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ACTIVITY OF PLANT EXTRACTS ON MULTI-DRUG RESISTANT HUMAN PATHOGENS

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ABSTRACT

The present study was conducted with a view to evaluate the therapeutic potentials of seven medicinal plant extracts against multi-drug resistant human pathogenic bacteria– Escherichia coli and Staphylococcus aureus by agar well diffusion method. Ethanolic extracts of Catharanthus roseus, Syzygium cumini, Ocimum sanctum, Asparagus racemosus, Acmella oleracea, Aloe vera and Punica granatum showed broad-spectrum antibacterial activity with inhibition zones ranging from 5 to 20mm. These medicinal plants contribute in human health care system. They are recommended in Ayurvedic texts for prevention and treatment of various diseases. They are also used successfully for the treatment of certain infectious diseases.

The outcome demonstrates antimicrobial potential of the plants and hence lends support for the use of them in traditional medicine in the treatment of bacterial infections. The results of this study were encouraging, despite the need for clinical studies to determine the real effectiveness and potential toxic effects in vivo.

Key words: Antimicrobial activity, plant extract, human pathogens, multi-drug resistant, Agar well diffusion method.

Introduction

Since the beginning of human civilization, medicinal plants have been used by mankind for their therapeutic values. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based traditional medicine system continues to play an essential role in health care, with about 80% of the world inhabitants relying mainly on traditional medicines for their primary health care. Prevention, protection and treatment of various diseases are the three major areas of concern in today's scenario. The microbial infection is one among them due to the production of multi-resistant bacteria in the clinical trials. Scientists are also focusing to treat such diseases without causing any side effects in the human body.

Plants are the largest drug stores ever known on Earth, by producing endless bioactive chemical compounds which have direct effects on animal and human health (Abdallah, 2011). Today, most of the modern drugs (synthetic or semi-synthetic) are initially produced from natural products such as medicinal plants prescribed in the ancient traditional medicine (Sukanya *et al.*, 2009). Atropine, Ephedrine, Digoxin, Morphine, Quinine, Reserpine and Tubocurarine are few examples of medicines invented from the knowledges of the traditional medicine (Gilani and Atta-ur-Rahman, 2005). Traditional medicine is still used as the primary health care system for up to 80 % of the world population. Most of them are in the developing countries. This popularity of traditional medicine is due to the better cultural acceptability, compatibility and lesser side effects (Kamboj, 2000). Recently, the interest in medicinal plants is growing, since many plant species have been recognized to have medicinal benefits and positive impact on human health, such as anti-inflammatory, antibacterial, hypolidemic, anticarcinogenic, anti-oxidant and many other (Cai *et al.*, 2004). Infectious diseases remain among the leading causes of death; more than 25% of annual deaths worldwide are related to infectious diseases (Morens *et al.*, 2004). Due to the failure of modern antibiotics to

overcome these infectious diseases, attention has been drawn to medicinal plants. Numerous plants have been reported worldwide having antimicrobial activities, most of these activities could be attributed to the bioactive phytochemical ingredients of these plants (Abdallah *et al.*, 2012). Accordingly, screening for antimicrobial properties in order to extract the curative compounds from these plants is of crucial importance.

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. One of the vital activities possessed by these medicinal plants is antimicrobial. The scarcity of infective diseases in plants is in itself an indication of the successful defense mechanisms developed by them. The substances that can either inhibit the growth of bacteria or kill them, with no toxicity or minimum toxicity to host cells are considered candidates for developing new antimicrobial drugs. Some of the bioactive compounds could hinder the life processes of disease-causing bacteria, either by itself or in combination with other therapeutic agents. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants. There have been numerous documentations found in the medical literature concerning the significance of traditional medicinal plants as alternatives to synthetic antibacterial medications. Most of these published works came from many countries that are still practicing the use of herbal medicine for the treatment of various diseases for practical and economic reasons. These studies are valuable resources for local medical scientists who seek to explore and substantiate the antibacterial activities against MDR bacteria. Knowledge on the different antimicrobial assays and the plants' bioactive compounds are vital for the design of future studies.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by micro-organisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant.

