Vierh is commonly called a gray mangrove (Fig. 1b). It belongs to the family Avicenniaceae. The genus *Avicennia* had worldwide distribution. Growth varies from tall trees to shrubby individuals with broad trunks. The bark is smooth and white. *A. marina* (Forssk.) Vierh plant grows to a medium height of 3–11 m. The bark of the plant displays a pattern of peeling, smooth, and flaky yellowish-green sections. The pneumatophores in the roots are slender, measuring 10–15 cm in length and having pointy tips. The leaves are glossy and tough, with a yellowish-green color on top and a pale, dull color underneath [6]. The yellow petals of *A. marina*'s flowers are grouped tightly in cross-like inflorescences, each with a diameter of 4 mm. The fruits are oval, flattened, pale gray-green, 20–25 mm across, and have two valves [7].

METHODS

Collection of plant sample

The roots, stems, and leaves of *A. marina* (Forssk.) Vierh was collected from Manakudi village, Ramanathapuram district, Tamil Nadu, between latitude 9.65955 and longitude 78.950214 (Fig. 1a).

Taxonomic identification

The collected plant samples were sent to the Department of Pharmacognosy, SCRI, Arumbakkam, Chennai-6. and identified taxonomically as *A. marina* (Forssk.) Vierh (Requisition no. 535.07062301).

Plant sample preparation

The dirt of the collected plant samples was cleaned with running tap water followed by deionized water. Then, they were ground into a fine powder after being dried in the shade [6].

Crude extract preparation

The extract was prepared by adding the homogenous powdered plant sample to solvents such as methanol, ethyl acetate, and chloroform. About 10 g of each plant portion was extracted with 100000 μL of the solvent with continuous stirring for 72 h. After centrifugation, the filtrate was separated and dried. The dried sample was dissolved in DMSO for further studies [6].

Pathogenic bacterial strains used in the study

The selected pathogenic strains *L. monocytogenes* ATCC 19115 and *E. coli* ATCC 25922 were procured from Microbial Type Culture Collection and Gene Bank, Chandigarh. Pure colonies of the bacterial pathogens were inoculated into Muller Hinton agar broth and standardized to 0.5 McFarland turbidity standards.

Antibacterial activity using agar well diffusion method

Using sterile cotton swabs, the overnight-cultured pathogens were swabbed onto the Muller Hinton agar plates. A sterile borer was used to construct wells with a diameter of roughly 8 mm. About 50 μL of the solvent extract of the plant parts was added to the well. DMSO and chloramphenicol served as the positive and negative controls, respectively. Muller Hinton agar plates that had been inoculated were incubated at 37°C for 24 h. Triplicates were carried out for all the experiments, and the results were observed after 24 h of incubation [8].

Analysis of bioactive compounds in *A. marina* (Forssk.) Vierh by TLC

TLC is an analytical method to investigate the occurrence of natural biologically active compounds. The advantage of TLC is running more

Fig. 1: The location of sample collection (a) and the Mangrove plant *Avicennia marina* (Forssk) Vierh (b)

samples simultaneously at a low cost. Freshly prepared plant extract was used for TLC profiling [9]. The plant extract was spotted on the silica gel plate with a uniform thickness of 0.2 mm. Chromatography was developed by employing the solvent systems hexane: ethyl acetate (1:1), (3:7), and (7:3). The slides were observed at 240 nm in the ultraviolet (UV), and spots were visualized by spraying with p-p-anisaldehyde staining. The movement of the metabolites along with the solvent was measured (Rf value). The Rf value of each band was calculated and documented using the formula

$$\text{Retention factor} = \frac{\text{Distance travelled by the plant extract}}{\text{Distance travelled by the solvent}}$$

Statistical analysis

The arithmetic means of the readings of different extracts of various plant parts against the pathogens *L. monocytogenes* ATCC 19115 and *E. coli* ATCC 25922 was analyzed by one-way analysis of variance (ANOVA) calculator and Tukey Honestly Significant Difference, and the F-value was calculated.

