

## **Antibacterial activity of ethanolic extracts of *Prosopis juliflora* against gram negative bacteria**

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### **ABSTRACT**

*Prosopis juliflora*, a multipurpose dry land tree or shrub introduced to Kenya due to concern about desertification, deforestation and fuelwood shortages, has become invasive, forming dense, impenetrable thickets, associated with unfavorable impacts on human economic activities. It has soothing, astringent, antifungal and antiseptic properties and is commonly used to treat eye conditions, open wounds and dermatological ailments. An assessment of antibacterial activity of ethanolic extract of root (REE) and leaves (LEE) of *P. juliflora* against clinical isolates of *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) was carried out using paper disc diffusion method. The results of investigation showed that all the extracts had inhibitory effect on the growth of all the isolates. Only chloramphenicol, erythromycin and minocycline were effective against all the bacterial strains tested and there was no significant difference ( $P > 0.05$ ) between the activity of REE and LEE at the highest concentration compared to the activity of chloramphenicol, erythromycin and minocycline. All the bacterial strains exhibited susceptibility to erythromycin and minocycline while Penicillin, methicillin and ampicillin were the least effective antibiotics. Both LEE and REE possess saponins, tannins and alkaloids; phytochemicals whose antimicrobial properties are well documented and therefore could be attributed to the observed antibacterial activity exhibited by these extracts. Results from this study strongly validate use of *P. juliflora* in the management of bacterial infections.

**Key words:** Antibacterial, Phytochemicals, Invasive, Resistance

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### **INTRODUCTION**

Traditional medicine is widely practiced in Kenya, where this has been documented by ethnobotanical surveys [1-2]. The high cost of conventional drugs and/or inaccessibility to modern healthcare facilities has led to over reliance on traditional medicine since it is affordable and available to rural people. On the other hand, even when modern health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective [3-4].

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value and some of them are also used for prophylactic purposes [5]. A medicinal plant is any plant which, in one or more of its organs, contain substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human or animal body [6].

Infections associated with bacterial pathogens are among some of the indications treated using traditional remedies in Kenya [2]. Bacterial infections are prevalent due to various factors such as the HIV/AIDS pandemic, poor hygiene, overcrowding and resistance to conventional antimicrobials. Natural products of higher plants may provide a new source of antimicrobial agents with novel mechanisms of action [7].

Gram-negative bacteria, such as *Escherichia coli*, are present in the human intestine and cause lower urinary tract infections and septicemia [8]. Hence, we report here the effect of ethanolic extracts of root and leaves of *P. juliflora* on pathogenic strains of Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) by zone inhibition assays. Their effects were compared to various conventional drugs, namely Penicillin, chloramphenicol, methicillin, ampicillin, erythromycin, minocycline and lincomycin and which have different mechanisms of action.

*Prosopis juliflora* (Sw.) DC (Fabaceae) is an evergreen tree native to South America, Central America and the Caribbean. *Prosopis* species are generally fast-growing, drought-resistant, nitrogen-fixing trees or shrubs adapted to poor and saline soils in arid and semi-arid zones [9]. *P. juliflora* was first introduced to Kenya in 1973 for the rehabilitation of quarries and to safeguard the existing natural vegetation from overexploitation, but over the years, *Prosopis* has spread outside the designated plantation areas, adversely affecting natural habitats, rangelands and cultivated areas in many parts of the country [10]. Because this is an exotic plant species that was introduced into the country, its use as a phytomedicine is not widespread and therefore the aim of this study was to determine the antibacterial activity of leaves and roots ethanolic extracts of *P. juliflora*.

## MATERIALS AND METHODS

### 2.1 Sample collection, preparation and extraction

Leaves and root bark samples of *P. juliflora*, obtained from Endao, Marigat district, in Baringo County of Kenya were botanically identified and authenticated by a field officer from Kenya Forestry Research Institute, Marigat station and a taxonomist from Botany Department of Jomo Kenyatta University of Agriculture and Technology, where voucher specimens were also deposited. The collected materials were washed thoroughly in water, chopped; air dried for two week, pulverized in electric grinder and exhaustively extracted using 80% ethanol. The extracts were concentrated *in vacuo*, dried and stored at 4°C until required for bioassay.

### 2.2 Antibacterial Assay

#### 2.2.1 Test microorganisms

The two Gram negative bacteria (*P. aeruginosa* and *E. coli*) were obtained from the Botany Department of the Jomo Kenyatta University of Agriculture and Technology, Kenya. The bacterial isolates were first sub-cultured in a nutrient broth (Oxoid) and incubated at 37°C for 18 h.

#### 2.2.2 Preparation of culture medium and inoculation:

Mueller Hinton Agar (MHA) medium (38 g) was mixed with 1000 ml of sterile distilled water and sterilized by autoclaving at 120°C for 20 minutes. Under aseptic conditions, in the laminar flow hood 15 ml of MHA medium was dispensed into pre-sterilized petridishes to yield a uniform depth of 4 mm.

#### 2.2.3 Antibiotic susceptibility testing

Antibacterial activity of the ethanolic extracts of root and leaves was evaluated by the paper disc diffusion method on MHA plates [11-12]. Bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto MHA (Oxoid) plates (diameter: 15 cm). Sterilized paper discs (diameter 6 mm), soaked in known concentrations of the crude extracts of *P. juliflora* in DMSO were applied over each of the culture plates previously seeded with the 0.5 McFarland. Antibiotic discs of Penicillin(1µg), Chloramphenicol(30 µg), Methicillin(5 µg), Ampicillin(10 µg), Erythromycin(15 µg), Minocycline(30µg), and Lincomycin(2 µg) were used as positive control while sterilized paper discs without extracts or antibiotics were used as negative controls for the bacteria. The cultures were then incubated at 37°C for 18 h. Antibacterial activity was determined by measurement of zone of inhibition around each paper disc. The experiment was performed in triplicate for the various concentrations and the results expressed as mean  $\pm$  standard deviation.