Consequently, new infections can occur in hospitals resulting in high mortality. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotics, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. 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. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few decades, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannin.

Medicinal plants

Plants are the largest drug stores ever known on Earth. Today, most of the modern drugs (synthetic or semi-synthetic) are initially produced from natural products such as medicinal plants prescribed in the ancient traditional medicine (Sukanya *et al.*, 2009). Infectious diseases remain among the leading causes of death. More than 25% of annual deaths worldwide are related to infectious diseases (Morens *et al.*, 2004). Due to the failure of modern antibiotics to overcome these infectious diseases, attention has been drawn to medicinal plants. Accordingly, screening for antimicrobial properties in order to extract the curative compounds from these plants is of crucial importance. There has been renewed interest in screening high medicinal plants for novel biologically active compounds. There is a great demand of fruit juices in treatment of various illnesses such as arthritis, heart

diseases and muscle aches and drug addiction (Tedesco et al., 2001). Extraction of bioactive molecules from medicinal plants facilitates pharmacological studies leading to a synthesis of a more potent drug with a reduced toxicity (Beuchat *et al.*, 1994, Das *et al.*, 1999). Plant based extracts can be extracted from any part of plant like barks, leaves, fruits, seeds and fruit rinds (Parekh J and Chanda, 2007). Medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity (Pandey and Chowdhry, 2006). The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments.

Following are the medicinal plants whose effects were studied against some clinically isolated bacteria:

- Catharanthus roseus
- Syzygium cumini
- Ocimum sanctum
- Asparagus racemosus
- Acmella oleracea
- Aloe vera
- Punica granatum

Material and Methods

Plant Samples

Plants were collected from different sites of Suddhowala Dehradun (Uttarakhand, India) during March 2016. Voucher specimens were deposited in the respective department of botany for proper identificationand were authenticated by a taxonomist. The plant materials used in this study consisted of*Catharanthus roseus,Syzygium cumini, Ocimum sanctum, Asparagus racemosus, Acmella oleracea, Aloe vera, Punica granatum*. The parts which were used are: Leaves, stems, flowers, roots, young branches, and also fruits.

Table: List of medicinal plants used to evaluate antimicrobial activity

Botanical name	Englishname	Family	Plant part(s)	Collection time	Origin
Catharanthus roseus	Madagascar periwinkle	Apocynaceae	LF, FL, YB	10/03/2016	Botanical garden
Syzygium cumini	Jamun	Myrtaceae	LF, FR	10/03/2016	Sal forest
Ocimum sanctum	Holy basil	Lamiaceae	LF, YB	10/03/2016	Suddhowala locality
Asparagus racemosus	Satavari	Asparagaceae	LF, YB, FR	10/03/2016	Campus
Acmella oleracea	Electric daisy	Asteraceae	LF, FL, S	14/03/2016	Sal forest
Aloe vera	Aloe	Asphodelaceae	LF, FL	14/03/2016	Campus
Punicagranatum	Pomegranate	Punicaceae	LF, FL, RF	14/03/2016	Local orchard

(LF: leaves, FL: flowers, FR: fruits, RF: raw fruits, RT: roots, S: stems, YB: young branches, NI: not informed)

Bacteria:

Human clinical bacterial isolates of presumptive *Staphylococcus aureus* and *Escherichia coli* were collected during March 2016 from Synergy Hospital, Dehradun, India and aseptically transferred to Department of Botany Laboratory, Baba Farid Institute of Technology (BFIT) for further studies. After collection, all the isolates were labeled, sub cultured and placed in an incubator for further use.

Culture media and Chemicals

Types of media were required for carrying out this study, Nutrient broth, Agar. Also ethanol and distilled water was used for extraction process.

