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ANTIBACTERIAL AND ANTIOXIDANT EFFECTS OF *ZANTHOXYLUM ARMATUM* DC EXTRACTS

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Abstract

Antibacterial and antioxidant activities of three different extracts (acetone, methanol, and n-hexane) of *Zanthoxylum armatum* fruits and leaves were investigated. The maximum inhibition zone was shown by methanolic leaf extract against *K. pneumonia* (20.51 ± 0.93). The minimum inhibition zone (12.01 ± 0.93) was exhibited by methanolic fruit extract against *S. typhi*. The highest antioxidant activity was recorded at 1000 ppm. Methanolic leaf extract was the most antioxidant (89.8%) among all the extracts, followed by methanolic fruit extract (88.6%). The methanolic and acetonic extracts showed good antibacterial activity as compared to n-hexane extracts and n-hexane was less antioxidant.

Introduction

The genus *Zanthoxylum* of the family Rutaceae is economically important due to its nutritional, industrial and therapeutic values (Seidemann 2005). *Z. armatum* is a short tree or a shrub that grows under dry conditions. This plant is found at low foothills at a height of about 800 m to 1500 m and is found in different areas of Pakistan (Shinwari *et al*. 2006). It is also extensively found in several countries like Bangladesh, Bhutan, China, India, Indonesia, Japan, Korea, Laos, Myanmar, Nepal, Pakistan, Taiwan, Thailand and Vietnam (Vashist *et al*. 2016).

The most frequently used part of this plant is fruit. Fruit is utilized as carminative, and also for treatment of toothache and heartburn (Abbasi *et al*. 2010) as well for curing cold and cough, cholera, fever, headache, bronchitis, tonsillitis, asthma, colic vomiting, dizziness, indigestion, fibrosis, high altitude sickness, skin diseases, limb numbness, dysentery, diarrhea and dental problems (Islam *et al*. 2009, Bisht and Chanotiya 2011). Its fruits and seeds can be utilized as a flavoring agent (Barkatullah *et al*. 2014). Powder of about 5 - 6 seeds is taken with warm water, two times a day for a week to treat malfunctioning of the liver, cold, stomach pain and constipation (Rai and Pokharel 2006). Generally, leaves play an essential role in catching fishes. A mixture of leaves is taken orally to cure stomach discomfort and leukoderma (Manandhar 2002). A lotion made from leaves is used for the treatment of scabies, snake bite and also expels worms from the body (Wangkhem *et al*. 2011).

At present, antibiotic resistance of bacteria has caused worrying situations, and there is a continuing need to develop alternative therapeutic agents. Therefore, antimicrobial drugs from medicinal plants are being developed for the treatment of infectious diseases. The free radicals are formed inside the body by natural mechanisms (Halliwell 2011). On the other hand, if these free radicals are produced in large amounts, it will cause oxidative stress (Elahi *et al*. 2009). Thus in the present study the antibacterial activity of *Z. armatum* extracts against selected pathogenic bacteria and the antioxidant activity were investigated.

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Materials and Methods

Fresh leaves and fruits of *Z. armatum* were collected from Haripur, Khyber Pakhtunkhwa (KP) and was identified by a taxonomic expert from the Department of Botany, Government Postgraduate College, Haripur.

Plant samples (fruits and leaves) were washed separately under running tap water to remove dirt particles and sand and placed in the shade for about 15 days to completely dry.

