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Antibacterial activity of two medicinal plants: *Withania somnifera* and *Curcuma longa*

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Abstract:

In vitro aqueous and ethanol extracts of Withania somnifera (leaves and fruits) and Curcuma Longa were evaluated for antibacterial activities against Basillus subtillis, Staphylococcus aureus, Escherichia coli and S.epidermitis. The inorganic extract of W. somnifera leaves showed more antibacterial activity as compared to the organic fraction. While both the organic and inorganic phases of fruit extract of W. somnifera showed antibacterial activity against all the tested microorganisms. S.epidermitis and B.subtillis were inhibited by

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inorganic fraction of fruit extract. Organic fraction of Curcuma Longa showed antibacterial activity, while there was no activity of inorganic fraction of this medicinal plant. Gentamicin showed lesser activity as compared to inorganic fraction of fruit extract of W.somnifera against all the tested microorganisms. Therefore, these plants may be used to investigate the active components of the different fractions as an important future prospective.

Key words: Withania somnifera, Curcuma longa, antibacterial activities, active components

Introduction

Man interest in plants goes back to the time immemorial of the early civilization, for basic needs namely food, shelter and clothing. He was dependent on plants. Plants do play an important role as a fulcrum in any ecosystem and also contribute to the welfare of mankind by providing food fiber, fuel, timber, medicine etc. [1] The Medicinal plants is a biosynthetic laboratory, not only for chemical compounds but also a mass of the compound from the beginning of time, herbs have been used for healing purposes and most of the world's people are still using herbs as remedies for various diseases. Synthetic drugs and new synthesized compounds with their known side effects have not been able to replace herbal medicines. All over the world there is a revival of interest in medicinal botanic. [2] Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. Many of the herbs and spices used by humans to season food yield useful medicinal compounds. [3] Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. The scope of herbal medication sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts. [4] The WHO estimated that more than 80% population of the world for some aspect of primary health care use herbal medicines. [2] It is

estimated that about 35,000 to 70,000 plants species are used as medicinal plants among 422127 species of plants. [5] A large numbers of therapeutic agents have been originated from screening of historically pharmacological natural or synthetic compounds. The most productive tool in discovering new biologically active molecules in area of antibiotics is the random screening. [6,7] In the west 25%of the pharmaceutical products are plants derived. [8] Germany is the most advanced country in the west in use of herbal products share about 50% of the total European market of herbal drugs and 70% of German physicians prescribe herbs[8].In Pakistan 80% of the people population belonging to the rural areas still depends upon the herbal medicines. [1] *Withania Somnifera* is also known as Ashwagandha nightshade family. *Withania Somnifera* grows commonly in the Eastern Cape and it is used to treat the tuberculosis, arthritis and cancer. [9] *Curcuma longa* (Turmeric) is one of the important medicinal plants that belong to Zingiberaceae family. [10]. It is well known for its medicinal properties and is widely used as a spice and colouring agent. [11] The present study was aimed to analyze the variation in the antibacterial activity of *Withania Somnifera* and *Curcuma Longa* and to compare the patterns of antibacterial activity of these medicinal plants with other pharmaceutical products. The present work was carried out in Microbiology laboratory at National Institute of Health Islamabad Pakistan.

Materials and Methods

Sample collection

The plants of *Withania somnifera* were collected from the streets of Shahzad town Islamabad, Pakistan and dry rhizomes of *Curcuma Longa* were collected from district Bannu, Khyber Pakhtunkhwa, Pakistan. The sampling was carried out during the month of June and July. The plant species were

identified by referring standard morphological characteristics features according to flora of Pakistan.

Test Microorganisms

The following four bacterial pathogenic strains were taken: *Bacillus subtillis, Staphylococcus aureus, Escherichia coli and Staphylococcus epidermitis.* These bacterial strains were obtained from stock culture of Drugs Control Department N.I.H. Islamabad.