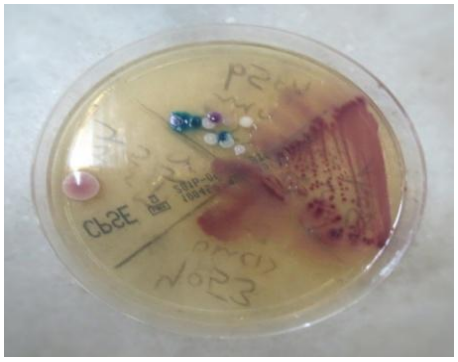

Fig.: Cultured*Escherichia*

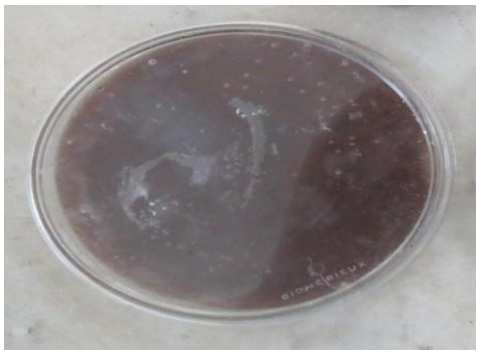

Fig.: Cultured Staphylococcus aureus

The following steps were taken to determine the activity of plant extracts against multi-drug resistant pathogens:-

- Preparation of plant extracts
- Preparation of the nutrient agar medium and plating
- Agar well diffusion method
- Antimicrobial activity of plant extracts to clinical isolates

Preparation of plant extracts

A total of seven plant extracts were used in this study to screen their antibacterial activity**.** The fresh parts of plants such as young leaves, root, flower, rhizome or petiole were collected and washed with distilled water. The plant parts were dried and then powder was made from them using a blender. 1.5 grams of each of the sample have been taken in a beaker with 3.5 ml of 96% ethyl alcohol. It means, ratio of plant parts and alcohol is 1:3 (w/v). Then each of the individual beakers has been kept for 60 hr at normal room temperature. After 48 hrs, the extracts have been filtered and stored in sterile volumetric flasks with sample numbers and preserved in refrigerator for further use.

Preparation of the nutrient agar medium and plating

500 ml of the nutrient agar medium was prepared. A nutrient agar medium 500 ml is prepared from nutrient broth (6.5 gram) and agar powder (12.5 grams) by dissolving them in distilled water and making the volume upto 500 ml. The nutrient agar medium after dissolving properly was transferred into an Erlenmeyer flask and autoclaved for 15 minutes. After autoclaving, the nutrient agar medium was plated onto the petri-plates carefully. The petri plates were kept as such for few minutes till the medium began to solidify for further use.

Agar well diffusion method

Agar well-diffusion method was done to determine the anti-microbial activity. Nutrient agar medium plates were swabbed with broth culture of respective bacteria (spreading). Wells (10 mm diameter and about 2 cm apart) were made in each of

these plates using sterile cork borer. Five different dilutions (0.25%, 0.50%, 0.75%, 1.0% and a control) were made from each of the plant extract and then placed in test tubes. About 0.3ml of each dilution of the plant solvent extract was added into each well using a sterile syringe and allowed to diffuse at room temperature for 2hrs. The control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37^0c for 18-24 hrs for bacterial pathogens. The diameter of the inhibition zone (mm) was measured. Duplicates were maintained and the experiment was repeated.

Figure: Creating wells using a cork borer

Antimicrobial activity of plant extracts to clinical isolates

The antimicrobial potential of all the experimental plants was evaluated according to their zone of inhibition against various pathogens. The results revealed that all the extracts are potent antimicrobials against all the microorganisms studied. The diameter of the zone of inhibition was measured by measuring scale.

Results and Discussions

Emergence of multi-drug resistance in human pathogenic bacteria as well as

Figure: Plating of the nutrient-agar medium

undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. The study was conducted with a view to investigate the antimicrobial properties of

some medicinal plant extracts against some human clinical bacterial isolates (*Escherichia coli* and *Staphylococcus aureus*)**.** Among the seven samples tested in the present study, seven crude plants extracts namely *Catharanthus roseus, Syzygium cumini, Ocimum sanctum, Asparagus racemosus, Acmella oleracea, Aloe vera, Punica granatum* were found to exhibit potential antimicrobial properties against the isolated human clinical bacterial isolates.

