Pharmacognostic and phytochemical studies of *Zanthoxylum armatum* DC

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Abstract: The pharmacognostic and phytochemical investigations on the leaf, stem bark, and fruit of *Zanthoxylum armatum* were carried out. Macroscopic study of leaf, stem bark, and fruit of *Z. armatum* revealed some of the characteristic features like size, shape, color, odor and taste of the crude drug. Distinguishing fragments were identified from the powder drug. Leaf, bark, and fruit powder of this plant have higher values of total, acid insoluble, and water soluble ash as compared to the powder of this plant exhausted with ethanol and n- hexane. Extraction values in methanol were highest for leaves (20.10%) and fruit (11%), while bark showed highest value in chloroform (8.5%). The extractive values varied among other parts with different solvents. Fluorescence analysis of the *Z. Armatum* leaf, stem bark, and fruit powder with various reagents showed characteristic coloration at day and under UV light. Quantitative phytochemical screening revealed the presence of many bioactive constituents in leaves, such as alkaloids (15.60±0.10 mg/g), sterols (71.60±0.10mg/g), saponins (21.57±0.12mg/g), tannins (34.43±0.21mg/g), phenols (11.66±0.33mg/g) and flavonoids (13.68±0.66mg/g). Alkaloids (19.60±0.10mg/g), sterols (33.83±0.29mg/g), saponins (14.78±0.10mg/g), tannins (28.62±0.13mg/g), phenols (16.48±1.33mg/g) and flavonoids (18.33±1.22mg/g) were reported form the bark, while fruits were reported to have alkaloids (25.07±0.21mg/g), sterols (164.92±0.14mg/g), saponins (28.60±0.10mg/g), tannins (35.5±0.5mg/g), phenols (21.68±0.44mg/g) and flavonoids (22.8±1.33mg/g). *Z. armatum* is an important medicinal plant, traditionally used for various ailments. This study will be helpful in the future pharmacognostic standardization of this important plant.

Keywords: *Zanthoxylum*, pharmacognostic, organoleptic evaluation, extra active values.

INTRODUCTION

Zanthoxylum armatum is a small xerophytic tree or shrub. Leaflet blades usually with prickles. Leaves are compound, imparipinnate with 3-7 foliolate and pellucid-punctate. Petiole and rachis are winged. Leaflets are sessile, elliptic to ovate-lanceolate with crenate or entire margins. Flowers are born axiliary, minute and polygamous. Calyx is 6-8-acute lobed, while petals are absent. Male flowers have 6-8 stamens with rudimentary ovary. Female flowers possess 1-3 carpels and 1-3 locular ovary. Fruit is drupe and small with red color, splitting into two when ripe. Seed are rounded and shining black (Ali and Nasir, 1989).

Z.armatum prefers semi shady environment or no shade for growth. It grows wild in foothills starting from about 800m up to 1500m in Malak and, Swat, Dir, Hazara, Buner, Muree hills, and Rawalpindi (Shinwari *et al*., 2006). In Pakistan, it is known as Dambrary, Tamur (Urdu) and Dambara (Pashtu). Its fruits and seeds are edible and used as potherb species. The plant is used for treatment of pneumonia and tick infestation (Iqbal *et al*., 2010). Young shoots are used as toothbrush and useful for curing gum diseases. Fruit is used for toothache, dyspepsia, stomachache, and as a carminative. Seeds are used as condiment and flavoring agent. Wood is used to make walking stick (Abbasi *et al*., 2010; M. Arshad and Ahmad, 2004; Muhammad Arshad and Ahmad, 2005). Powdered fruit, mixed with Menthaspp and table salt is eaten with boiled egg for chest infection and other digestive problems (Islam *et al*., 2009). Recently we have tested the leaves and fruits of this plant for various pharmacological activities including antipyretic action (Barkatullah *et al*., 2011).

Pharmacognosy is the science, which provides infrastructure for the evolution of novel medicines. It is a long-established pharmaceutical science, which has played an alternative role in the finding, characterization, manufacturing, and standardization of plant material as

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well as phytomedicines regarding their65morphological, anatomical and biochemical characteristics (Kaplan, 2001; Kinghorn, 2002)(Gokhale *et al*., 2008).

Z. Armatum has significant medicinal value, traditionally applied in several ailments. This study will furnish valuable information for the pharmacognostic evaluation as well as for pharmaceutical preparations from this plant.