Extraction and isolation of compound from leaves and fruits of *W. somnifera*

Fresh leaves and fruits of *Withania somnifera* were collected and washed under running tap water, shade dried and used for extraction. These separate parts of plants were kept in room for drying for 15 to 20 days at 37 °C. The dried leaves and fruits of *W.Somnifera* were crushed by electric grinder. Then 20 g crushed leaves were taken in 1 liter conical flask. The crushed leaves were soaked in 500 ml ethanol and hot water separately. These solvents were used for the extraction of active compound. The specimens were kept for about 15 days at room temperature, examined and shaked it daily. The specimens were filtered through filter papers and soluble extract were collected in 500 ml flask. The solvents were evaporated by rotatory evaporator at 50 °C for one hour. So finally 5 grams of crude extract of leaves was obtained in small bottles. Then crude extract were stored in refrigerator at 4 °C for further use.

Extraction and Isolation of Compound of *Curcuma longa*

The dry rhizomes were washed first by removing rhizoids, removing roots, soil and dirt. These were then kept in room for 20 days at 37 °C for drying. The rhizomes were then crushed with electric grinder. Then 20 gram of this powder was

taken in 1 liter conical flask. The powder was soaked in 500 ml ethanol and hot water separately. These solvents were used for the extraction of active compound. The specimens were kept for about 15 days at room temperature, examined and shaked it daily. The specimens were filtered through filter papers and soluble extract were collected in 500 ml flask. The solvents were evaporated by rotatory evaporator at 50 °C for one hour. So finally 5 grams of crude extract of turmeric powder was obtained in small bottles. Then crude extract were stored in refrigerator at 4 °C for further use.

Preparation of test plates and Loading of extract in wells

Nutrient medium was used for the growth of bacteria. 50 ul of culture was poured into each Petri plate using a micropipette. The Petri plate was placed on turn table and culture was evenly spread on the medium using sterile steel spreader. Agar well diffusion method was used and wells were made in the agar by using a cork borer, diameter size of 8 mm. The cork borer was sterilized before used. Ten wells were made in each Petri plate, nine at the periphery and one in center. The wells were loaded with the 0.5 ml of plant extract with the help of micropipette. Gentamicin 30 ug was used as control antibiotic for comparing the activity with the plant extracts. The petri plates were then incubated at 35 °C in the incubator for 24 hours. After 24 h of incubation, the digital Vernier caliper was used to measure the zone of inhibition for each plant extract and the results were recorded. Each experiment was repeated three times. The solvents used for extraction were checked in pure form for antibacterial activity as control. Actual zone of each plant extract was calculated by subtracting the control solvent activity from the extract activity.

Results and Discussion

Leaves of *W. somnifera*

Inorganic fraction of *W.somnifera* showed antibacterial activity, while organic fraction had no antibacterial activity .*S. aureus* was inhibited by water extract with an inhibition zone of 19.2 mm. *S.epidermitis* was inhibited by the water extract with a zone of 19.5 mm while E.coli was inhibited by the water extract with inhibition zone of 20.2 mm *B. subtillis* was inhibited by the water extract with inhibition zone of 15.2 mm (Table 1). Mahesh and Satish (2008) used the methanol extracts of leaf of *Tinospora cordifolia, Ziziphus mauritiana, Withania somnifera, Acacia nilotica* and *Sida cordifolia* that showed considerable bioactivity against *Staphylococcus aureus,, Xanthomonas axonopodis pv. Malvacearum, Bacillus subtilis, Pseudomonas fluorescens* and *Escherichia coli.* The leaf extracts of these plants also showed antifungal activity against *Fusarium verticillioides, Dreschlera turcica* and *Aspergillus flavus. B. subtilis was* highly inhibited by the leaf extract of *A. nilotica* and *S. cordifolia.* All the tested bacteria were highly inhibited by root and leaf extract of *S. cordifolia*. The bark and leaf extract of *A. nilotica* showed considerable bioactivity against the fungi *A. flavus*, *F. verticillioides* was inhibited by the methanol extract of *Sida cordifolia*. [12] Kumar and Vinoth (2011) examine the Leaf and root samples of *Withania somnifera* for their antimicrobial potential against some human pathogenic bacteria (*Escherichia coli, Bacillus* and *Shigella*) fungi (*Aspergillus niger* and *Trichophyton rubrum*) growth inhibition was observed in different volumetric concentrations of this extract. [13] Leaf sample showed higher antimicrobial activity than the root sample. These results confirm the antimicrobial property of *W. somnifera* leaf and root support the traditional use of the plant in therapeutic use against microbial infections.

