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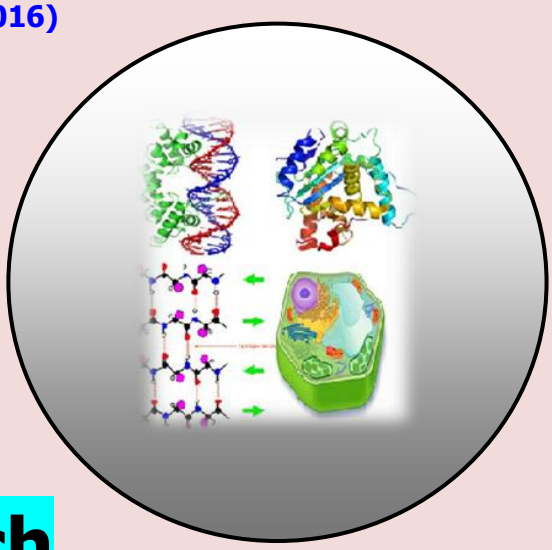

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An Investigation of *Zanthoxylum armatum* Dc. Leaves for Antibacterial, Antioxidant and Enzyme Inhibitory Activities

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ABSTRACT

The present study was designed to investigate the leaf extracts of Zanthoxylum armatum DC. For their antibacterial, antioxidant and enzyme inhibitory (α-amylae and urease) activities. The antibacterial activity of leaf extracts in methanol, acetone and aqueous solvents was determined in vitro against medically important bacteria (Escherichia coli, Yersinia pestis, Bacillus cereus, Pseudomonas aeruginosa,Listeria monocytogenes and Staphylococcus aureus)following agar-well diffusion method using different concentrations (25, 50, 75 and 100%). Results exhibited low to significant antibacterial activity against tested bacterial strains. Leaf extract prepared in methanol was found to be more effective against selected pathogenic bacteria as compared to acetone and aqueous leaf extracts. Further, leaf extracts inhibited gram-positive bacteria more efficiently than gram-negative bacteria. Furthermore, the antioxidant capacity of different extracts of this plant was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and reducing power tests. The plant showed moderate DPPH radical scavenging and reducing power. In addition, all the extracts of Z. armatum were reported to possess anti-alpha amylase and anti-urease activity of greater than 10% in all the solvents used at a concentration of 1 mg/mL. Thus this study provides scientific evidence to the traditional uses of this plant in the treatment of obesity, diabetes, ulcers, kidney stones etc. besides in curing bacterial and oxidative-stress related diseases.

Keywords: Zanthoxylumarmatum; Agar well diffusion; DPPH; reducing power; α-Amylase and Urease.

INTRODUCTION

Zanthoxylum armatum DC. (syn. *Zanthoxylum alatum*)is a deciduous shrub or small tree belonging to the family Rutaceae and generally grows in well drained alluvial, black soil. It is widely distributed in India, from Kashmir to Bhutan at an altitude of about 2,500 m and also occurs throughout North East India. Plant also finds wide distribution in Nepal, China, Philippines, Malaysia, Japan, Taiwan and Pakistan at altitudes ranging from 1,300-1,500 m (Kanwal et al., 2015). The valleys and thickets in the mountains, wasteland and the under-storey of mixed forests are customary locations of this species (Kala et al., 2005). In its natural habitat, it grows up to 6 m in height with dense foliage and branched-flattened prickles. Leaves are often compound, 4-20 cm

long, imparipinnate, rachis winged, serrate with gland dots and aromatic, containing a flavour like lime and mint. Minute yellow flowers arise in the leaf axils. Flowers have 6-8 acute sepals. Petals are highly reduced or absent. Seeds are rounded, shining black and about 3 mm in diameter (Hartley, 1996).

In traditional health care system, the plant is valuable because of stomachic, carminative and anthelmintic properties of itsbark, fruits and seeds. Z. *armatum*fruits, seeds and stem bark are used in the treatment of various ailments such as asthma, bronchitis, indigestion, varicose veins, diarrhoea, rheumatism, dyspepsia, cholera and toothache (Kanjilal, 1997).Because of deodorant, disinfectant and antiseptic properties of fruits, they are used in dental troubles and their lotion for scabies. They are also used to ward off certain insects like houseflies (Gaur, 1999).*Z. armatum* extracts have shown to possess antifungal, antibacterial, antioxidant, hepatoprotective, anti-diabetic and anti-inflammatory activities (Rynjah et al., 2017).Most of the studies have concentrated on *Z. armatum* stem bark, fruit and seed extracts. Therefore, we planned to investigate its leaf extract in different solvents for its antibacterial, antioxidant and anti-alpha amylase and anti-urease activities.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Z. armatum*were plucked and collected from Singholi area of District Sirmaur, Himachal Pradesh, India. The plant material was then brought to the laboratory for further analysis.

