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ANTIMICROBIAL ACTIVITY OF VARIOUS PLANT EXTRACTS AGAINST BACTERIAL PATHOGENS ISOLATED FROM URINARY TRACT INFECTION PATIENTS

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Abstract Medicinal plants produce several antibacterial compounds. These plants are well known for their medicinal value and are widely used in community for the treatment of various diseases. In this study 5 different medicinal plants (*Azadirachta indica*, *Ficus religiosa*, *Acacia nilotica*, *Linum usitatissimum* and *Rosa damascene*), traditionally used in medicine, were subjected to preliminary screening for antimicrobial potential against four pathogenic microorganisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus species)* isolated from patients with urinary tract infections (UTI). Multi drug resistant (MDR) gram negative pathogenic microorganisms were selected out of isolated UTI pathogens. Aqueous extracts of each plant were prepared and tested for their antibacterial potential using agar well diffusion method. The results indicated that all plant extracts exhibit antibacterial activity against test pathogens. *Azadirachta indica* showed excellent and broad spectrum activity as compared to rest of four plant extracts. This study highlights the need to exploit the antibacterial potential of these plants for development of new antibiotics.

Key words: Medicinal plants, MIC, MBC, UTI

Introduction

Bacteria are ubiquitous pathogens causing various types of infections including urinary tract infections [1], nosocomial bloodstream infections [2], wound infections [3], brain abscess [3], asthma [4], community-acquired pneumonia [5] and skin infections [6]. These infections including urinary tract infections can result in fatal consequences if they are not treated properly or are left untreated. To treat all kinds of bacterial infections antibiotics are used worldwide; however, bacteria are gradually becoming resistant against these antibiotics. Furthermore, during recent years, misuse of antibiotics is calling for an accelerated search for novel antibacterial therapeutic agents [3]. Most of anti-microbial drugs are natural products derived from plants [7]. Since beginning of human civilization on this planet, plants are playing marvelous role in maintaining and improving human health. The production of antimicrobial agents by plants has opened new avenues for the discovery of novel natural products that can serve as substitutes for current antibiotics [8]. Although plant extracts have great potential in the treatment

of infectious diseases caused by resistant superbugs, but it is surprising that less than 5% plant species have been analyzed for production of antimicrobial compounds, while rests of the 95% of plants still need to be analyzed [9].

Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. In recent years, keeping in view of the need of new therapeutic agents, this interest is growing dramatically [10]. Medicinal plants are considerably useful and economically suitable. They contain active constituents that are used in the treatment of many human diseases [11]. The plant extracts used in traditional medicine have been developed and proposed to treat chronic and infectious diseases[12]. Medicinal herbs practiced in traditional folk medicine in Pakistan have been screened for the presence of antibacterial activity. In past, research was mostly carried out on phenolic extracts of medicinal plants. Due to the indiscriminate use of antimicrobial drugs, the microorganisms have developed resistance to many antibiotics. This has created immense clinical problem in the treatment of infectious diseases [13]. In addition to this problem, antibiotics have several adverse effects which include hypersensitivity, depletion of beneficial gut flora, immunosuppression and allergic reactions [14]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. The present investigation represents a preliminary screening on various plant extracts for the isolation and identification of biologically active compounds having antimicrobial activity. These compounds can be of significant importance in improving the shelf-life of food products because majority of these compounds are safe for human consumption and provide hurdle to the growth of food borne pathogens and spoilage bacteria. Various studies have reported that medicinal plants produce a large number of secondary metabolites with antimicrobial effects on pathogens [15]. The main purpose of the current study was to assess the inhibitory activities crude extracts of selected medicinal plants against some human pathogens that cause urinary tract infections. Medicinal plants included in study were *Azadirachta indica* (Neem), *Ficus religiosa* (Peepal), *Acacia nilotica* (Kikar), *Linum usitatissimum* (Alsi) and *Rosa damascena* (Gulab). The present study mainly focused to determine activity against multidrug resistance bacteria isolated from UTI.

Materials and Methods

Collection of Plant Materials

Plants were collected from various areas of Lahore, Pakistan; these plants were confirmed up to species level by the Department of Botany, University of the Punjab, Lahore, Pakistan. Each specimen was labeled, numbered and annotated with the date of collection.