Fruit of *Withania somnifera*

Inorganic and organic fractions of *W.somnifera* fruit showed antibacterial activity. *S. aureus* was inhibited by water extract with a zone of 21.2 mm. Ethanolic extract showed antibacterial activity against *S. aureus* with an inhibition zone of 16.2 mm. *S.epidermitis* was inhibited by water extract with a zone of 28.0 mm while ethanolic extract showed inhibitory zone of 15.6 mm. *E.coli* was inhibited by ethanolic extract with an inhibition zone of 27.0 mm while ethanolic extract gave inhibition zone of 17.1 mm. *B. subtillis* was inhibited by water extract with an inhibition zone of 28.1 mm and the ethanolic extract gave inhibition zone of 16.7 mm (Table-1 and Figure-1). Antibacterial activity of Gentamicin was checked as positive control against mentioned clinical isolates. Extract in buffer phosphate (ph 8) show antibacterial activity. *Staphylococcus aureus* inhibited in buffer phosphate extract with a zone of 24 mm. *S.epidermitis* inhibited in buffer phosphate extract with a zone of 26 mm. *E.coli* inhibited in buffer phosphate extract with inhibition zone of 24.0 mm. *B. subtillis* inhibited in buffer phosphate extract with inhibition zone of 25.4 mm (Table-1). In vitro antibacterial activity of methanol and aqueous extracts of some medicinal plants were screened against multi-drug resistant bacteria including *Proteus mirabilis, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumonia, S.aureus* and *E.coli* isolated from clinical specimen by Rajendran and Ramakrishnan (2009). [14] The higher antibacterial activities were showed by methanol extracts of *W.somnifera* compared to other plant extracts tested.

Bioactivity of *Curcuma Longa*

Antibacterial activity of *Curcuma Longa* was checked against mentioned clinical isolates. Organic fraction of *Curcuma Longa* showed antibacterial activity, while there was

no activity of inorganic fraction of this medicinal plant. *Staphylococcus aureus* inhibited in ethanolic extract with a zone of 25.2 mm (Table-1). Water extract did not show antibacterial activity against *Staphylococcus aureus.* The ethanolic extract inhibited *S.epidermitis* with a zone of 26.00 mm and water extract showed no inhibitory zone. *E.coli* inhibited in ethanolic extract with inhibition zone of 22.2 mm and water extract give no inhibition zone. *B.subtilis* inhibited in ethanolic extract with inhibition zone of 18.5 mm and water extract give no inhibition zone.

Tables and Figures

Table-1: Antibacterial Activity of *Withania somnifera* (Leaves and fruit) and *Carcuma Lunga* along with positive control (Gentamicin)

Sample	**Solvent**	**Zone of Inhibition (mm)**			
		Staphylococcus aureus	***Staphylococcus epidermitis***	***Escherichia coli***	***Bacillus subtilis***
***Withania somnifera* Leaves**	Ethanol	-	-	-	-
	Aqueous	19.2	19.5	20.2	15.2
***Withania somnifera* Fruits**	Ethanol	16.2	16.6	17.1	16.7
	Aqueous	21.2	28	27	28.1
Curcuma Longa	Ethanol	25.2	26	22.2	18.5
	Aqueous	-	-	-	-
Positive Control (Gentamicin)	Buffer Phosphate	24	26	24	25.4

