

IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME SELECTED INDIAN PLANTS

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Abstract— Medicinal plant extracts prepared with various selected solvents from four species, *Glycyrrhiza glabra*, *Piper bittle*, *Azadirachta indica* and *Moringa olifera*, were screened for antimicrobial activity by using well diffusion method. Plant extracts showed strong antimicrobial action against microbes, among the plant extracts the extracts of *Piper bittle* shows maximum antimicrobial activity against all microbes. *Moriga* extract gave antimicrobial activity only against *Staphylococcus aureus* while all other strains were resistant to this extract. *Escherichia coli* was resistant to all extracts except *Piper* extract.

Key Words- Antibacterial, Inhibition, Medicinal Plants, Methanol, Ethanol, Acetone.

INTRODUCTION

Plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments (Grabley and Thiericke, 1999). From ancient times, plants are rich source of effective and safe medicines. In recent years there has been focus on plants with antimicrobial activity. There are many published reports on the effectiveness of traditional herbs against microorganisms and as a result, plants are still recognized as the bedrock for modern medicine to treat infectious diseases (Evans et al., 2002). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena, 1997; Nimri et al., 1999; Saxena and Sharma, 1999). Some foods contain naturally occurring substances showing antimicrobial activity. Some spices are known to contain cinnamic aldehyde, allicin in garlic and alliin in onion. These substances can be used for protection against microorganisms (Chang, 1995). It has been reported that the higher plants have shown to be a potential source for the new antimicrobial agents (Mitscher et al., 1987). The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used. Antimicrobials therefore, may have a significant clinical value in treatment of resistant microbial strains (Eloff, 1988). Besides, the antimicrobial activities, plant oils and extracts have formed the basis of many applications including raw and

processed food preservation, pharmaceuticals, alternative medicine, and natural therapies (Hammer et al., 1999). Frankel et al. (1996) and Mau et al. (2001) also reported that the use of herbal drugs increased instead of synthetic drugs. Although Dıgrak et al. (2001), Sarac and Ugur (2007), Poyrazoglu et al. (2009), Karatas and Ertekin (2010) and so on were investigated about antibacterial activity of Natural products can be selected for biological screening based on ethnomedical use of plants, because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is found in “Rigveda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing. Medicinal plants are a source of great economic value all over the world. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids etc. that are present in these plants (Esin Poyrazo lu Çoban and Halil Biyik., 2010).

Antimicrobials of plant origin have enormous therapeutic potential Human infections particularly those involving microorganisms i.e. bacteria, fungi, viruses, they cause serious infections in tropical and subtropical countries of the world (Akroum S, Bendjeddou D, Satta D, Lalaoui K ,2010). In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases (N. Boulououar, A. Marouf, A. Cheriti, and N. Belboukhari, 2012). In general, bacteria have the genetic ability to transmit and acquire resistance for drugs, which are utilized as therapeutic agents.

Over the past twenty years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from traditional medicinal plants have been tested. Many reports have shown the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine

to attain new principles. The increasing interest on traditional ethno medicine may lead to discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals, nutraceuticals, cosmetics and food supplements. In this regard, plants have given western pharmacopoeia about 7000 different pharmaceutically important compounds and a number of top-selling drugs of modern time, e.g. quinine, artemisinin, taxol, camptothecin, etc.. Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens (Vento S & Cainelli F, The need for new Antibiotics, 2010)

MATERIALS AND METHODS

Experimental Section

All the chemicals and reagents used were laboratory grade. Glass wares used were from borosil. The solid media and broth used for microbial culture were from Hi-Media Pvt. Limited, Bombay, India. Except *Glycyrrhiza* roots all plants were obtained from biotech park, Lucknow. *Glycyrrhiza* roots were purchased from the local market.

Microorganism strains-Two strains of gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and one strain of gram negative bacteria (*Escherichia coli*). Three strains of fungi namely *Alternaria*, *Aspergillus* and *Penicillium* (All microorganisms obtained from Biotech Park, Lucknow) were used.

Preparation of Extracts

Fresh plants were collected from Biotech park and outside sources. Leaves of *Moringa oliefera*, *Azadirachta indica*, *Piper betle* and root of *Glycyrrhiza glabra* were dried in tray dryer at 45°C for 2 days. The dried leaves were then powdered by grinder and stored in air tight bags till extraction. Dried powdered material extracted using acetone and ethanol as solvents by using polytron homogenizer. 25 g of powder was dissolved in 150 ml of solvent and left for overnight. Next day extraction was performed by using polytron homogenizer. Solvents were removed under high pressure using rotatory evaporator. The dried crude extracts were dissolved in DMSO and stored at 4°C.

