Medicinal plants combating against human pathogens: A review

Kosar Batool1 • Sabira Sultana1* • Naveed Akhtar1 • Hafiz Muhammad Asif1 • Naheed Akhtar2 • Khalil Ahmad1 • Aymen Owais3

1University College of Conventional Medicine, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan.
2Department of Pharmacy, Faculty of Health and Medical Sciences, The University of Poonch, Rawalakot, AJ & K, Pakistan.
3Jinnah University for Women, Karachi-74600, Pakistan.

*Corresponding author. E-mail: drsabirachishti12@gmail.com. Tel: 03026768718.

Abstract. The extensive acceptance of traditional medicine as an alternative form of healthcare and the alarming increase in the incidence of new and re-emerging infectious diseases bring about the need to investigate new medicinal plants. Another concern was the increase resistance of the antibiotics in current clinical use. Anti-microbial agents are irrefutably one of the most important therapeutic discoveries of the 20th century. However, with the beginning of ‘antibiotic era’ barely five decades old, mankind is faced the global problem of emerging resistance in virtually all microbes. During the last decade, the use of traditional medicine has gained popularity around the world. Recently, the herbal medicines serve the health needs of about 80% of the world’s population, especially for millions of people in the rural areas of developing countries. In recent years, many natural antibiotics have been in use in variety of infectious diseases, mostly bacterial and fungal. The increasing incidence of drug-resistant microorganisms is a big threat to successful therapy of microbial diseases. Therefore, there is an urgent need of the time to search new antimicrobial compounds characterized by diverse chemical structures and mechanisms of action. The use of plant compounds as antimicrobial agents is interesting strategy for discovering bioactive products that in upcoming future could become useful therapeutic tools. Thus, it is an important task for the researcher to find out alternative medicine against dreadful human pathogens. This review can help researchers to explore herbs to further extents to find out the alternatives against the drug resistant human pathogens.

Keywords: Antimicrobial activity, antibacterial activity, traditional medicine, pathogens, medicinal plants, bioactive compound.

INTRODUCTION

Medicinal plants have been used as a source of relief from illness for over five millennia, so no doubt it is an art as old as mankind. WHO (2008) found that more than 80% of the world's population rely on traditional medicine for their primary healthcare needs. The extensive acceptance of traditional medicine as an alternative form of healthcare and the alarming increase in the incidence of new and re-emerging infectious diseases bring about the necessity to investigate new medicinal plants. Another concern is the development of resistance to the antibiotics in current clinical use. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Vital and Rivera, 2009).

Herbal drugs have become increasingly popular and
their use is widespread. Their efficacy in treating different ailments induced by microorganisms yet needs to be explored. Different medicinal plants have been used for years in daily life to treat disease all over the world. More than 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Dey et al., 2010).

Different ethnic groups have contributed in the development of research on natural products; these contacts help in increasing knowledge about the close relationship between the chemical structure of a certain compound and its biological properties. Due to these reasons, medicinal plants are important sources for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents (Ushimaru et al., 2007).

Many efforts have been made to find out new antimicrobial compounds from various kinds of natural sources such as micro-organisms, animals, and plants. One of such resources is plants that were used in folk medicines. Systematic screening of these plants may result in the discovery of new effective compounds. The increasing prevalence of multidrug resistant in strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections, which adds the need to search for new infection-fighting strategies (Janovska et al., 2003). Plant based antimicrobial drugs represent a vast available source for medicines and further investigation of plant antimicrobials needs to occur. Antimicrobials of plant origin have great therapeutic potential. Human infections mostly involve microorganisms, that is, bacteria, fungi, viruses, that cause serious infections in tropical and subtropical countries of the world. In previous years, multiple drug resistance in human pathogenic microorganisms has developed due to arbitrary use of commercial antimicrobial drugs commonly used in the treatment of such diseases. Bacteria have genetic ability to develop and transmit resistance to drugs, which are utilized as curative agents. The problem of microbial resistance is growing and the manner for the use of antimicrobial drugs in the future is still uncertain. So, actions must be taken to reduce this problem, for example, to control the use of antibiotic, and 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. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens (Girish, 2008).

Bacteria can produce resistance to antibiotics by four general mechanisms, which include, the inactivation or modification of the antibiotic drug structure, an alteration in the target site of the antibiotic that reduces its binding capacity, the modification of metabolic pathways to avoid the antibiotic effect, the reduced intracellular antibiotic accumulation by decreasing permeability and/or increasing active efflux of the antibiotic.

The investigation of many indigenous plants for their antimicrobial properties has yielded useful results. The plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or virus (El astal et al., 2005).

The phenomenon of antibiotic resistance exhibited by the pathogenic microorganisms has led to the need for screening of several medicinal plants for their potential antimicrobial activity. Plant produce a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry and 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 and out of the 400,000 plant species on Earth. Only a small number has been systematically investigated for their antimicrobial activities. However, there has not been often effective collaboration between the traditional and western medical therapeuticists, largely due to the perception that the use of traditional and herbal medicines has no scientific basis. According to World Health Organization (WHO, 2000) medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Sharma et al., 2009).

ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS

The effect of plant extracts on bacteria has been studied by a number of researchers in different parts of the world. Hussain et al. (2010) evaluated the antibacterial, antifungal and insecticidal activities of the crude extract of Polygonum persicaria, Rumex hastatus, Rumex dentatus, Rumex nepalensis, Polygonum plebejum and Rheum australe. Six bacterial species were used, of which Citrobacter freundii, Escherichia coli, Enterobacter aerogenes and Staphylococcus aureus were the most susceptible bacterial species to crude extract with MICs 16, 5.0, 25 and 0.156 mg/ml, respectively. Among the tested fungal species Fusarium solani, Aspergillus flavus and Aspergillus niger were more susceptible to crude extracts with MICs 0.75, 2.15, and 1.75 μg/ml, respectively. The crude extracts of R. dentatus and R. nepalensis show significant insecticidal activity against Sitophilus oryzae, P. persicaria and P. plebejum show significant insecticidal activities against Tribolium castaneum. The above selected plants were shown by in vitro assays to be a potential source for natural antifungal, antibacterial and insecticidal agents (Hussain et al., 2010).
Dorman and Deans, (2000) studied antimicrobial agents from plants contains volatile oils. The volatile oil of *Piper nigrum* L. (Piperaceae), *Syzygium aromaticum* L. (Myrtaceae), *Pelargonium graveolens* Geraniaceae, *Myristica fragrans* (Myristicaceae), *Organun vulgare* (Lamiaceae) and *Thymus vulgaris* L. (Lamiaceae) were assessed for antibacterial activity against 25 different genera of bacteria. These included animal and plant pathogens, food poisoning and spoilage bacteria. The volatile oil exhibited considerable inhibitory effects against all the organisms under test while their major components demonstrated various degrees of growth inhibition (Dorman and Deans, 2000).

Singh et al. (2010) studied the ethanolic extracts of *Stevia rebaudiana*, *Murraya koenigii*, *Psidium guajava* and *Hibiscus roasanensis*. He concluded that these plants could be a possible source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant strains of microorganisms from community as well as hospital settings. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties. We can even modify the variety of our plant sample by the recombinant biotechnology method (Singh and Joshi, 2010).

Hussain et al. (2011) designed a study which purpose was to observe the antibacterial activity of aqueous methanolic extracts of 10 plants against 2-gram negative bacteria (*Pasteurella multocida*, *Escherichia coli*) and 3-gram positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Corynebacterium bosis*) by using disc diffusion method. The minimum inhibitory concentration (MIC) was determined by agar well diffusion method and agar dilution method. All the bacteria were susceptible to different plant extracts. *Lawsonia inermis*, *Embellia ribes* and *Santalum album* showed antibacterial activity against all the tested bacteria. The extract of *Santalum album* showed maximum antibacterial activity of the 10 plant extracts used. *Bacillus cereus* and *Pasteurella multocida* were the most sensitive bacteria against most of the plant extracts. It is clear from the results of the present study that the plant extracts have great potential as antimicrobial compounds against bacteria. However, there is a need of further research to isolate the active ingredients for further pharmacological evaluation (Hussain et al., 2011).

Islam et al. (2008) conducted a study to identify plant species having potential antimicrobial principles, antibacterial activity from 5 indigenous plant species which were screened from 16 plant species were examined using agar disc diffusion method against ten bacteria (*Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae* and *Bacillus megaterium*). The plants were extracted using 95% ethanol and showed inhibitory effect against gram-positive and gram-negative bacteria. But gram-positive bacteria are more sensitive than gram-negative bacteria. The largest zone of inhibition (23 mm in diameter) was recorded against *Bacillus subtilis* with the leaf extract of *Blumea lacera* (Islam et al., 2008).

Tipu et al. (2006) has given a detailed account of medicinal properties of different plants. According to these workers, these plant act as antibacterial, antioxidant, anticarcinogenic, antifungal, analgesic, insecticidal, and growth promoters. These plant extracts compete with the synthetic drugs. Majority of medicinal plants do not have the residual effects. *Azadirachta indica*, *Zizyphus valgaris*, *Ocimum gratissimum* and *Atlanta monophylla* have strong antibacterial activity, whereas *ocimum* plant has strong antioxidant, anticarcinogenic, antifungal, analgesic and antipyretic properties. Leaves of *Azadirachta indica* are used for feeding and reducing the parasitic load of animals (Tipu et al., 2006).

Kelmanson et al. (2000) evaluate the aqueous, methanolic and ethyl acetate extracts of 14 plants used in traditional Zulu medicine for treatment of ailments of an infectious nature, and they were screened for antibacterial activity. Most of the activities detected were against gram-positive bacteria. Tuber bark extracts of *Dioscorea sylvatica* showed activity against gram-negative *Escherichia coli*, and extracts of *Dioscorea dregeana*, *Chelianthe viridis* and *Vernonia colorata* were active against *Pseudomonas aeruginosa*. The highest antibacterial activity was found in extracts of *C. viridis*, *D. dregeana*, *D. silvatica*, *Melianthus comosus* and *V. colorata*. In general, methanolic extracts exhibited higher activity than aqueous and ethyl acetate extracts (Kelmanson et al., 2000).