The dried sample was pulverized into a fine powder using an electric grinder. The leaves and fruits powder were separately soaked in several solvent systems including acetone, methanol and n-hexane by using the ratio of 60 gm/500 ml, 500 ml of methanol and 250 ml of acetone and n-hexane for about 15 days. The extracts were filtered by using Whatman filter paper No. 1. After 15 days, the filtrate was concentrated under reduced pressure using a rotary evaporator. The extract was prepared at Chemistry laboratory of Government Postgraduate College Haripur. The extract was stored at a low temperature for further study. The human pathogenic bacteria were isolated from different diseased samples comprising blood, sputum, urine, pus from patients of different ages and gender in Pathology Lab of Pakistan Institute of Medical Sciences (PIMS), Islamabad. The collected microorganisms were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus* spp., *Salmonella typhi*, *Escherichia coli*, Methicillin-resistant strain and *Streptococcus* spp. The experimental work for determining antibacterial activity was carried out at Welwrd Pharmaceuticals Industry Hattar, Haripur. The antibacterial activity of various extracts of *Z. armatum*, well diffusion method was used along with specific changes described by Rahman *et al*. (2015). Nutrient agar was used for culturing bacteria in the Petri plates which were kept at room temperature for solidification. The selected bacterial colony was picked up and then dissolved in saline water. The dissolved colony was spread over the surface of solidified agar and then 5 mm diameter wells were made and sterilized pair of forceps was used for the removal of agar plug. Different extracts were poured into the wells with the help of micropipettes. The Petri plates were incubated at 37°C for 24 hrs. The inhibition zone (mm) was determined around the well using Vernier caliper.

Four different antibiotics, namely cefixime (200 µg/ml), ceftriaxone (200 µg/ml), vancomycin (200 µg/ml) and ciprofloxacin (200 µg/ml) were evaluated by agar diffusion method for the selected bacterial strains.

The diphenylpicrylhydrazyl (DPPH) free radical scavenging activity of *Z. armatum* extracts was carried out by using the protocol of Asma *et al.* (2011) with minor modifications. DPPH (4 mg) was dissolved in 100 ml of several organic solvents (methanol, acetone, and *n*-hexane) to obtain a stock solution. The 3 ml stalk solution was added to each tube and various concentrations of plant extracts (10, 100 and 1000 ppm) were added. The mixture in the test tube was continuously shacken in the dark for about 30 min and the absorbance was measured at 517 nm by using a spectrometer (Irmeco Germany, model U2020). Ascorbic acid was used as a positive control and scavenging activity calculated by a formula:

$$\% = \frac{\text{Abs of control} - \text{Abs of extract} \times 100}{\text{Abs of control}}$$

Each experiment was repeated three times, and the mean ± standard deviation (M ± Sd) was calculated.

Result and Discussions

The results of the present study showed fluctuation among different extracts. The acetonic and methanolic extract showed an excellent antibacterial effect, while n-hexane extract exhibited a

minimum antibacterial effect against tested bacterial pathogens. In the extract, the methanol leaf extract had the highest inhibition rate against *K. pneumoniae* (20.51±0.93 mm), and the methanol extract had the lowest inhibition rate against *S. typhimurium* (12.01±0.93 mm), as shown in the Table 1. The plant extracts exhibited moderate antibacterial activity against tested bacterial pathogens in comparison to the standard antibiotics. This statement is also justified by the results of (Negi *et al*., 2012). According to Wazir *et al.,* (2014) the zone of inhibition of methanolic leaf extract against K. pneumoniae was 19±0mm. **Table 1.**

Table 1. Inhibition zone (mm) of different *Z. armatum* extracts against human pathogenic bacteria.

Bacterial strains	Leaf Extracts			Fruit Extracts		
	n-hexane	Acetonic	Methanolic	*n*-hexane	Acetonic	Methanolic
K. pneumoniae	0 ± 0	18.41 ± 0.18	20.51 ± 0.93	14.01 ± 0.77	19.35 ± 0.35	14.48 ± 0.57
S. aureus	0 ± 0	15.05 ± 1.59	15.61 ± 0.74	13.26 ± 0.78	15.69 ± 1.13	0 ± 0
S. aureus (M)	0 ± 0	16.93 ± 0.96	0 ± 0	0 ± 0	14.72 ± 0.83	0 ± 0
P. aeruginosa	0 ± 0	16.42 ± 0.93	0 ± 0	0 ± 0	14.3 ± 0.95	0 ± 0
E. coli	0 ± 0	15.20 ± 0.9	0 ± 0	13.76 ± 0.70	12.36 ± 0.96	0 ± 0
S. typhi	14.36 ± 1.13	18.54 ± 1.07	16.42 ± 0.87	0 ± 0	15.38 ± 1.43	12.01 ± 0.93
Streptococcus	15.17 ± 0.83	14.39 ± 0.71	16.43 ± 0.97	0 ± 0	12.82 ± 0.79	13.82 ± 2.09
Enterococcus	0±0	17.22 ± 1.14	13.25 ± 1.05	15.27 ± 1.09	17.48 ± 1.01	14.4 ± 1.06