In the present investigation, the inhibitory effects of ethanol extracts of in vivo leaves from the above mentioned seven plants were evaluated against bacterial strains. These plants are known to have healing properties and are used for treatment of various diseases in people. The antimicrobial activity was determined using agar well diffusion method. The activity was quantitatively assessed on the basis of inhibition zone.

Measurement of antimicrobial activity using Agar well diffusion method

The antimicrobial potential of all the experimental plants was evaluated according to their zone of inhibition against various pathogens. The results revealed that all the extracts are potent antimicrobials against all the microorganisms (*Escherichia coli* and *Staphylococcus aureus*) studied. The antibacterial activity of the extracts and their potency was assessed by the presence or absence of inhibition zone as shown in charts below.

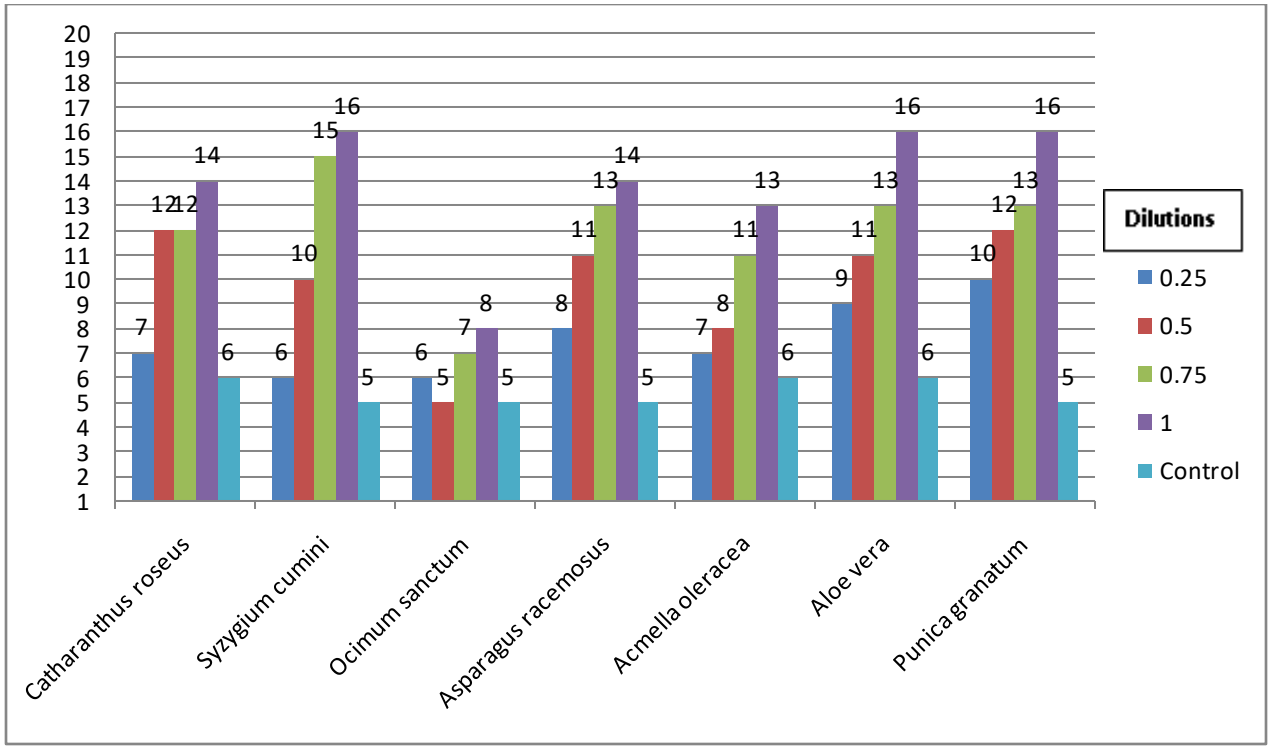

Figure: Chart showing the antibacterial activity of seven potent medicinal plant extracts against *Escherichiacoli* (Reading 1)