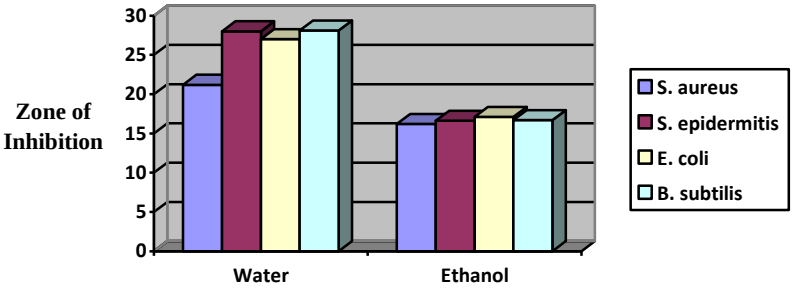

Figure 1: Antibacterial Activity of *Withania somnifera* Fruits

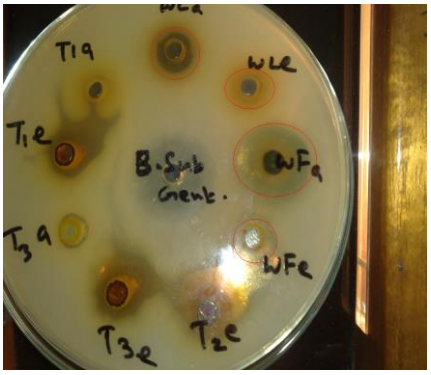

Figure 2: Zone of Inhibition against *B.subtilis*. Circles indicate the inhibition zones by different plant extracts

Conclusion

Plants extracts have great potential as antibacterial compounds against bacteria. It is concluded from the present study that the aqueous and ethanolic extract of *Withania somnifera* and ethanolic extract of *Curcuma Longa* might be exploited as natural drugs for the treatment of many infectious diseases caused by these organisms.

BIBLIOGRAPHY:

Ahmad, M., M. Qureshi, A. khan and M. Saqib. 2003. "Ethnobotanical studies of some cultivated plants of chhahh region (District Attock)." *Pakistan. Scient. Khyb*. 16 (2):109-121. [4]

Ahmad, T. 2004. "Medicinal Herbs and Products." *Pharma professional services Karachi, Pakistan*. 1: 15-18.[1]

Chattopadhyay, I., K. Biswas, U. Bandyopadhyay and R.K. Banerjee. 2004. "Turmeric and curcumin: Biological actions and medicinal applications." *Curr. Sci*. 87: 44-53. [10]

Gerhartz, W., Y.S. Yamamota, F.T. Campbell, R. Pfefferkorn and J.F. Rounsaville. 1985. Ullmann's, *Encyclopedia of Industrial*. [6]

Gilani, A. H. 2004. "Role of Ethnopharmacology in development of modern medicine." In *International symposium medicinal plants: Linkage beyond national boundaries*, edited by: Shinwari, Z. K., T.Watanabe, M.Ali and R. Anwar. Kohat University of Science and Technology Kohat, 192-197. [8]

Gill, M. A. 2003. "Cultivation of Medicinal and Aromatic Herbs: Experience of IMHSC."In *Ethnomed plants*, edited by P. C. Trivedi and N. K. Sharma, 1. [3]

Hassan, A., M. A. Khan and M. Ahmad. 2007. "Authencity of folk medicinal plants of Pakistan, taxonomic and Chemical Methods." 1-5. [5]

Kroschwitz, J.I. and M. Howe-Grant. 1992. *Kirk-Othmer encyclopedia of chemical Technology*. 2: 893. [7]

Kumar, S. and V. Kumar. 2011. "Evaluation of *Withania somnifera* (Dunal). (Solanaceae) Leaf and Root Extracts as an Antimicrobial Agent-Highly Medicinal Plants in India." *Asian J. Exp. Biol. Sci.* 2(1): 155-157. [13]

Luthra, P.M., R. Singh and R. Chandra. 2001. "Therapeutic uses of *Curcuma longa* (Turmeric)." *Indian J. Clin. Biochem*. 16: 153-160. [11]

Mahesh, B. and S. Satish. 2008. "Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens." *World. J. Agri. Sci.* 4: 839-843. [12]

Rajendran, N.K. and J. Ramakrishnan. 2009. "*In Vitro* evalution of antimicrobial activity of crude extracts of medicinal plants against multi-drug resistant pathogens." *Biyoloji Bilimleri Araptyrma Dergisi* 2 : 97-101. [14]

Shinwari, Z. K., Watanabe, T. Rehman and T. Yoshikawa. 2006. *A pictorial guide to medicinal plants of Pakistan.* [2]

Van Wyk B.E., B. Van Oudshoorn and N. Gercke. 1997. "Medicinal Plants of South Africa." *Briza, Pretori* 304. [9]