Year:**2013**, Volume: **1**, Issue:**1**
First page: (**6**) Last page: (**11**)
ISSN: **2321-2187**
Online Available at **www.florajournal.com**

International Journal of Herbal Medicine

Pharmacognostical and Phytochemical Screening of Leaf Extract of *Zanthoxylum armatum* DC

Kamalesh Upreti[1*], Amit Semwal[1], Kumud Upadhyaya[1], Manoj Masiwal[1]

1. Gyani Inder Singh Institute of Professional Studies, Rajpur, Dehradun, India.
[E- mail: upkuupku@gmail.com]

Zanthoxylum armatum DC belongs to family Rutaceae is a sub deciduous aromatic shrub or small tree up to 5 m high. Bark pale brown rough croky; leave alternate imparipinnate, flowers small pale yellow and bisexual. Tender shoots are eaten as vegetable, suitable with pork and considered a good vegetable by Mishings. It is presumed that cooking with pork deworms, specially tap worms, by this vegetable. The Photomicrographic evaluation revealed interesting microscopic characteristic. The transverse section of the midrib showed schizolysogenous cavity which is a characteristic of family Rutaceae. Double layer of palisade and presence of sclerenchyma around vascular bundle is distinct identification characteristic. Also add to this is the presence of rosette shaped calcium oxalate crystals and prismatic crystals which are clearly visible in the parenchyma around vascular bundle. The leaves have anisocytic stomata which are surrounded by 4-6 straight walled subsidiary cells. Phytochemical Screening of Aqueous and Ethanolic extract of leaf of *Zanthoxylum armatum* DC showed the presence of Alkaloid, Glycoside, Carbohydrate, tennins, Amino Acids, Sterols and Terpenoids. The loss on drying of the powder of *Z. armatum* was found to be 0.15%. Theash value of powder of *Z. armatum* DC leaf was determine as total ash, water soluble ash and acid insoluble ash was found to be 10.27%, 6.53% and 12.5% respectively. The Extractive Value of *Z. armatum* was found to be 14.66% and 10.33% in Ethanolic and Aqueous extract.

Keyword: *Zanthoxylum armatum* DC, Aqueous and Ethanolic Extract, Ash Value, Extractive Value.

1. Introduction

The genus *Zanthoxylum armatum DC* belongs to the family Rutaceae and is distributed in the temperate and subtropical Himalayas. It is a large genus of aromatic prickly trees or shrubs. *Zanthoxylum armatum DC* (Rutaceae) [1,2,3], called 'Timer' in Kumaun (Uttarakhand). It grows as a large prickly shrub particularly in Uttarakhand. The major chemical constituents are alkaloids, glycosides, carbohydrates, tannins, amino acids, sterols and terpenoids. In Uttarakhand, the plant is traditionally used for various medicinal purposes.The root is used in toothache, stomachache, fever, rheumatism, paresis, boils and as an insecticide and pesticide. The fruit is used in the treatment of stomachache, cough, colic vomiting, diarrhea, and paresis and as an aromatic, stimulant and pesticide [4,5,6,7]. The small branches, seeds and stem bark are prescribed in fever, diarrhea and cholera as well as in treatment of toothache. Leaves 6-14 inch long, rachis armed with prickles beneath. Leaf rachises adaxially and midvein of leaflet blades adaxially pubescent [8,9,10]. zanthoxylum armatum dc is used in the treatment of asthma, bronchitis, cholera, fever, fibrosis's, indigestion, rheumatism, skin diseases, toothache [11,12].

2. Materials and Method:-

2.1 Powder Microscopic Examination: Glycerol reagent as per WHO guideline was prepared. Small amount of powder was taken on the slide to this mixture was added and covered with cover slip and examined under microscope.

2.2 Microscopic Evaluation:

Microscopic evaluation is a step towards authentication of internal structure of the vegetable drug to establish proper identification by revealing tissue arrangement. This is done by identifying internal structures such as epidermis, collenchyma, vascular bundles, types and arrangement of vascular bundles, sclerenchyma, crystals and any other specific features that lie there in. For this purpose a transverse section or longitudinal section by either free hand or using microtome, may be prepared. For

the present work, free hand sections were prepared as follows:

2.3 Free Hand Sectioning:
The midrib of the leaf was cut using a sharp razor including a small portion of lamina. The portion of midrib was put between the pith and fine sections were cut with the help of a sharp razor. The sections so obtained were cleared using chloral hydrate solution.