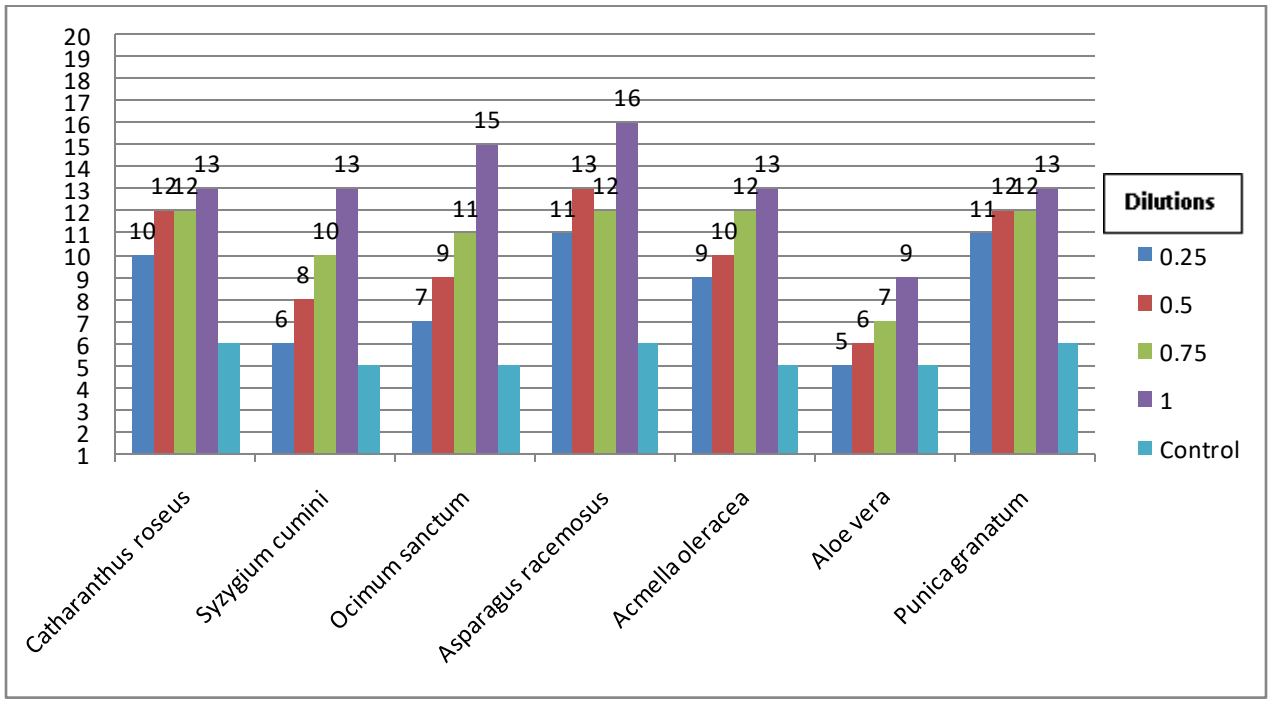

Figure: Chart showing the antibacterial activity of seven potent medicinal plant extracts against *Staphylococcusaureus*(Reading 1)

