Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Botany

Elixir Appl. Botany 97 (2016) 42260-42262

ISSN: 2229-712X

Antimicrobial activity of Acacia Nilotica against Various Clinical Isolates

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ARTICLE INFO

Article history:

Received: 14 May 2016;

Received in revised form:

10 August 2016;

Accepted: 20 August 2016;

Keywords

Antimicrobial,

Acacia nilotica,

Bacillus subtilis,

Phytochemical screening.

ABSTRACT

Aim of present study was to evaluate *in vitro* antimicrobial activity of crude extracts of *Acacia nilotica*. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 6 to 22 mm and exhibited appreciable activity against all the clinically important bacterial and fungal species. Overall maximum Inhibition zone (22mm) was observed in extract of methanol and chloroform (75:25) against *Bacillus subtilis*and minimum (4mm) in extract of methanol and chloroform (50:50) against *Dreschlera avenacea*. Phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids and flavonoids in the extracts. The antimicrobial effect of the extract was compared with the standard drugs (Ciprofloxacin for bacterial and ketokenazole for fungus). Crude extracts of *A.nilotica* inhibited the growth of various bacteria and fungi thus showed its broad spectrum antimicrobial potential, which may be employed in the management of microbial infections.

Introduction

Plant based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials is needed as antimicrobials of plant origin have enormous therapeutic potential. They are potent in the treating infectious diseases while simultaneously mitigating many of the side effects of synthetic antimicrobials (Iwu*et al.*, 1999). They may act as lead compounds for the pharmaceutical industry or as the base for the development of new antimicrobials (Aiyelaagbe, 2001; Aiyegoro*et al.*, 2008).Antibiotics provide the main basis for the therapyagainst various microbial infections. Excessive irrational use of antibiotics has created various microorganisms as multidrug resistance (Perez *et al.*, 1990) so, there is a need of formulation of new antimicrobial agents. Since ancient times natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs.

Acacia nilotica is found in Asia, Australia and Africa. It is widely cultivated in the Indian subcontinent, also found on lateritic soil in the Himalayan foothills in India.Banso (2009) as well as Mashram et al., (2009) reportedthe antimicrobial activity of ethanol extracts of the stem-bark against human pathogenic microbes. Mahesh et al., (2008) reported antifungal activity of methanol extracts and aqueous extract of A. nilotica. Methanol extract also possess antiviral activity(Mohamed et al., 2010).The root extracts of A. nilotica showed potent antimalarial activities (Ali et al., 2010).

Materials and Methods

Antimicrobial Activity

Antimicrobial activity of *Acacia nilotica* (Stem-bark)was studied with different ratios of chloroform and methanol extracts. Four bacterial and three fungal strains were selected for the primary screening.

Microorganisms Used

Clinical laboratory bacterial isolates of Pseudomonas flurorescens, Staphylococcus aureus, Bacillus subtilis, Escherichia coli and fungal isolates viz. Aspergillus niger, Fusarium oxysporium and Dreschlera avenacea were collected from the stock cultures of Microbiology Laboratory, SMS Medical College Jaipur, India.

Preparation of Extract

The crude extracts (both methanol and chloroform) in different ratios viz. 25: 75 (Sample 1), 50: 50 (Sample 2) and 75:25 (Sample 3) were obtained by macerating dried plant powder in respected solvents and kept on a rotary shaker for 24 h. The extract was filtered, centrifuged at 5000 g for 15 min. and dried under reduced pressure. The extract was stored at 4ºC in airtight bottles.

Culture and Maintenance of Bacteria

Bacteria pure culture were grown in Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) in distilled water at 15 lbs psi for 25-30 min) and incubating at 37°C for 48 h. Each bacterial culture was further maintained on the same medium after every 48 h of transferring.A fresh suspension of test organism in saline solution (usually 0.47%) was prepared from a freshly grown agar slant before every antimicrobial assay

Determination of Antibacterial Assay

In vitro antibacterial activity of the crude extracts was studied against gram positive and gram negative bacterial strains by the agar well diffusion method (Perez *et al.*,1990). Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium .The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL. The Mueller Hinton agar was melted and cooled to 48-50ºC and a standardized inoculum (1.5×108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37ºC. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well.

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The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values are presented.

Determination of Antifungal Assay

Antifungal activity of the experimental plant was investigated by agar well diffusion method (Bonjar*et al.,* 2005). The fungal strains were subcultured on Potato dextrose agar (PDA) dextrose agar, (Merck, Germany) and respectively incubated at 37°C for 24 h and 25 °C for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 ml of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). All experiments were made in triplicate and means were calculated.

Results and discussion

The antimicrobial activity of different extracts of A. nilotica were tested against four bacterial strains (Staphylococcus aureus, Pseudomonas fluorescens, Escherichia coli and Bacillus subtilis) and three fungal strains (Aspergillus niger, Fusarium oxysporium and Dreschlera avenacea

The Inhibition Zone (IZ) was measured by antibiotic zone reader (Table 1). Individually against *E. coli* maximum zone was in sample 3 (10mm) and it was at par in samples 1 and 2. *S. aureus* was found to be resistant against all the samples. Against *Dreschlera avenacea* sample 1 gave maximum zone (8mm) and in sample 1 it was minimum while no activity in sample 3. However against *B. subtilis* maximum zone was observed in sample 3 (22mm) and minimum in sample 2, while in *A. niger* case was reverse as only sample 3 gave zone of inhibition (6mm). Plants synthesize variety of phytochemicals as part of their normal metabolic activities. Chemical profile of a single plant may vary over a time, as it reacts to changing conditions. Plant scientists and natural products chemists are combing the flora for the phytochemicals and lead compounds, which could be developed for treatment of various diseases. In 2010 a survey of 1000 plants was done out of which, 156 clinical trials for evaluation of their pharmacological activities and therapeutic applications gave encouraging results (Cravotto*et al.,* 2010). This led to the new search for drugs and dietary supplements derived from plants. During the last 10 years pace of development of new antimicrobial drugs has slowed down, while prevalence of resistance has increased multifold (Akinpelu and Onakoya, 2006). The problem of microbial resistance of growing and outlook for the use of antimicrobial drugs in future is still uncertain therefore, action must be taken to reduce this problem, such as controlling the use of antibiotics and carrying out research for better understanding of genetic mechanism of resistance. This prompted to evaluate plants as source of potential chemotherapeutic and antimicrobial agent along with their ethnomedicinal use (Prashanth*et al.,* 2006).

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Table 1. Antimicrobial activities of various extracts of *A. nilotica (Stem-Bark)*.

Test organisms	Plant parts and zone of inhibition (IZ)			Standard antibiotics
	Sample1	Sample2	Sample3	C/K
Bacteria				
E.coli	6±0.18	6±0.18	10±0.38	22
S. aureus	NA	NA	NA	22
P. fluorescens	16±0.53	22±0.81	18±0.55	22
Bacillus subtilis	20±1.10	18±0.55	22±0.81	22
Fungi				
Dreschlera avenacea	8±0.19	4±0.08	NA	20
A.niger	NA	NA	6±0.18	20
Fusarium oxysporium	8±0.19	10±0.23	NA	20

IZ=Inhibition zone (in mm)
NA= NoActivity,
C= Ciprofloxacin, K= Ketokenazole
Where Sample 1 is 25: 75
Sample 2 is 50: 50
Sample 3 is 75:25.

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