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Bioassay-Guided Evaluation of *Smilax perfoliata* Leaf Extracts for Antibacterial Activity against Gram-Positive and Gram-Negative Pathogens

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Abstract: *The present study evaluated the antibacterial potential of Smilax perfoliata leaf extracts prepared using different solvents against selected Gram-negative bacterial pathogens. Aqueous–ethanolic, methanolic, and acetone extracts were assessed for their inhibitory activity against Escherichia coli and Pseudomonas aeruginosa using the agar well diffusion method, and the results were compared with a standard antibiotic. The aqueous–ethanolic extract exhibited no inhibitory activity against E. coli, whereas measurable zones of inhibition were observed with methanol and acetone extracts (10 mm each). In contrast, P. aeruginosa showed moderate sensitivity to all plant extracts, with zones of inhibition ranging from 10–12 mm, indicating better susceptibility compared to E. coli. The standard antibiotic demonstrated significantly higher antibacterial activity against both test organisms, producing zones of inhibition of 18 mm for E. coli and 15 mm for P. aeruginosa. The variation in antibacterial efficacy among solvents highlights the influence of extraction medium on the recovery of bioactive phytoconstituents. Overall, the findings suggest that Smilax perfoliata leaves possess moderate antibacterial activity against Gram-negative bacteria, particularly when extracted with organic solvents, supporting their potential as a natural source of antimicrobial agents.*

Keywords: Smilax perfoliata; antibacterial activity; Gram-negative bacteria; solvent extracts; zone of inhibition; phytochemicals.

I. INTRODUCTION

Medicinal plants possess the capacity to host endophytic microbes, attributed to their bioactive compounds. [1]. Plants play a crucial role in the development and sustenance of human civilization. [2] Based on the latest assessment of Over 80 individuals in developed nations depend on traditional medicines for their health needs. Sixty individuals utilize vitamins and phytomedicines for their health benefits at various intervals.[3]. A global issue is emerging regarding the treatment of hospital and community-acquired illnesses caused by multidrug-resistant bacterial pathogens, as existing antibiotic regimens are ineffective against them. Consequently, antibiotic resistance poses a significant threat to human health, with a progressive increase in illness and mortality rates attributed to prevalent bacterial infections. Recent observations indicate that resistance genes are emerging due to the incorrect and excessive usage of antibiotics. [4] Plants are a prolific source of secondary metabolites and innovative medicinal molecules that promote human health with manageable unwanted effects [5]. Plants possessing therapeutic characteristics are becoming increasingly significant in the food and pharmaceutical sectors due to their roles in illness prevention and treatment. Plant extracts and their constituents are recognized for demonstrating various biological activities, particularly in the realms of antimicrobial, antifungal, antibacterial, and antioxidant properties. Compounds that demonstrate the ability to impede pathogenic activity while exhibiting minimal toxicity to host cells may be regarded as potential candidates for the advancement of

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novel antimicrobial agents [6]. The recent advancement of functional foods and pharmaceutical products derived from medicinal and edible plants, particularly fruits and vegetables, has enhanced various facets of life, including the mitigation of physical ailments, the decrease in reliance on synthetic antibiotics, and the extension of life expectancy [7]. The researchers have consequently undertaken an investigation to isolate antimicrobial compounds derived from natural sources, with a particular emphasis on plant materials. These natural antibiotics have been utilized within the realm of ethnomedicine and exhibit bio-compatibility. Alongside natural antimicrobials, there has been a growing interest within the research community regarding natural antioxidants for application in food and medicinal materials. This shift aims to substitute synthetic antioxidants, which are facing restrictions due to their potential carcinogenic properties.