Abdalla et al. (2013) described chemical and biological screening of crude ethanolic extract from four species, *Ozoraa insiginis* Del., Oliv; (Anacardiaceae), *Ximenia americana* L; (Olacaceae), *Boscia salicifolia* Oliv; (Capparidaceae), and *Terminalia brownii* Fresen; (Combretaceae). The four species were selected for this study according to ethnobotanical literature and their significant as traditional medicine in Alangasana area, in West of Sudan (Abdalla et al., 2013).

The four species revealed high availability of tannins, saponins, steroids, flavonoids, and terpenoids. Alkaloids were present only in *O. insiginis*. The antimicrobial activity of the ethanolic extracts from the barks of the four species was performed to determine quantitatively the presence or absence of inhibition zones, and measurement of zone diameter. *O. insiginis* extract showed good activity against *Pseudomonas aeruginosa*. Whereas *X. americana* extract exhibited significant activity against *Staphylococcus aureus* and low activity against *Pseudomonas aeruginosa*. Consequently T. brownii showed significant activity against *S. aureus*, and good activity against *P. aeruginosa*. B. salicifolia exhibited no activity against all
types of tested bacteria.

Britto et al. (2011) evaluated the methanol and aqueous extracts of leaves of six different medicinal plants, *Acalypha indica*, *Aerva lanata*, *Phyllanthus amarus*, *Phyllanthus emblica*, *Cassia auriculata* and *Caesalpinia pulcherrima*, were used for the investigation of antibacterial studies. In antibacterial screening performed by disc diffusion method against two types of bacteria namely *Xanthomonas campestris* (plant pathogen) and *Aeromonas hydrophila* (human pathogen), it was found that the methanol extracts of all the plant samples showed significant activity against the two tested bacteria. The methanol extracts of *Acalypha indica*, *Aerva lanata* and *Phyllanthus amarus* exhibited clear zone of inhibition against the tested microorganisms. Among these three samples, the MIC value of *Aerva lanata*, determined by serial dilution technique, was found to be 32 and 64 μg/ml against *X. campestris* and *Aeromonas hydrophila* respectively (Britto et al., 2011).

Kone et al. (2004) evaluated the sixty-seven crude ethanol extracts from 50 plants (31 families), were screened for in vitro activity against Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Bacillus subtilis*) bacteria. Thirty-one extracts showed antibacterial activity only on gram-positive bacteria. Of these, 10 extracts from 10 plant species had a promising level of activity against bacteria including strains resistant to antibiotics such as aminosides, penicillin M, macrolides, lincosamide and streptogramin B. The most active was *Erythrina senegalensis* DC (Fabaceae) followed by *Bobgunnia madagascariensis* (Desv.) J.H. Kirkbr. & Wiersema (Caesalpinaceae), *Waltheria lanceolata* R. Br. ex Mast (Sterculiaceae), *Uapaca togoensis* Pax (Euphorbiaceae), *Ximenia americana* L. (Olacaceae), *Khaya senegalensis* (Ders.), *A. Juss.* (Meliaceae), *Lannea acida* (Anacardiaceae), *Cissus populnea* Guill. & Perr (Vitaceae), *Keetia hispida* (Benth.) Bridson (Rubiaciae) and *Ficus thonningii* (Miq.) (Moraceae). This is the first report of the antibacterial potency of these 10 plant species on a range of bacteria. The results provided evidence that some of the studied plants might indeed be potential sources of new antibacterial agents, also against some antibiotic-resistant strains (Kone et al., 2004).

Soniya et al. (2013) studied the medicinal plant extracts prepared with selected various solvents from ten species, *Murraya koenigii*, *Syzygium aromaticum*, *Piper nigrum*, *Ocimum tenuiflorum*, *Laurus nobilis*, *Cinnamomum zeylanicum*, *Phyllanthus niruri*, *Cuminum cyminum*, *Trilobatum* sp. and *Hibiscus rosasinensis* were screened for antibacterial activity against bacterial pathogens by using disc diffusion method. The antibacterial activities of the extracts (methanol and aqueous) were gave maximum inhibition zone when compared with other solvent extracts. Plant extracts showed strong antibacterial action against pathogens, among the plant extracts the methanol extracts of *Ocimum tenuiflorum* and *Syzygium aromaticum* against *B. subtilis* and *Cuminum cyminum* against *E. coli* while aqueous extracts of *Piper nigrum* gave maximum inhibition against *Proteus* sp. However, no antibacterial activities noted in methanol extracts of *Hibiscus rosasinensis* against *E. coli* and *P. aeruginosa* (Soniya et al., 2013).

