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Comparative *in vitro* activity of ethanol and hot water extracts of *Zanthoxylum armatum* to some selective human pathogenic bacterial strains

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Key words: Antibacterail activity; ethanol extract; hot water extract; zone of inhibition; *Z. armatum*.

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Abstract

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Pakistan has a great wealth of *Z. armatum* which has valuable use as oil not only in Pakistan but all over the world. The present study was carried out in vitro to determine Comparative activity of *Z. armatum* leaf extracts against Pathogenic bacterial strains by using leaf Hot water and Ethanol extracts. Antimicrobial activity of *Z. armatum* was evaluated by well diffusion methods. The highest zone of inhibition produced by Ethanol extract as compared to Hot Water. *Escherichia coli* 15mm and 2 mm, *Enterococcus faecalis* 14mm and 2 mm, *Pseudomonas aeruginosa* 33 mm and 3 mm, *Klebsiella pneumoniae* 28 mm and 2mm, *Staph. Aureus* 12 mm and 2 mm and *Salmonella typhimurium* 28 mm and 2 mm on Ethanol and Hot water extract respectively. This study concludes that Ethanol extract has more effect then Hot water extract and further work should be done to investigate and find out the active Chemical compounds responsible for the biological activity and to find out the accurate mechanism of action by which extracts exert their antimicrobial effect to recognize which can be used in drug development for safe health care services.

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Introduction

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Nowadays Antimicrobial activity of medicinal plant has turn out to be a worldwide concern. This problem is of great issue especially in Developing countries because due to these infectious diseases. There is a continuous and urgent need to discover new antimicrobial compounds for new infectious diseases. 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 (Majid *et al.,* 2013). Although hundreds of plants species have been experienced for antimicrobial properties, the vast majority have not yet been adequately examined for their possible antimicrobial activity against verities of infectious microorganism (Balandrin *et al.,* 1985).

The *Z. armatum* perennial plant belongs to the family Rutaceae and is distributed in the temperate and subtropical Himalayas. It is a large genus of aromatic prickly trees or shrubs (Ranawat *et al.,* 2013). The pleasing astringent taste and stimulating boost it provides is so deep-pervasive that its potential health remuneration and medicinal properties are often overlooked. Ongoing scientific exploration points that the certain potential health benefits derived from oil have important implications on human health. The fruits and seeds are employed as an aromatic tonic in fever, dyspepsia, carminative, stomachic, anthelmintlc and expelling roundworms. The volatile oil is employed as an antidiarrehal, antiseptic, deodorant and anticataerhal. Almost all parts of the plant are aromatic and hence, supposed to possess essential oil. The essential oil composition can provide much more knowledge regarding the medicinal proper-ties and active constituents of this plant. The pharmaceutical companies generally use *timur* fruit for making different types of toothpaste (Waheed *et al.,* 2011).

The beneficial medical effects of plants materials typically results from the combination of secondary metabolites present in plant. Main compounds present in *Z. armatum* are Tannis and Phenols, Alkaloids, Flavoniods, steroid, Resin and fatty acid gum and Anthraquinones Glycoside (Joshi *et al.,* 2009; Mehta *et al.,* 2013).

Extracts of *Z. armatum* leaves contain Tannis and Phenols and Flavoniods components with activity against a wide spectrum of microbes. In modern era, herbal has drawn attention to identified and isolate easy accessible antimicrobials agent from natural source to cure various infectious diseases (Ullah *et al.,* 2011). Joshi *et al.,* (2009) found that *Z. armatum* have a wide range antimicrobial activity against deferent pathogenic bacteria. The extract of *Z. armatum* have high potential antimicrobial activity against *Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis, Staphylococcus aureus, Pseudomonas* spp, *Proteus* spp, *Salmonella* Typhi*, Escherichia coli, Shigella dysenteries* and *Klebsiella pneumoniae* (Guleria and Kumar, 2006; Ullah *et al.,* 2009; Joshi *et al.,* 2009; Ranawat *et al.,* 2013).

The beneficial medical effects of plants materials typically results from the combination of secondary metabolites present in plant. The most activity of concerned with some compound Like Alkaloids also contain *Z. armatum* when its extracts is heated in boiling water it's denature and lose its activity (Mehta *et al.,* 2013). The present study was conducted to investigate the comparative activity ethanol and hot water extracts of *Z. armatum* against gram positive and gram negative ATCC (American Type Cell Culture) 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.

Materials and methods

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

Plant materials

Healthy, disease free, mature *Z. armatum* Plant was collected directly from local region of Tehsil Ugaye District Mansehra, Khyber Pakhtunkhwa and leaf was separate from Plant and brought to Microbiology Research Laboratory, Department of Microbiology, Hazara University, Mansehra. 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.

