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Research Article

Antimicrobial Activity of some Medicinal Plants against Human Pathogenic Bacteria

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Abstract

Medicinal plants are found to deliver a promising health benefits probably due to the secondary metabolites which are responsible for various activities of such plants. Antimicrobial activities of some important Indian medicinal plants are analyzed in present study using methanol, ethanol and acetone extracts of each plant. Methanol extract along with ethanol extract were found equally potent against human pathogenic bacteria by using agar well diffusion method and measuring the inhibition zone of activity ranging from 5mm – 20 mm diameter. Emblica officinalis showed maximum zone of inhibition (29 mm) against Pseudomonas aeruginosa, methanol extracts of Tabernaemontana undulata and Phyllanthous amarus have highest antimicrobial activity on B. subtilis (22 mm) and Pseudomonas aeruginosa (27 mm). ethanol extracts of Emblica officinalis on four different human pathogenic organisms using agar well diffusion method have showed maximum zone of inhibition (31 mm) against Pseudomonas aeruginosa.

Keywords: *Medicinal plants, antimicrobial activity, agar well diffusion.*

Introduction

Since the time unmemorable, plants have been a valuable source of natural products for maintaining humans. But gradually, the use of phytochemicals for pharmaceutical purpose has been increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Showkat Ahmad, 2012). The use of crude extracts of plants parts and available phytochemicals of known antimicrobial properties can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries aiming at antimicrobial traits (due to secondary metabolites) in some medicinal plants to prove such efficiency. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Farzana *et al*., 2014). This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants.

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However, very little information is available on such activity of medicinal plants and out of the 4 lakh plant species on earth, only a small number has been systematically investigated for their antimicrobial activities (Verma and Ramteke 2009). Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity. They have also tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal activities (Doughari and Obidah,2008). Some plants such as, Ocimum gratissimum, *Eugenia uniflora* have been reported to be rich in volatile oils which contain up to 75% thymol having an antimicrobial effect and is mainly used in the treatment of diarrhea and ear infection in human beings, besides it also has antimicrobial properties against *Staphylococcus* sp., *Escherichia coli,* and *Shigella* sp (Fadeyi, and Alcapan,1989). A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases as urinary tract infections, gastrointestinal disorders, respiratory disease, and cutaneous infections (Sarita *et al*., 2019).

Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The primary benefits of using plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic

benefits and more affordable treatment. Hence keeping the all above in mind the present study has been planned to evaluate antimicrobial activities of Indian medicinal plant extracts on human pathogenic bacteria.

Materials and Methods

Collection of plants samples

A total of nine plants have been used in the present studied and they were collected from the field. They are enlisted with their common names in Table 1. As the bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season climate and particularly by the growth phase. Leaf is one of the highest accumulated plant part of such compounds and people are generally preferred it for therapeutic, purposes some of the active compounds inhibit the growth of disease causing microbes either single or in combination (Hassawi and Kharma 2006).

Table 1. Medicinal plants used along with common names

S.No.	Scientific name	Common name
1	*Aegle marmelous*	Baelpathar
2	*Tinospora CordifoliaFam*	Giloe
3	*Azardirchata–indica*	Neem
4	*Ocimum sanctum*	Tulsi
5	*Mentha pipertia*	Pippermint
6	*Tabernaemontana undulata*	Chandni
7	*Emblica officinalis*	Amla
8	*Vincearosea/ catharanthus Roseus*	Sadabahar
9	*Phyllanthous amarus*	Bhumiamla

Preparation of plants extracts

The leaves of all the plants were used to prepare different extracts for the present study. The plants were collected and washed under running tap water to remove soil and dust particles followed by rinsing with 70 % ethanol. They were then dried to lose their moisture for 3-4 days at 40^0C and then blended to form a fine powder and stored in airtight vial.

The methanol extract was prepared by dissolving 2gm of powdered plant material in 20 ml of ethanol. The contents were kept under shaking condition for 48 hrs. Then the extract was filtered via Whattman filter paper no. 1 and then the extract was stored for further studies. The ethanol and acetone extracts were prepared in the same manner as like that of methanol extract.

Collection of microbial culture

Four bacterial cultures were used for the study undertaken. The culture names are as follows along with their MTCC number.

- *Staphylococcus aureus* (MTCC 96)
- *Bacillus subtilis* (MTCC 121)
- *Escherichia coli* (MTCC 1625)
- *Pseudomonas aerginosa* (MTCC 741)

The microbial strains were revived in nutrient broth (Hi-media) for obtaining actively dividing bacterial population. The plating was achieved by pour plate method.

Assay of Antimicrobial activity using Agar well diffusion method

The bacterial suspension (1 ml) was added to sterile petri plate followed by addition of 20 ml of sterilized nutrient Agar media. The media and suspension were then gently mixed for uniform bacterial growth. The plates were then kept undisturbed for about 20 minutes for solidification. After solidification, plates were incubated for 48 hours at 37ºC. Three wells were punctured in each agar plates using sterile gel borer to study antimicrobial activity of each of methanol, acetone and ethanol extract on bacterial strain in same plate. After puncturing wells, poured 50µl of different plant extracts in each of three well. Now plates were allowed to stand on the bench for 1 hr for proper diffusion and thereafter incubated at 37^oC for 24 h. The resulting inhibition zones were measured in millimeters (mm).

