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Research Article

In vitro Antibacterial Activity of *Alangium salvifolium* and *Alangium lamarckii* against Human Pathogenic Bacterial Species

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A B S T R A C T

Introduction: The plants *Alangium salvifolium* and *Alangium lamarckii* have been utilised to treat various ailments in traditional folk medicine. The current study aims to examine the antibacterial efficacy of extracts of bark of *Alangium salvifolium* and *Alangium lamarckii*.

Methods: In this study, the extracts of bark of selected plants using solvents methanol, ethanol and petroleum ether were subjected to evaluation of their antibacterial activity. The serial dilution method was employed to determine the minimum inhibitory concentration (MIC) for each plant extract. The agar well diffusion method was used to assess antibacterial activity against clinically significant gram-positive and gram-negative bacterial strains, including *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, and Enterobacter cloacae.*

Results: The methanolic extract of the bark of *Alangium salvifolium* and *Alangium lamarckii* exhibited the maximum antibacterial effect when compared with the ethanolic and petroleum ether extracts. The methanolic extract of the bark of *A. salvifolium* showed the maximum zone of inhibition of 26.07 ± 1.31 mm against *Pseudomonas aeruginosa*. Methanolic extract of bark of *A. lamarckii* showed the maximum zone of inhibition 27.00 ± 1.20 mm against *Pseudomonas aeruginosa*.

Conclusion: Therefore, the bark extracts of *Alangium salvifolium* and *Alangium lamarckii* exhibited an antibacterial effect against selected human bacterial pathogens. So, the present study concluded that both of these plants may serve as prospective source materials for the development of new antibacterial agents.

Keywords: Antibacterial Activity, Bark, *Alangium salvifolium*, *Alangium lamarckii*, Human Pathogens

Introduction

Asia's herbal medicine represents the long history of the human race with nature. According to reports from the World Health Organization (WHO), over 80% of the global population depends on traditional medicine to meet their basic healthcare needs. Chronic as well as infectious diseases can be treated with a broad range of compounds in plants.[1] The exact medicinal value of a plant relies on the specific chemical substance that further has a major physiological action on the human body. These are called bioactive compounds and are majorly classified as alkaloids, tannins, flavonoids and phenolic compounds.[2] Most of the compounds are secondary metabolites. Till now about 12,000 phytocompounds have been discovered, but this is likely less than 10% of the total number of secondary metabolites in plants.

In most cases, the substances act as the plant defence mechanism against predators such as microorganisms, insects and herbivores. Microbial illnesses are a serious health concern and a major contributing factor to rising morbidity and mortality rates globally. Human infections, especially those that affect the skin and mucosal surfaces, are a severe issue, particularly in tropical and subtropical poor nations.[3] For the development of new antibacterial medications, chemicals that may inhibit infections while being relatively benign to host cells may be considered possibilities.[4] Natural products are compounds with biological activities that are derived from natural sources. Natural products have long been used in the development of contemporary medicines and as an alternative to conventional medical care.

The antibiotic resistance of microorganisms has been a constant concern for the past few years. The vast chemical diversity of plant metabolites is the best natural reservoir for research for developing antibacterial compounds.[5] Medicinal plants serve as the best source for pharmaceutical product production. Traditional healers have utilised the root of *Alangium salvifolium* to cure skin malignancies by local application. Its vasodilator action lowers blood pressure when taken orally. In India, many plant parts are employed as traditional medicines. The monogeneric plant genus *Alangium* belongs to the Alangiaceae family. The size of *Alangium salvifolium* varies from a diminutive shrub to an elongated arborescent form that is between 3 and 12 feet tall. Madagascar, Western Africa, Southern and Eastern Asia (Indonesia, Philippines, China, Malaysia and India), tropical Australia, the islands of the Western Pacific Ocean and New Caledonia are its natural regions.[6] The leaf has asymmetrical edges and is alternate in arrangement. It can be lance-shaped, oblong, or oval, with 3 to 6 pairs of angled veins. The upper surface of the leaf is smooth, while the veins on the lower surface of the leaf are covered with soft hairs. The flowers are white or yellow colour that have a pleasant fragrance. Fruit drupe with one or two seeds and calyx lobes are on top.[7]

In India, the *Alangium salvifolium* plant is widely cultivated under the name Ankola. In Asia, leaves are utilised in the treatment of a variety of illnesses.[8] A decoction made from the entire plant, along with the fruit of the coconut palm, can be applied topically to treat boils. The stem can be used to treat vomiting and diarrhoea. Asthma and rheumatoid arthritis discomfort can be cured by leaves. Fruit juice is used to treat eye problems and is used as an expectorant, purgative, carminative, and antidote for poisoning. The roots have aperient properties and are used as a vermifuge to prevent intestinal worms.[9] So, with the above points in mind, the current study is aimed to investigate the antibacterial potential of extracts of bark of *Alangium salvifolium* and *Alangium lamarckii* against selected human bacterial pathogens using different solvent systems such as methanol, ethanol and petroleum ether.

