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IN VITRO STUDY OF ANTIBACTERIAL ACTIVITY OF *A. PANICULATA* AGAINST CLINICALLY IMPORTANT PATHOGENS

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Abstract
The objective of the study was to determine the presence of antibacterial activity in the aqueous crude aqueous extract of *Andrographis paniculata.* In this preliminary investigation, the leaves crude extract was subjected for screening against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* i.e., isolated from clinical samples from the pathology labs. The antibacterial activity was assessed by the presence or absence of inhibition zones and MIC values. *A. paniculata leaves* aqueous extract has potential antibacterial activities against *S. aureus* with MIC 1000mcg/disc *and P. aeruginosa* with MIC 50mcg/disc. *A. paniculata extract* showed no antibacterial activities towards gram negative *E. coli* and *K. pneumoniae*.

KEYWORDS: Plants, MIC, aueous extract, *S. aureus, Klebsiella pneumoniae, Pseudomonas aeruginsa, Escherichia coli*

INTRODUCTION
Medicinal plants are frequently used as remedies for many infectious diseases (Ahmed *et al.,* 1998). Its interesting to note that the search for new antimicrobials from the medicinal plants is still increasing with the emergence of antibiotic resistance development in the pathogens (Fyhrquist, *et al.*, 2002) Plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Dimayuga and Garcia, 1991). The control of bacterial infection has been remarkably effective since the discovery of antibacterial drugs (Rios and Recio, 2005). However, some of the pathogens rapidly become resistant to many of the first discovered effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics (WHO, 2002) has led to the search of new antibacterial agents in particular from medicinal plants. The screening of plant extracts has been of great interest to the researchers for the discovery of new drugs effective in the treatment of several diseases.

MATERIAL AND METHODS
Bacterial strains and Plant Material
S. aureus, Klebsiella pneumoniae , Pseudomonas aeruginsa, and *Escherichia coli* were obtained from the clinical samples in the local pathological laboratory at Udgir *Andrographis paniculata*, this medicinal plant species was selected on its traditional claim as having antibacterial properties for the *in vitro* antibacterial screening(Chopra *et al.*, 2002).

Plant extract Preparation
Dried *A. paniculata* leaves were extracted with water in a Soxhlet apparatus for approximately 12 hours.The resulting aqueous extract was filtered through Whatman paper No.1 and concentrated to obtain a crude residue (Gnanamani *et al.*, 2003; Eloff, 19908 and Jeevan ram, *et al.*, 2004).

Preparation of impregnated disc
Plant extracts were diluted in DMSO in a serial two fold dilution across a 96-well plate starting from 200 mg/ml. The concentration was then further diluted to16 fold in water correspondingly. Twenty micro liters from each of the well was then used to impregnate a blank sterilized disc. The final concentration used for the testing were from 1 mg/disc to 0.002mg/disc. The impregnated discs were dried at 37°C incubator for 18 to 24 hours and immediately used for the sensitivity test9 Srinivasan, *et al*, 2001, Karman, *et al*, 1998 and Immanuel, *et al*, 2004).

Bacterial Cultures
Isolated bacteria after morphological and biochemical confirmation were cultured onto blood agar plate and nutrient agar plate viz. incubated for 18 to 24 hours at 37oC. A single colony was then cultured in 5 ml Nutrient Broth for 4 hours at 37oC. The density of bacteria culture required for the test was adjusted to 0.5McFarland standard (1.0 x 108 CFU/ml) (Dharmarate *et al*., 1999)

Disc Diffusion Method
Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer *et al.*, (1966) to assess the presence of antibacterial activities of the plant extract. A bacterial culture (which has been adjusted to 0.5 McFarland standard) was used to lawn nutrient agar plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with a series of plant extracts were placed on the Nutrient agar surface. A standard commercial antibiotic disc was used as positive control. The standard antibiotic discs were Vancomycin 30 µg and Ampicillin 10 µg for *S. aureus* and *K. pneumoniae* respectively, Amikacin 30 µg was for *P. aeruginosa* and *E. coli* The negative control was DMSO (100%).The plate

was then incubated at 37°C for 18 to 24hours depending on the species of bacteria used in the test. After the incubation, the plates were examined for zone of inhibition. The zone of inhibition was then measured and recorded. The tests were repeated three times to ensure reliability (Zaidan, *et al.*, 2005, Bagachi, *et al.*, 1999 and Denni & Sadiq, 2002).

Determination of minimum inhibitary Concentration

Minimum Inhibition Concentrations (MIC's) was determined (Guerin-Faublee *et al.*, 1996). It was carried out by the diffusion test. Twelve discs of different concentration of the plant extract similar to the concentration used in the sensitivity tests against the four bacterial isolates as mentioned earlier. The lowest concentration that inhibits the growth of bacteria were noted and considered as the MIC value for each of the bacterial strain.

RESULTS

The antimicrobial activitty of *A. paniculata* aqueous extract against the four bacterial isolates observed were assessed by the presence of inhibition zones and MIC values as given in table.1. *A. paniculata* aqueous extract has potential antibacterial activities towards *S. aureus and P. aeruginosa* .No activity was observed for *E. coli* and *K. Pneumonia.*

TABLE 1: Antbacterial activity of *A. paniculata* leaves against the bacterial isolates

Sr. No.	Bacterial isolates	Zone of Inhibition(mm)	MIC mcg/disc
1	*S. aureus*	7.4	1000
2	*P. aeruginosa*	8.2	50
3	*E. coli*	NA	00
4	*K.pneumonia*	NA	00

DISCUSSION

The present study was carried out as a preliminary investigation on the antibacterial activity of *A.paniculata* aqueous extract against pathogenic bacteria isolated from clinical samples. *A. paniculata* water extract showed the potent activity towards *P. aeruginosa* in presence of antibacterial conc. at 50mcg/disc. Since, *A. paniculata s*howed potential activities towards *S aureus*, which causes infections including superficial skin lesion, localized abscesses and food poisoning. MRSA infections, now a days commonly are found in hospitals and also the emerging trend of drug resistance developing in the *S aureus* (Tassou, *et al.*, 2010).

The two bacterial isolates that were not susceptible to the plant extract were *E. coli* and *K. pneumoniae*. These could be due to the presence of a double membrane surrounding each bacterial cells of *E. coli* and *K. pneumonia which* excludes certain drugs and antibiotics from penetrating the cell, partially accounting for that is why gram-negative bacteria are generally more resistant to antibiotics than other gram-positive bacteria. Results showed that the aqueous extract of *A. paniculata* possesses potential antibacterial activity against *S. aureus* and *P. aeruginosa* (Gnan and Demello, 1999 and Fleisher, *et al.*, 2003). These findings will be helpful to many researchers.

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