Preparation of extracts

Leaves and seeds of *Azadirachta indica* (Neem), bark and fruit of *Ficus religiosa* (Peepal), flowers & fruits of *Acacia nilotica* (Kikar), seeds of *Linum usitatissimum* (Alsi) and flowers of *Rosa damascena* (Gulab) were collected, washed under tap water, air dried and powdered by grinding using an electric blender. Fine powder of *Azadirachta indica* (Neem) and *Ficus religiosa* (Peepal) was obtained by sieving followed by grinding. Flowers & fruits of *Acacia nilotica* (Kikar) was boiled in the water for 02 hours, filtered and extract was dried for fine powder. Seeds of *Linum usitatissimum* (Alsi) and flowers of *Rosa damascena* (Gulab) were grinded after keeping in water for 4 hours. The powder was stored in airtight bottles until required for further analysis.

After obtaining powder of all plant materials, 1% stock solution of each was prepared by dissolving 1g of powder in 100 ml of distilled water. Sterilization was achieved by filtration through Whatman filter paper no. 1(18) (Whatman, England). These stock solutions were stored at 4 °C.

Antimicrobial activity

Isolation of microbial strains

Microorganisms used in this study were *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus species.* For isolation of these pathogenic microorganisms, one hundred mid stream urine specimens were collected from patients of urinary tract infection (UTI), admitted in Doctors hospital and medical complex (DHMC) Lahore, Pakistan. Samples were taken before any antibiotic administration. These specimens were processed in

microbiology department as per standard guidelines. Antimicrobial susceptibility patterns of the isolates were determined using CLSI 2013 guidelines by kirby bauer disc diffusion method and most resistance strains were selected. Drugs used for *Escherichia coli, Klebsiella pneumoniae* and *Proteus species* were Amoxicillin/Clavulanic acid (30µg), Ampicillin/Sulbactam (30µg), Cefepime (30µg), Cefoperazone (75µg), Cefotaxime (30µg), Cefuroxime (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cephalexin (30µg), Cephradine (30µg), Cefaclor (30µg), Cefixime (30µg), Imipenem (10µg), Meropenem (10µg), Aztreonam (30µg), Amikacin (30µg), Gentamicin (10µg), Tobramycin (10µg), Doxycycline (30µg), Naladixic acid (30µg), Pipemedic acid (20µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Ofloxacin (5µg), Norfloxacin (10µg), Moxifloxacin (5µg), Nitrofurantoin (300µg), Fosfomycin (50µg), Cefoperazone+Salbactam (105µg), Piperacillin/ Tazobactam (110µg), Colistin (10µg), Polymyxin B (300µg), Tigecycline (15µg) and Ticarcillin/Clavulanic acid, while drugs used for *Pseudomonas aeruginosa* were Cefepime (30µg), Ceftazidime (30µg), Imipenem (10µg), Meropenem (10µg), Aztreonam (30µg), Amikacin (30µg), Gentamicin (10µg), Tobramycin (10µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Ofloxacin (5µg), Norfloxacin (10µg), Moxifloxacin (5µg), Piperacillin/ Tazobactam (110µg), Colistin, Polymyxin B (300µg), Tigecycline (15µg) and Ticarcillin/Clavulanic acid(85 µg). Selected strains of *Escherichia coli, Pseudomonas aeruginosa, Kleibsiella pneumoniae* and *Proteus species* were only sensitive to Fosfomycin, Colistin, Polymyxin B and Tigecycline only.

Diffusion method

The microbial growth inhibitory potential of the plant extracts was determined by using agar disc diffusion method [16]. One microorganism of each strain was selected to determine the antimicrobial activity of plant extracts. Inocula were prepared by mixing few microbial colonies in sterile nutrient broth and comparing the turbidity with the standard 0.5 McFarland solution [17] which is equivalent to 10^6–10^8 CFU/ml. One hundred microlitres of standard suspension of all selected isolates were inoculated by spreading on the surface of mueller hinton agar (Oxoid). Plants extracts were mixed with diluted and poured into inoculated plates by making wells in it. Plates were incubated at 37 ^{0}C overnight.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Although the results of the disc diffusion assay cannot be compared always to the minimum inhibitory concentration (MIC) data, the plant extracts which showed best antimicrobial activity against most of the microorganisms tested in the disc diffusion assay were further tested for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The micro dilution technique using 96-well micro-plate, as described by Eloff (1998), was used to obtain the MIC and MBC values of the crude extracts against microorganisms under study. Selected extracts were serially diluted two fold dilution for 100 µg/dl to 800 µg/dl in 96-well micro-plate with sterile distilled water, selected microorganisms form standard 0.5 McFarland solution were added and results were noted after incubation at 37°C for turbidity by visual inspection and sub culturing.