MATERIALS AND METHODS

Plant materials

Z. *armatum*was collected from Charkotli hills, Batkhela, Khyber Pakhtunkhwa, Pakistan. The plant was rinsed with water and dried. The dried plant material was then pulverized and separately macerated with ethanol and n-hexane for 14 days, with constant shaking. The procedure was repeated thrice before the extraction. Extract was filtered and the filtrate was concentrated *in vacuo*. Concentrated extract and powdered material of the plant were used as stock material for future experiments.

Macroscopic studies

Morphological characters of (leaves, stem, and bark) of the plant were carefully observed, following standard methodology (Trease, 2002; Wallis, 1985).

Physical characteristics

Powdered drug of *Z.armatum* (leaves, bark and fruits) was studied in detail forits physical characteristics, such as color, odor and touch etc., following standard methods (Trease, 2002; Wallis, 1985).

Powder microscopy

For powder microscopy, a pinch of fine powder was taken on a glass slide, treated separately with water, chloral hydrate and iodine solution. The microscopic observations were accomplished using 45X and 10X objective lenses. The detected fragments of the powder were identified and drawn on paper (Trease, 2002; Wallis, 1985).

Ash analysis

Total, acid insoluble, and water-soluble ash contents of the powdered drug were analyzed for crude, ethanol-exhausted and n-hexane-exhausted powder of bark, fruits, and leaves. Muffle furnace and silica crucibles were used for this process. Percent ash and total ash contents were calculated as follows (Hortwiz, 1980; Jarald and Jarald, 2007; Wallis, 1985).

Weight of crucible = W_1
Weight of crucible + ash = W_2
Total ash (mg/g) = $W_2 – W_1$ of the sample= mg/g
% Ash = W_2-W_1/Wt. of sample × 100

The acid-insoluble ash was calculated in mg/g of air-dried material as follows (Hortwiz, 1980; Jarald and Jarald, 2007; Wallis, 1985).

Weight of crucible = W_1
Weight of crucible + sample = W_2
Acid insoluble ash = $W_2 – W_1$ = X mg/g

Water-soluble ash content (Y mg/g) of air-dried material was then calculated by subtracting this amount from total ash (Trease, 2002; Wallis, 1985).

Weight of crucible = W_1
Weight of crucible + sample = W_3

Weight of the sample = W_3 - W_1 = X mg/g
Water soluble ash = $W_2 – X$ = Y mg/g

Florescence study

The fluorescence analysis of the dried parts of the plant in whole (leaves, bark, and fruits) and powdered form (1g each) was carried out by observing the samples under day and UV light of both short and long wave lengths (Brain and Turner, 1975; Chase Jr and Pratt, 1949; Nikam *et al*., 2009; Trease, 2002). Powder of different plants were treated with different chemical reagents (50% HNO_3, 50% H_2SO_4, 50% HCl, NH_3 solution, NaOH in water, NaOH in ethanol, Picric acid, Iodine solution and 10% $FeCl_3$ solution) before exposure to rays.

65 ***Extractive values determination***

Extractive values of the drugs were calculated following (Ansari *et al*., 2007). 10g of the crude powdered drug was dissolved in 200mL of different solvents, in airtight bottles, for seven days with occasional shaking. Each extract was filtered that was dried in rotary evaporator. Each process was repeated thrice; combining the extract of each solvent, their percent extractive values were calculated as under Percent (%) extractive value (W/W) = Wt. Of Extract/Wt of Sample× 100

Quantitative chemical analysis

Quantitative analysis of the selected parts of the plant were carried out to determine the quantity alkaloids, sterols, tannins, saponins, phenols and flavonoids.

Alkaloids determination

Alkaloids, in all selected parts, were quantitatively determined (Harborne, 1998). Two grams of the ethanolic extract were treated with 100mL of 10% acetic acid in a beaker that was covered and kept for 4 hours. The mixture was then concentrated on a water bath to 1/4th of its original volume, to which concentrated ammonium hydroxide solution was added drop wise, for precipitation. This precipitate was collected on a pre-weighed (W_1) Whatman filter paper, and washed with dilute ammonium hydroxide solution. The residue along with filter paper was dried, weighed (W_2) and amount of alkaloids in mg/g as well as in percentage was calculated as.

