

Research Article **Evaluation of Ethnopharmacological and Antioxidant Potential of Zanthoxylum armatum DC.**

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Zanthoxylum armatum DC. (syn. *Z. alatum* Roxb.) is an important medicinal plant commonly called Timur or Indian prickly ash. The ethnopharmacological study of *Z. armatum* revealed the use of different plant parts for curing various ailments including cholera, chest infection, fever, indigestion, stomach disorders, gas problems, piles, toothache, gum problems, dyspepsia, as carminative, antipyretic, aromatic, tonic, and stomachic. Keeping in view the medicinal potential of the plant, the antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, reducing power, and phosphomolybdate assay using different concentrations (7.81 μ g/mL-250 μ g/mL). Ascorbic acid was taken as standard. The results indicated that the free radical scavenging activity ranged from 40.12% to 78.39%, and the reductive potential ranged from 0.265 nm to 1.411 nm while the total antioxidant activity ranged from 0.124 nm to 0.183 nm. The antioxidant potential evaluated by three assays increased in a concentration dependent manner and ascorbic acid showed better antioxidant activity than leaf extract. Results obtained through different tests confirmed redox protective activities of *Zanthoxylum armatum*. Further in vitro and in vivo research should be performed, so this plant can be further utilized in drug development.

1. Introduction

Free radicals are the reactive oxygen species (ROS) like superoxide anion radical, hydroxyl radical, nitric oxide radical, singlet oxygen, hypochlorite radical, hydrogen peroxide, and various lipid peroxides [1, 2]. Free radicals are the compounds that are produced by the body naturally [3]. The lesser production of free radicals in the body is responsible for normal physiological functioning but their higher concentration or decreased antioxidant level leads to oxidative stress [4]. This oxidative stress is mainly responsible for inducing many chronic and degenerative diseases like Alzheimer's disease, arthritis, Parkinson's disease, stroke, atherosclerosis, cancers, diabetes mellitus, ageing, ischemic heart disease, immune suppression, chronic inflammatory diseases, and neurodegenerative diseases [3, 5-7]. In spite of the natural production of free radicals in the body some other factors are also responsible for the increased level of these radicals which include smoking, alcohol, environmental pollution, ionizing radiations, and chronic diseases [3].

Antioxidants are the vital chemical compounds which can bind to free radicals, hence, preventing the deleterious effect of these radicals on healthy body cells [3]. Artificial antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) commercially available are harmful and unstable as compared to natural antioxidants. Therefore, the trend has been shifted towards antioxidants from natural sources [8]. The natural products like vegetables and fruits, herbs, sprouts, cereals, seeds, and edible mushrooms can be used as a vital source of antioxidants so the damaging effects of free radicals could be minimized [9]. The antioxidant potential is usually attributed to the phytoconstituents, that is, phenols, flavonoids, anthocyanin, flavones, isoflavones, lignans, coumarins, catechins, and iso catechins present in the plants [10–12].

The plant domain had a great contribution in providing the health benefits to man when no concept of synthetic medicines and surgical management existed [13]. Worldwide, it is a fact that indigenous communities are highly knowledgeable about plants and other natural resources on which they are greatly dependent [14]. Medicinal plants possess a variety of secondary metabolites that make them a potential natural source of disease combating and preventing compounds and attracted the attention of a lot of researchers in identifying the naturally occurring compounds of plants responsible for various medicinal properties [15]. The different phytochemicals responsible for healing potential of plants include alkaloids, tannins, flavonoids, and phenolic compounds [16].

Zanthoxylum armatum DC. (syn. Z. alatum Roxb.), a large spiny shrub or small tree, is medicinally significant commonly called Timur or Indian prickly ash. It is found throughout India, from Kashmir to Bhutan at altitudes up to 2,500 m. It is also widely distributed in Taiwan, Nepal, China, Philippines, Malaysia, Japan, and Pakistan at altitudes of 1,300–1,500 m [17].

The objectives of the present study were to record the ethnomedicinal uses of *Z. armatum* from different areas of Pakistan and evaluation of in vitro antioxidant activity by different assays.

2. Materials and Methods

2.1. Plant Material and Extraction. The plant material was carefully cleaned with distilled water and dried under shade. Dried material was pulverized to fine powder. For extraction purpose cold maceration method was used in which ground plant material was soaked in methanol for 7 days at room temperature. For filtration of plant material Whatman filter paper no. 1 was used and then to get dry residue, filtrate was evaporated through rotary evaporator. To obtain maximum yield of the plant extract the above process was repeated three times. For performing antioxidant assays plant extract was dissolved at a concentration of 1 mg/mL in 95% methanol and then this solution was diluted to prepare the series concentration.