Chemicals

All agars and chemicals used in this study were of highest grade of purity and were purchased from Oxoid (UK), Fluka Chemika, and Difco Laboratories, USA.

Leaf extraction

Ethanol and Hot Water extracts

The Adebayo and Ishola (2009) method of extraction was used. Ten grams powdered samples of leaf was soaked in 100 ml Hot Water and Ethanol 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.

Preparation of Media and Culturing of Microorganisms

To determine the activity of different plant extracts microorganisms were grown on Nutrients agar media. Media preparation was done according to manufacturer's instructions.

Nutrient agar

Nutrient Agar was enrichment medium for the growth of microorganisms. Medium was prepared by adding 13g of dehydrated powder using electrical balance into 1 liter of distilled water. PH was adjusted by electrical pH meter at 7.4 and was boiled to dissolve completely.

Media sterilization

All Media were sterilized by using automatic autoclave (SANYO) at 121°C for 15 minutes.

Media pouring and drying

Media was poured in pre-sterilized glass Petri plates of 90mm in Laminar Flow Hood which was sterilized by overnight exposure of UV light and disinfected with 70% ethanol solution. Media plates were kept open for half an hour in the Laminar Flow Hood for drying and solidifying media.

Test microorganisms

The in-vitro activity of the extracts was assayed against the bacterial strains. All the ATCC (MicroBioLogics) against gram positive and Negative 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 °C.

Inoculation of test organisms

100µl of 1McFarland bacterial suspensions were aseptically introduced and spread using pre-sterilized cotton swabs on surface of Nutrient Agar plates.

Wells preparation by cork borer

Agar well diffusion techniques as described by Adeniyi *et al.,* (1996). Wells of 6mm diameter with sterile cork borer were aseptically punched in the 90mm Nutrient Agar plates.

Evaluation of antimicrobial activity

Antimicrobial activity of *Z. armatum* leaf extract was tested using agar well diffusion method. With the help

of sterile micropipette tips *Z. armatum* leaf extract (cold water) 100µl were poured into the wells. The plates were incubated at 370C for 24 hours. After incubation, the diameter of the resulting zone of inhibition was measured with the help of Digital Vernier Caliper (Mitutoyo) and the average values were recorded. Each antimicrobial assay was performed three times. Mean values were reported in this report.

Data analysis

All data were measured average value of three replicates and standard error (±). Results were subjected to Microsoft excel 2007 and through SPSS 16.0 using Chi square test.

Results

In the present study, the antimicrobial activity of the Hot water and Ethanol extracts against gram negative and gram positive bacterial strains and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values.

Comparative Activity of Ethanol Extract and Hot Water

The Hot water and Ethanol extracts of investigated plant species *Z. armatum* in which Ethanol extracts showed high range of antimicrobial activities against all tested ATCC bacterial strains then Hot water extracts. Results of the antimicrobial activity obtained using the well diffusion assay is summarized in Table 1 and Figure 1, 2, 3, 4, 5, 6 and 7.

Table 1. Zone of inhibition against the tested organisms by ethanol extract and Hot water by well Diffusion Method.

Z. armatum Zone of inhibition against the tested organisms by Hot water and ethanol extract by well Diffusion Method.

	Spp	ATCC®	Ethanol extract	Hot water extract
1	*Escherichia coli*	25922	15 ± 1 mm	2 ± 2 mm
2	*Enterococcus faecalis*	35824	14 ± 1 mm	2 ± 2 mm
3	*Pseudomonas aeruginosa*	74303	33 ± 1 mm	3 ± 2 mm
4	*Klebsiella pneumoniae*	45321	28 ± 1 mm	2 ± 2 mm
5	*Staph. Aureus*	6538	12 ± 1 mm	2 ± 2 mm
6	*Salmonella typhimurium*	14028	28 ± 1 mm	2 ± 2 mm

Discussion

Our present research work was planned to carry out the study on comparative activity of ethanol and hot water extracts of *Z. armatum* against to some selective Human pathogenic bacterial strains. The extracts of *Z. armatum* exhibited greater extend of antibacterial activities.

The antimicrobial activities of medicinal plants are qualified due to the presence of alkaloids, Steroid and Flavoniods (Burapedjo and Bunchoo, 1995; Fewell and Roddick, 1993). These reports and presence of flavoniod, alkaloids and Steroid in different extract of *Z. armatum* confirm it's prospective against all selected pathogens bacterial strain.