Essawi et al. (2000) checked out the antibacterial activity of organic and aqueous extracts of 15 Palestinian medicinal plants were carried against eight different species of bacteria: *Bacillus subtilis*, two *Escherichia coli* species, *Staphylococcus aureus* (mecillin resistant), two *S. aureus* (mecillin sensitive) species, *Pseudomonas aeruginosa*, and *Enterococcus fecalis*. Of the 15 plants tested, eight showed antibacterial activity. Each plant species has unique against different bacteria. The most active antibacterial plants against both gram-positive and gram-negative bacteria were *Thymus vulgaris* and *Thymus originum*. The organic and aqueous extract from the same plants showed different activities; the organic extract showed the same or greater activity than the aqueous extract. Finally, the hole-plate diffusion method showed larger activity than the disc diffusion method (Essawi et al., 2000).

Senthilmurugan et al. (2013) screened and studied the five different plant specimens belonging to different families for phytochemical constituents was performed using generally accepted laboratory technique for qualitative determinations. The constituents screened were saponins, combined anthraquinones, terpenoids, flavonoids, carotenoids, steroids, xanthones, coumarins, alkaloids, quinones, vitamin C. The distribution of these constituents in the plant specimens were assessed and compared. The medicinal plant studied were *Acalypha indica*, *Camellia sinensis*, *Plectranthus amboinicus*, *Curcuma longa*, *Rawvoffia tetraphylla*. All the plant specimens were found to contain terpenoids, xanthone proteins, coumarins and vitamin C. They also contain saponins (except *Curcuma longa*), combined anthraquinones (except *Acalypha indica*, *Camellia sinensis*, *Curcuma longa*) flavonoids (except *Acalypha indica*, *Camellia sinensis*), coumarins (except *Acalypha indica*, *Curcuma longa*), and steroids (except *Plectranthus amboinicus*, *Rawvoffia tetraphylla*). Quinones were found in one out of the five specimens. Some of the medicinal plant seemed to have potential as source of useful drugs. Though the one percent extracts of all the plants showed some degree of antimicrobial activity, it was significant in *Acalypha indica*, *Camellia sinensis*, *Plectranthus amboinicus*, *Curcuma longa*, and *Rawvoffia tetraphylla*. The extract of *C. sinensis* and *Acalypha indica* was most effective against *Enterobacter faecalis* (ZI = 3 cm and ZI = 1.7 cm) and *C. sinensis* and *A. indica* was most effective against *Staphylococcus aureus* (ZI = 2.1 cm) (Senthilmurugan et al., 2013).

Samy et al. (2000) evaluated the series of 30 Indian folklore medicinal plants used by tribal healers to treat
infections, were screened for antibacterial properties at 10 mg/ml concentration by using disc diffusion method against Bacillus subtilis, Escherichia coli, Klebsiella aerogenes, Proteus vulgaris, Pseudomonas aerogenes and Staphylococcus aureus. Twenty plant species showed activity against one or more species of bacteria used in this assay; among them the leaf extracts of Cassia occidentalis and Cassia auriculata exhibited significant broad spectrum activity against B. subtilis and S. aureus. Ten of plant species were not found active against all tested bacteria. These results were compared with results obtained using standard antibiotics, chloramphenicol (30 µg/disc) and streptomycin (30 µg/disc) which served as a reference for inhibition zone diameter (Samy et al., 2000).

Brantner et al. (1994) evaluated the aqueous extracts of plants used externally for the treatment of infected skin lesions were tested for their antibacterial potential. The results indicated that about 60% of the plant extracts tested exhibited some level of antibacterial action (Brantner et al., 1994).

Rios et al. (2005) analyzes the past, present and future of medicinal plants, both as potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infection agents. In the past few decades, the search for new anti-infection agents has occupied many research groups in the field of ethnomedicinal plants (Rios et al., 2005).

Singh and Jain (2011) evaluated the antibacterial activity of alcoholic and aqueous extracts of fourteen medicinal plants collected from Uttarakhand, North India by Agar diffusion method against medically important bacteria viz. B. subtilis, K. pneumonia, E. coli, Enterobacter aerogenes, Pseudomonas aeruginosa, S. aureus and P. mirabilis. Kanamycin was used as standard drug for antibacterial activity. Out of fourteen plant extracts, alcoholic extract of Curcuma longa showed the best antibacterial activity (Singh and Jain, 2011).

Gandhiraja et al. (2009) conduct a study. In his study, the active phytocomponents of Mimosa pudica were revealed using phytochemical analysis. The antimicrobial activity of Mimosa was studied using well diffusion method. The activity was tested against Aspergillus fumigatus, Citrobacter divergens and Klebsiella pneumonia at different concentrations of 50, 100 and 200 µg/disc and the results showed significant activity against bacterial strains (Gandhiraja et al., 2009).