2.2. Chemicals. All the chemicals used in the work were of analytical grade. The chemicals include methanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), sulphuric acid, ammonium molybdate, sodium phosphate, phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, distilled water, and ascorbic acid.

3. Evaluation of Antioxidant Activity

3.1. DPPH Radical Scavenging Assay. The DPPH assay was carried out following the protocol with some alterations [18]. 24 mg of DPPH was dissolved in 100 mL of methanol to prepare the stock solution and then stored at 20°C until needed. To prepare the working solution, DPPH solution was diluted with methanol to attain an absorbance of 0.980 \pm 0.02 using the spectrophotometer at 517 nm. Then 3 mL of the solution was taken in a test tube from working solution and dissolved with 100 μ L of the plant extract at varying concentrations (7.81–250 μ g/mL). The solution in the test tubes was well shaken and incubated in the dark at room temperature for 15 min. Then the absorbance was taken at 517 nm. The scavenging activity was estimated based on

the percentage of DPPH radical scavenged as the following equation:

Scavenging effect (%)
=
$$\left[\frac{(\text{control absorbance} - \text{sample absorbance})}{(\text{control absorbance})}\right] \times 100.$$
 (1)

 IC_{50} value is the effective concentration that could scavenge 50% of the DPPH radicals. Ascorbic acid standard was used as positive reference.

3.2. Phosphomolybdate Assay. The total antioxidant activity of plant sample was determined by the phosphomolybdenum method according to the protocol of [19]. 0.1 mL of plant sample solution was taken in test tubes and dissolved in 1 mL of reagent solution containing 0.6 M sulphuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate. Then the test tubes were covered with silver foil and incubated in a water bath at 95°C for 90 min. Allow the sample to cool at room temperature, after that the absorbance of the mixture was measured at 765 nm against a blank. Ascorbic acid was used as standard. Higher absorbance indicates higher total antioxidant potential.

3.3. Reducing Power Assay. The reducing ability of the plant sample was evaluated according to the procedure of [20]. 2 mL of extract solution, 2 mL of phosphate buffer (0.2 M, pH 6.6), and 2 mL of potassium ferricyanide (10 mg/mL) were mixed and then incubated at 50° C for 20 min. After that 2 mL of trichloroacetic acid (100 mg/L) was added to the mixture. A volume of 2 mL from the above mixture was dissolved with 2 mL of distilled water and 0.4 mL of 0.1% (w/v) ferric chloride in a test tube. After 10 min reaction, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates a high reducing power.

3.4. Statistical Analysis. All the measurements were taken three times and the results obtained were expressed as mean \pm standard deviation (SD). The results were further analyzed by ANOVA (analysis of variance) and Tukey's test using SPSS 16.0 software. At P < 0.05 the values were considered to be significant. To calculate the IC₅₀ values linear regression analysis was used.

4. Results and Discussion

The practice of indigenous medication by different populations throughout the world has proved to be a tremendous route to the discovery of many important modern drugs. Although the advent of allopathic medicine somewhat diminished the importance of traditional medicinal systems, yet these medicinal systems have, in recent years, staged a comeback.

The present study deals with the review of pharmacological uses of *Z. armatum* from different areas of Pakistan. The ethnopharmacological uses along with local name in particular area, part used, formulation, and area of research are enlisted in Table 1.

The review revealed that the most frequently used part of Z. armatum is fruit, followed by young branches (twigs) and seeds. In Dir Kohistan valley fruits are used against indigestion and cholera [21]. The fruits are used as carminative, for treating dyspepsia and stomachache, and branches are used as toothbrush to treat toothache in Galliyat areas [22]. It was reported that in Abbottabad fruits in combination with other plant parts are used against indigestion and cholera while branches are used to treat toothache and gum problems [23]. In Tehsil Kabal (Swat) the fruit is used for the treatment of stomach disorders [18]. In Buner seed is used against fever and cholera and increases saliva secretion, and fruit is used against toothache and stomachache and as carminative while young shoots are used for treating gum diseases [24]. In another study, it was seen that in Swat fruit is used for stomach disorders and as antipyretic [25]. In Lesser Himalayas fruit is used to treat stomach disorders, gas problems, and piles while young shoots are effective for treating toothache and gum problems [26]. In valley Alladand (Malakand) the stem is used as miswak against gum diseases, fruit is used as carminative and stomachic, and seeds are used for treating cholera, fever, and toothache as aromatic and tonic [27].