Results and Discussion

The antimicrobial activity of ethanol methanol and acetone extracts of nine Indian medicinal plants were investigated using agar well diffusion method against selected human pathogens named *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. The medicinal plant extracts used against the pathogenic organisms showed varied degree of antimicrobial activity against the pathogens.

Antimicrobial activity of methanol extract

The antimicrobial activity of methanol extract using agar well diffusion method, *Emblica officinalis* showed maximum zone of inhibition (29 mm) (fig.1) against *Pseudomonas aeruginosa* followed by *B.cerues* (23 mm), and *E. coli* (12 mm) (Table 2). Similarly, the methanol extracts of *Tabernaemontana undulata* and *Phyllanthous amarus* have highest antimicrobial activity on *B. subtilis* (22 mm) and *Pseudomonas aeruginosa* (27 mm) respectively (Table 2). Whereas the *Azardirchata-indica* and *Mentha pipertia* have showed the minimum activity (1mm) against *Pseudomonas aeruginosa (*fig.2*)* and *B.subtilis* respectively. The overall observation indicated that the methanolic extracts of *Ocimum sanctum, Azardirchata – indica* and *Mentha pipertia* have lesser impact on the four different human pathogens when compared to other three species (*Phyllanthous amarus*, *Tabernaemontana undulata, and*

Emblica officinalis) of medicinal plants. The methanol extracts of *Phyllanthous amarus* showed maximum zone of inhibition (24 mm) against *E.coli* followed by *Pseudomonas aeruginosa* (27mm) (fig.1) and Staphylococcus *aureus* (16 mm). The extract of *Catharanthus roseus* showed minimum zone of inhibition against *E. coli* (6 mm), moreover the collective analysis indicated that the extract of *Mentha pipertia, Ocimum sanctum, Azardirchata-indica* and have lesser rate of antimicrobial activity when compared to other species of plants used in the study (Table 2).

Table 2 Antimicrobial activity of methanol extract of medicinal plants against human pathogenic bacteria using agar well diffusion method

Name of medicinal plants	Microbial Strain			
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*
Aeglemarmelous	*+++*	*+++*	*+++*	*Not Found*
TinosporaCordifoliaFam	*++*	*++*	*++*	*++*
Azardirchata - indica	*Not Found*	*+*	*+*	*+*
Ocimum sanctum	*+++*	*Not Found*	*Not Found*	*Not Found*
Menthapipertia	*+++*	*Not Found*	*+*	*+*
Tabernaemontanaundulata	*++*	*+++*	*+++++*	*++*
Emblicaofficinalis	*+++++*	*Not Found*	*++++*	*+++*
Vincearosea/ catharanthus Roseus	*+++*	*++*	*++*	*++*
Phyllanthousamarus	*+++++*	*++++*	*Not Found*	*++*

+(less than 5 mm), ++ (5-10 mm), +++ (10-15 mm), ++++ (15-20 mm), and +++++ (higher than 20 mm) are zone diameter respectively

Table 3 Antimicrobial activity of ethanol extract of medicinal plants against human pathogenic bacteria using agar well diffusion method

Name of medicinal plants	Microbial Strain			
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*
Aeglem armelous	*++*	*+++*	*++++*	*Not Found*
Tinospora CordifoliaFam	*Not Found*	*Not Found*	*Not Found*	*Not Found*
Azardirchata - indica	*+++*	*+*	*+*	*+*
Ocimum sanctum	*+++*	*Not Found*	*Not Found*	*Not Found*
Mentha pipertia	*+++*	*+*	*+*	*+*
Tabernaemontan aundulata	*++*	*+++*	*+++*	*++*
Emblica officinalis	*+++++*	*++*	*+++*	*Not Found*
Vincearosea/ catharanthus Roseus	*+++*	*+++*	*++++*	*++*
Phyllanthous amarus	*+++*	*+++*	*Not Found*	*+++*

+(less than 5 mm),++(5-10 mm),+++(10-15 mm),++++(15-20 mm), and +++++(higher than 20 mm)are zone diameter respectively

Antimicrobial activity of ethanol extracts

The antimicrobial activity of ethanol extracts of *Emblica officinalis* on four different human pathogenic organisms using agar well diffusion method have showed maximum zone of inhibition (31 mm) against *Pseudomonas aeruginosa* (Fig.1) followed by *Staphylococcus aureus* (18 mm), and *E. coli* (25 mm) (Table 3). The collective analysis of antimicrobial activity of ethanol extract indicated that among the nine medicinal plants used in the study *Phyllanthus amarus* and *Emblica officinalis* have better impact ranged from 9 to 23 mm on all the four species of pathogenic bacteria when compared to rest of the plant species Whereas, in case of agar well diffusion method, the ethanol extract *Phyllanthous amarus* of showed the maximum zone of inhibition (14 mm) (Table 3) against *E. coli* followed by *Staphylococcus aureus* (15 mm) (Table 3) with the extract of *Vincearosea/cathranthas roses*. The extract of *Azadiarachta-indoica* showed the minimum zone of inhibition (1mm) (Table 3) against *E.coli* while the collective analysis indicated similar trend of impact obtained in case of well diffusion method. In some plant extract no antimicrobial activity was found.