2.4 Staining:
The cleared sections were transferred to a watch glass containing staining solution (Safranin 1% solution). The sections were allowed to stain for 2-3 minutes. The sections were then transferred to a watch glass containing plain distilled water to wash away excess of stain. The sections were then transferred to a clean micro slide and observed under microscope. If any excess of stain was observed the same was removed by washing the section with dil. HNO_3.

2.5 Microphotography:
The photographs were taken with the help of a Microscope (Model-Leica DMILHC Bio) fitted with a camera (CANNON-DC 150).

2.6 Physico-chemical Analysis:
The parameter studied were total ash, acid insoluble ash, alcohol soluble extractives and water soluble extractives.

2.7 Recommended procedure:-
2.7.1 Loss on Drying:
The powdered leaves of *Zanthoxylum armatum* was dried in the oven at 100- 105^0c to constant weight.

2.7.2 Total Ash:
Place about 2-4g of the ground air-dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 450°C until it is white, indicating the absence of carbon. Cool in a desiccator and weigh. Ash value can be calculated by using formula:-

$$\text{Ash value} = \frac{\text{Initial Weight} - \text{Final Weight} \times 100}{\text{Initial Weight}}$$

2.7.3 Water soluble Ash:
The total ash obtained above was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter –paper, washed with hot water and ignited to constant weight at low temperature. The weight of the insoluble matter was subtracted from the weight of total ash, represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug. The result was calculated with reference to the air dried drug.

2.7.4 Acid Insoluble Ash:
The total ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on tarred grouch crucible, washed with hot acidulated water, ignited, cooled and weighed. The percentage acid insoluble ash was calculated with reference to the air dried drug. The same procedure was repeated with other ash obtained.

2.8 Determination of Extractive Value
This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material.

3. Method:-
3.1 Hot extraction:
3.1.1 Water Soluble Extractive Value: Place about 15gm of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Add 300ml of water and weigh to obtain the total weight including the flask. Shake well and allow standing for 1 hour. Attach a reflux condenser to the flask and boil gently for 6 hour; cool and weigh and filter rapidly through a dry filter. Dry the extracted powder in oven till the weight is constant. Calculate the content of extractable matter in mg per gm of air-dried material.

3.1.2 Alcohol Soluble Extractive Value Place about 15gm of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Add 300ml of water and weigh to obtain the total weight including the flask. Shake well and allow to stand for 1 hour. Attach a reflux condenser to the flask and boil gently for 6 hour; cool and weigh and filter rapidly through a dry filter. Dry the extracted powder in oven till the weight is constant.
Calculate the content of extractable matter in mg per gm of air-dried material by using this formula:-

$$\text{Extractive value} = \frac{\text{Initial weight-Final weight} \times 100}{\text{Initial weight}}$$

3.1.3 Phytochemical Screening:
Phytochemical screening of the extract give general idea regarding the nature of chemical constituents present in the crude drug.

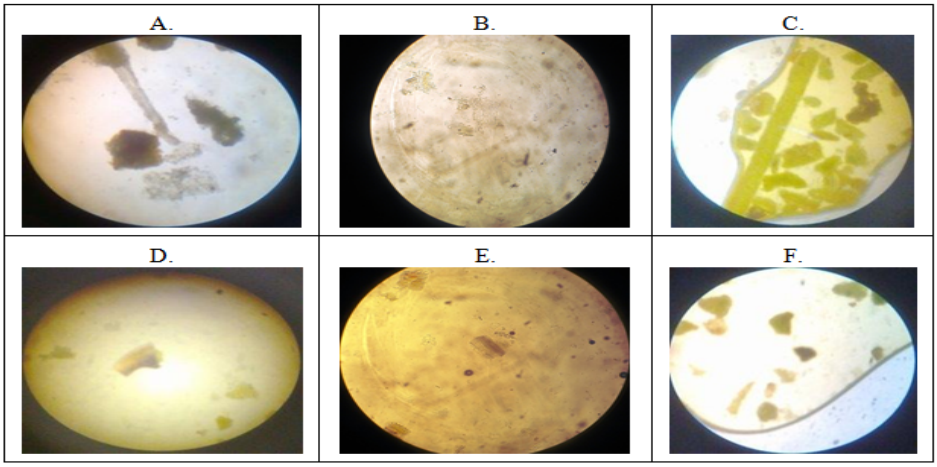
3.2 Preparation of Extracts

a) **Aqueous Extract**:- 10gm of leaf powder was extracted with 150ml of water andplant extract was taken in different test tube and required amount of solvent added in each test tube. Then it was subjected to different chemical reagents and tests and observation was noted.

b) **Ethanolic Extract:-** 10gm of leaf powder was extracted with 150ml of ethanol andplant extract was taken in different test tube and required amount of solvent added in each test tube. Then it was subjected to different chemical reagents and tests and observation was noted.