In the above results, (0 ± 0) indicates no sensitivity, (11 - 14 mm) indicates low sensitivity and (15 - 25 mm) indicates high sensitivity: (*Staphylococcus aureus* Methicillin-resistant strain).

The antibacterial activity of different antibiotics showed that all of them have good antibacterial activity except ceftriaxone, and ceftriaxone was inactive against *E. coli* (Table 2). The highest area of ceftriaxone was against *Streptococcu*s (25.90 ± 0.95 mm), and the lowest area of ciprofloxacin was against *Staphylococcus aureus* (12.97 ± 0.62 mm) **(**Table 2).

Table 2. Inhibition zone (mm) of different antibiotics against human pathogenic bacteria.

Bacterial strains	Antibiotics			
	Cefixime (µg/ml)	Ceftriaxone (µg/ml)	Vancomycin (µg/ml)	Ciprofloxacin (µg/ml)
K. pneumoniae	18.73 ± 0.65	19.58 ± 1.00	22.49 ± 0.78	25.23 ± 1.16
S. aureus	21.08 ± 1.47	15.57 ± 1.30	20.46 ± 1.39	19.6 ± 1.67
S. aureus (M)	18.37 ± 1.22	14.30 ± 1.60	16.87 ± 1.18	12.97 ± 0.62
P. aeruginosa	20.41 ± 0.79	14.4 ± 0.91	22.55 ± 0.85	18.53 ± 0.98
E. coli	16.24 ± 1.13	0 ± 0	23.72 ± 1.22	17.76 ± 1.45
S. typhi	19.62 ± 0.9	14.41 ± 1.41	22.89 ± 0.96	20.57 ± 1.38
Strepto spp.	18.22 ± 2.90	25.90 ± 0.95	24.22 ± 1.88	23.91 ± 1.75
Entero spp.	18.42 ± 1.03	16.1 ± 0.36	23.61 ± 1.22	20.4 ± 0.60

In the above results, (0 ± 0) indicates no sensitivity, (11 - 14 mm) indicates low sensitivity and (15 - 25 mm) indicates high sensitivity.

Antioxidant activity of three different extracts (acetonic, methanolic and n-hexane) of fruits and leaves of Z. armatum was determined by DPPH free radical scavenging method. Absorbance at different concentrations (10ppm, 100ppm and 1000ppm) were recorded at 517nm. Highest absorbance was recorded at 1000ppm. Ascorbic acid was taken as a reference which showed the highest antioxidant activity. After ascorbic acid, the highest antioxidant activity was shown by methanolic leaf extract (89.8%), then methanolic fruit extract (88.6%), then acetonic leaf extract (71.9%), then acetonic fruit extract (69%). The n-hexane leaf and fruit extracts are less antioxidant as compared to methanolic and acetonic extracts. **Table 3**.

Table 3. Antioxidant activity of different extracts of *Z. armatum.*

Conc. (ppm)	Standard % DPPH scavenged	Leaf extract % DPPH scavenged	Fruit extract % DPPH scavenged
Acetonic extract			
10	66.4	18.4	11
100	71.9	56.3	46
1000	88.5	71.9	69
Methanolic extract			
10	69.4	55	53
100	80	69	66
1000	94	89.8	88.6
n-hexane extract			
10	57.6	27.5	17
100	69.8	42.3	31.8
1000	76.3	58	44.9