Determination of cellular toxicity using sheep erythrocytes

The method described by Hammer (1994) was employed to study cellular toxicity. 10-fold serial dilution of the extracts was made in phosphate buffered saline. A total volume of 0.8 ml of each dilution was taken in separate eppendorf tubes. A negative control tube (containing saline only) and a positive control tube (containing tap water) were also included in the analysis. Fresh 1 % sheep erythrocytes were added to each tube, to give a final volume of 1.0 ml. Solutions were incubated at 37°C for 30 min. Then all tubes were centrifuged for 5 min and observed for hemolysis.

Results and Discussion

The present study investigated the antimicrobial potential of medicinal plants against common gram negative urinary tract pathogens. Most resistant strains of *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus species* were selected. These strains were resistant to all available drugs used except Fosfomycin, Colistin, Polymyxin B and Tigecycline and were therefore very suitable for the screening of medicinal plants for

extracts having novel antimicrobial compounds. Efforts have been devoted over the past years to the search of new antimicrobial compounds from natural sources [18].

Five crude plant extracts, which were examined for their antimicrobial activity against four pathogenic microorganisms, showed good antimicrobial potential (Figure 1). *Azadirachta indica* (Neem) showed best antibacterial activity against multi drug resistant pathogens isolated from UTI patients.

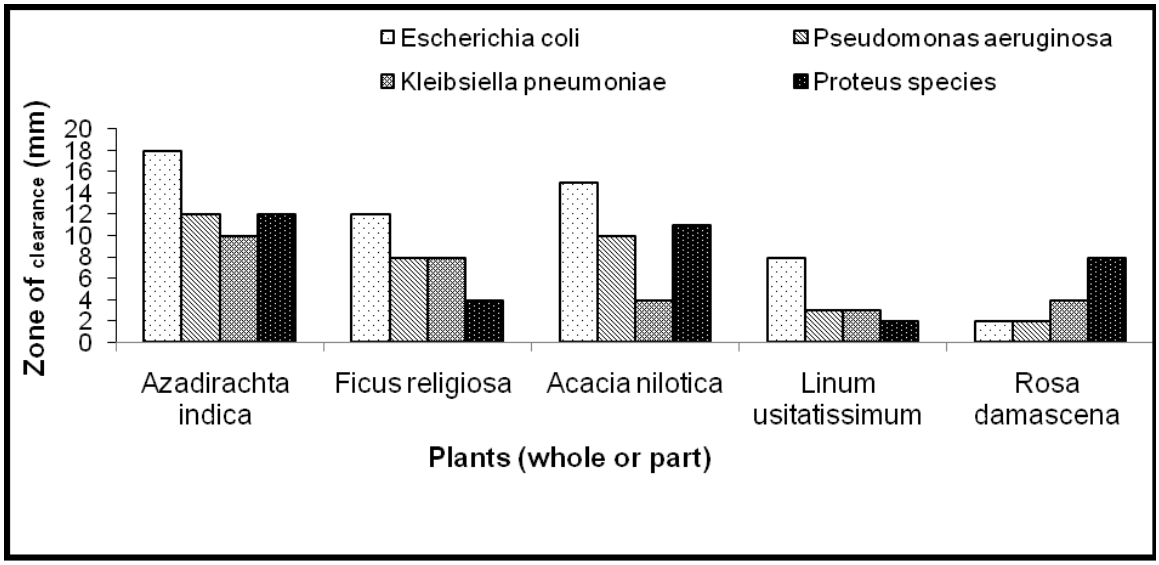

Figure 1: Antimicrobial activity of 5 different crude plant extracts against four pathogenic microorganisms.