Phytochemical screening of different parts (seed, seed oil, fruit, bark, leaf oil, dry fruit, carpel, and aerial parts) of *Z. armatum* indicated the presence of important phytoconstituents like phenolics, terpenoids, flavonoids and their glycosides and benzenoids, alkaloids, coumarins, lignins, sterols, amino acids, fatty acids, and alkenic acids [28–33].

n-Hexane and ethanol extracts of leaves, bark, and fruit of *Z. armatum* showed remarkable antibacterial activity against various Gram-positive and Gram-negative bacteria [34]. There is a direct relation between saponin content and plant's antibacterial activity. The above results given by various researchers may explain the use of different parts of *Z. armatum* for the treatment of cholera because cholera is a bacterial disease and the plants showing significant antibacterial activity could possibly be used for this purpose and alkaloids and saponins present in *Z. armatum* might be responsible for antibacterial activity [35, 36].

The traditional use of leaf and root extract of *Clutia abyssinica* against stomachache and constipation was reported and correlated to the presence of increased anthraquinones in the plant [37]. Phenolics like anthraquinones have also been used as purgative. The scientific research revealed the use of alkaloid containing plants in medicines for curing fever and headache [38]. The use of *Z. armatum* parts as antipyretic or in treating fever may be explained by the presence of alkaloids in the plant.

The review on the medicinal uses of various parts of *Z. armatum* by the local inhabitants clearly demonstrates that the plant has strong medicinal potential. Literature review showed that in Lesser Himalayas leaves of *Z. armatum* are used as food but not for medicinal purposes. The leaves are mixed in cooked food (sag) made of *Rumex*, *Ficus*, and *Cichorium intybus* [26]. In all the other areas leaves are neither used as food nor for treating any kind of disease. Keeping in



FIGURE 1: Correlation curve of scavenging activity (%) of ascorbic acid.



FIGURE 2: Correlation curve of scavenging activity (%) of leaf extract of *Z. armatum*.

view the use of *Z. armatum* leaves as food, the antioxidant potential of methanolic leaf extract was evaluated in order to confirm its medicinal properties.

4.1. DPPH Radical Scavenging Assay. The antioxidant potential of leaf extract of *Z. armatum* in methanol at various concentrations (7.81–250 μ g/mL) was estimated using DPPH radical scavenging assay (Figure 2). The results were stated in terms of percentage of inhibition or scavenging activity as shown in Table 2. The free radical scavenging potential of leaf extract ranged from 40.12 to 78.39% while in case of ascorbic acid from it ranged from 43.50 to 83.50% (Figure 1 and Table 2). The results depicted that DPPH radical scavenging activity of leaf extract and standard increased in a concentration dependent manner. This finding is in accordance with [8]. Generally higher percentage of inhibition at higher

	TABLE 1. LOCAL HALLS	part used, and cumpp	maninaconogical ases of 2. withmain mount anneren	r ar cas of 1 arostart.	
Botanical name/family name	Area of research	Local name	Part used/formulation	Ethnopharmacological uses	References
	Dir Kohistan Valley, KPK	Dambara	Fruits in powdered form are taken with boiled egg Fruit is taken by mixing with <i>Mentha</i> spp. and	Chest infection	[21]
			salt Seed	Cholera, fever, and increasing saliva	
	Buner, KPK	Dambara	Fruit	secretion Carminative, stomachache, and toothache	[24]
Zanthoxylum armatum	Tehsil Kabal, Swat District, KPK	Dambara	Young shoots are used as toothbrush Fruit	Gum diseases Stomach disorders	[18]
DC/Rutaceae	Abbotabad District, KPK	Timer	Dried fruit of <i>Z. armatum</i> in powder form, dried leaves of <i>Mentha longifolia</i> , seeds of <i>Trachyspermum ammi</i> , and black salt is taken	Indigestion, cholera	[23]
			with water three times a day for 3-4 days.	-	,
	Swat, KPK	Dambara	Twigs used as toothbrush Fruit	Toothache, gum problems Antipyretic, stomach disorders	[25]
	Galliyat areas, District Abbotabad	Timber, Timmer	Fruit Branches	Dyspepsia, stomachache, carminative Toothache	[22]
	Lesser-Himalayas	Timber	Fruit in powdered form Young branches used as toothbrush	Stomach disorders, cholera, gas problem, and piles Toothache, gum problems	[26]
	Valley Alladand Dehri, District	Dambaun	Seeds	Aromatic, tonic, cholera, fever, and toothache	
	Malakand	Dallibara	Fruit Stem is used as miswak	Carminative, stomachic Gum diseases	[27]

TABLE 1: Local names, part used, and ethnopharmacological uses of Z. armatum from different areas of Pakistan.