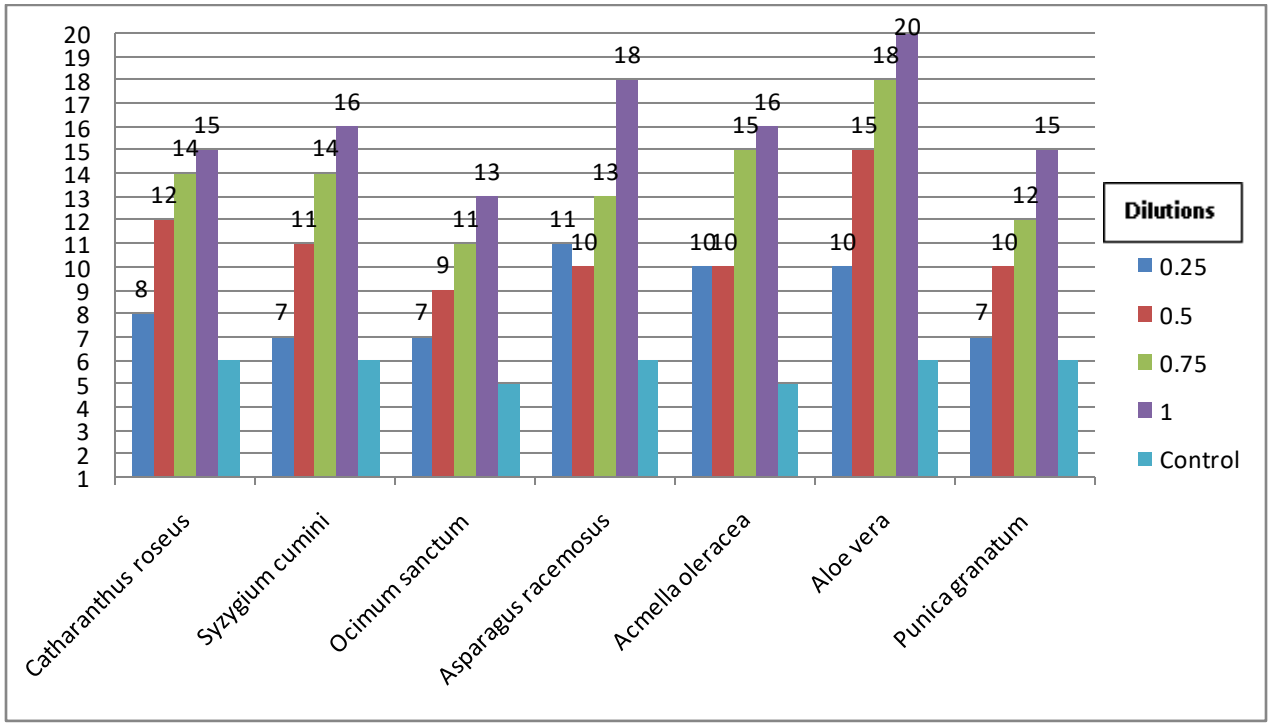

Figure: Chart showing the antibacterial activity of seven potent medicinal plant extracts against *Escherichiacoli* (Reading 2)