Amount of alkaloid (mg/g)= X/Wt. of sample % alkaloids= X/Wt. of sample× 100
X= Weight of alkaloids = W_2-W_1

Saponins determination
Two grams of the ethanolic extract was dissolved in 20mL of distilled water and transferred to a 250mL separating funnel. 20mL of diethyl ether was added to the funnel and was vigorously shaken. Ether layer was drained out and the aqueous layer was mixed with 60mL n-butanol. Precipitates of saponins were formed. The saponins were collected on pre-weighed (W_1) Whatman filter paper, which was then washed twice with about 10mL of 5% aqueous NaCl solution. The precipitate was then dried in oven (40°C) to constant weight (W_2) and the saponin contents were calculated in mg/g as well as percentage of the initial weight of the sample (Edeoga *et al*., 2005).

Amount of saponin (mg/g) = X/Wt. of sample
Saponin (%) = X/Wt. of sample× 100
As, X=Weight of saponin = W_2-W_1

W_1 =Weight of filter paper, W_2=Weight of filter paper residue

Tannins determination
Percent tannins in the respective samples were determined following standard methods (Van Buren and Robinson, 1969). 2g of ethanolic extract was dissolved in 75mL distilled water to form a suspension, which was filtered and treated with saturated solution of lead acetate to get tannins as lead- tinnate precipitate. The residue was then dissolved in 20mL of distilled water to which dilute sulfuric acid was added (for removal of lead sulphate). The mixture was filtered and the tannins were collected on a pre-weighed Whatman filter paper (W_1). Filter paper was then dried in hot air oven at 60ºC. The amount of tannins was estimated by taking the weight of the filter paper again and tannins content in mg/g as well as percentage of the initial weight of the sample.

Amount of tannins (mg/g) = X/Wt. of sample
Tannins (%) =X/Wt. of sample × 100
As, X =Weight of tannins = W_2-W_1, W_1 =Weight of filter paper, W_2=Weight of filter paper+ residue

Sterol determination
Percentage of sterol was determined by dissolving 2 gram of respective sample in 75mL of distilled water, to which 25mL of 10% KOH was added. This treatment converted the chlorophylls into water-soluble salt called chlorophyllins. This mixture was then extracted thrice with 75mL petroleum ether in separating funnel. Ether fraction was transferred into a pre weighed (W_1) flask, concentrated on hot water bath, dried and cooled in desiccator. The flask was weighted (W_2) again and sterol contents in mg/g as well as percentage were calculated (Huang *et al*., 2010).

Amount of sterols (mg/g) = X/Wt. of sample
Sterols (%) = X/Wt. of sample × 100
As, X = Weight of sterols, W_1 = Weight of flask, W_2= Weight of flask residue

Total phenol determination
About 2 gram of ethanolic extract was treated with 20mL of Folin-Denis reagent, 30mL of 20% Na_2CO_3 and diluted by a factor of 100 with distilled water. The resulting mixture was kept as such at room temperature for 30 minutes and was filtered then. With Spectronic 20D (Milton Roy), the absorbance was measured at 770 nm against the blank. The total phenol contents of each sample were calculated by comparing with a standard curve of tannic acid using as blank (McDonald *et al*., 2001).

Total flavoniod determination
One gram of extract was dissolved in 10mL of ethanol, from which 0.5mL were mixed with 1.5mL of methanol, 0.1mL of 10% aluminum chloride, 0.1mL of 1M potassium acetate and 2.8mL of distilled water. After 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to100µg/mL in methanol (Chang *et al*., 2002).

STATISTICAL ANALYSIS

The data were analyzed statistically using arithmetic mean and standard deviation (Saeed *et al*., 2010).

RESULTS

Leaf, stem bark and fruit of *Z.armatum* were evaluated for pharmacognostic features including macroscopy, microscopy and physicochemical study.

Macroscopy
Macroscopic or organoleptic study revealed that *Z. armatum* has compound, bifacial leaf with prominent midrib, sometime provided with spines. The leaflet is lanceolate in shape with slightly dentate margins and acute apex (fig. 1) having a pleasant odor and astringent taste. The other macroscopically characteristic features of the leaf are summarized in table 1. The barks also have some diagnostic macroscopic characteristics such as size, shape, color, odor and taste etc. which may be considered as standard pharmacognostic markers (Sharma *et al*., 2009). Various macroscopic features of the stem bark of the *Z. armatum* were worked out (table 2). It is cylindrical irregular curved in shape, the outer surface of which was dark brown while the inner was light brown in color (fig. 2). The organoleptic features of fruit are presented in

table.3. It is small drupe with spherical ovoid shape (fig. 3) with reddish brown color and astringent in taste.