Well Diffusion Method

The antimicrobial activity of the extracts was determined by well diffusion method (NCCLS, 1997) in petri plates containing Nutrient Agar (NA) and Potato Dextrose Agar (PDA) medium (20 mL media/plate), respectively. The wells

(6 mm in diameter) were separately filled with plant extracts which had previously been inoculated with the selected test microorganisms. Ampicillin was used as a positive reference for bacteria while Gentamicin for fungi. Wells without samples were used as a negative control. Plates were kept at 4°C for 1h. The plates were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for fungal strains. Antimicrobial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters (including diameter of well) the test organisms comparing to the controls.

Determination of Antibacterial Activity

The extracts were individually tested against a panel of microorganisms selected. Bacterial strains were cultured overnight at 37°C in nutrient agar (NA). Wells of appropriate size were made in solid agar media already inoculated with bacterial strains. Different concentrations of extracts were injected into these wells and then kept in incubator for appropriate time. The diameters of zone of inhibition observed were measured.

Determination of Antifungal Activity

The well diffusion method (NCCLS, 1997) was modified. Potato dextrose agar (PDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in 0.1% saline. The wells (6 mm in diameter) were filled with different concentration of plant extracts which had previously been inoculated with the selected test microorganism. Fungal plates were incubated at 37°C for 72 h. The diameters of zone of inhibition observed were measured.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of microbes was determined using well diffusion method (James H. Jorgensen¹ and Mary Jane Ferraro, 2009). Four wells were made on each plate with help of 6 mm diameter cork borer. Dimethylsulfoxide (DMSO) was used as negative control in one well while in other three wells different conc. of plant extracts were added. One plate in which only media was present is used as negative control and one plate with spore and media is used as positive control.

RESULTS

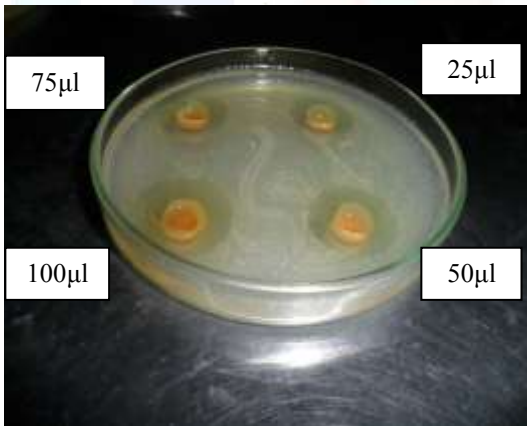
Antimicrobial Activity

The antimicrobial activity was determined by measuring the diameter of zone of inhibition recorded. The results obtained in the evaluation of the antimicrobial activity of the different plant extracts against selected microbes are listed in the table-1

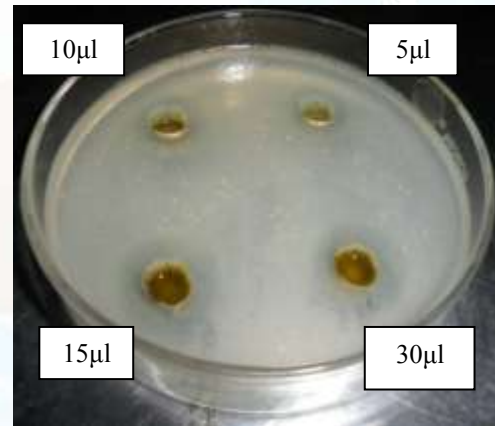
Table1- Antimicrobial activity of different plant extracts against selected microbes

| SI no. | Plant extracts name Test Organism/s | Inhibition zone diameter in mm | | | | | | |
|--------|--|--------------------------------|------------------------|------------------------------|------------------------------|---------------------------|------------------------------|------------------------------|
| | | <i>Piper</i> (Acetone) | <i>Piper</i> (Ethanol) | <i>Glycyrrhiza</i> (Acetone) | <i>Glycyrrhiza</i> (Ethanol) | <i>Moringa</i> (Methanol) | <i>Azadirachta</i> (Ethanol) | <i>Azadirachta</i> (Acetone) |
| 1 | <i>S.aureus</i> | 15mm | 12mm | 15 mm | 13mm | 8mm | 11mm | 12mm |
| 2 | <i>E.coli</i> | 1mm | 8mm | 0 | 0 | 0 | 0 | 0 |
| 3 | <i>B.subtilis</i> | 13mm | 13mm | 12mm | 15mm | 0 | 14mm | 15mm |
| 4 | <i>A.niger</i> | 18mm | 16mm | 0 | 15mm | 0 | 15mm | 0 |
| 5 | <i>Altermeria</i> | 15mm | 15mm | 18mm | 15mm | 0 | 0 | 0 |
| 6 | <i>Penicillium</i> | 16mm | 17mm | 18mm | 15mm | 0 | 0 | 0 |