Processing of plant material

Leaves of *Z.armatum* were washed thoroughly under tap water and then with 2% Mercuric chloride. Thereafter the leaves were cut into smaller pieces for quick drying and shade dried for 15-20 days. The dried plant material was crushed into fine powder with the help of pestle mortar and stored in an air tight container at room temperature.

Preparation of plant extracts in different solvents

5 g dried leaves of *Z. armatum* were taken in separate Erlenmeyer flasks to which 50 mL of required solvents i.e. methanol, acetone and water (aqueous) were added. The flasks were covered with aluminium foil and allowed to stand for about 3-5 days for extraction. The extracts were then filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected, weighed and finally stock solution of concentration 50 mg/mL was prepared.

Procurement of bacteria

Different strains of bacteria namely *Bacillus cereus* (MTCC 1272)*, Escherichia coli* (MTCC 912)*, Listeria monocytogenes* (MTCC 657)*, Pseudomonas aeruginosa* (MTCC 741)*, Staphylococcus aureus* (MTCC 1144)and *Yersiniapestis* (MTCC 4912) have been procured from Indira Gandhi Medical College, Shimla and Department of Microbiology & Biotechnology, Himachal Pradesh, University Shimla (India) for screening antibacterial properties of plant extracts.

Maintenance and preservation of pure culture

The collected pathogens were revived in nutrient broth medium and stored in nutrient agar slants at 4°C. Pure cultures of all the bacteria were maintained on nutrient medium broth and preserved in refrigerator. Sub-culturing was done at regular intervals in order to maintain the cultures.

Evaluation of antibacterial activity by agar-well diffusion method

Leaf extracts prepared in methanol, acetone and aqueous solvents were screened using agar well diffusion method used by Kannanet al.(2009) and Hemashenpagam and Selvaraj (2010) with slight modifications. Nutrient agar medium i.e. Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 5 g, Agar 20 g and Distilled Water 1000 mL was used throughout the investigation. The medium was autoclaved at 121.6^{o}C for about 30 minutes and poured into Petri plates. A 100 µL of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were seeded with 25, 50, 75 and 100% concentration of prepared plant extracts. The Petri plate taken as a control contained pure solvent only. The Petri plates were incubated at 37±2^{o}C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the zone of inhibition (ZOI) around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition zone diameter using positive control as standard.

Growth inhibition percentage (%) = $\left(\frac{\text{Control} - \text{Test}}{\text{Control}}\right) \text{x}100$

Where, Control = average diameter of bacterial colony in control.

Test = average diameter of bacterial colony in treatment sets.

Antioxidant Activity Test

DPPH radical scavenging activity assay

The free radical scavenging activity of leaf extracts of *Z. armatum* was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as proposed by Blois (1958) with some modifications. Briefly, to 1 mL of different concentrations (20, 40, 60, 80 and 100 µg/mL) of plant extract, 1 mL of DPPH (0.1 Mm in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as a reference standard. Mixture of 1 mL methanol and 1 mL DPPH solution without plant extract was taken as control. All the experiments were carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The inhibition percentage was calculated using the following formula:

DPPH scavenging effect (%) = $\left(\frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}}\right) \times 100$

Where, $A_{control}$is the absorbance of control

$A_{sample}$is the absorbance of sample

Graphs were plotted against inhibition percentage verses concentration of plant extract/standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was calculated using the following equation given below:

$IC_{50} = \frac{50 - Y - Intercept}{\text{Slope}}$

Reducing power assay

The reducing power was measured according to the method described by Oyaizu (1986) with slight variations. Different concentrations of plant extracts i.e. 20, 40, 60, 80 and 100 µg/mLin 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for about 20 minutes. A portion (2.5 mL) of Trichloroacetic acid (10%) was added to the mixture and then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with the distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Ascorbic acid was used as a standard. Phosphate buffer (pH 6.6) was used as a blank solution. Higher absorbance of the reaction mixture indicated greater reductive potential of plant extract. Experiment was performed in triplicates to determine reducing power percentage calculated by using the formula:

Reducing power percentage (%) = $\left(\frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}}\right) \times 100$

Where, $A_{control}$is the absorbance of control

$A_{sample}$is the absorbance of sample

Graphs were plotted against inhibition percentage v/s conc. of plant extracts or standard ascorbic acid in order to find out the values of slope and y-intercepts. Finally IC_{50} value for each extract and ascorbic acid was evaluated.