Antimicrobial activity of acetone extracts

The observation antimicrobial activity, acetone extracts of medicinal plants on human pathogenic species using agar well diffusion method showed that the extracts of *Emblica officinalis , Phyllanthous amarus* have more impact on *Pseudomonas aeruginosa* (31mm) (Fig.1) and *Tabernaemontana undulata*on *B. subtilis* (18 mm) (Table 4). The acetone extract of *Azardirchataindica* have showed minimum zone of inhibition on *E.coli* (10 mm). The overall observation of antimicrobial activity of acetone extract of nine medicinal plants (using agar well diffusion method) indicated that the *Emblica officinalis, Phyllanthous amarus and Aegle marmelous* have more impact than the remaining four species of plants.

Table 4 Antimicrobial activity of acetone extract of medicinal plants against human pathogenic bacteria using agar well diffusion method

Name of medicinal plants	Microbial Strain			
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*
Aegle marmelous	+++	+++	++++	*Not Found*
Tinospora CordifoliaFam	*Not Found*	*Not Found*	*Not Found*	*Not Found*
Azardirchata - indica	*Not Found*	+	*Not Found*	+
Ocimum sanctum	++	*Not Found*	*Not Found*	*Not Found*
Mentha pipertia	+++	+	+	+
Tabernaemontana undulate	++	++	++++	++
Emblica officinalis	+++++	*Not Found*	++	++++
Vincearosea/ catharanthus Roseus	*Not Found*	*Not Found*	*Not Found*	*Not Found*
Phyllanthous amarus	+++	+++	*Not Found*	+++++

+(less than 5 mm),++(5-10 mm),+++(10-15 mm),++++(15-20 mm), and +++++(higher than 20 mm)are zone diameter respectively

Fig. 1 Antimicrobial activity (zone of inhibition) of methanol (29mm), ethano (29mm) and acetone (31mm) extracts of *Emblica officinalis* against *Pseudomonas aerginosa*.

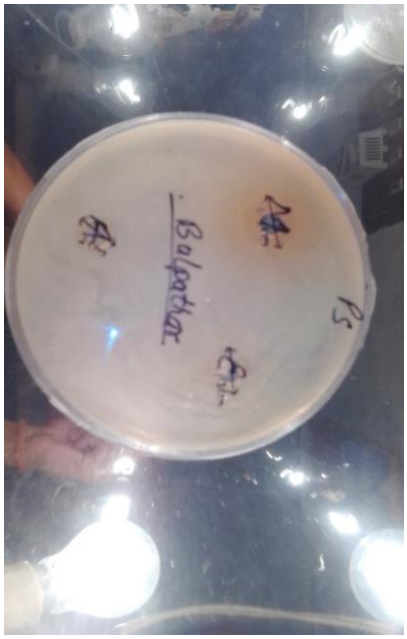

Fig. 2 Antimicrobial activity (zone of inhibition) of methanol (14mm), ethanol (10mm) and acetone (13mm) extracts of *Aeglemarmelous* against *Pseudomonas aerginosa*.

Infectious diseases are the major cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. Such increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infections. This situation provided the impetus to the search for new antimicrobial substances from various source like medicinal plants.

The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment.

In this present study, preliminary screening for antimicrobial activity showed, that the methanol extract of *Emblica officinalis* exhibited maximum inhibitory zone (29mm) (Table 2) against Pseudomonas sp. While the ethanol and aqueous extracts of *Tinospora CordifoliaFam*, *Azardirchata indica*, *Ocimum sanctum* and *Mentha pipertia* showed least inhibitory activity. The antimicrobial assay by agar-well diffusion method revealed that methanol extract of medicinal plants exhibited broad spectrum activity against tested isolates as compared to ethanol and acetone extracts.

Conclusion

It may be concluded from this study that medicinal plants have antimicrobial activity against pathogens. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. This can explain the rationale for the use of the plant in treating infections in traditional medicine. The medicinal plants could be a veritable and cheaper substitute for conventional drugs since many of the plants are easily obtainable and the extract can easily be made via a process of maceration or infusion. It is essential that research should continue to isolate

and purify the active components of this natural herb and use in experimental animals.

In vitro propagation of medicinal plants with enriched bioactive principles and cell culture methodologies for selective metabolite production is found to be highly useful for commercial production of medicinally important compounds. Advances in plant cell cultures could provide new means for the cost-effective, commercial production of even rare or exotic plants, their cells, and the chemicals that they will produce. Knowledge of the biosynthetic pathways of desired compounds in plants as well as of cultures is often still rudimentary, and strategies are consequently needed to develop information based on a cellular and molecular level.

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