RESULTS AND DISCUSSION

The search for new antibacterial compounds continued to be challenging for microbiologists worldwide. Medicinal plants with antimicrobial properties were explored for novel antibacterial compounds. The mangrove plants also show an inhibitory effect on the pathogenic bacteria. These plants are unexplored due to their extreme environments. Mangroves that grow in tropical and subtropical saline coastal environments are investigated nowadays for novel chemotherapeutic agents. Because of their widespread presence in the mangrove ecosystems of India and around the world, the plant species belonging to the genus *Avicenniaceae* are of utmost relevance to researchers. *A. marina* (Forssk.) had a phytochemical profile that revealed the presence of several organic components and secondary metabolites with pharmacologic properties [9].

Antibacterial activity of various extracts of the plant parts

The ethyl acetate, methanol, and chloroform extracts of the stem, leaf, and roots of *A. marina* (Forssk.) Vierh showed the least inhibitory action against *E. coli* ATCC25922. The *A. marina's* leaf's methanol extract showed a minimum zone of inhibition against *L. monocytogenes* ATCC 19115. In contrast, the chloroform and ethyl acetate leaf extracts showed minimal inhibition zone. The ethyl acetate and the chloroform extract of the root and stem showed a minimal area of inhibition ranging from 3 mm ± 1 mm. The root extracts of all the solvents showed better inhibition against *L. monocytogenes* ATCC 19115. Of these, the methanol root extract of the *A. marina* (Forssk.) Vierh showed a maximum inhibition zone of 9 mm against *L. monocytogenes* ATCC19115. The negative control DMSO showed no zone of inhibition against both pathogens. The positive control chloramphenicol recorded an inhibitory zone of 10 mm against *L. monocytogenes* ATCC 19115 and 11 mm against *E. coli* ATCC 25922.

In this study, the root of the *A. marina* (Forssk.) Vierh showed a better antibacterial effect against *L. monocytogenes* 19155 compared to the other plant portions, and the extract of methanol demonstrated higher antibacterial effectiveness than the other solvent extracts.

Research has demonstrated the extracts from the stem (ethyl acetate, ethanol, petroleum ether, benzene) of *A. marina* were active against *Salmonella paratyphi*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Bacillus subtilis* [10]. According to studies, *Pseudomonas aeruginosa*, *E. coli*, *B. subtilis*, and *S. aureus* were all inhibited by the ethanol extracts of *A. marina* roots, and chloroform extracts demonstrated antibacterial activity against *S. aureus*, *P. aeruginosa*, and. *E. coli. A. marina* leaf ethyl acetate extracts demonstrated antibacterial activity against *S. aureus* and *E. coli* [6]. *A. marina* (Forssk.) leaf extracts demonstrated more noteworthy antibacterial efficacy against multidrug-resistant *E. coli*, *S. aureus*, and *Klebsiella* spp [11].