Materials and Methods

Study Period

The present study was conducted for six months duration from January 2022 to May 2022. During this study period, the collection, identification and preparation of plant materials were carried out. The plant was identified by Dr.R.Murugan, Associate Professor and Head, Department of Botany, Government Arts College (Autonomous), Kumbakonam – 612 002, Tamilnadu, India.

Plant Extract Preparation

Alangium salvifolium and *Alangium lamarckii* barks were collected from the banks of river Cauvery near Kumbakonam, Thanjavur District, Tamil Nadu, India. The collected barks of plants were washed with water, rinsed with distilled water and finally dried under shade for 15 days at room temperature. The dried material of each plant was made into fine powder. Each plant material was extracted with the solvents methanol, ethanol and petroleum ether by using the Soxhlet apparatus. The solvent extracts were kept at 40–45 °C using a hot air oven till the solvent was completely evaporated from it. It is confirmed that there was observed no solvent smell from the extract. Finally, the dark brown residue was obtained and used for further antibacterial study.

Bacterial Strains

The antibacterial potential of plant extracts was tested against pathogenic strains, including the gram-positive bacterium *Staphylococcus aureus* MTCC 3160 and gram-negative bacteria *Klebsiella pneumoniae* MTCC 7028, *Pseudomonas aeruginosa* MTCC 7602, *Proteus mirabilis* MTCC 9242, and *Enterobacter cloacae* MTCC 8544. These

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strains were sourced from the Microbial Type Culture Collection and Gene Bank (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial cultures were regularly sub-cultured on the same medium and stored at 4 °C until the time of use.

Preparation of Media and Sterilisation

Antibacterial susceptibility was assessed using the agar well diffusion method on solid agar media. Nutrient agar (28 g/L) was selected for the antibacterial assay, while a serial dilution assay was employed to determine the minimum inhibitory concentration (MIC). To evaluate MIC, bacterial cell growth was supported by preparing a 2% nutrient broth suspension. All prepared media were sterilised by autoclaving at 121 °C for 20 minutes.

Minimum Inhibitory Concentration (MIC)

In an MIC assay, a test organism is exposed to a series of drug dilutions to identify the minimum concentration needed to inhibit bacterial growth. The MIC represents the lowest concentration of an antibacterial agent required to visibly prevent bacterial growth under specific conditions. MIC-based assays, including macro (test tubes), micro (microtiter plates) broth dilutions, and agar dilution, are widely accepted standards for evaluating bacterial sensitivity to inhibitors. Different concentrations of the extract were added to 10 mL of nutrient agar broth (v/v), and each test tube was inoculated with 10 µL of the target bacterial suspension. The cultures were incubated at 37 °C for 3–5 days, and the MIC was recorded as the concentration at which no visible growth occurred.

Antibacterial Activity

Antibacterial activity was evaluated using the agar well diffusion method. Nutrient agar plates were swabbed with bacterial broth cultures using sterile cotton swabs, and wells were created in the agar with a sterile cork borer. Into each well, 100 µL of three different extracts were added, allowing them to diffuse for two hours at room temperature. Control trials included plates with bacterial inoculum but without plant extracts. Plates were then incubated at 37 °C for 18–24 hours for bacterial growth. Zones of inhibition (in mm) were measured, with each experiment performed in triplicate. For each sample, measurements were taken in three fixed directions, and average values were recorded.[10-14]

Statistical Analysis

The experiment was carried out thrice, and the results are represented as mean ± standard deviation.

Results and Discussion

A key source of pharmacological molecules for human health and well-being is traditional medicinal plants. Significant antibacterial activities are seen in plant extracts for therapeutic use. Minimum Inhibitory Concentration (MIC) of extracts of bark of *A. salvifolium* and *A. lamarckii* against selected microorganisms *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Enterobacter cloacae* was determined using different concentrations (10 µg, 20 µg, 30 µg, 40 µg, 50 µg, 60 µg and 70 µg) of extracts in 10 mL of nutrient broth. For this, no visible growth was observed and the results are presented in Tables 1 and 2. The inhibition of bacterial growth (no visible growth) increased with the increased level of concentration of extracts of bark of *A. salvifolium* and *A. lamarckii*. The bark extracts of *A. salvifolium* and *A. lamarckii* exhibited MIC at 40–60 µg or less against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Proteus mirabilis*. However, the petroleum ether extracts of the bark of *A. salvifolium* and *A. lamarckii* exhibited MIC at 60–70 µg against *Enterobacter cloacae*. So, the MIC of extracts of bark of *A. salvifolium* and *A. lamarckii* was more than 40 µg against selected bacterial species.

