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Bactericidal Efficacy Of Different Plant Parts Of *Calotropis Procera* Against Pathogenic Bacteria

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ABSTRACT: *Calotropis procera* is a medicinal plant known for its rich phytochemical composition and therapeutic potential. The present study evaluates the antibacterial activity of *Calotropis procera* extracts against three pathogenic bacteria: *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. The plant extracts exhibited significant inhibitory effects against both Gram-negative and Gram-positive bacteria, indicating broad-spectrum antibacterial potential. The observed activity may be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and phenolics. These findings support the traditional use of *Calotropis procera* and suggest its potential as a natural source for developing effective antibacterial agents.

Key words: *Calotropis procera*, antibacterial activity, alkaloid, flavonoid

I.INTRODUCTION

India has two major hot spots and rich in biodiversity of flora and fauna. Many plants have been contributed in Indian tradition medicinal practices like Ayurveda and Siddha. Almost all plant families have many herbal plants. Asclepiadaceae is one such family**,** now often classified under the Apocynaceae family (specifically the subfamily Asclepiadoideae), is a diverse group of flowering plants, many of which are known for their medicinal and ornamental uses. The family is famous for its unique floral structure and its role in the production of important alkaloids and other bioactive compounds.

Several plants in this family, particularly those in the *Calotropis* genus, have been used traditionally in herbal medicine. For example, milkweed has been used to treat ailments such as coughs, wounds, and even as a purgative. However, many of these plants contain toxic compounds like cardiac glycosides and alkaloids, so they must be used with caution.

Calotropis procera L., commonly known as aak, grows abundantly in Rajasthan. It is found in waste lands and grows as a weed in cultivated fields. *Calotropis* is used as a traditional medicinal plant (Rastogi and Mehrotra, 1991; Oudhia and Dixit, 1994; Oudhia, 1999) with unique properties (Oudhia and Tripathi, 1998). Traditionally *Calotropis* is used alone or with other medicines (Caius, 1986) to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhea (Das, 1996). The plant is poisonous can lead to blindness if its juice is put in to the eyes. The milky exudates from the plant are corrosive. It is said to have mercury like effects on the human body and is sometimes referred as vegetable mercury. Calotropin a compound in the latex is more toxic than strychnine which is responsible for the cytotoxicity of *Apocynum cannabinum* (Pathyusha, 2012). Plant is also using as a source of methane, through anaerobic fermentation for bio fuel production (Ashwani, 2009). *Calotropis procera* demonstrates significant antimicrobial properties against a wide range of bacteria, fungi, and potentially some viruses. The bioactive compounds found in the plant, such as alkaloids, flavonoids, terpenoids, and glycosides, are believed to contribute to its ability to inhibit microbial growth. In the present work, a systematic study of potent antibacterial activity of *C. procera* has been carried out.

II. MATERIAL AND METHODS

Collection of plant material: The plant materials have been collected from already growing mature plants of selected *Calotropis procera* L. from Bada Math, Bhainsawa, Jaipur, Rajasthan . Approximate one Kilogram of fresh plant materials were collected in poly bags including stem, leaves, flower and fruit of C. *procera*.

Preparation for extraction: After collection the plant parts were washed thoroughly with tap water to remove any dirt or impurity on the surface and then given a final wash with distilled water. The washed plant parts were first chopped into small pieces and then allowed to shed dry completely. The plant parts were ground to fine powder with the help of mixture grinder. The powdered material was then weighed and stored in an air tight container in refrigerator until the practical starts.

Sources of test organisms

For the antibacterial experiment, the bacterial strains Gram –ve strain *Escherichia coli* (MTCC 1652),and Gram +ve strain *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 0441) and *Pseudomonas aeruginosa* (MTCC 0741) were selected and procured from the Microbiology Lab, Jaipur, India.

Culture of test microbes

Bacteria was grown on Nutrient Agar Medium (NA) was prepared using 8% Nutrient Broth. Respective medium (10 to 15 ml) was poured into each petri dish in Laminar air flow. Once the medium had solidified, the test plates were inoculated with the prepared bacterial suspension. This was performed by using a sterile spreader.

Agar well diffusion method (Bauer *et al.*, 1996) was performed for bactericidal assay. Zone of inhibition exhibited by both standard were recorded for calculation of activity index (AI). Three replicates of each test extract were examined and the mean values were then referred.

STATISTICAL ANALYSIS

The results of the antimicrobial activity of different extracts are expressed as mean ± standard deviation of the response of 3 replicates determinations per sample. Results were analyzed statistically by using Microsoft Excel.

RESULT AND DISCUSSION

In the present investigation, a comprehensive antimicrobial experiment of sequential fractions of *C. procera* has done against the selected bacterial strains. Extractions of different plant parts (Leaves, stem and flowers) in different organic solvents (Petroleum ether, Chloroform, Acetone, Ethanol and Aqueous) were screened for antibacterial activity.

Escherichia coli, *Staphylococcus aureus*, and *Bacillus subtilis* were the indicator bacteria. The activity was analyzed on the basis of potential of an extract in inhibiting the growth of tested bacteria (Table1, Figure 1-3). The antimicrobial activity was determined by the disc diffusion method.

