

Research Paper—



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ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACTS OF MEDICINAL PLANTS AGAINST BACTERIAL SPECIES



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A B S T R A C T

In the present work an attempt has been made to carry out screening for the preliminary antibacterial activity of different plants used in Indian folk medicine. Some of folk medicinal plants such as Acacia nilotica, Withania somnifera, Ziziphus mauritiana, Tinospora cordifolia are commonly used for herbal preparations in the treatment of gonorrhoea, leucorrhoea, diarrhoea, dysentery, diabetes, aphrodisiacs, diuretics as a skin ointment hepatoprotectant, hepatotoxicity etc. By and large, all the extracts possessed antimicrobial properties with the MIC of the extracts.

Keywords – Antimicrobial, Bacterial species, Minimum inhibitory concentrations.

Introduction

For centuries plants have been used throughout the world as drugs and remedies for various diseases (UNESCO, 1996). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants (Bhavnani, S.M. and Ballow, 2000). One such resource is folk medicines and systematic screening of these may result in the discovery of novel effective compounds (Janovska, D., Kubikov, K. and Kokosk, L, 2003). The widespread use of herbal remedies and healthcare preparations such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times (Farombi EO, 2003). Over 50% of all modern clinical drugs are of natural product origin. (Stufulness M, Douros J., 1982) Natural products play an important role in drug development programs in the

pharmaceutical industry. (Baker JT, Borris RP, Carte B et al., 1995). There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom.

Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy PS, Jamil K, Madhusudhan P et al., 2001).

More and more researchers find that food and their individual constituents perform similar fashion to modern drugs and sometimes better without the dreaded side effects. The use of herbs and medicinal

plants as the first medicines is a universal phenomenon. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties (Wainright M., 2001) Considering the above aspects, an attempt has been made to carry out the screening for preliminary antibacterial activity of different plants used in Indian folk medicine.

Material and Methods

Preparation of plant (leaf and bark) extract -

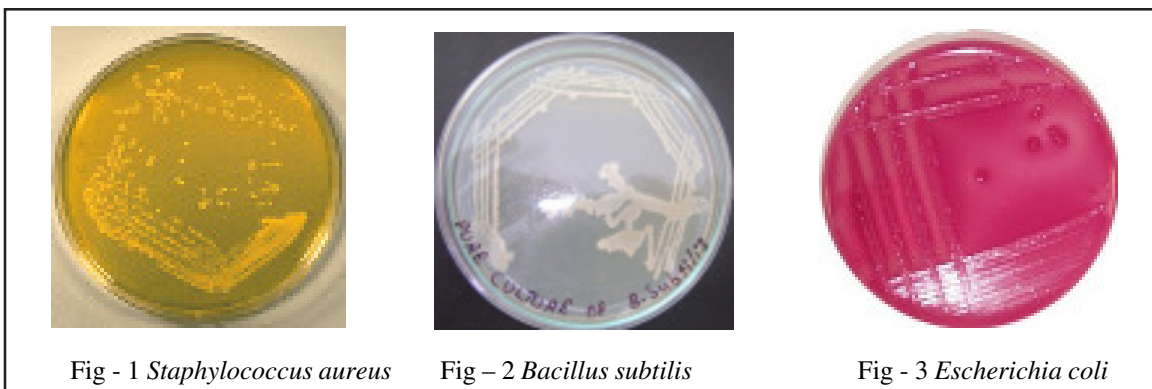
The plant parts to be used were washed with tap water then with distilled water and allowed for drying. It was further wiped with 70% alcohol and again dried. Grinded the plant parts with the help of pistle and mortar to form paste. 0.5 gm from each of it was taken and dissolved into 5 ml of methanol. These plant extracts were centrifuged at 10,000 rpm for 10 minutes. The supernatants were transferred into other tubes and the pellet was discarded. Stored into refrigerator at 4°C till use.

Preparation of innoculum - The microbial strains (*Bacillus subtilis*, *Staphylococcus aureus* and

Escherichia coli) used in this investigation was locally isolated from the clinical samples (Pus and Urine samples). Their identification and confirmation was performed on the basis of selective medium and biochemical tests. For use in experiments, the organisms were sub-cultured in their respective selective medium.

Preparation of Selective Medium - Dehydrated chemically defined media (Hi Media Laboratories Limited, Mumbai) will use in preparation of the culture media as per manufactures instructions. For *B. subtilis* - Bacillus Differentiate Agar Media, for *S. aureus* - Mannitol Salt Agar Media and for *E. coli* - Macconkey's Agar Media were taken in the flask, stirred well to dissolve. This medium was dispensed into culture flasks, autoclaved at 121°C at 15 lb pressure for 15 min. Then allowed to cool at room temperature and poured in petridish. After solidification the medium was streaked with samples collected. Then the plates were incubated at 35°C to 37°C for 24 - 48 hrs. The desired colonies were collected and preserve as pure culture in nutrient broth.

Pure Cultures of the Micro Organisms



Screening of antimicrobial activity of extract - The antimicrobial activity of the plant extracts against the selected microorganisms was evaluated by the Agar Disc Diffusion Method. The discs of Whatmann filter paper no.1 were cut in 5mm diameter. The stock solution 0.5 gm/5 ml of methanolic concentration was made by dissolving 0.5gm of sample in 5 ml of methanol. 10µl of this stock solution was poured on to the discs. Now the discs were sterilized in hot air oven for

1 hour each on three successive days. Discs were stored at room temperature till use.