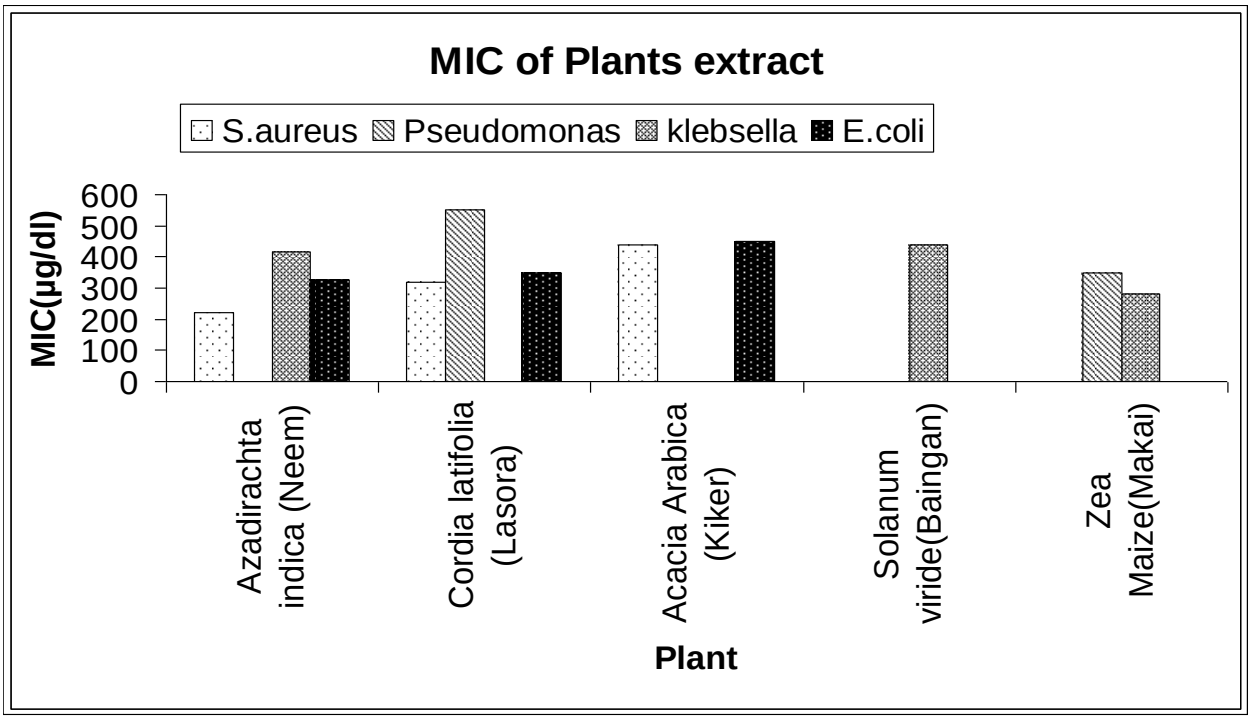

Figure 2: Minimum inhibitory concentration (MIC) of 5 different plant extracts against 4 pathogenic microorganims isolated from Urinary Tract Infection (UTI) patients.

After determining the antimicrobial activity of plant extracts, MIC and MBC were determined using the micro well plate method. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) are accepted and well used criterion for measuring the susceptibility of microorganisms to antimicrobial compounds. The results (Figure 2) showed that antimicrobial activity of *Azadirachta indica* was excellent for *Escherichia coli* and *Proteus* species, while antibacterial activity of *Rosa damascena* was least against *Escherichia coli* and *Proteus* species. Pritima and Pandian (2008) has also supported our results about the antibacterial potential of *Azadirachta indica* against *E.coli* and Proteus species. On the other hand, *Rosa damascene* showed good antibacterial activity against *klebsiella pneumoniae* and *Pseudomonas aeruginosa*. This result has great significance as these two microorganisms were found to be very consistent in their high MIC and MBC values with other four plant extracts. However, this plant extract was found to be least significant for *Escherichia coli* and Proteus species.

Last experiment involving the sheep RBC showed that plants extract showed no signs of cytotoxicity. A lot of research has been conducted to examine the antimicrobial potential of herb and shrubs. Most researches have shown excellent antimicrobial potential of *Azadirachta indica* [19]. Our study showed concordant results with researches conducted in past and therefore *Azadirachta indica* is an excellent candidate for alternative antimicrobial treatment avoiding the development of drug resistance and side effects of antibiotics.

In addition to *Azadirachta indica,* other four plants have also shown good antibacterial potential while it must be considered that all plant extracts are not equally effective for all microorganisms. This difference indicates diverse nature of antibacterial compounds present in different plant extracts.

In recent years, the focus of researchers is to isolate and characterize antimicrobial compounds from these plant extracts. However, this is a very time consuming process and needs to be followed by *in vivo* studies and clinical trials. The aim of the present study was also to compare different plant extracts and highlight best plant extract for future development of antibiotics. However, different factors including method of extraction of plant extract, inoculum size and temperature must be considered in mind which affects the values of MIC and MBC [20].