E.coli is a rod-shaped, Gram-negative bacteria commonly found in the lower intestines of humans. While most strains are harmless and contribute to a healthy intestinal tract by producing vitamins and aiding digestion, certain pathogenic strains can cause severe illness. *E. coli* is a leading cause of urinary tract infections (UTIs), bacteremia, and neonatal meningitis. In the present investigation, In case of leaves extractions, pet. ether extraction exhibits prominent activity among all other solvents. Here, zone of inhibition (ZI) is 20±0.98mm and activity index is 0.76. in stem extraction, bioactive compounds in acetone solvent exhibited most efficacy against *E.coli* with 24±0.98 mm ZI and high AI=0.92.

Whereas, in case of flower sample, chloroform extract is most effective with ZI=22±2.0mm; AI=0.91.

Staphylococcus aureus is a highly versatile Gram-positive cocci bacterium that exists both as a common inhabitant of the human body and a dangerous opportunistic pathogen. Approximately 30% of the global population is persistent carriers, primarily colonizing the nostrils (anterior nares), skin, and throat. It also leads food poisoning caused by ingesting heat-stable toxins in contaminated food; symptoms include rapid onset of nausea and vomiting.

In both leaves and stem samples, pet. ether extract is most effective with ZI 22±2.98mm and 24±2.21mm respectively. While, again chloroform extract is most effective for flower samples similar as *E. coli*.

Bacillus subtilis, known as the hay bacillus or grass bacillus, is a Gram-positive, rod-shaped bacterium. While typically non-pathogenic, it can act as an opportunistic pathogen in immunocompromised individuals, potentially causing infections such as endocarditis or meningitis in rare cases.

Leaves extracts exhibited good efficacy against *B. subtilis*. In all sequential extracts, pet. ether showed maximum zone of inhibition 24±1.37mm with 0.91 activity index. Ethanolic extract of stem exhibited equal zone of inhibition to standard. It indicates potential efficacy against bacterial strain. In case of flowers, antibacterial activity is not much effective. Here also ethanol extract and pet ether extract are effective with 20mm zone of inhibition. Antimicrobial activity (Kishore and Chopra , 1997, Morcelle *et al.*, 2004, Rastogi and Mehrotra, 1993, Doshi *et al.*, 2011).

Figure 1: Antimicrobial efficacy of *C. procera* against *E. coli.*

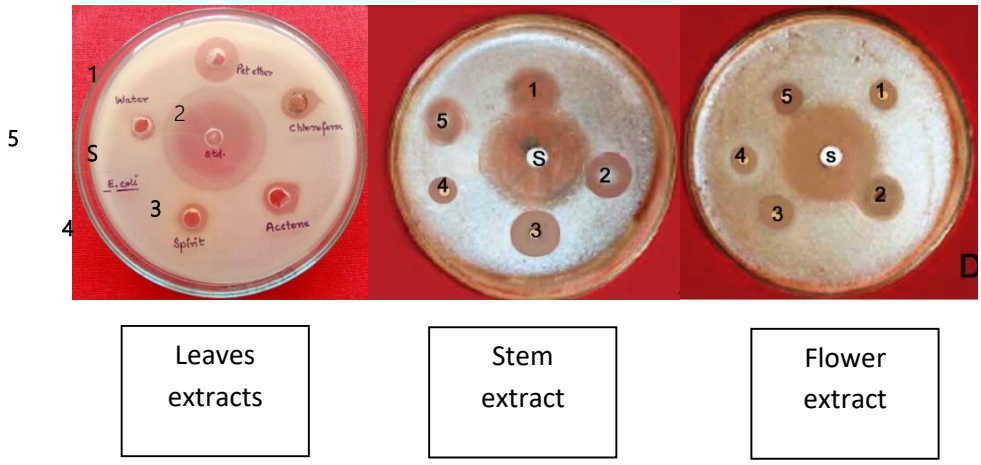

Figure 2: Antimicrobial efficacy of *C. procera* against *Staphylococcus aureus.*

