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Antibacterial Efficacy of Twenty Two Medicinal Plants Crude Extracts against Six Human Pathogenic Bacterial Strains of Hazara Davison Khyber Pakhtunkhwa

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Abstract:

Ethanolic, Methanolic, Cold water and Hot Water extracts of twenty two medicinal plants in Hazara Division were tested for antibacterial activity against six human pathogenic bacterial strains using well diffusion method. Total of twenty two medicinal plants were studied in the present investigation. In which seventeen medicinal plants were show moderate to good antibacterial activity against tested bacterial strains. Which were included Camellia sinensis, Solanum jacquinii (Solanaceae), Datura alba (Solanaceae), Withania somnifera, Swertia chirata (Gentianaceae), Solanum xanthocarpum (Solanaceae), Ricinus communis (Euphorbiaceae), Cannabis sativa (Cannabaceae), Ephedra vulgaris (Ephedraceae), Brassica nigra (Brassicaceae), Centella asiatica (Umbelliferae), Solanum nigrum (Solanaceae), Primula vulgaris (primulaceae), Cedrus deodara, Zanthoxylum armatum (Rutaceae), Mentha piperita (Lamiaceae). Other five medicinal plant which included Colycynthis vulgaris (Cucurbitaceae), Ziziphus jujuba (Rhamnacae), Carica papaya (Caricaceae), Ricinus communis. (Euphorbiaceae) and Solanum xanthocarpum show no antibacterial effects. Hot water extracts of all medicinal plants show no activity against any test organisms. Medicinal plants are growing abundantly in Hazara division and other part of Pakistan. There is also need for further investigation to explore the flora of Hazara Division and screening for new antimicrobial agents and fortitude of their Phytochemical studies to establish the types of compounds responsible for the activity and to recognize which can be used in drug development for safe health care services.

KEY WORDS:

Medicinal Plants, Antimicrobial Activity, Bacteria, Hazara Division.

INTRODUCTION:

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Plants have been used throughout the world by human beings as drugs and remedies for various diseases since time immemorial and nowadays Antimicrobial activity of medicinal plant has turn out to be a worldwide concern (Akbar *et al*., 2014). Conventionally, this treasure of information has been passed on orally from generation to generation without any written article (Perumal and Ignacimuthu,

1998, 2000). This trouble is of great issue especially in Developing countries because due to infectious diseases. Reduced susceptibility to antibacterial drugs is continuously increasing which is attributed to indiscriminate use of broad-spectrum antibacterial and immunosuppressive agents (Dean, 1996). There is a continuous and urgent need to discover new antimicrobial compounds for current new infectious diseases. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants (Cordell, 2000) and screening of these plants may result in the discovery of new effective compounds. Therefore, researchers are increasingly revolving their concentration to traditional medicine and probing for new leads to develop better drugs against wide range microbial infections including bacterial and fungal and adds urgency to the search for new infection fighting strategies (Tomoko *et al*., 2002). There is a need to search for new infection-fighting strategies to control microbial infections. Several studies have been carried out on various medicinal plants screening for their antimicrobial activity (Akbar *et al*., 2014; Zamin *et al.,* 2014, 2013; Majid *et al*., 2013; Firas *et al.*, 2008; Yoshikawa *et al.*, 2008; Morales *et al.*, 2008).

Past two decades, antibacterial efficacy of various plants and its parts like leaves, stem, seeds root, and flowers have been well recognized for some of the medicinal plants (Nandagopal *et al*., 2007; Parekh and Chanda, 2007) and just about 20% of the plants found in the worldwide have been submitted to biological or pharmacological test, and a considerable number of new antibiotics are obtained (Mothana and Lindequist, 2005).

The present investigation, we choose Twenty Two potential medicinal plants used conventionally in folks and as Unani medicines in Pakistan (Hazara Davison) and evaluated them for potential antimicrobial efficacy in order to confirm their use and to detect the new sources of antimicrobial agents.

MATERIALS AND METHODS

This research work was conducted at the Microbiology research Laboratory, Department of Microbiology, Hazara University Mansehra, Pakistan.

Plant material and extract preparation

Healthy, disease free, mature *Camellia sinensis, Solanum jacquinii Willd. (Solanaceae), Datura alba Nees. (Solanaceae), Withania somnifera (L.), Swertia chirata C.B.Clarke (Gentianaceae), Solanum xanthocarpum Schrad. Wendl. (Solanaceae), Ricinus communis L. (Euphorbiaceae), Cannabis sativa L. (Cannabaceae), Ephedra vulgaris Rich. (Ephedraceae), Brassica nigra (L.) W. D. J. Koch. (Brassicaceae), Centella asiatica (L.) Urb. (Umbelliferae), Solanum nigrum Linn. (Solanaceae), Colycynthis vulgaris (Cucurbitaceae), Ziziphus jujuba Mill. (Rhamnacae), Carica papaya Linn. (Caricaceae), Ricinus communis L. (Euphorbiaceae), Solanum xanthocarpum, Primula vulgaris (primulaceae), Cedrus deodara, Zanthoxylum armatum (Rutaceae), Mentha piperita L. (Lamiaceae) and Ficus carica (Moraceae)* were collected from different region of Harry Pur, Dhodhial, Mansehra, Balakot, Battgram, Shinkiary, Abbotabad, Nawa sher, Bafa, Qlandar Abad, Hazara University, Uagye of Hazara Division and brought to Department of Microbiology, Hazara University, Mansehra Laboratory. The leaves were cleaned with tap water. After cutting the leaf into small pieces, they were air dried in room temperature for 7 days, and then dried leaves were crushed into a fine powder by blender machine. All species were identified in the Herbarium of Hazara University, Mansehra, Pakistan. The Adebayo and Ishola (2009) method of extraction was used. Ten grams powdered was soaked in 100 ml cold water, Ethanol, Methanol and Hot water each in 250ml sterile flask and rotated on shaker at 150 rpm for 24 hours at room temperature. The extract was filtered through a muslin cloth and then centrifuged at 4400 rpm for 7 minutes. The supernatant were collected and the pellet was discarded. These steps were repeated three times.