Enzyme Inhibitory Activity Test

α-Amylase inhibition assay

The α-amylase inhibitory activity of *Z. armatum* leaf extracts was determined by some modifications in the method proposed by Giancarlo et al. (2006). The starch solution (1% w/v) was prepared by boiling and stirring 1 g of potato starch in 100 mL of sodium phosphate buffer for about 30 minutes. The porcine pancreatic α-amylase enzyme (EC 3.2.1.1; purchased from Sigma Aldrich-3176) was obtained by mixing 0.01 g of α-amylase in 10 mL of sodium phosphate buffer (pH 6.9) containing 0.0006 Mm sodium chloride. The extracts were dissolved in DMSO to give concentrations from 0.2 to 1.0 mg/mL. The colour reagent was a solution containing 0.1 g of 3,5-dinitrosalicylic acid with 2.99 g sodium potassium tartrate in 0.16 g sodium hydroxide and phosphate buffer (10 mL). 50 µL of each plant extract and 150 mL of starch solution along with 10 mL of enzyme were mixed in a 96-well plate and incubated at 37°C for 30 minutes. Thereafter, 20 mL of sodium hydroxide and 20 mL of colour reagent were added and the closed plate was placed in a 100°C water bath. After 20 minutes, the reaction mixture was removed from the water bath and allowed for cooling. In this way, α-amylase activity was determined by measuring the absorbance of the mixture at 540 nm using a UV-VIS spectrophotometer.

Blank samples were used to adjust the absorption of the mixture, where the enzyme was replaced with the buffer solution. Also, a control reaction was used, whereby the plant extract was replaced with 50 mL of DMSO and the maximum enzyme activity was measured. Acarbose solution was used as a positive control in the concentration range of 0.2-1.0 mg/mL. The assay was performed in triplicate and the mean absorbance was used to calculate the percentage of α-amylase inhibition. The inhibition percentage of α-amylase was assessed by the following formula:

$$\% \text{ α-Amylase Inhibition} = \left(\frac{\Delta A\text{control} - \Delta A\text{sample}}{\Delta A\text{control}}\right) \times 100$$

Where, $\Delta A_{control} = A_{test} - A_{Blank}$

$\Delta A_{sample} = A_{test} - A_{Blank}$

The concentration of the extract (inhibitor) required for 50% of enzyme inhibition (IC_{50}) for all the crude extracts was determined from corresponding dose-response curves of inhibition percentage v/s inhibitor concentration and compared to acarbose, a known inhibitor of α-amylase. The % Inhibition was plotted against the concentration of sample and a logarithmic regression curve was established to calculate the IC_{50} value for each sample which is the concentration of the given sample required to inhibit the activity of urease enzyme by 50%. Data were expressed as mean ± standard deviation (SD).

Urease inhibition assay

The enzyme inhibition was determined through catalytic effects of urease on urea by measuring change in absorbance in the absence and presence of inhibitor at 640 nm using UV-VIS spectrophotometer. The leaf extracts were tested for their urease inhibitory activity at a concentration range of 0.2 to 1.0 mg/mL (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL). For urease inhibition assays, after addition of 10 mL of phosphate buffer to accurate weight of enzyme, sonication was performed for about 60 seconds, followed by centrifugation and the absorbance of upper solution was measured at 280 nm. By using equation A= εbc, where c is the concentration of solution (mol/L), b is the length of the UV cell and ε represents molar absorptivity, one can calculate the concentration of initial urease solution. After proper dilution, the concentration of enzyme solution was adjusted to 2 mg/mL. Reaction mixture containing 1.2 mL of phosphate buffer solution (10 mM potassium phosphate, 10 mM lithium chloride and 1 mM EDTA, pH 8.2 at 37^{o}C), 0.2 mL of urease enzyme solution, and 0.1 mL of test compound was subjected to incubation for about 5 minutes. After pre-incubation 0.5 mL (66 mM) of urea was added to the reaction mixture and incubated for 20 min. Urease activity was determined by measuring the ammonia released during the reaction by modified spectrophotometric method as described by Weatherburn (1967). Briefly, 1 mL phenol reagent (1% w/v sodium nitroprusside) and 1 mL alkaline reagent (1% w/v NaOH and 0.075% active chloride NaOCl) were added to each test tube. The control contained all the reagents except the sample. The increase in absorbance at 640 nm was measured after 30 minutes. Finally the inhibition percentage was determined using the formula:

$$\% \text{ Urease Inhibition} = \left(\frac{Ac - As}{Ac}\right) \times 100$$

Here A_s is the absorbance of the sample under study

A_c is the absorbance of the control

Each experiment was repeated thrice and average value was calculated. Thiourea was used as a positive control. Data were expressed as mean ± standard deviation (SD). Furthermore, IC_{50} values were determined from the dose response curves.