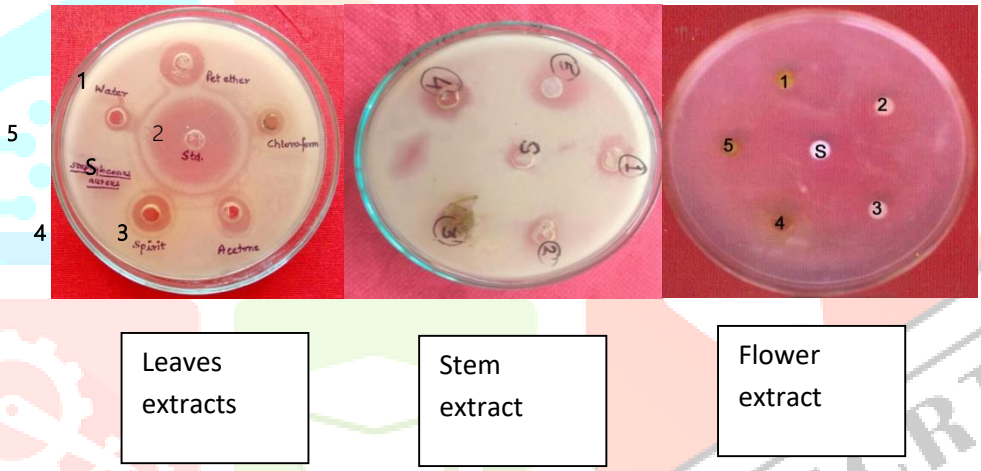

Figure 3: Antimicrobial efficacy of *C. procera* against *Bacillus subtilis.*

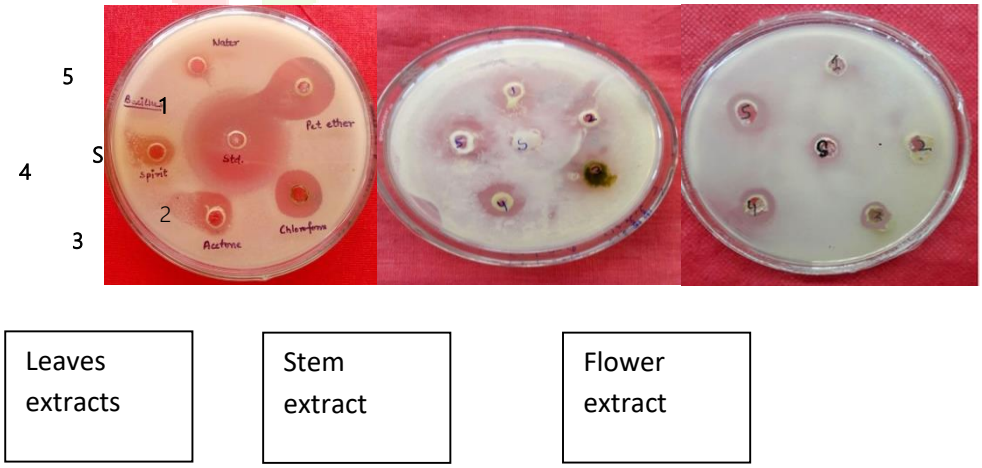

Table 1: Antibacterial activity of different plant parts of *Calotropis procera* L.

Bacterial strain	**Plant part**	**Activity**	**Sequential extraction**					**Standard**
			Petroleum ether	**Chloroform**	**Acetone**	**Ethanol**	**Aqueous**	
Escherichia coli	Leaves	ZI	20±0.98	18±0.23	16±1.33	18±0.21	12±1.45	26.6±2.61
		AI	0.76	0.69	0.61	0.69	0.46	
	Stem	ZI	20±0.11	22±1.33	24±0.98	18±0.23	20±0.89	24.5±0.23
		AI	0.83	0.91	0.92	0.75	0,83	
	Flowers	ZI	18±2.11	22±2.0	20±2.90	18±1.90	16±2.45	24.8±1.12
		AI	0.75	0.91	0.83	0.75	0.66	
Staphylococcus aureus	Leaves	ZI	22±2.98	16±0.43	20±0.23	18±0.89	16±1.45	26.2±1.66
		AI	0.91	0.61	0.76	0.69	0.61	
	Stem	ZI	24±2.21	22±0.33	20±0.23	18±3.33	20±2.42	20.0±2.0
		AI	1.2	1.1	1.0	0.9	1.0	
	Flowers	ZI	22±1.12	24±3.43	20±1.34	18±3.13	22±1.66	24±2.90

		AI	0.91	1.0	0.83	0.75	0.91	
Bacillus subtilis	Leaves	ZI	24±1.37	22±2.00	20±1.88	20±2.70	18±1.21	26±4.33
		AI	0.92	0.91	0.76	0.76	0.9	
	Stem	ZI	20±2.65	18±3.21	20±0.88	22±0.43	20±3.09	22±2.11
		AI	0.90	0.81	0.90	1.0	0.90	
	Flowers	ZI	20±3.09	18±0.34	20±0.33	16±1.32	18±4.21	20±1.88
		AI	1.0	0.9	1.0	0.8	0.9	

(-) Not measurable activity, *IZ = Inhibition zone (in mm) including the diameter of disc (6 mm)(-) Not measurable activity 18.32±0.33

Gentamycin = 10µg/disc

$$\text{Activity index} = \frac{\text{Inhibition area of the test sample}}{\text{Inhibition area of the Standard}}$$

CONCLUSION

The present study concludes that *Calotropis procera* exhibits significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. The inhibitory effects observed suggest the presence of potent bioactive compounds capable of suppressing both Gram-negative and Gram-positive bacteria. Among the tested organisms, variations in sensitivity indicate differential modes of action of the plant extracts. These findings highlight the therapeutic potential of *Calotropis procera* as a natural antibacterial agent. Its effectiveness supports further phytochemical investigations and pharmacological evaluations aimed at developing eco-friendly, plant-based alternatives to conventional antibiotics for managing bacterial infections.

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