Test microorganisms

The in-vitro efficacy of the Twenty Two plants extracts was assayed against the bacterial strains. All the ATCC (MicroBioLogics) against gram positive bacteria *S. aerious ATCC®6538, Escherichia coli ATCC®25922, Salmonella typhimurium ATCC®14028, Klebsiella pneumoniae ATCC®, Pseudomonas aeruginosa ATCC®74303 and Enterococcus faecalis* ATCC®35824. Which were kindly provided by Dr. Malik Mujaddad Ur Rehman, Assistant Professor, and HOD Department of Microbiology, Hazara University, Mansehra strains were maintained on Nutrient Agar Tubes at 4 oC. For the sub culturing of the microorganisms nutrient agar and sabouraud dextrose agar were used; and was autoclaved at 121°C for 15 min. The final pH was 7.0 = 0.2. All the culture media were prepared and treated according to the

manufacturer guidelines (DIFCO). The inoculums were prepared in N/saline or broth.

Antibacterial activity

The agar plate well diffusion method for Ethanol, Methanol, Cold water extracts and Water Extracts were employed for the determination of antibacterial activity, in which the wells are used as a reservoir of the sample dilutions and the standard dilutions. The reservoir containing the sample dilutions were brought into contact with the inoculated medium and after incubation for 18 to 24 h, the diameter of the clear zones of inhibition around the reservoirs was measured with vernier caliper (Rios *et al*., 1988). However, in case of no activity of the sample, no zone of inhibition will develop. The concentration of the extracts employed was 100 mg/ml. The dilution medium for the positive controls was sterile distilled water. The test was carried out by triplicate.

RESULTS

Ethanolic, Methanolic, Cold Water and Hot Water extracts of twenty Two medicinal plants from twenty one families used in the Unani system of medicines were screened for their antimicrobial activity against six commonly prevalent human Pathogenic gram negative and gram positive bacteria's particularly *S. aerious ATCC®6538, Escherichia coli ATCC®25922, Salmonella typhimurium ATCC®14028, Klebsiella pneumoniae ATCC®, Pseudomonas aeruginosa ATCC®74303 and Enterococcus faecalis* ATCC®35824. Ethanolic, Methanolic, Cold Water extract of seventeen plants show moderate to good antimicrobial activity against test organisms except *S. aerious ATCC®6538*, which show high resistance to all extracts. While other five medicinal plants have no activity. Hot water extracts of all medicinal plants show no activity against any test organisms. The cold extract and Ethanolic extracts of *Camellia sinensis, Mentha piperita L. (Lamiaceae) and Ficus carica (Moraceae) and Ficus carica (Moraceae)* have good antimicrobial efficacy. The remaining thirteen plants have moderate activity (Table 1).

DISCUSSION

In our present investigation, Ethanolic, Methanolic, Cold water and Hot water extracts of Twenty two medicinal plants were tested against six common human pathogenic bacterial strains. The use of these plants in Pakistan for treating with the most frequent medicinal uses being astringent, anthelmentics, antispasmodic, laxative, stomachic, diuretic etc has been already reported.

In other studies conducted in Turkey, Cambodia, Yemen, Peru, Brazil, Mexico and Cameron and Pakistan screening of antibacterial activity were conducted. In these studies, either the number of extracts were fewer than our study or the microorganisms were less in number against whom the antibacterial activity was performed (Akbar *et al*., 2014; Majid *et al.,* 2013; Turker and Usta, 2008; Chea *et al*., 2007). The antimicrobial activity of crude extracts of medicinal plants used in traditional Indian medicine was tested against five important pyogenic bacteria. They are *S. aureus, E. coli.* The most effective antimicrobial plant was identified as *Glycyrriza glabr*a followed by *Dathura metal, Coccinia grandisand Camellia sinensis* (Bagyalakshmi *et al*., 2009).