Fig. 1: *Z. armatum* leaf a. Adaxial surface, b. Abaxial surface

Fig. 2: *Z. armatum*bark a. Adaxial surface, b. Abaxial surface

Fig. 3: *Z.armatum* fruit

Powdered drug microscopy

Microscopic evaluation is indispensable in the initial identification of plant materials by studying small fragments of crude or powdered drug (Jarald and Jarald, 2007). Powdered drug microscopy of *Z. armatum* leaf, stem bark and fruit was carried out in the present study.

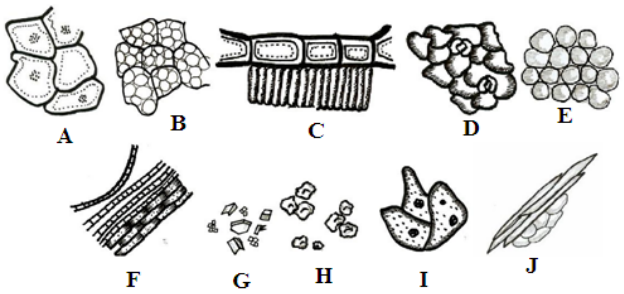

Fig. 4: *Z. armatum* leaf powder. a. upper epidermal cells; b.-c. upper epidermal cells, with palisade cells attached, surface view; c. upper epidermal cells with palisade cells attached, side view; d. lower epidermal cells with stomata; e. spongy mesophyl cells in surface view; f. vessels with elongated parenchymatous cells; g. single and aggregate crystals of calcium oxalate; h. starch grains; i. collenchymatous cells with calcium oxalate crystals; j. fibers from vein with parenchymatous cells.

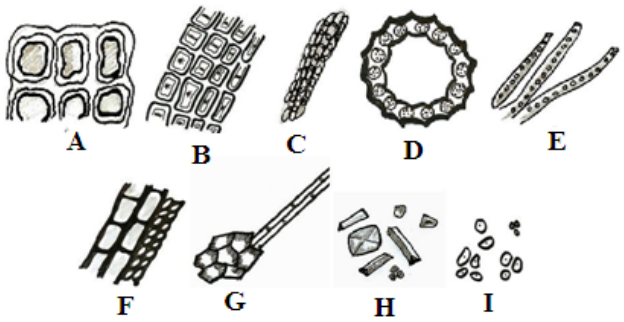

Fig. 5: *Z. armatum* bark powder fragments. a. closely fitted cork cells; b. collenchymatous cells with calcium oxalate crystals; c. fragments of medullary rays; d. isolated sclerieds; e. fitted sieve elements; f. phloem parenchyma with attached medullary ray cells; g. fibers to which parenchymatous cells are attached; h. different types of calcium oxalate crystals; starch grains.

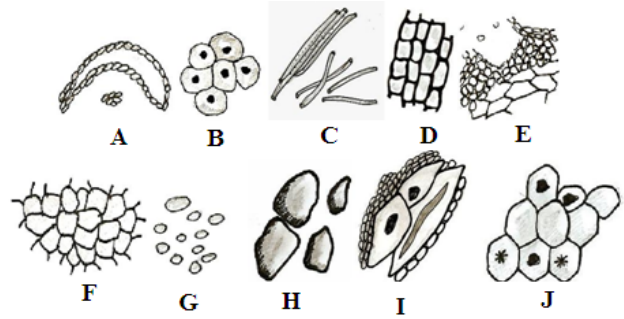

Fig. 6: *Z.armatum* fruit powder. a. group of parenchymatous cells from mesocarp; b. a group of parenchymatous cells; c. fibers; d. thick walled

parenchymatous cells from endocarp.; e. a fragment from fruit wall with broken oil gland; f. Inner epidermal cells of pericarp; g. starch grains; h. Sclerids from epicarp; i. elongated sclerieds, with small parenchymatous cells from epicarp; j. endocarp cells with calcium oxalate crystals.