The present study suggests that the Ethanol extract of *Z. armatum* have a board spectrum of antimicrobial activity as compared to Hot Water extract, Because the alkaloids present in the secondary compound are denature in heated water, so current results confirmed it, although the extent of susceptibility could diverse between different microorganisms. The antibacterial activity found in this current conducted research may be attributed to the occurrence of secondary metabolites either independently or in recipe of various types of chemical composition present in the plant products. The plant active substances were soluble in organic solvents so plant extracts obtained more activity than commercial antibiotics (Boer *et al.,* 2005).

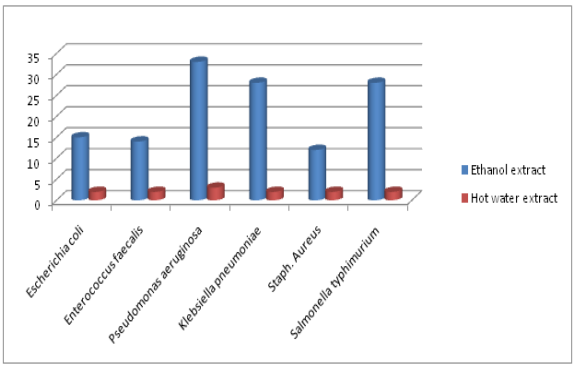

Fig. 1. Comparative activity of Ethanol extract and Hot water extract against tested organism.

Fig. 2. Zone of Inhibition of *Z. armatum armatum* against *E. coli*.

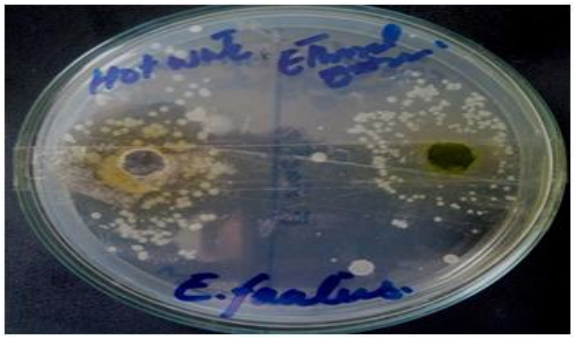

Fig. 3. Zone of Inhibition of *Z.* against *E. faecalis*.

Fig. 4. Zone of Inhibition of *Z. armatum armatum* against *Klebsiella pneumonia*.

Results of this study showed that the potential usefulness of *Z. armatum* in the treatment of various pathogenic or infection diseases as it may help in the innovation of new chemical classes of antibiotics or drugs that could serve as selective agents for the protection of human health and may provide life tools for the study of bacterial diseases or infection. In current study the leaf extracts of *Z. armatum* show great extant to different human pathogenic bacteria strains. *Z. armatum* Zone of inhibition against the tested organisms by Hot water and ethanol extract by well Diffusion Method showed *Escherichia coli* 15mm and 2 mm, *Enterococcus faecalis* 14mm and 2 mm,

Pseudomonas aeruginosa 33 mm and 3 mm, *Klebsiella pneumoniae* 28 mm and 2mm, *Staph. Aureus* 12 mm and 2 mm and *Salmonella typhimurium* 28 mm and 2 mm on Ethanol and Hot water extract respectively.

Fig. 5. Zone of Inhibition of *Z.* against *Pseudomonas aeruginosa*.

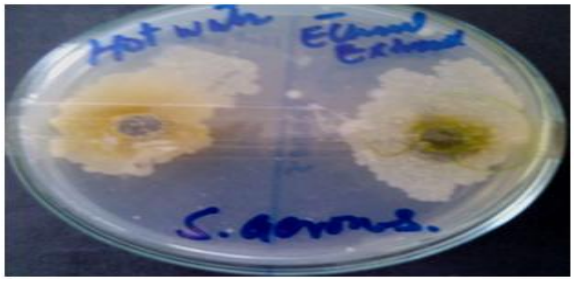

Fig. 6. Zone of Inhibition of *Z. armatum armatum* against *Staph. aureus*.

Antibacterial agents currently available in the market are limited due to their toxicity, low effectiveness and prove expensive in case of prolonged treatment. The discovery of a potent remedy from plant origin will be a great advancement in microbial infection therapies. Therefore, there is needed to develop new antibacterial agents which can satisfy the present demand.

Fig. 7. Zone of Inhibition of *Z.* against *Salmonella typhimurium*.

Conclusion

This study concludes that Ethanol extract has more effect then Hot water extract and further work should be done to investigate and find out the active Chemical compounds responsible for the biological activity and to find out the accurate mechanism of action by which extracts exert their antimicrobial effect to recognize which can be used in drug development for safe health care services.

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Competing interests

The author and co-authors have no competing interests.

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