RESULTS

Antibacterial activity analysis

The antibacterial activity of *Z. armatum* leaf extracts was determined by agar-well diffusion method. The results of the antibacterial assay are shown in Table 1. The screening revealed that the methanol leaf extract of *Z. armatum* was quite effective against *S. aureus*showing considerable diameters of zone of inhibition (22.35±1.30 mm at 100%, 19.90±0.63 mm at 75%, 17.50±0.41 mm at 50% and 13.50±0.70 mm at 25%) and showed minimum inhibition against *E. coli* (12.43±0.42 mm at 100%, 10.45±1.50 mm at 75%, 8.20±1.70 mm at 50% and 0.00±0.00 mm at 25%)**.** The acetone leaf extract was found to be most effective against *L. monocytogenes*(19.65±0.22 mm at 100%, 16.10±0.71 mm at 75%, 13.25±0.66 mm at 50% and 11.00±0.52 mm at 25%)and showed no inhibition against *E. coli*, *Y. pestis*and *S. aureus*. Aqueous leaf extract inhibited the growth of *S. aureus* only with ZOI ranging from 11.00-19.75mm at different concentrations. It is evident from the results that plant extracts showed greater inhibition in gram-positive bacteria as compared to gram-negative bacteria.

Antioxidant activity analysis

In the present study, extracts of *Z. armatum* in three different solvents (methanol, acetone and aqueous) were tested for their free radical scavenging ability by using DPPH assay and it was observed that the plant extracts showed moderate potency for scavenging free radicals as shown in Table 2. The extracts were tested on a concentration range i.e. 20, 40, 60, 80 and 100 µg/mL and it was reported that the activity altogether increased with increase in concentration of plant extracts (Fig. 1). The methanol leaf extract showed highest DPPH scavenging activity in the range of 12.30-32.00%. Similarly, DPPH scavenging activity ranged from 11.10-25.00% in case of acetone leaf extract whereas aqueous leaf extract exhibited minimum free radical scavenging activity (11.24-23%). Furthermore, antioxidant potential of *Z. armatum* was evaluated by reducing power assay (Table 2). Here, methanol extract showed highest reducing power (12.30-36.00%) followed by acetone leaf extract (10.25-32.00%). The percent reducing power in case of aqueous leaf extract was reported in the range of 10.00-27.00%. It is interesting and worthy mentioning here that the methanol extract which had the highest reducing power also possessed the highest radical scavenging activity.

Enzyme inhibitory activity analysis

The leaf extracts of *Z. armatum* were tested for their enzyme inhibitory activity against α-amylase and urease at varying concentrations i.e. 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL (Table 3). The plant extracts showed concentration dependent inhibition of enzyme as shown in Fig. 3 and 4. The methanol extract was reported to be the best extract for α-amylase inhibition (22.70-54.00%). For urease inhibition, acetone leaf extract proved to be better extract and inhibition ranged from 3.15-16.90% at different conc. used.

DISCUSSION

Antibacterial activity analysis

Agar-well diffusion method is widely used for screening the antimicrobial activity of plant extracts. The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular since there will be a confluent lawn of growth. In our study, maximum diameter of zone of inhibition (ZOI), (22.35 mm) was reported for the methanol extract against *S. aureus*while for its acetone extract maximum diameter of zone of inhibition was 19.65 mm against *L. monocytogenes*.Aqueous extract did not inhibited bacterial species except*S. aureus*. Thus organic solutions (methanol, acetone and) showed better antibacterial activity and this might be due to the less solubility of the active constituents in aqueous solutions, which results in less or no antibacterial effect on the bacterial isolates at lower concentration (Safiullah et al., 1995). Moreover, gram-negative bacteria are found to be more resistant than gram-positive bacteria to different plant extracts. These observations are likely to be the result of the differences in cell wall structure between gram-positive and gram-negative bacteria, with gram-negative bacteria outer membrane acting as a barrier to many environmental substances (Rabe and Staden, 1995; Pages et al., 2008). The inhibitory activity of plant extracts against both gram-positive and gram-negative bacteria may be an indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. Further detailed studies on the isolation and purification of bioactive chemical components can reveal the exact potential of *Z. armatum* extracts to inhibit growth of gram-positive and gram-negative pathogenic microbes. Our results correlate with the findings of Guleria et al. (2013)where essential oil obtained from leaves showed significant antibacterial activity against *B. subtilis*, *S. aureus* and *E. coli*with zones of inhibition ranging from 15-21 mm.Negi et al. (2012) also investigated essential oil of *Z. armatum* for antibacterial activity. The results showed that the gram-positive bacteria (*S. aureus*and *S. faecalis*) exhibited more sensitivity to the essential oil than gram-negative bacteria. The antibacterial activity of *Z. armatum* leaf extracts could be associated with its main constituents such as bornyl acetate, cymene, γ- terpinene, limonene, linalool, caryophyllene, α-terpineol, α-terpinolene, and β-pinene, some of which are well known to possess significant antimicrobial activity (Kim et al., 1995; Yang et al., 2008).