Similarly, Akbar *et al*. (2014) reported the antibacterial activity of ethanolic extract of leaves of *Z. armatum*, the inhibition zone against *K. pneumoniae* was 28 ± 1 mm, whereas in the present study the inhibition zone of methanolic leaf extract was 20.51 ± 0.93 mm. The results of Yasmin *et al*. (2015) were slightly different from the present findings, the methanolic extract of *Z. armatum* leaves possess moderate antibacterial activity against *K. pneumoniae* (14.0±1.0mm). This variation might be due to bacterial strains. In the present study, the *n*-hexane leaf extract remained inactive against *K. pneumoniae*, which is similar to the findings of Negi *et al*. (2012). Joshi *et al*. (2009) reported that *K. pneumoniae* was the most resistant microorganism towards the ethanolic extract of *Z. armatum*. The zone of inhibition of ciprofloxacin was 25.23 ± 1.16 mm while according to Negi *et al*. (2012) it was 18.6 mm.

In the present study, acetonic fruit extract and methanolic leaf extract possesses significant activities against *S. aureus* (15.69 ± 1.13 and 15.61 ± 0.74 mm). Tanushree *et al*. (2017) reported that *Z. armatum* extracts were most effective against *S. aureus* with MIC value of 125 µg/20 µl. Similar results were also reported by Joshi *et al*. 2009. In the present study, the *n*-hexane leaf extract remained inactive against *S. aureus*, which is similar to Barkatullah *et al*. (2011). The inhibition zone of ciprofloxacin in the present study was 19.6 ± 1.67 mm while it was 23.67 ± 0.33 mm as reported by Barkatullah *et al*. (2011). This variation was due to the varied concentration of ciprofloxacin. In the present study, the *n*-hexane extracts showed minimum inhibitory activity against the bacterial pathogens but Barkatullah *et al*. (2011) reported that n-hexane extracts of

fruit, leaves and bark possesses significant antibacterial activity against different types of Gram-negative and Gram-positive bacteria.

In the present study, *P. aeruginosa* is resistant against methanolic and *n*-hexane leaf as well as fruit extracts of *Z. armatum* which is similar to the findings of other workers (Joshi *et al.* 2009, Barkatullah *et al.* 2011, Yasmeen *et al*. 2015). Nidhi *et al.* (2013) and Priya *et al.* (2017) reported that the methanolic root and bark extracts of *Z. armatum* possess significant antimicrobial activity against *P. aeruginosa,* (14.33 ± 0.33 and 23.7 mm). It might be due to the presence of different types of phytochemicals in different parts of the plants that are responsible for various activities.

The acetonic leaf extract showed a significant inhibition zone (15.20 ± 0.9 mm) against *E. coli*. The present result is supported by Yasmeen *et al*. (2015). The *n*-hexane and methanolic leaf extract remained inactive against *E. coli.* This observation is similar to the findings of Negi *et al*. (2012). The free radical scavenging potential of methanolic leaf extract ranged from 55 - 89% while in the case of ascorbic acid it ranged from 69.4 - 94%. This result is also supported by the findings of Kanwal *et al.* (2015) The present study also revealed that as the concentration of the extract increased, the DPPH radical scavenging activity also increased, this statement is also justified by the earlier findings (Kamboj *et al*. 2014, Kanwal *et al*. 2015). The results of the present study indicate that higher concentration is associated with strong antioxidant ability which might be due to an increased proportion of flavonoid and phenolic contents in them. Wazir *et al.* (2014) also found that the DPPH free radical scavenging activity of methanolic extract of *Z. armatum* and compared it with ascorbic acid and found that the free radical scavenging activity of the methanolic extract was 70% while that of ascorbic acid was 96.5%.

The free radical scavenging activity of acetonic leaf extract was 18.4 - 71.9% at the concentration of 10 - 1000 ppm, which is analogous to the findings of Yasmeen *et al.* (2015). The free radical scavenging activity of *n*-hexane leaf extract ranged from 27.5 - 58%, and *n*-hexane fruit extract ranged from 17 - 44.9% at 10 -1000 ppm. The antioxidant activity of this extract was not found before.

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