The tested plant extracts exhibited antibacterial activities by zones of inhibition revealing a positive correlation with the activity of standard drug tetracycline (30 µg/100 µL). The findings showed that each extract has an effective antibacterial activity against each type of pathogen examined. Methanolic and ethanolic extracts exhibited the maximum level of inhibition (compared to petroleum ether extract). The negative control (DMSO) showed no zone of inhibition, indicating no antibacterial activity. Tetracycline (30 µg), used as a positive control, produced inhibition zones at varying levels against the tested human pathogenic bacteria. Tetracycline exhibited zones of inhibition ranging from 20.70 ± 1.77 mm to 26.03 ± 0.58 mm across all bacterial species included in this study.

The antibacterial activity of methanolic, ethanolic, and petroleum ether extracts of bark of *A. salvifolium* was analysed and the results are represented in Table 3. For all the tested bacterial strains, methanolic and ethanolic extracts of *A. salvifolium* showed maximum antibacterial activity. The results indicated that most of the active components (compounds responsible for antibacterial activity) in this plant are expected to be soluble in polar solvents. The methanolic extract exhibited a maximum zone of inhibition against *P. aeruginosa* as 26.07 ± 1.31 mm at the concentration of 100 µg/mL and a minimum zone of inhibition of 13.10 ± 1.30 mm against *S. aureus* at the concentration of 50 µg/mL. The decreasing order of the degree of susceptibility of methanolic extract of *A. salvifolium is P. aeruginosa > P. mirabilis > K. pneumoniae > E. cloacae > S. aureus.* Similarly, ethanolic extract showed a maximum inhibition zone of 25.10 ± 1.46 mm against *E. cloacae* at the concentration of 150 µg/mL and 23.97 ± 1.15 mm against *P. aeruginosa* at the concentration of 150 µg/

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mL and a minimum zone of inhibition against *P. mirabilis* as 12.13 ± 1.15 mm at the concentration of 50 µg/mL. The degree of susceptibility of ethanolic extract of *A. salvifolium* is in the decreasing order of *E. cloacae* > *P. aeruginosa* > *S. aureus* > *K. pneumoniae* > *P. mirabilis*. The petroleum ether extract showed a maximum zone of inhibition of 16.13 ± 1.42 mm against *S. aureus* at the concentration of 150 µg/mL and a minimum zone of inhibition of 9.13 ± 1.15 mm against *P. mirabilis* at the concentration of 50 µg/mL. The degree of susceptibility of petroleum ether extract of *A. salvifolium* is in decreasing the order of *S. aureus* > *P. aeruginosa* > *E. cloacae* > *K. pneumoniae* > *P. mirabilis*. More specifically, petroleum ether extract represented higher susceptibility to all bacterial strains.

The antibacterial activity of methanolic, ethanolic, and petroleum ether extracts of the bark of *A. lamarckii* was analysed and the results are represented in Table 4. The methanolic extract showed the maximum zone of inhibition against *P. aeruginosa* as 27.00 ± 1.20 mm at the concentration of 150 µg/mL and the minimum inhibition zone against *K. pneumoniae* as 11.07 ± 1.31 mm at the concentration of 50 µg/mL. The decreasing order of the degree of susceptibility of methanol extract of *A. lamarckii* is *P. aeruginosa* > *E. cloacae* > *S. aureus* > *P. mirabilis* > K. pneumoniae. The ethanolic extract of *A. lamarckii* showed a maximum zone of inhibition against *Staphylococcus aureus* of 29.00 ± 1.30 mm at the concentration of 150 µg/mL, and a minimum zone of inhibition against *K. pneumoniae* of 11.33 ± 1.31 mm at the concentration of 50 µg/mL. The decreasing order of the degree of susceptibility of ethanol extract of *A. lamarckii* is *S. aureus* > *P. mirabilis* > *P. aeruginosa* > *E. cloacae* > K. pneumoniae. The petroleum ether extract of *A. lamarckii* showed the maximum zone of inhibition against *P. aeruginosa* as 19.97 ± 1.25 mm at a concentration of 150 µg/mL and the minimum zone of inhibition against *E. cloacae* as 9.73 ± 0.64 mm at a concentration of 50 µg/mL. The decreasing order of the degree of susceptibility of petroleum ether extract of *A. lamarckii* is *P. aeruginosa* > *S. aureus* > *E. cloacae* > *K. pneumoniae* > P. mirabilis.