Antioxidant activity analysis

The DPPH (2,2-diphenyl-1-picryl hydrazyl) activity assay is the most widely used assay to test the free radical scavenging ability of various plant extracts (Goze et al., 2000; Li et al., 2008). DPPH is a free radical, which in the presence of antioxidants, is reduced from a stable free radical, 2,2-diphenyl-1-picryl hydrazyl (purple) to a diphenylpicryl hydrazine (yellow). Reaction of DPPH with hydroxyl group (R-OH) and R-NO_2 produces 2-(4-hydroxylphenyl)-2-phenyl-1-picryl hydrazine and 2-(4-nitrophenyl)-2-phenyl-1-picryl hydrazine, respectively (Saha et al., 2008).

The amount of DPPH reduced is equivalent to the amount of antioxidants present in the study sample, thus the results obtained give a true reflection of the plant extract's ability as an antioxidant (Prasad et al., 2009). The leaf extracts of this plant were tested for their free radical scavenging ability by using DPPH scavenging assay. The methanol leaf extract showed highest (32.00%) DPPH scavenging activity at a concentration of 100 µg/mL. In all cases, leaf extract in methanol proved to be better source of antioxidants than the corresponding acetone and aqueous extracts. A pattern of increasing antioxidant activity with increasing polarity has been reported (Goze et al., 2000). Reducing power experiment is a good reflector of antioxidant potential of the plants. The reducing capacity of compounds serves as an important indicator of their potent antioxidant activity. The plant having high reducing power usually reported to carry high antioxidant potential too (Meir et al., 1995).

The reducing capacity of *Z. armatum* was investigated by measuring Fe^{3+}-Fe^{2+} conversion as given in Table 2. In this assay, ferric ions reduced to ferrous ions thereby changing the colour of the reaction mixture from yellow to bluish green. Reducing power of leaf extracts increased with the dose, however, extracts exhibited low reducing power than that of standard ascorbic acid (Fig. 2). The methanol extract showed more reductive ability than the acetone and aqueous extracts, which was capable for neutralizing the free radicals. *Z. armatum* showed 36.00, 32.00 and 27.00% reducing power for methanol, acetone and aqueous extracts respectively at 100 µg/mL. Karmakar et al. (2015) evaluated antioxidant activity of *Z. armatum* leaves by different *in vitro* standard methods like 2,2-diphenyl-1-picrylhydrazil radical (DPPH) and reductive assay. They reported that methanol, chloroform and petroleum ether extracts show the capacity to scavenge all the tested reactive species. Among all the mentioned extracts, methanol extract was found to have better antioxidant properties compared to chloroform and petroleum ether extract. Batool et al., (2010); Sati et al. (2011) and Mehta et al. (2012) also determined *in vitro* and *in vivo* antioxidant potential of *Z. armatum* by various assays and reported significant free radical scavenging activity for its different parts (mainly stem bark and fruit) in different solvents. The radical scavenging activity of the leaf extracts and fractions of *Z. armatum*may be due to the presence of its phenolic compounds as their hydroxyl groups confer radical scavenging ability (Dorman et al., 2003).

Table 1. Zones of inhibition produced by leaf extracts of *Zanthoxylumarmatum* at different concentrations.

Plant Extract	Concentration in %	Inhibition zone diameter (in mm)					
		E. coli	*Y. pestis*	*P. aeruginosa*	*B. cereus*	*L. monocytogenes*	*S. aureus*
Methanol	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	9.20±1.30	10.20±0.00	12.45±0.80	11.00±1.26	13.50±0.70
	50	8.20±1.70	10.90±0.56	12.40±0.55	13.85±0.98	12.70±2.00	17.50±0.41
	75	10.45±1.50	12.50±2.60	14.15±0.24	15.00±1.08	13.70±0.00	19.90±0.63
	100	12.43±0.42	14.00±0.00	16.10±1.00	18.20±06	15.45±0.65	22.35±1.30
Acetone	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	0.00±0.00	10.34±0.30	12.15±1.55	11.00±0.52	0.00±0.00
	50	0.00±0.00	0.00±0.00	11.95±1.82	14.30±1.00	13.25±0.66	0.00±0.00
	75	0.00±0.00	0.00±0.00	13.50±1.90	17.30±0.87	16.10±0.71	0.00±0.00
	100	0.00±0.00	0.00±0.00	15.70±0.90	19.00±1.56	19.65±0.22	0.00±0.00
Aqueous	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	11.00±0.20
	50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	13.00±0.00
	75	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	15.35±0.18
	100	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	19.75±2.65

Values are given as mean ± S.E. (Standard Error)

Enzyme inhibitory studies

In our study, it was observed that the plant extracts showed ≥30% α-amylase inhibition at a concentration of 1 mg/mL as shown in Table 3. At a concentration of 1 mg/mL, the activity of α-amylase was54.00, 39.90 and 44.33% for methanol, acetone and aqueous extract respectively.