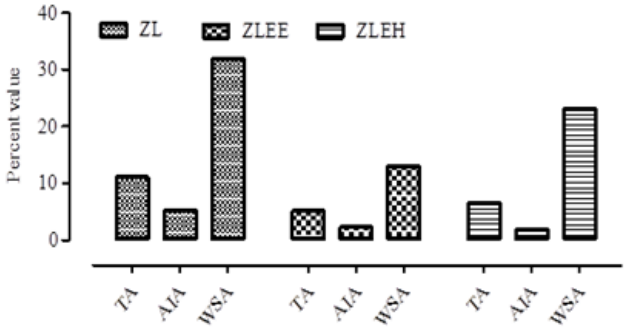

Fig. 7: Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of the leaves of *Z. armatum* leaves powder non exhausted (ZL) and exhausted with ethanol (ZLEE) and n-hexane (ZLEH).

Leaf powder drug microscopy

Z. armatum leaf powder was olive drab green with pleasant odor and some what astringent taste. Various fragments found in the leaf powder are given below (fig 4).

1. Upper epidermal fragments with irregular closely arranged thin walled cells, 19µ to 25µ, to which cylindrical palisade cells were attached from surface view and side view.
2. Lower epidermis fragments with irregular, curved thick walled cells containing stomata.
3. Spongy mesophyll cells in surface, 5µ in diameter.
4. Vessels with elongated parenchyma cells.
5. Single and aggregated Calcium oxalate crystals, 2 µ to 6 µ in diameter.
6. Rounded starch grains, 4µ to 8µ in diameter.
7. Collenchyma cells with calcium oxalate crystals.
8. Fibers from vein with attached parenchymatouscells.

These features can be used as a future reference for identification of the powdered drug of this plant. No trichomes were found in the leaf powder of *Z. armatum*.

Bark powder drug microscopy

Bark Powder of *Z. armatum* was grey brown in color with light pleasant odor and bitter taste (fig. 5). Following fragments were detected in the bark.

1. Closely fitted, thick walled cork cells.
2. Collenchymatous cells containing aggregates of calcium oxalate crystals.
3. Fragments of medullary rays.
4. Isolated sclerieds with calcium oxalate crystals.
5. Pitted sieve elements, 40-45 µ in length.
6. Thick walled phloem parenchyma, to which oval shaped medullary ray cells were attached.
7. Fibers, 75 to 95µ in length, to which parenchymatous cells are attached.
8. Calcium oxalate crystals of various types i.e. cluster, prismatic, elongated, round and rectangular.
9. Isolated and aggregated starch granules.
10. The present bark powder study could be valuable in the preparation of future monograph for appropriate evaluation and standardization of this plant.

Fruit powdered drug microscopy

Fruit is the main part used of *Z. armatum* and powder drug evaluation is essential to set parameters, that help in detection of adulteration. Fruit powder of *Z. armatum* was blackish brown with pungent smell and taste. Following are some of the fragments found in the fruit powder drug (fig. 6).

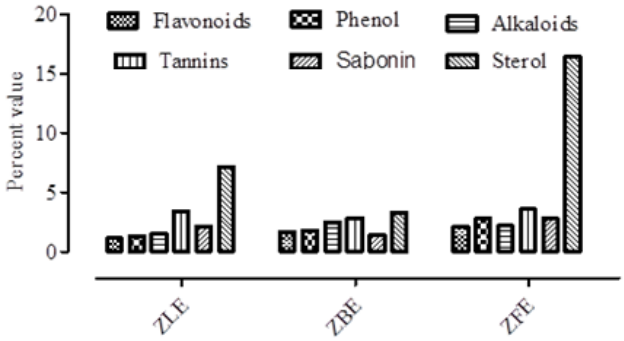

Fig. 8: Bars represent percents values of flavonoids, phenols, alkaloids, tannins, saponins and sterols in ethanolic extracts *Zanthoxylum armatum* leaves (ZLE), bark (ZBE) and fruit (ZFE).

1. Group of sclerenchymatous cells from mesocarp.
2. Thin walled closely packed parenchymatous cells.
3. Vascular tissue, consisting of elongated fibers.
4. Irregular, dark brown parenchymatous cells
5. Fragments from fruit wall with broken oil gland.
6. Inner epidermal cells of pericarp.
7. Rounded starch granules, 12-16µ in diameter.
8. Sclerieds from epicarp, irregular in shape.
9. Epicarp cells elongated in shape with small parenchymatous cells attached.
10. Rounded or polygonal endocarp cells with calcium oxalate crystals.