Abuhamdah et al. (2004) determined the antibacterial effect of crude methanolic extracts of six selected medicinal plants grown in Jordan (Paronychia argentea Lam., Inula viscosa L., Arbutus andrachne L., Asphodelus microcarpus, Peganum harmala L. and Aloysia citriodora Palau) against Bacillus subtilis, Staphylococcus aureus and Escherichia coli. The crude methanolic extract of P. argentea, A. andrachne, A. microcarpus had no antibacterial activity, crude extract of P. harmala showed good antibacterial activities against all the tested bacterial strains. MIC values for the seed and root extract against S. aureus were 0.375 and 1.5 mg/ml respectively while MIC values for seed and root extracts against B. subtilis were 0.375 and 6.25 mg/ml, respectively and also showed week activity against gram-negative bacteria. The crude methanolic extract of I. viscosa and A. citriodora was also active against bacterial strains S. aureus and B. subtilis and inactive against E. coli. MIC value for I. viscosa extract against S. aureus were 6.25 mg/ml and against B. subtilis 0.375 mg/ml. Meanwhile, MIC value for A. citriodora against S. aureus was 12.5 mg/ml and against B. subtilis 1.5 mg/ml. Results indicate the potential antibacterial activity of I. viscosa and A. citriodora towards gram-positive bacteria such as B. subtilis and S. aureus. The extracts phytochemical screening revealed the presence of terpenoids, flavonoids and phenolics. These preliminary results would be a guide in the selection of potential candidates for further pharmacological study and in search of new drug candidate for treatment of infections caused by Gram positive bacteria (Abuhamdah et al., 2004).

Sen and Batra (2012) studied antimicrobial efficiency of Melia azedarach L. Methanol, ethanold petroleum ether and water extracts were tested against eight human pathogens like bacteria: Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, using agar well diffusion method and Minimum inhibitory concentration. The alcoholic extract of M. azedarach showed maximum zone of inhibition and minimum inhibitory concentration against all the microorganisms. The minimum zone of inhibition and comparatively greater inhibitory concentration were determined in petroleum ether and aqueous extract of M. azedarach showing less antimicrobial activity against all the experimental strains. The spectrum of activity observed in the present study may indicate that these plants could be a possible source to obtain new and effective herbal medicines to treat infections, hence justified the ethnic uses of M. azedarach against various infectious diseases (Sen and Batra, 2012).

Girish et al. (2008) studied uses of plants in treatment of burns, dermatophytes and infectious diseases are common in traditional medicine. For this purpose, the antibacterial activities of aqueous and methanol extracts of some medicinal plants were determined in vitro by agar diffusion-method against some human pathogenic bacteria. The leaves of five different plants, belonging to the different family were studied for antibacterial activity. Powdered leaf materials of all selected plants were extracted with aqueous and methanol. The antibacterial screening of aqueous and methanol extract carried out in vitro on the following bacteria viz., Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis and Yersinia enterocolitica. It has been showed
that the methanol extracts had wider range of activity on these organisms than the aqueous extracts, which indicates that the methanol extracts of all selected plants may contain the active components. This study supports traditional medicines (herbal extracts) to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases (Girish et al., 2008).

Dogruoz et al. (2008) evaluated ten aqueous and one ethanolic extracts of medicinal plants used in Turkey, and they were evaluated for antimicrobial activity. Plant extracts were prepared using distilled water and 50% ethanol. It was found that three plant extracts from the 9 plants studied had antibacterial activity. These activities were produced by the aqueous extracts of Pistacia, Tilia argentea and Anthemis pungens. All of these plant extracts had antibacterial activity against E. coli. Also, Tilia argentea and Pistacia spp. inhibited the growth of Bacillus subtilis. In addition to these bacteria, Klebsiella pneumoniae, Staphylococcus aureus and environmental Aeromonas spp. strains were inhibited by T. argentea (Dogruoz et al., 2008).

Khond et al. (2009) studied antimicrobial activities of 55 plant extracts were evaluated against twelve microbial strains using micro-broth dilution assay. Twenty one extracts exhibited antimicrobial activity against the tested microorganisms in range of 0.20 to 6.25 mg/ml. Extracts from Madhuca longifolia, Parkia biglandulosa, Pterospermum acerifolium showed highest antimicrobial potential among the tested plants (MIC 0.20 to 12.5 mg/ml). Bio-assays showed presence of multiple specifically active compounds at different RF values in various plant extracts. Acetone and ethanol extracts of M. longifolia, P. biglandulosa and P. acerifolium showed greater antibacterial activity as compared to their water extracts and could be the potential source to develop new antimicrobial agents (Khond et al., 2009).

Sharma et al. (2009) investigated the antibacterial activity of 15 medicinal plants used by tribals against UTI causing isolates. The antibacterial activity of aqueous, ethanol and acetone extracts of Coriander sativum, Abutilon indicum, Boerhavia diffusa Andrographis paniculata, Plantago ovata, Bacopa monnieri, Bauhinia variegata, Flacourtia ramontchi, Embelia ribes, Euphorbia ligularia, Zinziber officinale, Terminalia chebula, Azadirachta indica, Ocimum sanctum and Cinnamomum cassia was determined against 33 UTI isolates, that is, Proteus mirabilis, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Enterobacter cloacae, Providencia pseudomallei, Pseudomonas aeruginosa and Klebsiella oxytoca by disc diffusion method. Our studies concluded that crude extracts of the selected plants especially the acetone and ethanol extracts exhibited significant activity against UTI pathogens. It can be concluded that these plants can be used to discover natural products that may serve as lead for the development of new pharmaceuticals addressing the major therapeutic needs (Sharma et al., 2009).