Preparation of Muller Hinton Agar Media for Screening - Dehydrated chemically defined media (Hi Media Laboratories Limited, Mumbai) were taken in the flask, stirred well to dissolve. This medium was dispensed into culture flasks, autoclaved at 121°C at 15 lb pressure for 15 min. Then allowed to cool at room

temperature and poured in petridish. Petri dishes were marked separately for *Staphylococcus aureus*, *Bacillus subtilis* and *E. coli*. After solidification, a sterile cotton swab was dipped in the standardized bacterial suspension and was spread evenly on the surface of medium to inoculate it. Medium was allowed to dry for 5 min. Test antibiotic disc were placed with a positive control on the surface of the medium with the help of sterile forceps or mechanical dispenser to allow for

proper diffusion of the extract to take place. Then the discs were placed keeping a proper distance among discs to check the effects of these plant extracts. Petri dishes were incubated in between 35°C to 37°C for 24 hrs. At the end of the incubation period plates were carefully observed for antibiotic sensitivity of the microorganism and the zone of complete growth inhibition was measured around each antibiotic disc with the help of a calliper or transparent plastic ruler.

Zone of inhibition by some of the microorganisms

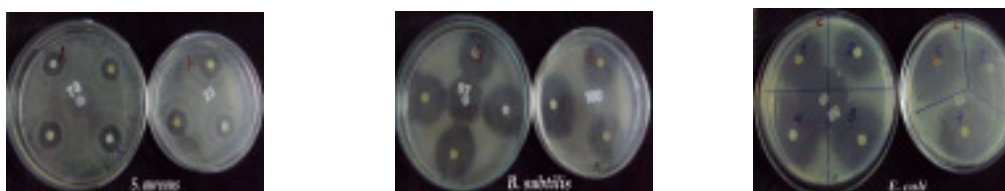


Table -1 Sensitivity pattern of bacterial strains to methanolic extracts

Plant Code	Plants used	Parts used	Zone of growth inhibition (in mm)		
			<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>
1.	<i>Acacia nilotica</i>	Leaf	7.5	15	16
2.	<i>Acacia nilotica</i>	Bark	8	12.5	15.5
3.	<i>Withania somnifera</i>	Leaf	8.5	13	17
4.	<i>Withania somnifera</i>	Bark	7.5	13	22
5.	<i>Ziziphus mauritiana</i>	Leaf	8.5	13.5	23
6.	<i>Ziziphus mauritiana</i>	Bark	9	18	20
7.	<i>Tinospora cordifolia</i>	Leaf	8	12.5	17.5

Table - 2 The MIC for the antibiotic used against the bacterial species

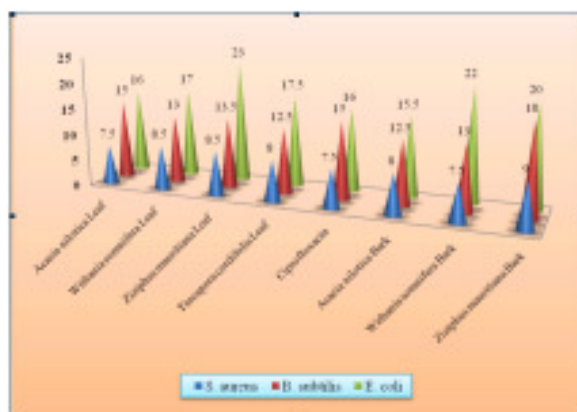
Antibiotic used	Bacterial strains used	Zone of growth inhibition (in mm)
Ciprofloxacin	<i>S. aureus</i>	7.5
Ciprofloxacin	<i>B. subtilis</i>	12
Ciprofloxacin	<i>E. coli</i>	15

Result and Discussion

In this study the results of the investigations show that the methanol extracts from the bark and leaf of *Acacia nilotica*, *Tinospora cordifolia*, *Withania somnifera* & *Ziziphus mauritiana* possess

antimicrobial activities against some of the tested organisms at a concentration of 0.5 mg/ml. The extracts were compared favourably with the standard antibiotic ciprofloxacin. The results indicated that the plant extracts has stronger activity than standard antibiotic

Graph: - Sensitivity pattern of bacterial strains to methanolic extracts



all plants showed promising activity against *Staphylococcus aureus*, *Bacillus subtilis* and *E. coli*. The tested plant extracts were most active against *E. coli* as compare to *Staphylococcus aureus* and *Bacillus subtilis*. The antibacterial activity may be

ciprofloxacin as shown in the table and snaps. Plant based products have been effectively proven for their utilization as a source for antimicrobial compounds. For instance, methanol extracts of *W. somnifera* was effective against *C. albicans* (Kambizi, L. and A.J. Afolayan, 2008). Out of the seven extracts tested for antibacterial activity all plants extracts showed antibacterial activity by inhibiting one or more microorganisms. The results of the antibacterial screening of the crude extracts of all species of plants are shown in the Table - 1. In this study all plants extracts are prepared in methanol. Methanol extracts exhibited a higher degree of antibacterial activity as compared with that of ethanolic and aqueous extracts (R. Nair, T. Kalariya, 2004). Among the plants screened, indicative of the presence of some metabolic toxins or broad-spectrum antibiotic compound. In this study methanol extracts of *Ziziphus mauritiana* produced the largest zone of inhibition against all the three bacterial strains.

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