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Antibacterial activity of root extracts of *Asparagus racemosus* Willd. against selected Gram-positive and Gram-negative bacteria

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Abstract

Medicinal plants continue to be an essential source of therapeutic compounds, especially in underdeveloped nations where traditional medicine is a major component of primary healthcare. The well-known medicinal plant *Asparagus racemosus* has a variety of pharmacological characteristics and is utilized in Ayurvedic, Siddha, and Unani systems. The current study assesses the antibacterial activity of *A. racemosus* root extracts made with various solvents (methanol, ethyl acetate, hexane, and distilled water) against specific strains of Gram-positive (*Staphylococcus aureus, Streptococcus mutans*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria. The disc diffusion method was used to measure antibacterial activity at 25, 50, and 100 µg concentrations. Comparable to the common antibiotic streptomycin, ethyl acetate extract demonstrated the strongest antibacterial activity among the studied extracts, demonstrating broad-spectrum potency against both Gram-positive and Gram-negative bacteria. While the water extract showed little inhibition, the methanol and hexane extracts demonstrated modest efficacy. The results support *A. racemosus* root's traditional medicinal use and future utility in drug development by indicating that it contains strong bioactive chemicals with significant antibacterial activity.

Key words : *Asparagus racemosus*, Antibacterial activity, Disc diffusion assay; Root extract, Phytochemicals, Terpenoids, Traditional medicine.

Since ancient times, medicinal plants have been used to treat illnesses and maintain health. In many developing nations, where traditional medicine is utilized by almost 80%

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of the population, medicinal plants continue to be a vital component of basic healthcare[22,24]. With more than 2,000 species known to have therapeutic uses, India is one of the richest sources of medicinal flora[20]. Herbal medicines are becoming more and more popular since they are more easily accessible, less expensive, culturally relevant, and have less negative effects than synthetic medications[14]. The fast development of multidrug-resistant pathogens and the decreased effectiveness of traditional antibiotics have led to a resurgence of interest in medicinal plants in recent years[12,21]. Through processes like membrane rupture, enzyme inhibition, and interference with nucleic acid production, secondary metabolites originating from plants, such as alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds, demonstrate strong antibacterial action[4,13,25]. Due to its galactagogue, immunomodulatory, adaptogenic, and reproductive health-promoting qualities, *Asparagus racemosus* Willd. (family Asparagaceae) is a well-known medicinal plant that is found throughout tropical and subtropical regions of India and is widely used in Ayurvedic, Siddha, and Unani systems[2,3,6,11]. According to phytochemical research, its roots include steroidal saponins, flavonoids, glycosides, and vital nutrients that support its various pharmacological actions, such as immunostimulatory, hepatoprotective, antioxidant, anti-inflammatory, and antimicrobial effects[7,8,15]. Nevertheless, there is still a lack of systematic testing of solvent-based root extracts against both Gram-positive and Gram-negative bacteria. Thus, the current study uses the disc diffusion method to evaluate the antibacterial properties of several solvent extracts of *A. racemosus* roots.

Collection and Identification of Plant Material :

Fresh *Asparagus racemosus* roots were gathered from the Agasthyarkoodam, Thiruvananthapuram district of Kerala. A certified taxonomist verified the authenticity of the plant material. To get rid of dirt and debris, the roots were thoroughly cleaned with running tap water and then distilled water.

Preparation of Plant Extracts :

The cleaned roots were ground into a fine powder using a mechanical grinder after being shade-dried at room temperature. Until extraction, the powdered material was kept in sealed containers. Hexane, ethyl acetate, methanol, and distilled water were used in a Soxhlet system for successive solvent extraction. A rotary evaporator was used to concentrate the extracts, which were then kept at 4 °C until further examination.

Test Microorganisms :

Two Gram-positive bacteria (*Staphylococcus aureus, Streptococcus mutans*) and two Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) were used to investigate the antibacterial activity. Every strain was kept at 4 °C on nutrient agar slants.

Culture Conditions :

Nutrient broth was used to subculture the bacterial cultures, which were then incubated for 24 hours at 37 °C. Prior to antimicrobial testing, the cultures' turbidity was corrected to meet the 0.5 McFarland standard.

Antibacterial Assay (Disc Diffusion Method):

Mueller-Hinton agar was used to assess antibacterial activity using the disc diffusion method. On inoculated agar plates, sterile discs with a diameter of 5 mm were impregnated with extract concentrations of 25, 50, and 100 µg. The positive control was 100 µg of streptomycin. Zones of inhibition were measured in millimeters after plates were incubated for 24 hours at 37 °C.