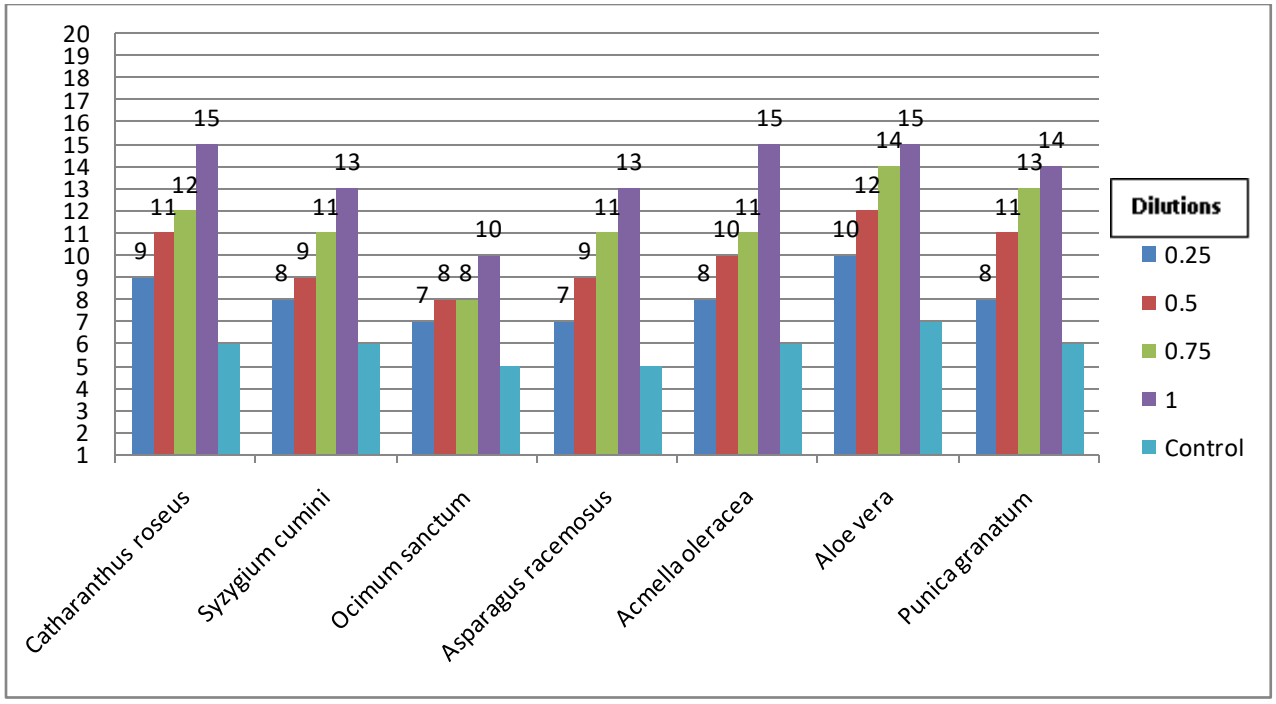

Figure: Chart showing the antibacterial activity of seven potent medicinal plant extracts against *Staphylococcusaureus*(Reading 2)

From the above results, it is clear that the leaf extracts exhibit antibacterial activity against both gram-positive as well as gram-negative bacteria. And also it is evident that the inhibition was concentration dependent.In ethanol extract, maximum inhibition zone diameter was obtained by *Punica granatum*(Reading 1) and *Aloe vera* in *Escherichia coli* (Reading 2). In the first reading, inhibition zones against *Escherichia coli* and *Staphylococcus aureus* were produced by the extracts of seven plants, in which *Punica granatum*and *Asparagus racemosus* appeared to be highly active respectively. In the second reading, inhibition zones were also produced by the extracts of seven plants, in which *Aloe vera* appeared to be highly active.

In the first reading, inhibition zones of the plant extracts with respect to these multi-resistant human pathogens, in decreasing order was as follows:

Punica granatum > Asparagus racemosus > Aloe vera > Catharanthus roseus >Syzygium cumini>Acmella oleracea>Ocimum sanctum

In the second reading, inhibition zones of the plant extracts with respect to these multi-

resistant human pathogens, in decreasing order was as follows:

Aloe vera >Acmella oleracea>Asparagus racemosus >Catharanthus roseus >Syzygium cumini> Punica granatum >Ocimum sanctum

With the increase in resistance of microorganisms to the currently used antibiotics and the high cost of production of synthetic compounds, pharmaceutical companies are now looking for alternatives. Medicinal plants could be those alternatives because most of them are safe with little side effects if any, cost less, and affect a wide range of antibiotic resistant microorganisms. The demand for new effective antimicrobials is urgent and of great importance in the clinical health. Allied with this demand is the need for assays to detect new and previously undiscovered antimicrobials from plant sources. From this study, the plant extracts were found tohave antibacterial activity against drug-resistantclinical bacteria. However, to explain the mode of action, the active phytocompounds of theseplants used against multidrug-resistantbacteria and their toxicity have to be determined by additional studies.