Physicochemical characteristics

Ash analysis

Ash analysis of powdered drug included total ash, acid insoluble ash and water-soluble ash tests for leaf, bark and fruit of *Z. armatum*. It is an important feature for detection of adulteration or the presence of inorganic matter such as metallic salts and/or silica (Jarald and Jarald, 2007) as well as for detection of exhausted materials in the drugs (14). Various ash values for genuine leaf, stem bark and fruit as well as exhausted with ethanol and n- hexane were calculated.

Table 1: Macroscopic features of *Z. armatum* leaf

S. No	Characteristic	Observation
1	Size	20 to 80mm in length and 12 to 25mm in width
2	Color	Upper surface dark green, Lower surface light green
3	Odor	Pleasant
4	Taste	Astringent
5	Phylotaxis	Spiral
6	Insertion	Ramel i.e. inserted on the branches
7	Leaf base	Symmetrical
8	Lamina Composition	Compound, Leaflets lanceolate
9	Petiole	Leaf stalk winged, Leaflet sessile
10	Incision	Dentate slight incision present
11	Venation	Reticulate and unicostate
12	Apex	Acute
13	Surface	Glabrous, thorny
14	Texture	Soft
15	Fracture	Short and smooth

Table 2: Macroscopic features of the bark of *Z. armatum*

S. No	Characteristic	Observation
1	Shape	Irregular, cylindrical curved
2	Dimension	2 to 5cm in length and up to 3 cm in width
3	Outer surface	Dark brown in color, rough texture, hard spines with corky base
4	Inner surface	Light brown in color, scaly with ridges, cavities may present
5	Odor	Light pleasant
6	Taste	Bitter
7	Fracture	Uneven and fibrous
8	Thickness	1-3mm

Table 3: Macroscopic features of the fruit of *Z.armatum*

S. No	Characteristic	Observation
1	Kind	Small drupes, pedicel present
2	Dimensions	6 to 10mm in diameter
3	Shape	Spherical ovoid
4	Insertion	Ramal i.e. present on the braches
5	Dehiscence	Splits into two halves at maturity i.e. bivalvate
6	Color	Reddish brown with oil gland spots
7	Taste	Astringent
8	Odour	Pungent
9	Texture	Soft
10	Marking	Ridges present
11	Sutures	Longitudnal
12	Seeds	One, black in color, hard in texture, ovoid in shape, raphe distinguished, micropyle present

Table 4: Ash analysis of *Z. armatum* leaf and bark

S. No	Powder	Total Ash	Acid insoluble Ash	Watersoluble
1	ZL	110.4	51.3	320.5
2	ZLEE	52.0	23.5	136.5
3	ZLEH	64.4	20.3	231.0
4	ZB	134.6	69.7	361.3
5	ZBEE	64.8	18.5	102.8
6	ZBEH	29.0	24.7	143.6
7	ZF	118.0	94.2	237.5
8	ZFEE	35.6	20.4	111.1
9	ZFEH	32.0	16.0	142.9

Table 5: Quantitative chemical analysis of *Z.armatum*. All values are mean ± SEM of three determination. All values are expressed in mg/g

S. No.	Extract	Flavonoids	Phenol	Alkaloids	Tannins	Saponin	Sterol
1	ZLE	13.68±0.66	11.66±0.33	15.60±0.10	34.43±0.21	21.57±0.12	71.60±0.10
2	ZBE	18.33±1.22	16.48±1.33	19.60±0.10	28.62±0.13	14.78±0.10	33.83±0.29
3	ZFE	22.8±1.33	21.68±0.44	25.07±0.21	35.5±0.5	28.60±0.10	164.92±0.14

Table 6: Fluorescence analysis of powder of leaves, bark and fruit of *Z. armtum* with different reagents