#### 2.2.4 Determination of Minimum Inhibitory Concentration (MIC)

To determine the Minimum Inhibitory Concentration (MIC) values, various concentrations that included 0.01, 0.1, 1, 10 and 100 mgmL<sup>-1</sup> were assayed against the test bacterial strains. The MIC was defined as the lowest concentration that inhibited any visible bacterial growth [13-14].

## RESULTS AND DISCUSSION

In recent years, the antimicrobial properties of medicinal plants have been increasingly reported in different parts of the world. It is expected that plant extracts demonstrating target sites other than those used by currently available antimicrobials will be active against drug resistant microbial pathogens [15]. The results for antibacterial assay are as shown in **Table 1** and **Table 2**.

Table 1: Antibacterial activity of root and leaves ethanolic extracts of *P. juliflora*

Strains		Minimum Zone of Inhibition in mm and MIC(mgmL <sup>-1</sup> )					
		Concentrations					MIC
		100 mgmL <sup>-1</sup>	10 mgmL <sup>-1</sup>	1 mgmL <sup>-1</sup>	0.1 mgmL <sup>-1</sup>	0.01 mgmL <sup>-1</sup>	
<i>E. coli</i>	REE	15.00±1.00	10.00±0.00	9.00±0.00	8.00±0.00	NI	10
	LEE	20.00±1.00	8.67±0.58	8.00±1.00	7.67±0.58	7.33±0.58	100
<i>P. aeruginosa</i>	REE	15.33±0.58	11.00±1.00	9.67±0.58	8.67±0.58	7.67±0.58	100
	LEE	9.33±0.58	8.67±0.58	8.00±0.00	7.67±0.58	7.33±0.58	>100

REE- Root Ethanolic Extract; LEE-Leaves Ethanolic Extract; MIC- Minimum Inhibitory Concentration

\*All the values are mean ± standard deviation of three determinations.

Table 2: Antibacterial activity of antibiotics used as positive control

Microbial strain	Minimum Zone of Inhibition in mm (Mean± SD) for various microbial strains						
	PEN	CHL	MET	AMP	ERY	MIN	LIN
<i>Escherichia coli</i>	NI	30	12	17	19	18	14
<i>Pseudomonas aeruginosa</i>	7	22	7	7	11	16	7

NI- NO INHIBITION; PEN- (PENICILLIN 1µ); CHL- (CHLORAMPHENICOL 30 µg); MET- (METHICILLIN 5 µg); AMP-(AMPICILLIN 10µg); ERY-(ERYTHROMYCIN 15µg); MIN-(MINOCYCLINE 30µg); LIN- (LINCOMYCIN 2 µg); SD- (Standard deviation)

The activity against *E. coli* and *P. aeruginosa* was concentration dependent. The antibacterial activity of the various concentrations of LEE and REE against the bacterial strains was not significantly different ( $p>0.05$ ). Only CHL, ERY and MIN were effective against the gram negative bacterial strains tested and there was significant difference between the activity of REE and LEE as compared to these three conventional antibiotics ( $p<0.05$ ). However, there was no significant difference ( $p>0.05$ ) between the activity of REE and LEE at the highest concentration compared to the activity of CHL, ERY and MIN. The activity of LEE against *E. coli* at the highest concentration was also not significantly different to those of CHL, ERY and MIN ( $p>0.05$ ). REE showed no activity against *E. coli* at the lowest concentration and generally, the activity of REE and LEE against *E. coli* and *P. aeruginosa* were not significantly different ( $p>0.05$ ).

*Escherichia coli* was susceptible to all the antibiotics except penicillin (1µ). Highest susceptibility was noted for chloramphenicol (ZI=30mm) while least susceptibility was noted for methicillin (ZI=12mm). *P. aeruginosa* on the other hand, exhibited susceptibility to all of the conventional antibiotics used except penicillin (1µ), with highest susceptibility noted for chloramphenicol (ZI=22mm) and lowest susceptibility to methicillin, ampicillin and lincomycin (ZI=7mm). All the bacterial strains exhibited susceptibility to erythromycin and minocycline while methicillin and ampicillin were the least effective drugs against the various strains used for the test.

A large number of constitutive plant compounds have been reported to have antimicrobial activity. Well known examples include phenols, unsaturated lactones, saponins, cyanogenic glycosides and glucosinolates [16-17]. Phytochemical analysis of ethanolic extracts of *P. Juliflora* revealed presence of alkaloids, tannins, saponins, flavonoids, sterols and triterpenes [18-21]. The presence of these phytochemicals in the investigated ethanolic extracts of *P. juliflora* would be responsible for the demonstrated antibacterial activity of the extracts. In this regard, the higher concentration of these phytochemicals in the ethanolic extracts may have been responsible for a relatively higher antibacterial activity demonstrated by the extracts on the tested bacterial strains. The results of the study support the traditional application of the plant and suggest the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents.

## CONCLUSION

Significant antibacterial activity against gram negative bacteria validates use of this plant in treatment of bacterial infections. However, further study is necessary for purification, separation, isolation and characterization of the active principles from the ethanolic extracts.

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