Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

IN VITRO & *IN VIVO* ANTIBACTERIAL COMPARITIVE STUDY IN *ACACIA NILOTICA* L.

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Received: 12 Feb 2011, Revised and Accepted: 18 May 2011

ABSTRACT

Present investigation was the first attempt which deals with the *in vivo* and *in vitro* comparative study of antimicrobial activity in *Acacia nilotica* L., a nitrogen fixing tree. The antimicrobial activity of callus as *in vitro* sample and naturally grown plant sample of *Acacia nilotica* L. were determined against four pathogenic microbes through broth dilution method. For obtaining the callus, the seeds were cultured on ½ MS medium under the *in vitro* condition. The cotyledonary nodal explants were taken from the *in vitro* seedlings and cultured in the MS medium supplemented in combination of 2,4-D (0.4 mg/l) with BAP (0.25 mg/l).The data proof antimicrobial activity of callus methanol extract showed great potential as source of antimicrobial agent. Results suggest that *in vitro* developed sample posses highest antimicrobial activity, least MIC range (1.9±0.02 mg/ml).Hence, developed *in vitro* cultivation technology would provide quality plant material for medicine.

Keywords: Antimicrobial activity, Microorganisms, Callus, Broth dilution, Cotyledonary node

INTRODUCTION

From ancient times, different parts of medicinal plants have been used to cure specific ailments. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants [1-3]. Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agents [4]. Therefore, on the basis of this, the present study was intended to screening the plant *Acacia nilotica* L. for the significant activities of antimicrobial activities. It is, commonly called as Babool or Kikar, belongs to the family leguminosae It is native to the drylands of tropic Africa and found in arid and semi-arid regions of India. Recently, antioxidant activity studied from bark and young leaves of Acacia nilotica L.[5]

MATERIAL AND METHOD

Mature seeds of *Acacia nilotica* L. were collected from Sirsi (Haatoj) District, Jaipur, Rajasthan. Prior to surface sterilization, seeds were treated in boiled water at 60°C for about 30 mins.and then soaked in distilled water for about 24 hrs. Then they were kept under running tap water for about 10-15 mins. followed by Washing with 1 % (v/v) Rankleen (Ranklem-India) for 2 mins. and rinsed with double distilled water for three times. Prior to inoculation, sterilized seeds were again sterilized with 0.1% (w/v) aqueous $HgCl_2$ for about 2 mins. followed by 2-3 rinsing with double distilled water in Laminar Air flow cabinet. These sterilized seeds were inoculated on half strength MS salts medium in cultured bottles. After 7-10 days, seeds germinated and gave rise seedlings. These *in vitro* seedlings were used as source of explants.

The Murashige and Skoog (MS) medium was prepared by adding 3%sucrose as a carbon source and 0.8% (w/v) agar as a solidifying agent. *In vitro* cotyledonary node (1.0 cm) of 20 day old seedlings inoculated as explants for callus induction on MS medium supplemented with a series of 2,4-D(0.4 mg/l) combination with BAP (0.2 mg/l).

The pH of medium was adjusted to 5.8 ± 0.2 before autoclaving at 1210C for 15 minutes at 15 lb/in2. 20 ml of molten agar medium was poured into a culture bottle and plugged with nonabsorbent cotton. All cultures were incubated in 16 h /8 h photoperiod under light intensity of 50 µE/ m2/s provided by cool, white and fluorescent light at 25 ± 2°C with 55% relative humidity. Each treatment performed using eight replicates and the experiment was repeated at least thrice.

The stem bark and leaves of *Acacia nilotica* L. (8 years old) were collected in April 2011 from Haatoj, Jaipur district, Rajasthan (India). The specimens were authenticated by the department of Botany, University of Rajasthan and the voucher specimen as *Acacia nilotica* L.(Voucher No. RUBL 20432) were deposited for future reference in the Botany Department Herbarium.

For the extraction plant samples were dried at room temperature and powdered via. mortar and pestle. Further, the plant samples were successively extracted with 80% ethanol (100 ml/ gm dry weight) on a water bath for 24 hrs [6] ethanol and methanol using soxhlet apparatus. The solvents were evaporated using a rotary vacuum-evaporator at 50ºC. The extracts used for the detection of anti-bacterial activity.

The four strains of pathogenic bacteria *Escherichia Coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), SMS Medical College, Jaipur, Rajasthan, India.

Antimicrobial assay

The antimicrobial activity of extract against four strains of pathogenic bacteria *Escherichia Coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) were determined by broth dilution method [7]. Extracts concentration was measured by spectrophotometer. A sample (100µL) of each concentration was pipetted into the corresponding well of a sterile microdilution tray. Bacterial suspensions from an overnight culture were standardized to 0.5 McFarland (1.5 X 108 CFU mL-1) using an API turbidometer. A 1:20 dilution was made to give a bacterial suspension of an approximately 6 X 106 CFU mL-1. A sample (10µL) of the bacterial suspension was added to each well giving a final suspension of 6 x 106 CFU mL-1. Tray was incubated at 37ºC for overnight. Next day these serial diluted samples were inoculated on Nutrient Agar and MacConkey Agar plate. Plates were further incubated at 37ºC for overnight. All experiment was run in duplicate. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of extract that showed no visible growth in broth microdilution tray and showed mild growth when sub cultured on a suitable solid medium.

RESULT AND DISCUSSION

The findings of the present study revealed that *Acacia nilotica* L., contain potent antimicrobial property against tested microbes. The results obtained in the broth dilution assay regarding the MIC

range of the tested microbes are shown in (Table 1). Outcome shows that *in vitro* sample, callus methanol extract having least MIC range which means it posses the most significant antimicrobial activity against microbial strains following bark and leaves extract (Table 1).

Table 1: Antibacterial screening of alcoholic extract of leaves, bark and calli of *Acacia nilotica* L. against different microorganisms

Microbial strains	Leaves EE MIC	Leaves ME	Bark EE range	Bark ME	Callus EE (mg/ml)	Callus ME
Escherichia coli(ATCC 25922)	3.50±0.03	3.75±0.05	2.0±0.01	1.87±0.05	1.9±0.02	1.85±0.07
Staphylococcus aureus (ATCC 25923)	3.50±0.03	3.75±0.07	2.0±0.03	1.87±0.02	1.9±0.09	1.85±0.01
Enterococcus faecalis(ATCC 29212)	3.50±0.05	3.75±0.01	2.0±0.06	1.87±0.03	1.9±0.06	1.85±0.05
Pseudomonas aeruginosa (ATCC27853)	3.50±0.01	3.75±0.03	2.0±0.02	1.87±0.05	1.9±0.02	1.85±0.03

MIC= minimum inhibitory concentration, EE= ethanol extract, ME methanol extract

Earlier, a few researches have been done on the account of comparative antimicrobial activities of this plant. However, no report available on the relative study of *in vivo* and *in vitro* samples. Therefore, present study deals first time to evaluate them.

From the above results and discussion it can be concluded that the *Acacia nilotica* L. exhibited the potent antioxidant & antimicrobial substances and a which may be responsible for its anti-tumor, anti-inflammatory and remedy for tuberculosis, malaria, leprosy, Hepatitis C virus as well as justify the basis of using this plant's extract as folkloric remedies.

In vitro cultivation technology would improve quality of raw material and so ultimately the efficiency of developed drug from them.

ACKNOWLEDGEMENT

The authors are thankful to Head, Department of Botany, University of Rajasthan, Jaipur, for providing basic research facilities. The authors are also sincerely thanks to the Head, SMS Medical College, Jaipur, Rajasthan, India for providing bacterial strains.

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