S. No.	Reagents	Day light	UV 256	UV310
1	ZF powder as such	Blackish	Dark slate blue	Dark brown
2	ZF powder +50 %HNO_3	Dark brown	Dark brown	Dark gray
3	ZF powder+Picric acid	Brownish	Brownish	Dark gray
4	ZF powder +NH_3	Dark yellow	Brown	Dark gray
5	ZF powder+H_2SO_4	Grey	Light brown	Black
6	ZF powder+NaOH	Yellowish brown	Yellow brown	Canker Brown
7	ZF powder+HCl	Yellowish brown	Brown	Brown
8	ZFpowder+NaOH+	Brown	Grayish	Grayish Brown
9	Ethanol ZF powder+Iodine	Yellowish brown	Grayish brown	Brown
10	ZF powder+$FeCl_3$	Grey brown	Brown	Canker Brown
11	ZL powder as such	Olive Drab	Brown	Dark Olive green
12	ZL powder +50 %HNO_3	Reddish brown	Brown	Dark brown
13	ZL powder+Picric acid	Greenish	Yellow	Brown
14	ZL powder +NH_3	Grey	Greenish	Blackish brown
15	ZL powder+H_2SO_4	Grey	Grey	Grey
16	ZL powder+NaOH	Dark brown	Green	Brown
17	ZL powder+HCl	Brown	Brown	Black
18	ZLpowder+NaOH+ Ethanol	Blackish	Blackish brown	Black
19	ZL powder+Iodine	Grayish	Brown	Grayish brown
20	ZL powder+$FeCl_3$	Light black	Dark green	Dark green
21	ZB powder as such	Dark Golden red	Yellowish brown	Brown
22	ZB powder +50 %HNO_3	Golden brown	Brown	Brown
23	ZB powder+Picric acid	Yellow	Grey	Brown
24	ZB Powder +NH_3	Yellow	Brown Yellowish	Grayish Brown
	ZB pwder+H_2SO_4		brown	
26	ZB powder+NaOH	Yellowish brown	Dark brown	Yellowish Brown
27	ZB powder+HCl	Dark brown	Reddish brown	Black
28	ZBpowder+NaOH+	Blackish	Dark brown	Dark brown
	Ethanol	brown		
29	ZB powder+Iodine	Brown	Brown	Black
30	ZB powder+$FeCl_3$	Yellowish brown	Green	Bluish brown

Fluorescence study

Plants contain a variety of chemical constituents and thereby exhibit different fluorescence phenomenon in ordinary day-light and under ultraviolet light. Some drugs have characteristic fluorescence under UV light, which is not observable in visible range of light (Ansari *et al*., 2007; Jarald and Jarald, 2007; Reddy and Chaturvedi, 2010). Fluorescence analysis is considered helpful for authentication and standardization of crude drugs. Many workers tried to authenticate crude drugs of plant origin including various parts of *Holopteleaintegrifolia* (Sharma *et al*., 2009), leaves of *Amaranthusspinosus* (Mathur *et al*., 2010), leaves of *Catunaregumspinosa* (Surabhiand Leelavathi, 2010) and *Hygrophilaauriculata* (Hussain *et al*., 2010). Fluorescence analysis of the Z. *armatum* leaf, stem bark and fruit powder, as a whole and with various reagents as well as their extracts in various solvents has been carried out (table 9 and 10).

Extractive values

Determination of extractive value is useful in the detection of exhausted or adulterated drugs (Singhal *et al*., 2010). Alcohol and water-soluble extractive values are indicative of the presence of adulterants, defective

Table 7: Fluorescence analysis of leaf, bark and fruit extracts of *Z. armatum*.

S. No	Part	Solvent	Day light	UV 256	UV310
1	Leaves	Ethanol	Dark green	Dark green	Greenish brown
		Methanol	Blackish Brown	Blackish Brown	Dark Brown
		Chloroform	Dark green	Blue green	Greenish brown
		Acetone	Blackish brown	Bluish black	Yellowish green
		Water	Dark Brown	Bluish black	Dark blue
		Hexane	Yellow green	Brownish green	Brownish green
		Butanol	Yellow green	Forest green	Olive drab
2	Bark	Ethanol	Brownish red	Brownish green	Brownish green
			Maroon	Forestgreen	Olive green
		Methanol	greenish		
		Chloroform	Forest green	Dark green	Yellowish green
		Acetone	Greenish brown	Lawn green	Lawn green
		Water	Blackish Brown	Bluish Green	Bluish blak
		Hexane	Dark green	Greenish brown	Dark green
		Butanol	Dark orange	Dark green	Dark green
3	Fruit	Ethanol	Orange red	Brownish green	Yellow green
		Methanol	Reddish orange	Dark Olive Green	Olive Drab
		Chloroform	Forest green	Dark green	Olive drab
		Acetone	Greenish brown	Olive green	Blue green
		Water	Brownish red	Light green	Greenish brown
		Hexane	Yellow green	Dark olive green	Brownish green
		Butanol	Lawn green	Lawn green	Yellowish green

Table 8: Percent extractive values of leaves, bark and fruit of *Z. armatum* with different solvents.