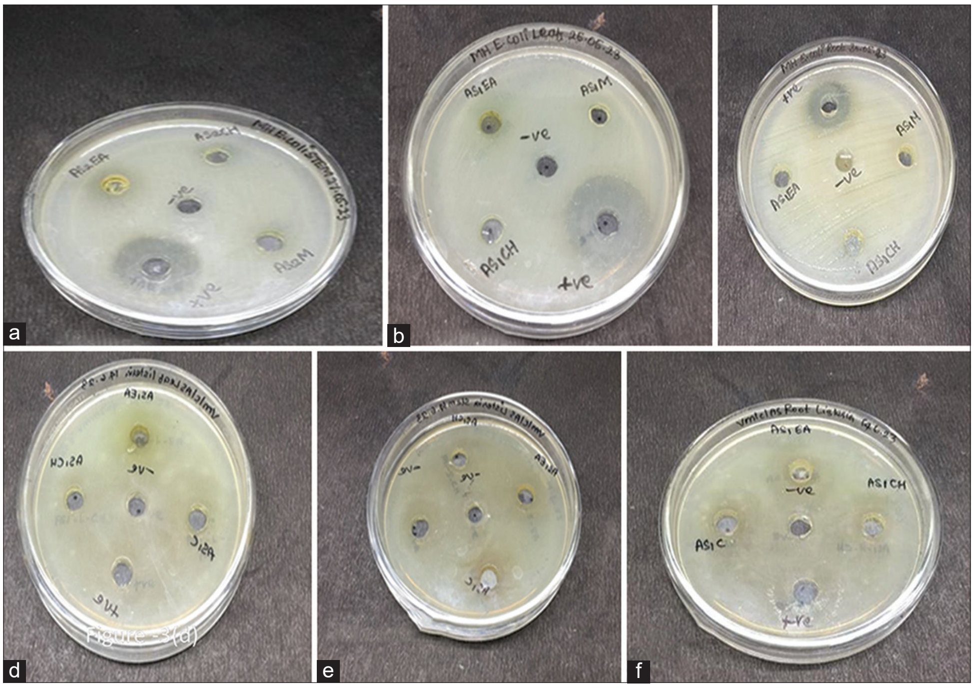

Fig. 2: The results of antibacterial activity of (a) stem extracts, leaf extracts, (b) root extracts, (c) of *Avicennia marina* (Forssk) Vierh. against *Escherichia coli* 25922, The results of antibacterial activity of (d) stem extracts, leaf extracts, (e) root extracts, (f) of *Avicennia marina* (Forssk) Vierh. against Listeria monocytogenes 19115

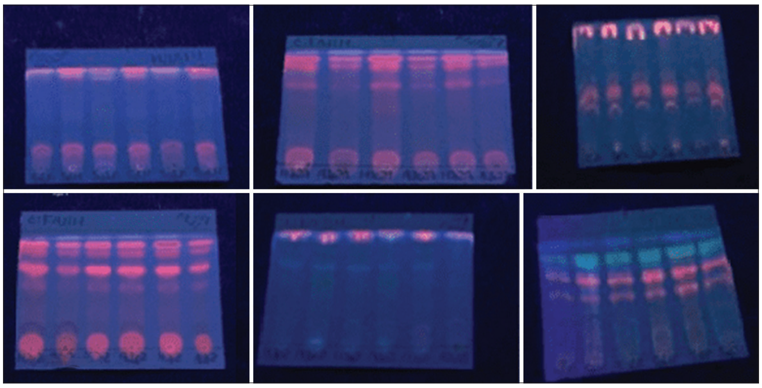

Fig. 3: The chromatogram of stem, root, and leaves of *Avicennia marina* (Forssk) Vierh

Table 1: Shows antibacterial activity of *Avicennia marina* (Forssk.) Vierh against *Listeria monocytogenes* ATCC 19115 and *Escherichia coli* ATCC 25922

S. No.	Part of the plant	Name of the organism	Solvents			Positive control	Negative control
			Methanol (mm)	Ethyl acetate (mm)	Chloroform (mm)	Chloramphenicol	DMSO
1	Leaf	*Listeria monocytogenes* ATCC 19115	3	1	1	10	-
		Escherichia coli ATCC 25922	1	1	1	11	-
2	Stem	*Listeria monocytogenes* ATCC 19115	3	2	2	10	-
		Escherichia coli ATCC 25922	1	1	1	11	-
3	Root	*Listeria monocytogenes* ATCC 19115	9	3	3	10	--
		Escherichia coli ATCC 25922	1	1	1	11	

Analysis of bioactive compounds using TLC

TLC of the methanol, chloroform, and ethyl acetate extract of the leaf, root, and stem of *A. marina* (Forssk.) Vierh was studied using the mobile phases hexane: ethyl acetate (1:1, 3:7, and 7:3). The developed plates were documented under UV 240 nm [12]. The chloroform extract of the leaf, root, and stem of *A. marina* (Forssk.) Vierh showed 10 and 7, 9 spots, respectively (Fig. 3). The ethyl acetate extract of leaf *A. marina* (Forssk.) Vierh showed 10 spots (Table 2) (Fig. 3). The methanol extract of the leaf of *A. marina* (Forssk.) Vierh revealed seven spots (Fig. 3).