In present study, the antibacterial activity of some medicinal plants against *S. aerious ATCC®6538, Escherichia coli ATCC®25922, Salmonella typhimurium ATCC®14028, Klebsiella pneumoniae ATCC®, Pseudomonas aeruginosa ATCC®74303 and Enterococcus faecalis* ATCC®35824 has been studied. Total of twenty two medicinal plants were studied in the present investigation. In which seventeen medicinal plants were show moderate to good antibacterial activity against tested bacterial strains. Which were included *Camellia sinensis, Solanum jacquinii Willd. (Solanaceae), Datura alba Nees. (Solanaceae), Withania somnifera (L.), Swertia chirata C.B.Clarke (Gentianaceae), Solanum xanthocarpum Schrad. Wendl. (Solanaceae), Ricinus communis L. (Euphorbiaceae), Cannabis sativa L. (Cannabaceae), Ephedra vulgaris Rich. (Ephedraceae), Brassica nigra (L.) W. D. J. Koch. (Brassicaceae), Centella asiatica (L.) Urb. (Umbelliferae), Solanum nigrum Linn. (Solanaceae), Primula vulgaris (primulaceae), Cedrus deodara, Zanthoxylum armatum (Rutaceae), Mentha piperita L. (Lamiaceae). Other five medicinal plant which included Colycynthis vulgaris (Cucurbitaceae), Ziziphus jujuba Mill. (Rhamnacae), Carica papaya Linn. (Caricaceae), Ricinus communis L. (Euphorbiaceae), and Solanum xanthocarpum* show no antibacterial effects.

As Hazara division (Pakistan) is rich in medicinal and aromatic plants, and have good potential of antimicrobial agents, these can be used as a replacement of antibiotics and as natural health care products in “conventional systems” of medicine prevalent in Pakistan.

CONCLUSION

Medicinal plants are growing abundantly in Hazara division and other part of Pakistan. There is also need for further investigation to explore the flora of Hazara Division and screening for new antimicrobial agents and fortitude of their Phytochemical studies to establish the types of compounds responsible for the activity and to recognize which can be used in drug development for safe health care services.

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Table 1. Antimicrobial activity of medicinal plants/Zone of inhibition.

Plant name	A				B				C				D				E				F			
	C	E	M	H	C	E	M	H	C	E	M	H	C	E	M	H	C	E	M	H	C	E	M	H
Camellia sinensis	++	+	+	0	+	++	+	0	+	+	+	0	+	+	+	0	+	+	0	0	0	+	+	0
Solanum jacquinii	0	+	+	0	+	+	0	0	+	+	+	o	+	+	+	0	+	+	+	0	0	0	0	0
Ziziphus jujuba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Datura alba	+	+	+	0	0	+	+	0	+	+	+	0	+	+	+	0	+	+	+	0	0	+	+	0
Withania somnifera	+	++	++	0	+	++	+	0	+	+	+	0	+	++	+	0	+	+	+	0	+	0	0	0
Swertia chirata	++	++	++	0	+	++	+	0	+	++	++	0	+	+	+	0	+	+	+	0	0	0	0	0
Solanum xanthocarpum	++	+	+	0	+	++	+	0	+	++	++	0	+	++	+	0	+	+	+	0	0	0	+	0
Colycynthis vulgaris	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carica papaya	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0
Ricinus communis	0	+	0	0	0	0	+	0	0	+	0	0	0	+	0	0	0	0	0	0	0	0	0	0
Mentha piperita L.	++	+	+	0	+	+	++	0	+	++	++	0	+	++	+	0	+	+	++	0	+	++	+	0
Zanthoxylum armatum	+	++	+	0	++	+	+	0		++	++	0	+	+	+	0	++	+	+	0	0	+	+	0
Cedrus deodara	+	+	+	0	+	++	+	0	+	++	+	0	+	++	+	0	+	+	++	0	0	+	0	0
Primula vulgaris	0	++	+	0	+	++	++	0	+	+	+	0	+	++	+	0	++	+	++	0	0	0	+	0
Ficus carica	0	0	0	0	0	+	0	0	0	+	0	0	0	+	0	0	0	+	0	0	0	0	0	0
Ricinus communis	++	++	+	0	++	++	++	0	+	++	++	0	+	++	+	0	++	+	++	0	+	+	+	0
Cannabis sativa L.	++	++	+	0	+	+	+	0	+	++	++	0	+	++	++	0	++	+	++	0	+	0	+	0
Ephedra vulgaris	+	+	+	0	+	++	+	0	+	+	++	0	+	+		0	+	+	++	0	0	+	0	0
Brassica nigra	+	+	+	0	+	++	+	0	+	+	+	0	+	+	++	0	+	+	++	0		+	0	0
Centella asiatica	+	+	+	0	+	+	+	0	+	++	++	0	+	+	+	0	+	+	++	0	+	+	0	0
Solanum nigrum	+	+	++	0	++	+	++	0	+	++	+	0	+	++	+	0	++	+	++	0	0	0	+	0

(A)*Escherichia coli* (B) *Salmonella typhimurium* (C) *Klebsiella pneumoniae* (D) *Enterococcus faecalis* (E) *Pseudomonas aeruginosa* (F) *S. aerious.* Key: - denotes zone of inhibition "+" 12 mm-15 mm, "++" 18 mm – 27 mm, "0" No activity and "C" Cold water extract, "E" Ethanolic extract, "M" Methanolic extract and "H" Hot water extract.