Table 2. Antioxidant activity of *Z. armatum* extracts at different concentrations.

DPPH radical scavenging activity (%)				
Concentration (µg/mL)	**Methanol extract**	**Acetone extract**	**Aqueous extract**	**Ascorbic acid**
20	12.30±0.08	11.10±1.05	11.24±0.30	35.24±0.50
40	16.15±1.00	14.92±2.10	13.05±2.00	50.54±0.42
60	22.87±0.56	17.23±0.65	16.00±0.00	62.35±1.20
80	25.27±0.23	21.23±0.36	19.67±0.80	74.14±0.00
100	32.00±1.90	25.00±1.20	23.00±0.00	83.26±2.20
IC_{50} (µg/mL)	178.50	249.05	283.00	41.44
Reducing power (%)				
Concentration (µg/mL)	**Methanol extract**	**Acetone extract**	**Aqueous extract**	**Ascorbic acid**
20	12.30±0.55	10.25±1.65	10.00±0.00	26.55±2.25
40	18.95±1.15	15.92±1.10	15.05±1.85	43.44±0.45
60	24.87±0.56	21.75±0.30	19.00±0.00	59.90±1.20
80	29.27±0.60	26.20±0.36	23.60±0.20	72.15±0.54
100	36.00±0.90	32.00±1.20	27.00±0.80	88.30±1.50
IC_{50} (µg/mL)	149.43	167.57	206.76	49.40

Values are given as mean ± S.E.

Table 3. Enzyme inhibitory activity of *Z. armatum* leaf extracts at different concentrations.

α-Amylase inhibitory activity (%)				
Concentration (mg/mL)	**Methanol extract**	**Acetone extract**	**Aqueous extract**	**Acarbose**
0.2	22.40±0.75	13.15±1.20	11.24±0.77	29.50±0.70
0.4	28.75±1.70	20.36±0.88	22.15±2.00	40.85±2.15
0.6	38.50±0.50	27.23±0.50	34.30±0.52	56.45±1.25
0.8	41.20±1.20	31.20±0.30	37.60±0.80	66.22±0.52
1.0	54.00±1.85	39.90±0.20	44.33±1.24	78.56±0.45
IC_{50} (mg/mL)	0.94	1.33	1.09	0.53
Urease inhibitory activity (%)				
Concentration (mg/mL)	**Methanol extract**	**Acetone extract**	**Aqueous extract**	**Thiourea**
0.2	2.40±0.08	3.15±1.66	1.24±0.30	28.38±0.78
0.4	6.15±1.75	4.32±0.80	2.05±2.05	41.58±0.55
0.6	8.55±0.56	7.23±0.56	4.30±0.55	56.30±1.20
0.8	11.20±0.20	11.23±0.30	7.67±0.80	69.20±0.50
1.0	14.00±1.90	16.90±0.20	10.33±0.65	81.26±1.25
IC_{50} (mg/mL)	3.54	3.00	4.37	0.51

Values are given as mean ± S.E.

The inhibitory activity increased with increasing the concentration of each plant extract in the range of 0.2-1.0 mg/mL (Fig. 3). The results further indicated that methanol extracts exhibit maximum inhibitory effects than other solvent extracts. This tends to show that the active metabolites of the different plant parts are better extracted with methanol than other solvents. Rynjah et al. (2017) designed a study to evaluate the anti-α-amylase or antidiabetic potential of *Z. armatum* leaves using *in vivo* and *in vitro* approaches. The results demonstrated that *Z. armatum* aqueous leaf extract possess antidiabetic property in both *in vivo* and *in vitro* condition in the range of 15.92-52.21% at concentration varying from 0.02-1.0 mg/mL.