In multiple developing nations, herbal medicinal systems continue to play a significant role in the treatment of various maladies. Ayurvedic medicine is prevalent in India, with around 85% of the population utilizing crude plant mixtures for the treatment of various diseases and ailments [8] It has been estimated that approximately 25 % of all prescription drugs currently in use are of plant origin. Natural products and secondary metabolites derived from living organisms, particularly of plant origin, exhibit significant potential in the treatment of human ailments, including cancer, coronary heart disease, diabetes, and infectious disorders. The World Health Organization states that 65-80% of the global population depends on traditional medicine for the treatment of various ailments [9]. The emergence of antimicrobial resistance and concerns regarding food safety have escalated into significant health issues for the public, governmental bodies, and regulatory organizations over the past twenty years [10].

Smilax, belonging to the *Smilacaceae* family, represents a substantial genus of climbing shrubs found across both tropical and temperate zones globally. *Smilax perfoliata Lour*. is distributed across multiple regions in India and possesses tuberous rhizomes [11]. It is a resilient, relatively well-equipped ascender. The stem serves as a tool for oral hygiene, promoting the fortification of the gums. The tender shoot is incorporated into curries and serves as a blood purifier [12]. Roots and stems are utilized for their anticancer properties, as well as for addressing dysentery and urinary issues [13]. The plant finds extensive application in the traditional culinary practices of various tribes in North-east India. The current investigation aimed to assess the antimicrobial properties of the leaves of Smilax perfoliata using methanol, acetone and hydroethanolic extracts, with the objective of substantiating its application in traditional culinary practices and medicinal uses.

II. MATERIAL AND METHOD

2.1 Collection of sample

Fresh *S.perfoliate leaves* were collected in January 2023 from forest region near Chicholi village located on Lakhandur Sakoli road dist. Bhandara, India. The woodland is dense here. The forest guard helped us gather *Smilex perfoliate* leaves. To remove dirt and fine particulate matter, the *Smilex perfoliate* section was washed twice with tap water and rinsed twice with double distilled water in the lab. After washing, all leaves were carefully sliced and stored in a shady area for 10–15 days.

2.2 Plant extract preparation

The extracts were obtained by immersing 10 g of finely ground plant material in 100 mL of different solvents, specifically Methanol, Acetone, and a 1:1 Ethano-aqueous solution, and were subsequently stored in a dark environment for the duration of 48 hours. The extract was prepared utilizing the maceration process. After 48 hours, the solution is subjected to centrifugation using a centrifuge machine at 1000 rpm for 30 minutes. The supernatant is then decanted and stored under cold conditions for further use.

2.3 Antimicrobial activity

The evaluation of the antibacterial efficacy of the test samples, alongside the standard (streptomycin), was conducted utilizing the agar well diffusion technique. The inoculum, comprising 10^6 cfu/ml of each bacterial culture—Bacillus subtilis (ATCC-19659), Staphylococcus aureus (ATCC-259), Escherichia coli (ATCC-16404), and Pseudomonas aeruginosa (ATCC-15442)—was uniformly distributed on Muller-Hinton agar plates using a sterile spreader with the bacterial suspension. Following this, wells were created in the agar medium utilizing a cork borer and subsequently filled

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with 100 µL of the test sample. Standard antibiotic discs, specifically streptomycin at a concentration of 10 mcg, were then positioned accordingly. Distilled water served as the control sample. Subsequently, the plates were permitted to undergo diffusion at ambient temperature for duration of 5 minutes. The plates were subsequently maintained in an upright orientation at a temperature of 37 °C for duration of 24 hours. Following the incubation period, the diameters of the zones exhibiting growth inhibition were quantified in millimeters.

III. RESULTS AND DISCUSSION

Plants engage in various interactions with numerous organisms within their natural ecosystems. Through these interactions, they synthesized compounds exhibiting antimicrobial properties. The antimicrobial activity of these compounds exhibits a range of levels [14]. Table no. 1 provides an overview of the findings regarding the antibacterial activity of the Smilex perfoliata hydroethanolic (1:1), methanolic and acetone leaf extract against two gram +Ve and Two gram –Ve bacterial strain.