Kumar et al. (2010) studied that in rural areas of West Bengal, India, several plants are commonly used as herbal medicine for the treatment of infectious diseases. Antibacterial activity of both aqueous and methanol extracts of the plants parts were used for screening. The plants screened were Psidium guajava, Andrographis paniculata, Terminalia arjuna and Adhatoda vasica. Antibacterial activity was tested against six strains of both gram-positive and gram-negative bacteria. The susceptibility of the microorganisms to the extracts of these plants was compared with each other and with selected antibiotics. The result showed that, the methanol extracts of selected medicinal plants exhibited high activity against the tested organisms rather than aqueous extract of those plants. So, the minimum inhibitory concentration (MIC) of the methanol extract of selected plants was studied. Extract from Terminalia arjuna showed higher antimicrobial activity than the extract of Psidium guajava. Abraham and Thomas (2010) investigated the antibacterial activity of Padathaali (Cyclea peltata) against three gram-positive and eight gram-negative bacterial strains. The dry crude non-polar and polar extract of whole plant of C. peltata, that is, petroleum ether, hexane, chloroform, ethyl acetate, acetone, methanol and aqueous extracts of five concentrations (1, 2, 5, 10 mg/ml), were used to investigate the antibacterial activity. NCCL standards were strictly followed to perform antibacterial disc susceptibility test using disc diffusion method. All the extracts showed varying degree of inhibitory potential against all the tested bacteria. Methanol extract of plant had higher inhibitory action against S. aureus, Streptococcus haemolytics, Klebsiella pneumonia and Proteus vulgaris. Acetone extract of plant showed maximum inhibitory action against K. pneumonia and S. haemolytics. The investigation showed the effectiveness of crude extract of this plant against tested bacterial strains. This study further suggests the use of whole plant extract in treating disease caused by tested microbial organisms (Kumar et al., 2010).

Prasannabalaji et al. (2012) evaluated the antibacterial activity of various solvent extracts of South Indian traditional medicinal plants Ocimum sanctum, Ocimum gratissimum, Aegle marmelos, and Adhatoda vasica leaves against clinical pathogens of human origin. The antimicrobial activities of different solvents crude extract of medicinal plants were tested by disc diffusion method against five bacterial pathogens: E. coli, S. aureus, S. typhi, Salmonella paratyphi and K. pneumoniae. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for evaluating the potential plant extract. The antibacterial results showed methanol extracts (0.4 g/ml) of Ocimum gratissimum and Ocimum sanctum showed maximum zone of inhibition (30 and 25.5 mm, respectively) against Salmonella typhi. MIC was tested at various concentrations from 0.625 to 0.039 mg/ml for all the plant
extracts. At the lowest concentration (0.039 mg/ml) tested, methanol extracts of *O. gratissimum* showed higher MIC against *S. typhi* and *Salmonella para typhi*; whereas the methanolic extracts of *O. gratissimum* showed potent activity against *S. aureus* at 0.078 mg/ml. Methanol extract (0.4 g/ml) of *Aegle marmelos* showed significant inhibitory activity of 22.5 mm and MIC value of 0.156 mg/ml against *E. coli* strain. The *Klebsiella* spp. was the most resistant strain of all and various concentrations *Adhatoda vasica* extract showed less activity against the tested pathogens. The present screening result demonstrated that the Indian traditional medicinal plants *O. sanctum*, *O. gratissimum*, *Aegle marmelos* methanol leaf extract has potent antibacterial activity, and the studied plants may be new source for novel antibacterial compound discovery for treating drugs resistant human pathogens (Prasannabalaji et al., 2012).

Bibi et al. (2011) checked the crude extracts and fractions of six medicinal important plants (*Arisaema flavum*, *Debregeasia salicifolia*, *Carissa opaca*, *Pistacia integerrima*, *Aesculus indica* and *Toona ciliata*) against three gram-positive and two gram-negative bacteria species using the agar well diffusion method. The crude extract of *P. integerrima* and *A. indica* were active against all tested bacterial strains (12 to 23 mm zone of inhibition). Other four plants’ crude extracts (*Arisaema flavum*, *Debregeasia salicifolia*, *Carissa opaca* and *Toona ciliata*) were active against different bacterial strains. The crude extracts showed varying level of bactericidal activity. The aqueous fractions of *A. indica* and *P. integerrima* crude extract showed maximum activity (19.66 and 16 mm, respectively) against *B. subtilis*, while the chloroform fractions of *T. ciliata* and *D. salicifolia* presented good antibacterial activities (13 to 17 mm zone of inhibition) against all the bacterial cultures tested. The methanol fraction of *P. integerrima*, chloroform fractions of *D. salicifolia* and *T. ciliata* and aqueous fraction of *Aesculus indica* are suitable candidates for the development of novel antibacterial compounds (Bibi et al., 2011).