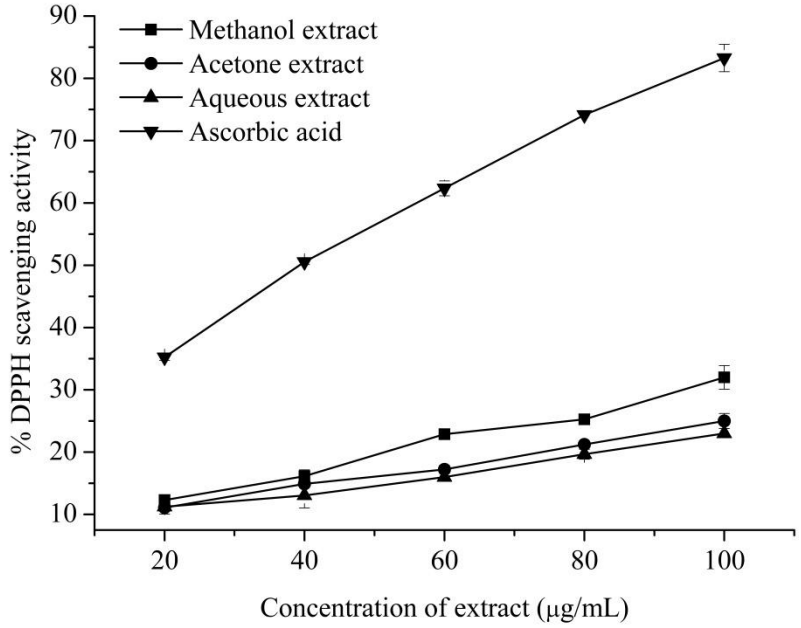

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Fig. 1.Percent scavenging (DPPH) activity of *Z. armatum*leaf extracts. The extracts were tested at a concentration range of 10-100 µg/mL. The DPPH scavenging activity increased with increase in conc. of plant extract. Vertical bars indicate mean±standard error of five replicates.

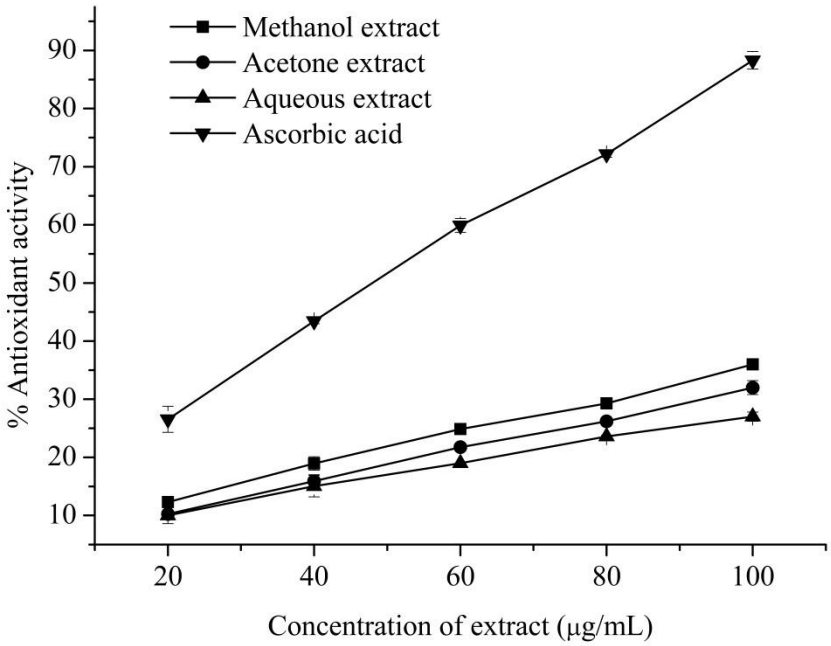

Fig. 2. Antioxidant activity percentage (reducing power) of *Z. armatum*leaf extracts. The extracts were tested at a concentration range of 10-100 µg/mL. The reducing power potential increased with increase in conc. of plant extract. Vertical bars indicate mean±standard error of five replicates.

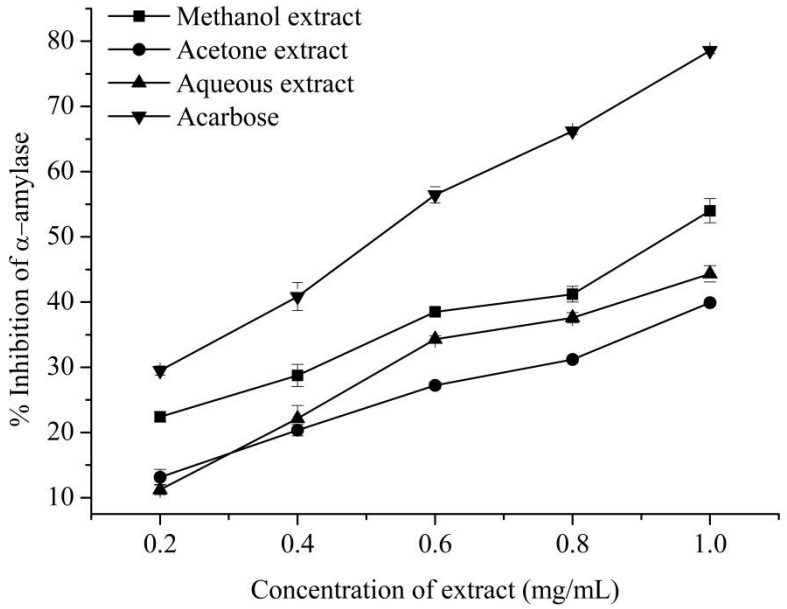

Fig. 3.α-amylase inhibition profile of different extracts of *Z. armatum* against porcine α-amylase. The extracts were tested at a concentration range of 0.2-1.0 mg/mL. The activity altogether increased with increase in conc. Vertical bars indicatemean±standard error of five replicates.