Table no. 1 : Zone of Inhibition in mm showing by Gram +Ve and Gram –Ve Bacteria against hydroethanol, methanol, acetone extract and standard

Sr.No.	Name of Bacteria		Zone of Inhibition (mm)			
			Solvent			Standard
			Aqueous + Ethanol	Methanol	Acetone	
1	Gram -Ve	*Escherichia coli*	0	10	10	18
2		*Pseudomonas aeruginosa*	10	12	11	15
3	Gram +Ve	*Bacillus subtilis*	11	13	10	22
4		*Staphylococcus aureus*	9	11	11	20

The antibacterial activity of the plant leaf extracts obtained using aqueous + ethanol, methanol, and acetone was evaluated against selected Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacterial strains by the agar diffusion method. The inhibitory effect was assessed by measuring the zone of inhibition (mm) and compared with a standard antibiotic.

The results revealed that antibacterial activity varied considerably with the type of solvent used and the bacterial species tested. Among Gram-negative bacteria, *Escherichia coli* exhibited no inhibition with the aqueous + ethanolic extract, while both methanolic and acetone extracts showed moderate activity (10 mm). The standard antibiotic produced a significantly larger zone of inhibition (18 mm), indicating higher potency. The resistance of *E. coli* to aqueous extract may be attributed to the presence of an outer lipopolysaccharide membrane, which acts as an effective permeability barrier against many phytochemicals [15].

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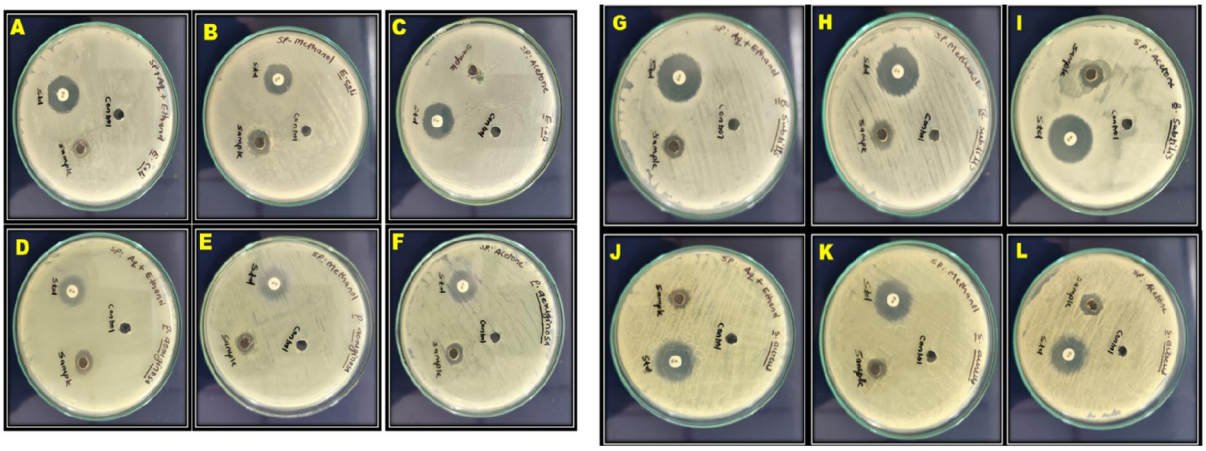

Fig no. 1: Zone of Inhibition of smilex perfoliata different extract against Gram+ve and Gram –Ve microorganism)

In contrast, *Pseudomonas aeruginosa* demonstrated susceptibility to all extracts, with the methanolic extract showing maximum inhibition (12 mm), followed by acetone (11 mm) and aqueous + ethanol (10 mm). The observed variation in sensitivity suggests differential interactions between bacterial cell components and extracted phytoconstituents. Similar findings have been reported where methanolic extracts showed enhanced activity due to efficient solubilization of antimicrobial secondary metabolites [16]. Gram-positive bacteria exhibited comparatively higher sensitivity to the extracts. *Bacillus subtilis* showed the highest inhibition with methanol (13 mm), followed by aqueous + ethanol (11 mm) and acetone (10 mm). *Staphylococcus aureus* also displayed moderate inhibition across all extracts, with methanol and acetone producing equal zones (11 mm). The increased susceptibility of Gram-positive bacteria can be explained by the absence of an outer membrane, allowing easier penetration of bioactive compounds into the cell wall [17].