S. No	Part	Solvent	% Extracts
1	Leaves	Ethanol	19.70
		Methanol	20.10
		Chloroform	6.10
		Acetone	5.20
		Water	14.80
		Hexane	4.80
		Butanol	7.10
2	Bark	Ethanol	7.20
		Methanol	7.40
		Chloroform	8.50
		Acetone	2.60
		Water	8.00
		Hexane	8.10
		Butanol	3.30
3	Fruit	Ethanol	10.80
		Methanol	11.00
		Chloroform	6.10
		Acetone	7.30
		Water	9.30
		Hexane	9.40
		Butanol	9.00

processing and poor quality of the drug, while petroleum ether soluble extractive value indicates lipid contents present in crude drug (Gokhale *et al*., 2008; Madhavan *et al*., 2009). Percent extractive values of leaf, stem bark and fruit of Z. *armatum* were determined using different solvents including ethanol, methanol, chloroform, acetone, distilled water, butanol and n-hexane.

Quantitative phytochemical screening

Bioactive constituents like alkaloids, sterol, saponins,

tannins, phenols and flavonoids were quantitatively evaluated in leaf, bark and fruit of *Z. armatum*. The results revealed the presence of bioactive constituents in leaves. Alkaloids (15.60±0.10mg/g), sterols (71.60±0.10 mg/g) saponins (21.57±0.12mg/g), tannins (34.43±0.21 mg/g), phenols (11.66±0.33mg/g) and flavonoids (13.68±0.66mg/g) were some of the biologically active compounds reported. The bark contained alkaloids (19.60±0.10mg/g), sterols (33.83±0.29mg/g) saponins (14.78±0.10mg/g), tannins (28.62±0.13mg/g), phenols (16.48±1.33mg/g) and flavonoids (18.33±1.22mg/g), while the fruits contained alkaloids (25.07±0.21mg/g), sterols (164.92±0.14mg/g) saponins (28.60±0.10mg/g), tannins (35.5±0.5mg/g), phenols (21.68±0.44mg/g) and flavonoids (22.8±1.33mg/g) (table 8). Comparative statistics of these bioactive constituents in the leaves, bark and fruit of *Z. armatum* is shown in fig. 8.

DISCUSSION

Macroscopy

Macroscopic or organoleptic study revealed that *Z. armatum* has compound, bifacial leaf with prominent midrib, sometime provided with spines. Powdered drug microscopy of *Z. armatum* leaf, stem bark and fruit was carried out in the present study.

In the present study sclerieds, starch grains, endocarp with calcium oxalate aggregates are some of the important fragments that might be helpful in authentication of Z. *armatum* fruit powder drug.

Physicochemical characteristics

Various physicochemical characteristics including ash analysis, fluorescence study, extractive values and quantitative phytochemical screening of leaf, stem bark and fruit powder of *Z. armatum* were carried out. Leaf, bark and fruit powder of *Z. armatum* have higher values of total ash, acid insoluble ash and water-soluble ash than their respective powders that were exhausted with ethanol and n-hexane (table 4). fig. 7a, 7b and 7c provide a comparative look of percent ash values for exhausted and non-exhausted leaf, bark and fruit powders of *Z. armatum*. The present ash studies of *Z. armatum* will be of immense importance for the evaluation of this valuable drug. The florescent study was carried out using UV 256 and UV 300. Leaf, bark and fruit powders, as whole and with various reagents as well as their extracts exhibited variations in color. The fluorescence analysis can be used as a diagnostic tool for detecting adulterants, not only in the whole drugs, but also in their powders. Highest extractive value for leaves was found in methanol (20.10 %), while for bark and fruit the highest value was in chloroform (8.5%) and methanol (11.00%) respectively. In other solvents, all the parts showed variable values (table 11). This suggests that extractive values determination is an important tool for evaluation of drugs and for detection of variety of intentional and unintentional adulterations in drugs. The quantitative analysis of *Z. armatum* showed that this plant might be a rich source of bioactive constituents for future uses in pharmaceutical preparations.

CONCLUSION

Z. armatum is an important medicinal plant, traditionally used for various ailments. This study will provide valuable information for the pharmacognostic standardizationas well as for pharmaceutical preparations from this plant. Further studies are required to explore this plant phytochemically and pharmacologically, which could prove this plant as cheaper and more easily accessible source of valuable drugs.

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