In one instance, according to Biffa, *M. stenopetala* leaf methanolic extract significantly inhibited the growth of *S. aureus*.[16] Further, Seleshe and Kang stated that the ethanolic leaf extract of *M. stenopetala* exhibited antibacterial activity against *S. aureus*.[17] Antibacterial activity can vary depending on the time of harvest,[18] the stage of plant development,[19] and the extraction technique[20].

The majority of the studies screened the plant extracts for the analysis of phytocompounds like flavonoids, anthraquinones, alkaloids, tannins, phenols, and saponins. The growth of microorganisms was found to be inhibited by these bioactive compounds that were obtained from traditional medicinal plants. Medicinal plants contain a diversity of bioactive secondary metabolites including flavonoids, phenolics, terpenoids, alkaloids, coumarins and tannins has showed bactericidal and bacteriostatic against a number of human pathogens.[21,22]

The hunt for novel treatments for pathogens which are resistant to many medications has led to the discovery of potential plant-based drugs for treating illnesses caused by microorganisms.[23,24] Additionally, there is a synergistic impact between antibacterial plant extracts and frequently administered antibiotics.[25–27] The worldwide increase of multidrug resistance in both community and healthcare-associated bacterial infections has impaired the current antimicrobial therapy, warranting the search for other alternatives.[24] Therefore, it is necessary to search the other alternatives that can potentially be effective in the treatment of these problematic bacterial infections. The usefulness of plant extracts for antimicrobial therapy has been observed to be a promising remedy since ancient times in Chinese medicine and in Ayurveda, Arabic, and Unani medicine.

Table 1.Minimum Inhibitory Concentration (MIC) of Methanolic, Ethanolic and Petroleum Ether Extracts of Bark of *A. salvifolium* against Selected Bacterial Species

Name of Solvent	Concentration of Plant Extract in 10 mL Broth (µg)	Appearance of Growth of Microorganisms				
		Pseudomonas aeruginosa	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Enterobacter cloacae*
Control	-	+	+	+	+	+
Methanol	10	+	+	+	+	+
	20	+	+	+	+	+
	30	+	+	+	+	+
	40	+	+	+	+	+
	50	-	-	+	-	+
	60	-	-	-	-	-

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Ethanol	10	+	+	+	+	+
	20	+	+	+	+	+
	30	+	+	+	+	+
	40	+	+	+	+	+
	50	-	-	+	-	+
	60	-	-	-	-	-
Petroleum ether	10	+	+	+	+	+
	20	+	+	+	+	+
	30	+	+	+	+	+
	40	+	+	+	+	+
	50	-	+	+	+	+
	60	-	-	-	-	+
	70	-	-	-	-	-

+ Appearance of visible growth of bacterium (turbid tube), – No appearance of visible growth of bacterium (clear tube)

Table 2.Minimum Inhibitory Concentration (MIC) of Methanolic, Ethanolic and Petroleum Ether Extracts of Bark of *A. lamarckii* against Selected Bacterial Species

Name of Solvent	Concentration of Plant Extract in 10 mL Broth (µg)	Appearance of Growth of Microorganisms				
		Pseudomonas aeruginosa	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Enterobacter cloacae*
Control	-	+	+	+	+	+
Methanol	10	+	+	+	+	+
	20	+	+	+	+	+
	30	+	+	+	+	+
	40	+	+	+	+	+
	50	+	-	+	-	+
	60	-	-	-	-	-
Ethanol	10	+	+	+	+	+
	20	+	+	+	+	+
	30	+	+	+	+	+
	40	+	+	+	+	+
	50	-	+	+	-	+
	60	-	-	-	-	-
Petroleum ether	10	+	+	+	+	+
	20	+	+	+	+	+
	30	+	+	+	+	+
	40	+	+	+	+	+
	50	+	+	+	+	+
	60	+	+	-	+	+
	70	-	-	-	-	-

+ Appearance of visible growth of bacterium (turbid tube), – No appearance of visible growth of bacterium (clear tube)

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Table 3.Antibacterial Activity of Methanolic, Ethanolic and Petroleum Ether Extracts of Bark of *A. salvifolium* against Selected Bacterial Species