4

Sr. no.	Concentration (µg/mL)	Methanol leaf extract inhibition (%)	Ascorbic acid inhibition (%)
1	7.81	40.12 ± 0.43	43.50 ± 0.55
2	15.62	45.56 ± 0.75	50.30 ± 0.59
3	31.25	48.23 ± 0.75	55.25 ± 0.65
4	62.5	57.28 ± 0.57	65.50 ± 0.49
5	125	69.73 ± 0.63	74.20 ± 0.22
6	250	78.39 ± 0.48	83.50 ± 0.44
		$IC_{50} (\mu g/mL) = 2.6$	$IC_{50} (\mu g/mL) = 2$

TABLE 2: Antioxidant activity of Z. armatum leaf extract in methanol with different concentrations using DPPH assay.

TABLE 3: Antioxidant activity of Z. armatum leaf extract in methanol with different concentrations using reducing power assay.

Sr. no.	Concentration (µg/mL)	Methanol leaf extract absorbance (nm)	Ascorbic acid absorbance (nm)
1	7.81	0.265 ± 0.06^{a}	0.454 ± 0.05^{a}
2	15.62	$0.391 \pm 0.08^{ m ab}$	0.517 ± 0.05^{a}
3	31.25	$0.558 \pm 0.07^{ m ab}$	$0.872 \pm 0.00^{ m b}$
4	62.5	$0.767 \pm 0.10^{\rm bc}$	$1.409 \pm 0.09^{\circ}$
5	125	$0.861 \pm 0.10^{ m bc}$	2.332 ± 0.10^{d}
6	250	$1.411 \pm 0.46^{\circ}$	2.860 ± 0.02^{e}

All values stated as mean \pm standard deviation (n = 3); values of the same column, followed by the same letter (a to e) are statistically significant (P < 0.05) as measured by Tukey's test.

concentration of various plant extracts is associated with higher antioxidant potential of a compound [39, 40]. The leaf extract in methanol and standard showed the highest antioxidant activity (78.39% and 83.50%, resp.) at 250 μ g/mL while it showed the lowest activity (40.12% and 43.50%, resp.) at 7.81 μ g/mL. The results imply that higher concentration is associated with strong free radical scavenging ability.

The IC₅₀ value for ascorbic acid and leaf extract was determined from calibration curve. The higher IC₅₀ value of the plant extract indicates higher antioxidant potential. The IC₅₀ value of leaf extract was found to be $2 \mu g/mL$. It appears that the free radical scavenging potential of the leaf extract was less efficient than ascorbic acid whose IC₅₀ was 2.6 $\mu g/mL$. This finding is in agreement with the previous findings [41]. Overall, the antioxidant potential of ascorbic acid was found to be better than leaf extract. The same findings were reported by [42, 43].

The increased contents of phenols and flavonoids in the plants are mainly responsible for strong antioxidant properties [44, 45]. Similarly, the presence of flavonoids and phenols in ethanol extracts of *Z. armatum* fruit, bark, and leaves with the highest contents in fruits (22.80 and 21.68 mg/g, resp.) was reported. Hence the strong DPPH radical scavenging potential of *Z. armatum* leaf extract might be due to the increased levels of phenol and flavonoid contents.

4.2. Reducing Power Assay. Reducing power is linked with the antioxidant potential of a compound so it may serve as a good indicator of antioxidant activity [46]. In this assay, the antioxidant substances of the sample reduce $Fe^{3+}/ferricyanide$ to $Fe^{2+}/ferrocyanide$ by giving an electron. Depending upon the reducing ability of the compound, the sample solution changes its color from yellow to various shades of blue and green. By estimating the formation of Pearl's Prussian blue at 700 nm, the concentration of ferric (Fe^{3+}) ions can be easily determined [47].