The severe side effects associated with the use of commercially available antibiotics, especially in immunocompromised patients and children; strongly recommend the use of natural products for the treatment of infections. Furthermore, the multi drug resistant pathogens causing nosocomial infections are a serious threat to the health of community. This study indicates that these crude plant extracts can be a good source of antimicrobial compounds and can lead to the development of novel broad spectrum antibiotics in future.

Conclusion

Development of resistance by the microorganisms to chemotherapeutic agents appears to be a continuous process since the discovery of antibiotics. Scientists have realized an immense potential in natural products from medicinal plants to serve as an alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity. Further work on isolation and characterization of active antimicrobial compounds from medicinal plants and their pharmacodynamic study would be highly beneficial for the management of severe life threatening infections.

References

1. Khan AU, Musharraf A. Plasmid-mediated multiple antibiotic resistance in Proteus mirabilis isolated from patients with urinary tract infection. Medical science monitor: international medical journal of experimental and clinical research. 2004;10:CR598-602.
2. Blot SI, Depuydt P, Annemans L, Benoit D, Hoste E, De Waele JJ, et al. Clinical and economic outcomes in critically ill patients with nosocomial catheter-related bloodstream infections. Clinical Infectious Diseases. 2005;41:1591-8.
3. Saeed S, Tariq P. Antibacterial activities of Mentha piperita, Pisum sativum and Momordica charantia. Pakistan Journal of Botany. 2005;37:997.

4. Lam S, Chan H, LeRiche JC, Chan-Yeung M, Salari H. Release of leukotrienes in patients with bronchial asthma. Journal of allergy and clinical immunology. 1988;81:711-7.
5. Grant EN, Lyttle CS, Weiss KB. The relation of socioeconomic factors and racial/ethnic differences in US asthma mortality. American journal of public health. 2000;90:1923.
6. Ma Y, Aymeric L, Locher C, Mattarollo SR, Delahaye NF, Pereira P, et al. Contribution of IL-17–producing γδ T cells to the efficacy of anticancer chemotherapy. The Journal of experimental medicine. 2011;208:491-503.
7. Poonkothai M, Saravanan M. Antibacterial activity of Aegle marmelos against leaf, bark and fruit extracts. Ancient science of life. 2008;27:15.
8. Donato F, Maurin D, Brun P, Delahaye T, Salati P. Constraints on WIMP Dark Matter from the High Energy PAMELA p/p data. Physical review letters. 2009;102:071301.
9. de Lemos JA, Blazing MA, Wiviott SD, Lewis EF, Fox KA, White HD, et al. Early intensive vs a delayed conservative simvastatin strategy in patients with acute coronary syndromes: phase Z of the A to Z trial. JAMA : the journal of the American Medical Association. 2004;292:1307-16.
10. Lentz DL, Clark AM, Hufford CD, Meurer-Grimes B, Passreiter CM, Cordero J, et al. Antimicrobial properties of Honduran medicinal plants. Journal of ethnopharmacology. 1998;63:253-63.
11. Sumathi P, Parvathi A. Antimicrobial activity of some traditional medicinal plants. Journal of Medicinal plants research. 2010;4:316-21.
12. Del Campo JA, García-González M, Guerrero MG. Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Applied microbiology and biotechnology. 2007;74:1163-74.
13. Davis RJ. MAPKs: new JNK expands the group. Trends in biochemical sciences. 1994;19:470-3.
14. Dick DM, Foroud T, Flury L, Bowman ES, Miller MJ, Rau NL, et al. Genomewide linkage analyses of bipolar disorder: a new sample of 250 pedigrees from the National Institute of Mental Health Genetics Initiative. The American Journal of Human Genetics. 2003;73:107-14.
15. Willett CG, Boucher Y, Di Tomaso E, Duda DG, Munn LL, Tong RT, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nature medicine. 2004;10:145-7.
16. Hagerman AE. Extraction of tannin from fresh and preserved leaves. Journal of Chemical Ecology. 1988;14:453-61.
17. McFarland J. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. Journal of the American Medical Association. 1907;49:1176-8.
18. Nielsen PV, Rios R. Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. International journal of food microbiology. 2000;60:219-29.
19. Stadler MB, Murr R, Burger L, Ivanek R, Lienert F, Schöler A, et al. DNA-binding factors shape the mouse methylome at distal regulatory regions. Nature. 2011.
20. Buznikov GA, Lambert WH, Lauder JM. Serotonin and serotonin-like substances as regulators of early embryogenesis and morphogenesis. Cell and tissue research. 2001; 305: 177-86.