Sattar et al. (2008) studied the antimicrobial activity of twenty wild plants growing in the western regions of the Kingdom of Saudi Arabia. The methanolic extracts from the aerial parts was determined using agar diffusion method. Eight microorganisms were used in this study, namely, *E. coli*, *P. vulgaris*, *P. aeruginosa*, *S. aureus*, *Sarcina lutea*, *Bacillus subtilis*, *Mycobacterium phlei* and *Candida albicans*. The results revealed that thirteen extracts exhibited a significant broad-spectrum antibacterial activity against both gram-positive and gram-negative bacteria. On the other hand, seven extracts showed only a narrow spectrum activity against gram-positive bacteria. All the tested extracts with the exception of one plant showed a significant antimycobacterium effect, while they have variable antifungal effects (Sattar et al., 2008).

GF et al. (2000) evaluated the antimicrobial activity of plant extracts, and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms. In addition, the possible synergistic effects when associated with antibiotics were studied. Extracts from the following plants were utilized: *Achillea millefolium* (yarrow), *Caryophyllus aromaticus* (clove), *Melissa officinalis* (lemon-balm), *Ocimum basilicum* (basil), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Syzygium joabolanum* (jambolan) and *Thymus vulgaris* (thyme). The phytochemicals benzoic acid, cinnamic acid, eugenol and farnesol were also utilized. The highest antimicrobial potentials were observed for the extracts of *C. aromaticus* and *S. joabolanum*, which inhibited 64.2 and 57.1% of the tested microorganisms, respectively, with higher activity against antibiotic-resistant bacteria (83.3%). Sage and yarrow extracts did not present any antimicrobial activity. Association of antibiotics and plant extracts showed synergistic antibacterial activity against antibiotic-resistant bacteria. The result obtained with *P. aeruginosa* was particularly interesting, since it was inhibited by clove, jambolan, pomegranate and thyme extracts. This inhibition was observed with the individual extracts and when they were used in lower concentrations with ineffective antibiotics (GF et al., 2000).

Selvamohan et al. (2012) investigated the methanol, ethanol and aqueous extracts of seven medicinal plants for activity against medically important bacteria such as *Staphylococcus* sp., *E. coli*, *Klebsiella* sp. and *Pseudomonas* sp. The *in vitro* antimicrobial activity was performed by agar well diffusion method and disc diffusion method. The ethanolic and aqueous extracts showed minimum antimicrobial activity when compared to methanolic extracts. The methanolic extract *Phyllanthus niruri* (stone breaker) showed the maximum activity against *Staphylococcus* sp. The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments (Selvamohan et al., 2012).

Bashir et al. (2012) studied many medicinal plants for their antimicrobial activity to discover new antimicrobial agents. Antimicrobial activity of aqueous and methanolic extracts of four ethnomedical plants such as *Acacia modesta*, *Thymus serpyllum*, *Syzygium cumuni* L. and *Olea ferruginea* were evaluated against five bacterial strains such as *S. aureus*, *E. coli*, *P. aeruginosa*, *S. epidermidis* and *B. subtilis* by disc diffusion method. The results showed that *Acacia modesta* showed maximum activity against *E. coli* with zone of inhibition (16.2 mm). *T. serpyllum* showed maximum activity against *B. subtilis* with zone of inhibition (13.3 mm) but no activity against *P. aeruginosa* and *E. coli*. *S. cumuni* L showed maximum activity against *S. epidermidis* with zone of inhibition (14.5 mm) but no activity against *E. coli*, *P. aeruginosa* and *B. subtilis*. Similarly *O. ferruginea* displayed maximum activity against *S. epidermidis* with zone of
inhibition (16.8 mm). Antimicrobial activity of plants was concentration dependent. Methanolic extracts of plants showed better activity than aqueous extracts (Bashir et al., 2012).

Bhardwaj and Laura (2009) studied the aqueous extracts of twenty plants which were screened by agar diffusion method for their antibacterial activity against Xanthomonas campestris pv. campestris, a causal organism of black rot of cabbage and cauliflower. X. campestris pv. Campestris was found most sensitive to the leaf extract of Camellia sinensis. Some of the other plants such as Acacia arabicae, Aegle marmelos, Acacia catechu, Achyranthus asper, Asparagus racemosus, Azadirachta indica, Callistemon lanceolatus and Acacia fernesiana, also showed inhibitory effect against the test bacteria (Bhardwaj and Laura, 2009).

Singh et al. (2012) checked the aqueous leaf extract of Euphorbia hirta, Erythrophleum suaveolens; and methanolic leaf extract of Thevetia peruviana showed antibacterial activity against extended spectrum beta lactamase (ESBL) producing bacteria E. coli, Pseudomonas, Klebsiella, MRSA (methylcillin-resistant staphylococcus aureus), Salmonella, and Proteus. Methanolic leaf extract of T. peruviana showed highest antibacterial activity against Klebsiella, E. coli (15 mm, 14 mm) respectively and significant against other. While Euphorbia hirta, Erythrophleum suaveolens showed least antibacterial activity against all these bacteria. The main purpose of the study is to eradicate the urinary tract infection problem across the world by using medicinal plants (Singh et al., 2012).