Antibacterial activity :

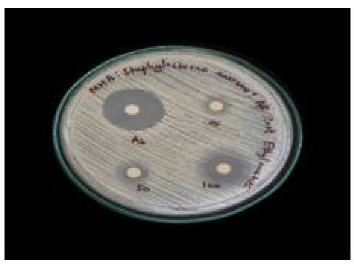
Using the disc diffusion method, the antibacterial activity of *Asparagus racemosus* root extracts made with methanol, ethyl acetate, hexane, and distilled water was assessed against *Staphylococcus aureus, Streptococcus mutans, Escherichia coli,* and *Pseudomonas aeruginosa*. The outcomes are shown in Figure 1 and Table-1. Out of all the solvents studied, ethyl acetate extract

Table-1, Antimicrobial activity of *Asparagus racemosus* root extract.
Zone of Inhibition (mm)

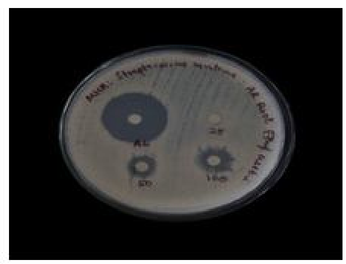
Extract	Concentration (µL)	*Staphylococcus aureus*	*Streptococcus mutans*	*Escherichia coli*	*Pseudomonas aeruginosa*
Methanol	25 µL	-	-	-	-
	50 µL	11	11	-	-
	100 µL	12	12	-	-
	Streptomycin (100 µg)	27	30	32	30
Ethyl acetate	25 µL	12	11	11	12
	50 µL	14	14	12	13
	100 µL	20	16	13	18
	Streptomycin (100 µg)	27	30	32	30
Hexane	25 µL	-	11	-	-
	50 µL	-	12	-	-
	100 µL	11	13	11	11
	Streptomycin (100 µg)	27	29	32	31
Distilled water	25 µL	-	-	-	-
	50 µL	-	-	-	-
	100 µL	-	11	-	-
	Streptomycin (100 µg)	27	30	30	30

exhibited the strongest antibacterial activity. Inhibition zones against all bacterial strains varied from 11 to 12 mm at a dose of 25 µg. At 100 µg, these zones greatly expanded, reaching 20 mm against *S. aureus,* 16 mm against *S. mutans*, 13 mm against *E. coli*, and 18 mm against *P. aeruginosa*. The existence of moderately polar bioactive chemicals with broad-spectrum antibacterial activity is indicated by this concentration-dependent increase[10,16].

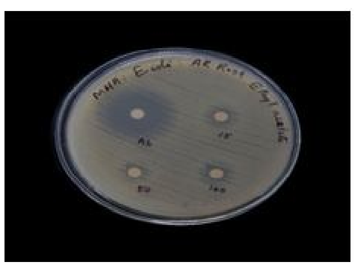
Methanolic extracts had moderate action mostly against Gram-positive bacteria, with inhibition zones of 11–12 mm against *S. aureus* and *S. mutans* at 50 and 100 µg. This was probably caused by the phenolics and flavonoids that were recovered from methanol[4,18]. With inhibition zones of 11–13 mm at 100 µg against all test species, hexane extracts had minimal antibacterial activity, indicating a lower contribution of non-polar chemicals to antibacterial efficacy[19]. Water is a poor solvent for extracting antimicrobial phytochemicals, as evidenced by the aqueous extract's limited efficacy and only suppression against *S. mutans* (11 mm) at 100 µg[14]. All bacteria were strongly inhibited (27–32 mm) by streptomycin (100 µg) (Table-1). Figure 1 supports a dose-dependent response and amply illustrates the better antibacterial activity of ethyl acetate extract in comparison to other solvent extracts. Due to variations in cell wall construction, Gram-positive bacteria were often more vulnerable than Gram-negative bacteria[13]. *A. racemosus* roots have been shown to exhibit similar antibacterial effects in the past[10,16], which supports both its potential as a natural antibacterial agent and its traditional medical use.



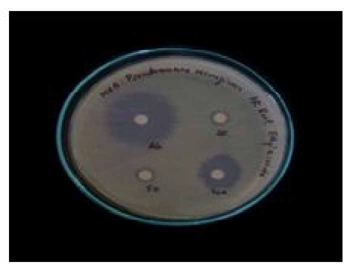
Staphylococcus aureus



Streptococcus mutans



Escherichia col



Pseudomonas aeruginosa

Figure 1. Shows the antimicrobial activity of Asparagus racemosus ethyl acetate root extract against Gram positive and Gram negative bacteria

The current investigation demonstrates the strong antibacterial activity of *Asparagus racemosus* root extracts against both Gram-positive and Gram-negative bacteria, especially in ethyl acetate extracts. The results confirm *A. racemosus's* traditional therapeutic usage and point to its potential as a natural source of antibacterial chemicals. It is necessary to conduct additional research on the active compounds' separation, characterisation, and mode of action.

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