Table 2: The results of thin-layer chromatography of leaves stem and roots of *Avicennia marina* (Forssk.) Vierh. using Hexane: Ethyl Acetate solvent (1:1, 3:7, and 7:3)

S. No.	Part of the plant	Solvent	Number of bands	Mobile phase					Spot identification UV-240 nm
			Compound	Hexane: Ethyl acetate 1:1	Compound	Hexane: Ethyl acetate 3:7	Compound	Hexane: Ethyl acetate 7:3	
1	Leaf	Ethyl acetate	Terpenoid saponin	0.575 0.975 1	Saponin	0.973	Pigment Flavonoids Phenolic acid Pigment Pigment Saponin	0.210 0.684 0,736 0.815 0.894 0.947	
2		Chloroform	Terpenoid Alkaloid Saponin	0.575 0.95 0.975	Saponin	0.973	Pigment Flavonoids Phenolic acid Pigment Pigment Saponin	0.236 0.657 0.736 0.815 0.894 0.947	
3		Methanol	Saponin	0.975	Saponin	0.948	Pigment Chlorophyll Flavonoids Phenolic acid Saponin	0.375 0.45 0.625 0.775 0.975	
1	Root	Ethyl Acetate	Saponin	0.921	Saponin	0.923	Terpenoid Pigment Alkaloids	0.5 0.8 0.95	
2		Chloroform	Saponin	0.921	Saponin	0.923	Chlorophyll Alkaloids Phenolic acid Alkaloid	0.475 0.675 0.775 0.95	
3		Methanol	Alkaloid Saponin	0.95 0.975	Saponin	0.925	Alkaloids Phenolic acid Pigment Saponin	0.675 0.783 0.891 0.972	
1	Stem	Ethyl Acetate	Pigment Saponin Alkaloid	0.225 0.9 0.95	Alkaloid Saponin	0.136 0.97	Pigment Pigment Saponin Alkaloid	0.8 0.85 0.9 0.95	
2		Chloroform	Saponin Saponin Alkaloid	0.225 0.9 0.95	Saponin Alkaloid	0.97 0.136	Saponin Saponin Phenolic acid Alkaloid	0.925 0.975 0.775 0.95	
3		Methanol	Saponin Saponin	0.948 0.974	Saponin	0.974	Flavonoids Phenolic acid Saponin Pigment Saponin	0.692 0.794 0.717 0.846 0.948	

Table 3: ANOVA table for antimicrobial activity of extracts of plant parts against *Listeria monocytogenes* 19155 and *Escherichia coli* 25922

Source	Source of variation	Degrees of freedom (d.f)	Sum of squares	Mean square	F-Ratio	P
Listeria monocytogenes 19155	Between groups	2	16.8889	8.444	3.1777	0.05966
	Within groups	24	63.7778	2.6574		
	Total	26	80.667	3.1026		
Escherichia coli 25922	Between groups	2	0.07407	0.03704	1	0.3827
	Within groups	24	0.8889	o. 03704		
	Total	26	0.963	0.03704		

ANOVA: Analysis of variance

TLC profile for alkaloids

The leaf in ethyl acetate extract showed the TLC profile for alkaloids with the Rf value of 0.95 (Table 2). The root of *A. marina* (Forssk.) Vierh in ethyl acetate, chloroform, and methanol extract showed the presence of alkaloids. The stem in chloroform and ethyl acetate extract revealed the existence of alkaloids [13].

TLC profile for flavonoids

The methanol, ethyl acetate, and chloroform extract from the leaves of *A. marina* (Forssk.) Vierh and the methanol extract from the stem of *A. marina* (Forssk.) Vierh showed the presence of flavonoids with the Rf value of 0.6 (Table 2).

