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Research Article

ANTIBACTERIAL ACTIVITY OF *CELTIS AUSTRALIS BY INVITRO STUDY*

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ABSTRACT

The Aqueous and Methanol crude extract of *Celtis australis*, traditionally used in Indian system of medicine was screened for its antibacterial activity against *S. aureus* and *P. aeruginosa*, using Cephotaxime as positive control. The antibacterial potential of the extract was evaluated by Disc diffusion method. The analysis showed that methanolic leaf extract had the highest activity against *S. aureus* at 200mg/ml concentration with 10.5±0.57mm zone of inhibition. Increasing the concentration of the extracts resulted in increased antibacterial potential for all methanolic and aqueous extracts tested. Both the strains were found to be resistant for Cefuroxime, Ampicillin and Tetracycline. The present study reveals that the selected plant would exert several beneficial effects by virtue of its antibacterial activity and could be harnessed as drug formulation.

Keywords: Antibacterial; Cefuroxime; *Celtis australis*; Cephotaxime; Disc diffusion; Traditional medicine.

INTRODUCTION

Plants have been valuable source of natural products for maintaining human health[1]. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs[2]. About 80% of population of developed countries use traditional drugs derived from the plants, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [3]. The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. It is known that more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine[4]. Plant sources provided a good source of anti-infective agents, which are cost-effective and have fewer side effects. Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants[5].

Celtis australis belonging to family Ulmaceae is a deciduous tree distributed to montane and submontane Himalaya[6]. The paste obtained from the bark of *C. australis* is effective remedy for bone fracture and also applied on pimples, contusions, sprains and joint pains[7]. The decoction of both leaves and fruits is used in the treatment of amenorrhea, heavy menstrual and inter-menstrual bleeding, diarrhea, dysentery and peptic ulcers. Since there is no report on antibacterial activity of *Celtis australis*, an attempt was made to evaluate the antibacterial activity of methanol and aqueous extracts of leaves by agar disc diffusion method.

MATERIALS AND METHODS

1. **Plant material:** The leaves of *Celtis australis* were collected from various areas of Kashmir.

2. **Extraction procedure:**

a). Aqueous Extract: For aqueous extract leaf powder was separately homogenized with sterile distilled water at 1:8 w/v ratio in a pestle and mortar and filtered through muslin cloth. The filtrate thus obtained was further strained through Whattman No. 1 filter paper. The extraction was carried out at room temperature.

b). **Organic Extract**: Organic extract of *Celtis australis* leaves was prepared by Soxhlet extraction method following[8].

The plant material was cut into small pieces, shade dried and made to coarse powder. Its weighed amount was packed in extraction thimble and placed in an extraction chamber which was suspended above the flask containing the solvent methanol and below a condenser. The flask was heated and the solvent evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the methanol extract was removed and methanol was evaporated by using rotary evaporator. The weight of the extract was measured and kept in refrigerator at 4°C to detect antibacterial activity.

3. **Microorganisms**

Two different strains were used for testing antibacterial activity included *Staphylococcus aureus (ATCC 25923)* and *Pseudomonas aeruginosa (*ATCC 27853). The test organisms used in this study were collected from National Jalma Institute of Leprosy and Other Mycobacterial Diseases, Agra, Dept. of Botany, R.B.S. College, Agra and Dept. of Microbiology, School of Life Sciences, Dr. B. R. Ambedkar University, Khandari Campus, Agra. The bacteria were cultured on nutrient agar slants. The cultures were maintained by subculturing periodically and preserved at 4ºC prior to use.

4. **Screening for antibacterial activity**

In vitro antibacterial activity of selected plant extract was tested by disc diffusion method[9].

For susceptibility testing, Plant extract was dissolved in suitable solvent, plant extract solution with different concentration (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) were prepared by serial dilution. Sterile discs having a diameter of 6 mm were impregnated with 25 µl of each serial dilution of extract solution. Some colonies from the pure culture were mixed in nutrient broth. This broth was inoculated on entire surface of nutrient agar plate with the culture moistened cotton swab. Plant extracts containing disc were placed on inoculated surface of agar plate with the help of sterile forceps. These plates were incubated for 24 hours at 37ºC. The diameter of the zones of inhibition around each of the disc was taken as measure of the antibacterial activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was measured in millimeter.

RESULTS AND DISCUSSION

❖ **Antibiotic drug sensitivity of *S. aureus* and *P. aeruginosa***

Staphylococcus aureus and *Pseudomonas aeruginosa* isolates were assessed for the antibacterial drug susceptibility by using disc diffusion method. Out of eight antibiotics *S. aureus* isolate was found to be resistant to Cefuroxime and Ampicillin, moderately sensitive to Ceftriaxone and highly sensitive to Co-trimoxazole, Gentamicin,

Ciprofloxacin and Tetracycline with 16, 14, 13 and 12mm zone of inhibition respectively. However, *P. aeruginosa* strain was resistant to Ampicillin, Cefuroxime, Tetracycline and Ceftriaxone and highly sensitive to Ciprofloxacin and Gentamicin with 15 and 12mm zone of inhibition respectively.