Reducing power assay was performed with different concentrations (7.81–250 μ g/mL) of methanol leaf extract and ascorbic acid and the results were recorded in terms of absorbance (Table 3). The absorbance of leaf extract and ascorbic acid ranged from 0.265 nm to 1.411 nm and 0.454 to 2.860 nm, respectively. The results indicated that absorbance of methanol leaf extract and ascorbic acid has a direct proportionality relationship to the concentrations. This finding is in agreement with [48]. The increased absorbance is an indication of increased reducing powers [39, 48] and increased reducing powers of various plant extracts indicated strong antioxidant potential [49]. The leaf extract and ascorbic acid exhibited maximum reducing ability and antioxidant capacity (1.411 nm and 2.860 nm, resp.) at the highest concentration $(250 \,\mu\text{g/mL})$ while it exhibited minimum reducing ability and antioxidant potential (0.265 nm and 0.454 nm, resp.) at the lowest concentration (7.81 μ g/mL). The results obtained were statistically significant at P < 0.05. The reducing capacity usually depends on the reductants present in a compound that exhibited antioxidant potential by breaking a free radical chain or donating a hydrogen atom [50]. The reducing power of reference compound (ascorbic acid) was found to be better than leaf extract at the same concentrations. These findings also correlate with the results of [51, 52].

4.3. Phosphomolybdate Assay. Phosphomolybdate assay has been reported to be effectively employed to evaluate the total antioxidant capacities of various plant extracts [53, 54]. In this assay molybdenum (VI) is reduced to molybdenum (V) in the presence of an antioxidant compound. During reduction a green colored phosphomolybdate (V) complex is formed whose presence can be tested spectrophotometrically at 765 nm [55].

Sr. no.	Concentration (µg/mL)	Methanol leaf extract absorbance (nm)	Ascorbic acid absorbance (nm)
1	7.81	0.124 ± 0.00^{a}	0.131 ± 0.01^{a}
2	15.62	$0.138\pm0.00^{\rm b}$	$0.144 \pm 0.01^{ m ab}$
3	31.25	$0.143\pm0.00^{\rm b}$	$0.160 \pm 0.00^{\rm ab}$
4	62.5	$0.152 \pm 0.00^{\circ}$	$0.207 \pm 0.03^{\rm b}$
5	125	$0.168\pm0.00^{\rm d}$	$0.284 \pm 0.00^{\circ}$
6	250	0.183 ± 0.00^{e}	$0.389 \pm 0.03^{\rm d}$

TABLE 4: Antioxidant activity of Z. armatum leaf extract in methanol with different concentrations using phosphomolybdate assay.

All values are stated as mean \pm standard deviation (n = 3); values of the same column, followed by the same letter (a to e) are not statistically different (P < 0.05) as measured by Tukey's test.

The results were expressed in terms of absorbance as shown in Table 4. The total antioxidant capacity of leaves extract ranged from 0.124 to 0.183 nm and in case of ascorbic acid ranged from 0.131 to 0.389 nm absorbance. The higher absorbance of the plant extracts is responsible for increased antioxidant activity [56]. The leaves extract with the highest absorbance (0.183 nm) at $250 \,\mu$ g/mL exhibited maximum antioxidant potential while extract with the lowest absorbance (0.124 nm) at 7.81 μ g/mL showed minimum total antioxidant potential. This finding is in agreement with the study of [57]. The absorbance of the leaves extract is found to be inferior to ascorbic acid (standard). The results obtained were statistically significant at *P* < 0.05.

Various scientists reported that phenols [56], carotenoids, flavonoids, and ascorbic acid [58] present in the plants are majorly responsible for strong total antioxidant activity. Phytochemical screening of *Z. armatum* indicated that various flavonoids, flavonol glycosides, alkaloids, lignans, phenolics, terpenoids, amino acids, fatty acids, and a number of other compounds have been isolated from different parts of the plant [17, 34, 59, 60]. Therefore, it can be said that the strong total antioxidant activity of *Z. armatum* leaves extract might be due to the presence of flavonoids, carotenoids, and ascorbic acid and phenols. This is in agreement with [21] which reported that strong total antioxidant activity possessed by citrus was owing to the presence of carotenoids, flavonoids, and ascorbic acid.

4.4. Conclusion and Recommendations. The results of the present study suggested that *Z. armatum* exhibited remarkable scavenging effects on DPPH and prominent reducing power and total antioxidant activity which confirmed that the use of this plant might be beneficial in preventing various diseases. Further in vivo antioxidant activity of the plant with different mechanisms and isolation, screening, and characterization of individual compounds responsible for various bioactivities is required.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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