Name of the Solvent	Concentration of the Plant Extract (µg/mL)	Diameter of Zone of Inhibition (mm)				
		Name of the Bacterial Species				
		Pseudomonas aeruginosa	*Staphylococcus aureus*	Klebsiella pneumoniae	Proteus mirabilis	Enterobacter cloacae
Methanol	50	24.07 ± 1.31	13.00 ± 1.30	13.63 ± 1.45	19.07 ± 1.50	13.90 ± 1.17
	100	26.07 ± 1.31	14.10 ± 1.25	15.07 ± 1.21	19.07 ± 1.50	15.97 ± 1.25
	150	25.87 ± 1.75	15.10 ± 1.30	18.10 ± 1.36	20.00 ± 1.40	14.00 ± 1.20
Ethanol	50	19.10 ± 1.10	14.00 ± 1.20	13.00 ± 1.30	12.13 ± 1.15	19.00 ± 1.20
	100	22.07 ± 1.31	15.00 ± 1.40	14.03 ± 1.15	14.03 ± 1.45	23.07 ± 1.40
	150	23.97 ± 1.15	19.07 ± 1.40	15.00 ± 1.40	18.90 ± 1.26	25.10 ± 1.46
Petroleum ether	50	10.00 ± 1.30	15.03 ± 1.15	09.30 ± 1.26	09.13 ± 1.15	09.30 ± 1.30
	100	11.03 ± 1.25	15.97 ± 1.25	10.00 ± 1.30	09.93 ± 1.40	09.33 ± 1.31
	150	13.90 ± 1.25	16.13 ± 1.42	12.07 ± 1.40	10.90 ± 1.36	10.03 ± 1.45
Negative control	DMSO	—	—	—	—	—
Positive control	Tetracycline (30 µg)	25.00 ± 0.51	20.70 ± 1.77	20.90 ± 1.35	26.03 ± 0.58	22.90 ± 0.25

Values are expressed as mean ± standard deviation of triplicates.

Table 4.Antibacterial Activity of Methanolic, Ethanolic and Petroleum Ether Extracts of Bark of *A. lamarckii* against Selected Bacterial Species

Name of the Solvent	Concentration of the Plant Extract (µg/mL)	Diameter of Zone of Inhibition (mm)				
		Name of the Bacterial Species				
		Pseudomonas aeruginosa	*Staphylococcus aureus*	Klebsiella pneumoniae	Proteus mirabilis	Enterobacter cloacae
Methanol	50	24.03 ± 1.35	14.90 ± 1.26	11.07 ± 1.31	11.97 ± 1.55	22.97 ± 1.25
	100	25.07 ± 1.31	16.97 ± 1.35	11.96 ± 1.40	13.13 ± 0.42	25.10 ± 1.36
	150	27.00 ± 1.20	16.70 ± 0.77	12.90 ± 1.35	15.03 ± 1.35	25.90 ± 0.36
Ethanol	50	22.20 ± 1.17	25.13 ± 0.12	11.33 ± 1.31	24.17 ± 1.15	12.23 ± 1.25
	100	23.00 ± 1.30	26.03 ± 1.25	12.93 ± 1.31	25.03 ± 1.35	13.97 ± 1.15
	150	26.00 ± 1.20	29.00 ± 1.30	14.03 ± 1.25	27.03 ± 1.15	14.97 ± 1.35
Petroleum ether	50	15.93 ± 1.40	09.03 ± 2.15	12.10 ± 1.36	11.00 ± 0.40	09.73 ± 0.64
	100	18.03 ± 0.15	11.03 ± 1.25	14.03 ± 0.15	13.03 ± 1.35	15.13 ± 1.32
	150	19.97 ± 1.25	18.00 ± 1.10	16.07 ± 1.21	16.07 ± 1.40	17.03 ± 1.06

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Negative control	DMSO	—	—	—	—	—
Positive control	Tetracycline (30 µg)	25.00 ± 0.51	20.70 ± 1.77	20.90 ± 1.35	26.03 ± 0.58	22.90 ± 0.25

Values are expressed as mean ± standard deviation of triplicates.

Conclusion

Based on the ethnobotanical literature, the present investigation supported the idea that medicinal plants have been used traditionally for their antibacterial properties. According to the findings, *A. salvifolium* and *A. lamarckii* bark extracts showed promising antibacterial efficacy against pathogenic bacterial species. Therefore, an in-depth study is required for compound isolation, toxicological research, and clinical trials on the potent compounds present in *A. salvifolium* and *A. lamarckii.*

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