❖ **Antibacterial Sensitivity**

The results of antibacterial activity have been summarized in Table 1. In Agar diffusion assay, the aqueous and methanol leaf extract of *Celtis australis* at different concentrations showed considerable activity against both the tested bacterial strains: *Staphylococcus aureus and Pseudomonas aeruginosa.* The strongest antibacterial activity was observed against *S. aureus* at 200mg/ml concentration with 10.5±0.57 mm zone of inhibition. 9.00 ± 0.82mm zone of inhibition was shown against *P. aeruginosa* at 200mg/ml concentration. However, 8.5± 1.86 and 7.5±1.00 mm zone of inhibition was observed in aqueous leaf extract against *S. aureus* and *P. aeruginosa* respectively. The antibiotic Cephotaxime exhibited 15 and 12mm zone of inhibition against *S. aureus* and *P. aeruginosa* respectively.

It is evident from the results that *S. aureus* was the most sensitive organism to leaf extract of *Celtis australis.* [10]reported the fatty acid composition and antimicrobial activity of *Celtis australis* L. fruits. The extract was found to be active against *B. subtilis* and *P. aeruginosa* with minimum inhibitory concentration of 250 and 125µg/ml, respectively. The degree of sensitivity increased with increase in inhibitory concentration. The study revealed that methanol was the promising solvent for antibacterial activity against both the pathogens. [11]reported *in vitro* antibacterial and antioxidant potential of medicinal plants used in the treatment of acne. They observed highest zone of inhibition (≥15) mm with methanolic extract of *Camellia sinensis* using disc diffusion method. The varying degrees of sensitivity of the bacterial test organisms may be due to the intrinsic tolerance of microorganisms. The activity of the plant against test organisms may be indicative to the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. Therefore this drug may perhaps be the suitable therapeutics option of an antimicrobial agent.

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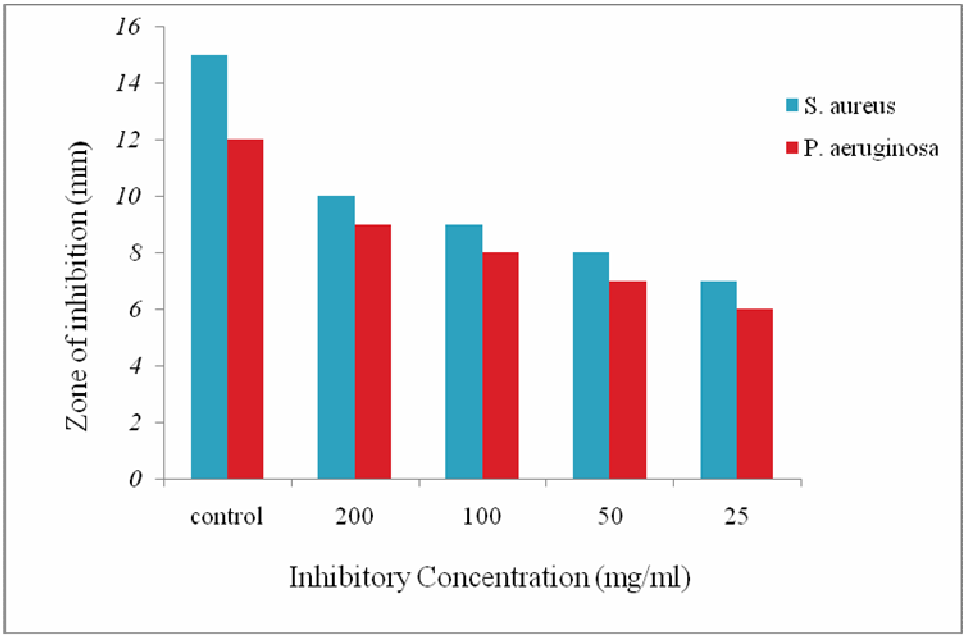

Fig. 1: It shows sensitivity of *Celtis australis* extracts against *S. aureus* and *P. aeruginosa* at different concentration.

Table 1: Shows antibacterial activity of *C. australis* leaf extract in Nutrient Agar disc diffusion assay.

Extract(m m)	Inhibitory concentration(mg/ml)	Zone of inhibition (mm) *S. aureus*	Zone of inhibition *P. aeruginosa*	Remarks
Aqueous	200	8.56±1.86	8.00±1.00	++
	100	7.53±1.52	7.56±1.52	++
	50	6.33±0.82	6.33±0.57	+
	25	–	–	–
	12.5	–	–	–
	6.25	–	–	–
Methanol	200	10.50 ± 0.57	9.00 ± 1.00	+++
	100	9.00±1.00	8.66 ± 1.52	++
	50	8.33±0.82	7.56 ±0.58	++
	25	7.66±1.52	6.33±0.52	++
	12.5	6.33±0.57	–	–
	6.25	–	–	–

± : Standard Deviation, **+++** : Significant activity, **++** : Activity but not significant, **-** : no activity.

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