Discussion

Medicinal plants are the most exclusive source of life saving drugs for majority of the world's population.They continue to be an important therapeutic aid for alleviating the ailments of human kinds. India has a rich and diverse flora of medicinal plants. Plants have been used as medicines by all cultures from arced times to the recent days. Medicinal plants play a vital role in human health care. The World Health Organization (WHO) predicts that the number of cases worldwide for diabetes is now 150 Billion, which will double by the year 2025. The results of the maximum antibacterial activity was identified with ethanolic leaf extracts of various medicinal plants and the antimicrobial activity of the ethanolic extracts might be due do the presence of the unique phytochemical constituents.The discovery of a potent remedy from plant origin will be a great advancement in microbial infection therapies. Use of natural products has been encouraged due to less or no side effects, cost effectiveness and development of resistance to conventional synthetic antibiotics. Hence, this study holds importance in using medicinal plants as an alternative source for treating various diseases. The bacterial resistance to different anti-microbial agents is considered as a major problem in medicine and public

health. Nowadays studies focused with new antibacterial targets which attenuating virulence factor and capacity of certain microorganism to eliminate the infection.

In the present investigation, different extracts of the seven medicinal plants were evaluated for exploration of their antimicrobial activity against both Gram negative and Gram positive bacteria. These plant species are highly recommended for further studies, in order to discover new antibacterial drugs able to stand in the face of the dramatic spreading multi-drug resistant (MDR) bacteria. The ongoing emergence and spread of multi-drug resistant (MDR) bacteria is an international public health issue and now the effective antimicrobial drugs for bacterial infections are limited few or even sometimes absent (Magiorakos *et al.*, 2012). This fact led to searching for new antimicrobial agents, hastily. The screening for antibacterial activity of medicinal plants has been spread all over the world and many plants showed different degrees of antibacterial activities, most of these plants are prescribed in traditional and folk medicine. Such studies are important, particularly in the developing countries, where the weakness of people's income and high cost of modern medicines makes medicinal plants becoming much popular.

Summary and Conclusion

Summary

Antimicrobial efficiency of seven ethno-medicinal plants (crude leaf extracts) were examined using ethanol as solvent and tested against two human pathogens- *Escherichia coli* and *Staphylococcus aureus*. All of the plants showed profound antimicrobial activity (> 5 mm inhibition zone). The organic extracts of these plants could be a possible source to obtain new and effective herbal medicines to treat infections, which may caused by multi-drug resistant (MDR) strains of microorganisms from community as well as hospital settings. The study for the first time justified the ethnic uses of plant parts against infectious diseases.This discovery leads to development of better treatment with herbal medicines to overcome the side effects caused by antibiotics. It has revealed the importance of natural products to control antibiotic resistant bacteria, which have been a threat to human health.The different plant extracts exhibited inhibitory effects on growth of microorganisms studied; though of distinct effect, these differences may be as a result of concentration of extracts used to determine the antimicrobial activity. Plants are unlimited source for treatment of diseases. There is growing interest in investigation of different plant species for

their potentials for therapeutic applications. The results obtained in this study add credence to the ethnomedicinal uses of theseplants for the treatment of pathogenic infections, microbial infections, a reservoir of new bioactive compounds and antimicrobial agenst in new drug therapy. It is, therefore highly essential that medicinal plants whose properties have not been fully characterized should form a top agenda of top management in developing nations whose citizens are sometimes unable to afford expensive orthodox medicine. It has further confirmed that the plant extracts could be used for the treatment of various diseases and microbial infections.

Conclusion

In conclusion, all of the plant extracts testedin this study had potential antibacterial activitiesagainst the reference strains. Our results supportthe use of these plants in traditional medicine andsuggest that some of the plant extracts possesscompounds with good antibacterial propertiesthat can be used as antimicrobial agents in thesearch for new drugs. Plants having potential pharmacological significance in comparison to their synthetic counterpart, directs a powerful natural tool to battle against diseases without compromising over the hazardous effects of the plant on the body. Majority of leads fail to cross the final approval due to one or another side effects, which rather than working as healing probe, work in exaggerating the problem further. The plant extractives studied could be an answer to the people seeking for better therapeutic agents from natural sources which are believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents.

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