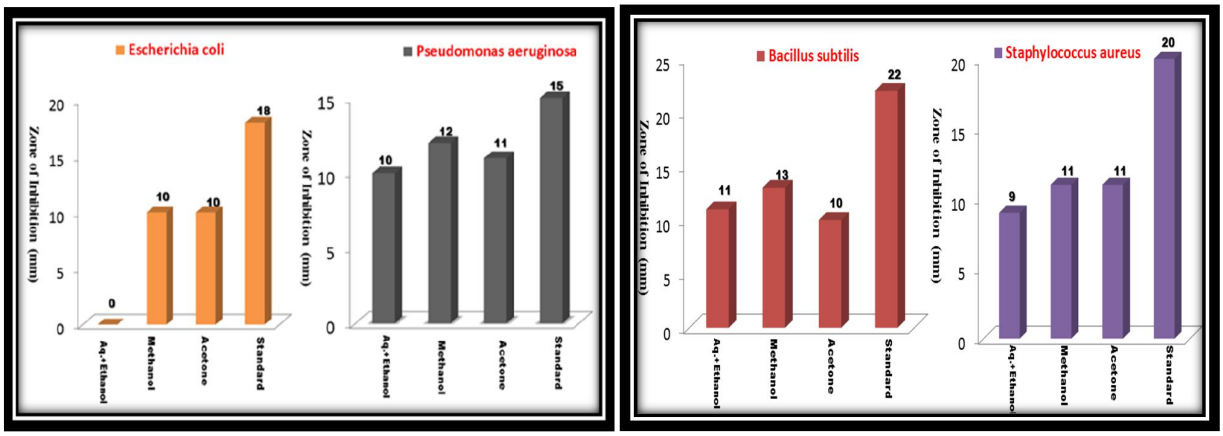

Fig no 2: Comparative graphical representation of various solvent smilex perfoliate leaves against *E.coli, P. aeruginosa, B. subtilis and S.aureus*

Although the standard antibiotic exhibited superior antibacterial activity against all tested organisms, the measurable inhibition produced by plant extracts confirms the presence of biologically active antimicrobial compounds. The consistently higher activity of methanolic extracts suggests that methanol is an effective solvent for extracting antibacterial phytochemicals such as flavonoids, tannins, phenolics, and alkaloids, which are known to disrupt microbial cell membranes and inhibit essential enzymatic processes [18,19]. Overall, the study highlights the promising

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antibacterial potential of the plant leaf extracts, particularly against Gram-positive bacteria, and supports their possible application as natural antimicrobial agents.

IV. CONCLUSION

The present investigation demonstrates that *Smilax perfoliata* leaf extracts exhibit notable antibacterial activity against both Gram-positive and Gram-negative bacterial strains, with the degree of inhibition varying according to the solvent used for extraction. Among the tested solvents, the methanolic extract showed comparatively higher antibacterial efficacy, indicating its effectiveness in extracting bioactive phytoconstituents. Gram-positive bacteria were more susceptible to the plant extracts than Gram-negative bacteria, which may be attributed to differences in cell wall structure. Although the standard antibiotic produced greater zones of inhibition, the observed antibacterial activity of *Smilax perfoliata* supports its traditional medicinal relevance and highlights its potential as a natural source of antimicrobial agents. Future studies should aim at the isolation, purification, and characterization of active antimicrobial compounds present in the leaves. Additionally, in vivo studies and toxicity assessments are necessary to establish the safety and therapeutic applicability of *Smilax perfoliata*–based formulations.

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