TLC profile for saponin

The ethyl acetate, methanol, and chloroform extracts from the leaf, root, and stem of *A. marina* (Forssk.) Vierh exhibited saponin, with Rf values ranging from 0.22 to 0.84, 0.92 to 0.94 to 0.97 (Table 2).

TLC profile for terpenes

Ethyl acetate and chloroform extracts from the leaves, along with ethyl acetate extracts from the roots of *A. marina* (Forssk.) Vierh demonstrated the occurrence of terpenes (Table 2).

Numerous secondary metabolites with potential medical uses, including flavonoids, saponins, steroids, alkaloids, triterpenes, and tannins, are abundant in mangroves. Approximately 80 mangrove species have been found to have medicinal potential worldwide, including antidiabetic, antibacterial, anticancer, and antiulcer activities. Mangroves yield beneficial medicinal substances from their leaves, roots, bark, and fruits, including limonoids, terpenoids, benzodioxole, lactones, and ketones [14]. Rich antioxidants have been found in the methanol leaf extract of *A. marina* (Forssk.) taken from Indian Sunderban mangroves [9]. *A. marina* (Forssk.) contains biological substances such as terpenoids, flavonoids, tannins, and phenolic acids, which have been used extensively as antimicrobial agents. Gas chromatography-mass spectrometry analysis has been used to identify bioactive compounds with antimicrobial activity, establishing the basis for using *A. marina* (Forssk.) as an antimicrobial agent [15].

Literature from the past has shown that tea is made from the bark of *A. marina* (Forssk.) Vierh treats ulcers, diarrhea, and rheumatic pain [16]. Biologically active compounds with different degrees of activity have been reported in mangrove plants. Studies have shown that alkaloids have varied medicinal properties. *A. marina* is a vital source of alkaloids [17].

Alkaloids in plants are parts of heterocysts that have one or more protonated nitrogen atoms. These compounds are responsible for biological activities that the host mediates. The color of flowers and leaves is due to the presence of flavonoids found in the chromoplasts of plants. These phenolic chemicals cause biological actions. Saponins are abundant in plants. They possess foaming qualities. They taste bitter and dissolve in water. They possess pharmacological qualities such as antipyretic and anti-inflammatory effects. Terpenes can turn plants poisonous or indigestible as a defense mechanism against herbivores and act as antibiotics to keep harmful bacteria away from the plants. Terpenes are utilized commercially as rubber, vitamins, and aromatic compounds for food and cosmetics. Studies proved that terpenoids contain antibacterial, antioxidant, and anticancer properties [13]. The mangrove plant A. marina (Forssk.) Vierh is a valuable source of bioactive phytochemicals such as esters, phenols, alcohols, steroids, carotenoids, triterpenes, and amino acids [18]. The plant exhibited promising antioxidant activity, which was attributed to its flavonoid compounds. Triterpenes exhibit radical scavenging activity, and fatty acids exhibit antimicrobial activities [19].

Statistical analysis for the antibiogram of plant parts against *L. monocytogenes* 19155 and *E. coli* 25922

Statistical analysis for the antibiogram of pathogens was performed with ANOVA. The test statistic F for *L. monocytogenes* 19155 is 3.1777, falling within the 95% acceptance region. The F-value of one falls within the accepted range for *E. coli* 25922. There is no notable variance in the averages of any two groups. It is assumed that the averages of each group are the same.

CONCLUSION

The present research indicated that the mangrove species *A. marina* (Forssk.) Vierh exhibits potential antibacterial properties and contains bioactive compounds. Building on these findings, the compounds will be purified for the development of antimicrobial drugs. Despite the fact that mangroves are a promising source of bioactive materials, their application remains restricted. In addition, the levels of secondary metabolites in mangrove species can differ significantly based on the specific species and their geographical location.

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AUTHOR 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 wrote the manuscripts.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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