Bacillus subtilis

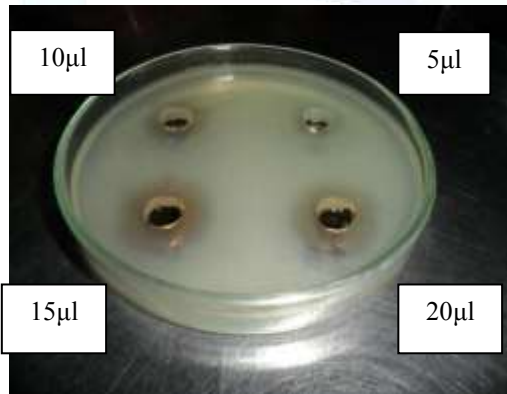


Glycyrrhiza (Acetone)

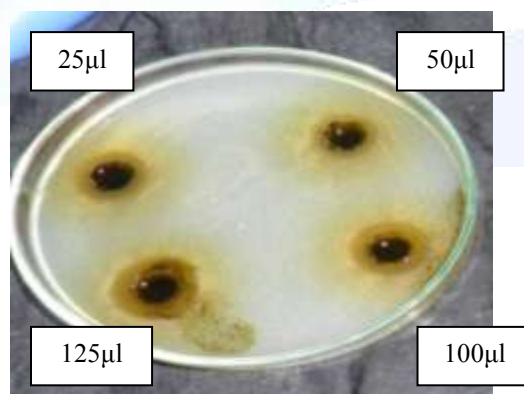


Azadirachta (Acetone)

S. aureus



Piper (Acetone)



Moringa (Methanol)

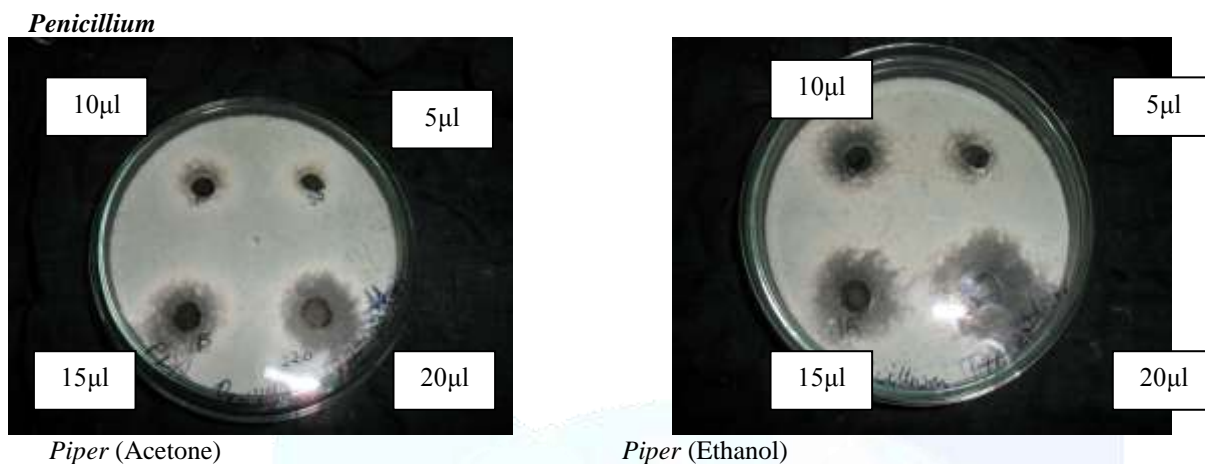


Figure 1: Pictures showing antimicrobial activity of plant extracts against different microbes

CONCLUSION

In this study the emphasis were on *S.aureus*, *E.coli*, *B.subtilis*, *A.niger*, *Penicillium*, *Alternaria*. According to the antibacterial assay *S. aureus* was the most susceptible bacteria to all the plant extracts, while *E.coli* was the most resistant of all the bacteria. There observations are likely to be the result of the differences in cell wall structure between gram +ve and gram -ve bacteria, with the gram -ve bacteria's outer membrane acts as a barrier to many environmental substances including antibiotics.

According to the anti fungal assay *A.niger* was the most susceptible for all the plant extracts while *Alternaria* and *Penicillium* were resistant. Our experiments concludes that piper extracts exhibiting the lowest MIC values piper shows activity against all selected microbes.

Thus it can be concluded from the above studies that piper has the maximum antibacterial activity. The noncytotoxic concentrations of plant extracts were used for antimicrobial activity tests Our findings suggests that the antimicrobial activity is not due to the cytotoxic activity of extracts. Antimicrobial activity was performed to different extents by the extracts of plants- *Piper*, *Glycyrrhiza*, *Azadirachta* and *Moringa*.

Many medicinal plants have been found effective in the cure of bacterial diseases. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics medicinal plants are now gaining popularity in the treatment of bacterial infections. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics. Extensive research in the area of isolation and characterization of the active principles of these plants are required so that better, safer and cost effective drugs for treating bacterial infections can be developed.

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