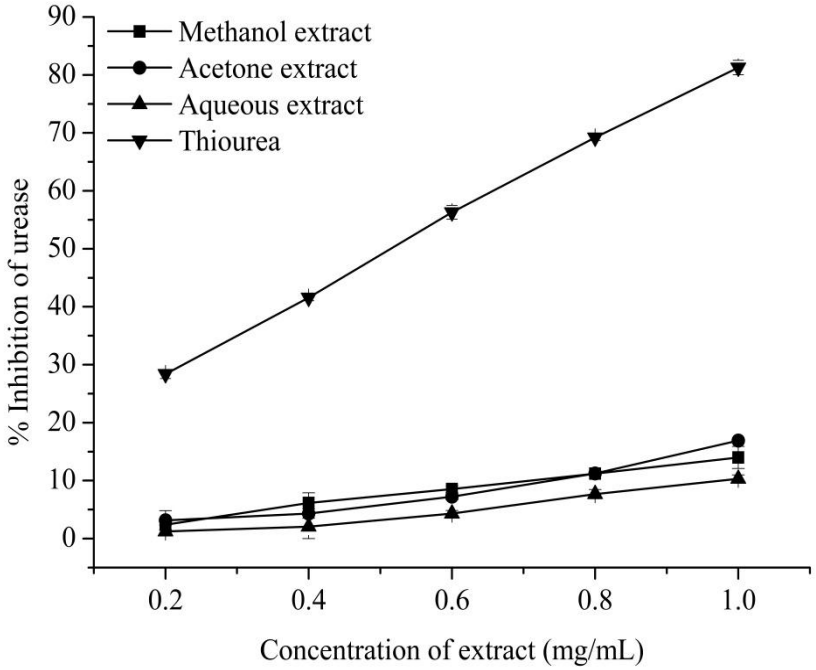

Fig. 4.Urease inhibition profile of different extracts of *Z. armatum* against porcine α-amylase. The extracts were tested at a concentration range of 0.2-1.0 mg/mL. The activity altogether increased with increase in conc. Vertical bars indicate mean ± standard error of five replicates.

There is not much work done on α-amylase inhibitory activity of leaf extracts of this plant in different solvents as per literature survey. Furthermore, the urease inhibitory activity of different plant extracts was studied against jack bean urease by using phenol hypochlorite method as compiled in Table 3 and shown in Fig. 4. All the extracts showed inhibition ≥10% at concentration of 1 mg/mL. All the three extracts of *Z. armatum* were reported to exert noticeable inhibitory effects on jack bean urease enzyme. Among these, acetone extract showed maximum inhibition in the range of 3.15-16.90% followed by methanol leaf extract. As per literature survey, there is no previous report found on α-amylase inhibitory activity of this plant. The different classes of chemical constituents reported from *Z. armatum* include flavonoids, alkaloids, terpenes, sterols, coumarins etc. which are responsible for its valuable medicinal properties (Alam and Saqib, 2017).

CONCLUSIONS

As a conclusion, it could be speculated that the plant *Z. Armatum* showed significant antibacterial activity at higher concentrations thereby confirming it as a good antibacterial agent. Methanol leaf extract was found to be more effective followed by acetone and aqueous leaf extracts. The higher inhibitory activity of methanol and acetone extracts can be attributed to the presence of higher amount of polyphenols as compared to aqueous extracts. This study suggests that the *Z. armatum* extracts possess potent antioxidant activity, which might be helpful in preventing or slowing the progress of various oxidative stress-related diseases. Further investigation on the isolation and identification of antioxidant components in this plant may lead to chemical entities with potential for clinical use. The current study also confirmed that the leaf extracts of *Z. armatum* possess anti-obesity and anti-ulcer potential through α-amylase and Jack-bean urease inhibitory activity since all the plant extracts exhibited inhibition at various concentrations. Therefore, the leaf extracts of this plant can be selected and screened for further investigations to discover their ultimate therapeutic potential.

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CONFLICT OF INTEREST

The authors hereby declare there is no conflict of interest that would prejudice the impartiality of this scientific work.

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