Gunalan et al. (2011) evaluated the antimicrobial efficacy of the ethanol extract of Bauhinia variegata leaves (EBV). Antibacterial activity was tested against both gram-positive and gram- negative bacteria. EBV showed varying degree of inhibitory potential against all the tested bacteria. EBV showed maximum inhibitory activity against Salmonella typhi (27 mm). This is followed by Vibrio cholera, K. pneumonia, E. coli and S. aureus. The least activity was observed for S. aureus (18 mm) (Gunalan et al., 2011).

Dusko et al. (2006) evaluated the water, ethanol and ethyl acetate extracts of 12 plants from the family Apiaceae, and they were screened for antibacterial activity against selected bacteria. The following plants were tested: Aegopodium podagraria, Angelica silvestris, Chaerophyllum bulbosum, Daucus carota subsp. carota, Foeniculum vulgare, Heracleum spondylium, Pastinaca sativa, Peucedanum cerasaria, Peucedanum oreoselimum, Pimpinella saxifraga, Sanicula europea and Torilis anthniscus. The antibacterial activities were tested in relation to the following bacterial species: Agrobacterium radiobacter pv. tumefaciens, Erwinia carotovora, Pseudomonas fluorescens and Pseudomonas glycinea. Antibacterial activity was determined by filter disc diffusion method. All the aromatic plants investigated showed antimicrobial activities against selected bacterial strains. 65.27% of all examined extracts showed antibacterial activities. The most active were the extracts of Torilis anthniscus especially in relation to Pseudomonas glycinea. Aegopodium podagraria, Daucus carota, Heracleum spondylium and Pimpinella saxifraga also showed significant antibacterial properties. The most sensitive bacteria were Pseudomonas glycinea followed by Agrobacterium radiobacter pv. tumefaciens, while the most resistant bacteria were Erwinia carotovora. Laboratory screening of plant extracts has given encouraging results indicating their potential uses in the control of selected bacteria (Dusko et al., 2006).

Sharma et al. (2010) studied the ethanolic extract of three medicinal plants viz., Acalypha indica, Ricinus communis and Euphorbia hirta were evaluated for their therapeutic potential as antimicrobial agent against six standard organisms, 3 bacteria (Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa) and 3 fungi (Aspergillus flavus, Fusarium oxysporum, Penicillium chrysogenum) by using Filter Paper Disc Method. Antimicrobial efficacy of callus cultures of E. hirta with inhibition zone of 16 mm was found to highest against F. oxysporum (fungus) and in A. indica with inhibition zone of 27 mm was found to maximum against K. pneumoniae (bacteria). On the contrary, in vivo analysis of antimicrobial efficacy of A. indica with inhibition zone of 15 mm were found to be highest against P. chrysogenum (Fungus) and E. hirta with inhibition zone of 25 mm was found to be highest against K. pneumoniae (Bacteria) (Sharma et al., 2010).

Cheruiyot et al. (2009) carried out a study to determine the antibacterial activity of plant extracts of Olea africana stem-bark, Psidium guajava leaves, Vernonia amygdalina leaves, Lantana camara leaves and Mangifera indica leaves which are used in folklore medicine to treat infections of microbial origin in Longisia region of Bomet District, Kenya. Methanol extracts were derived and screened. Standard cultures of Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Staphylococcus aureus ATCC 25923 were used in the study. The antibacterial tests used were the agar well diffusion assays at concentration 1 g/ml. Minimum Inhibition Concentration (MIC) was determined in the plant extract that showed some efficacy against the tested microorganisms. Gentamicin (10 µg) was used as a positive control. The methanol extracts showed weak antibacterial activity against the study organisms compared to Gentamicin. All extracts exhibited a significant bactericidal activity against S. aureus while L. camara and V. amygdalina lacked efficacy against P. aeruginosa and E. coli. O. africana and P. guajava presented the lowest MIC against S. aureus (62.5 and 250 mg/ml respectively). P. guajava and M. indica showed analogous MICs against P. aeruginosa (250 mg/ml). P. guajava exhibited a better MIC against E. coli (500 mg/ml). This in-vitro study corroborated the anti-
microbial activity of the selected plants used in folklore medicine. The plants could be potential sources of new antimicrobial agent (Cheruiyot et al., 2009).

CONCLUSION

The increasing incidence of drug-resistant microorganisms is a big threat to successful therapy of microbial diseases. Therefore, there is an urgent need of the time to search new antimicrobial compounds characterized by diverse chemical structures and mechanisms of action. The use of plant natural compounds as antimicrobial agents is an interesting strategy for discovering bioactive products that in upcoming future could become useful therapeutic tools. Plants are rich source of secondary metabolites, such as flavonoids, alkaloids, tannins and terpenoids, which have been found in vitro to have antimicrobial properties. Thus, it is an important task for the researcher to find out alternative medicine against dreadful human pathogens.

ACKNOWLEDGEMENT

The authors are thankful to Principal and Dean of Faculty of Pharmacy and Alternative Medicine, the Islamia University Bahawalpur, for their guidance, supervision and support during the entire research period.

REFERENCES


Singh K, Tiwari V, Prajapat R (2010). Study of Antimicrobial Activity of