4. Results

For the above experiment various results were found as:-



A- Traeheads, B- Parenchymantous Cells,C- Xylem Fibre,
D-Chollenchymatous Cell,E-Xylum Vessels, F- Covering Trichome

Fig 1: Powder Microscopy

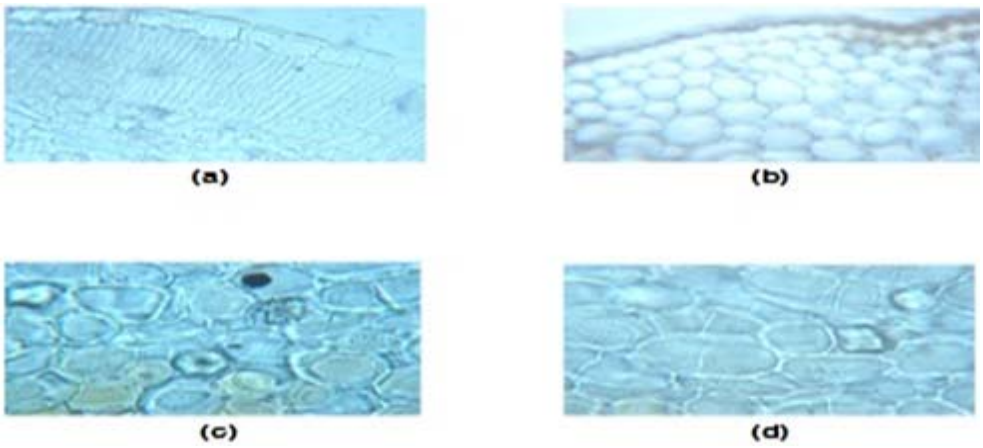


Fig 2: a) Double layer of palisade, b) Lower epidermis with collenchyma,
c) Rosette type Calcium oxalate crystals in parenchyma of midrib,
d) Prismatic crystals in parenchyma of midrib.

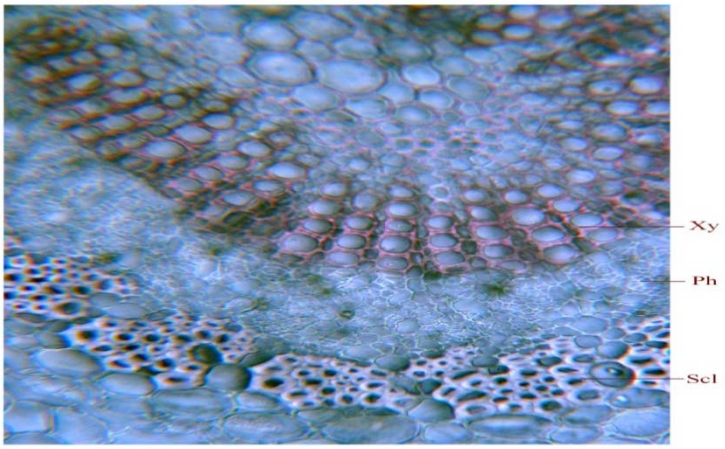


Fig 3: Vascular bundle with surrounding sclarenchyma, Scl-Sclerenchyma, Ph-Phloem, Xy-Xylem

5. Conclusion

Photomicrographic evaluation revealed interesting microscopic characteristic. The transverse section of the midrib showed schizolysogenous cavity which is a characteristic of family Rutacea. Double layer of palisade and presence of sclerenchyma around vascular bundle is distinct identification characteristic. Also add to this is the presence of rosette shaped calcium oxalate crystals and prismatic crystals which are clearly visible in the parenchyma around vascular bundle. The leaves have anisocytic stomata which are surrounded by 4-6 straight walled subsidiary cells. Phytochemical Screening of Aqueous and Ethanolic extract of leaf of *Zanthoxylum armatum* DC showed the presence of Alkaloid, Glycoside, Carbohydrate, tennins, Amino Acids, Sterols and Terpenoids.

The loss on drying of the powder of *Z. armatum* was found to be 0.15%. Theash value of powder of *Z. armatum* DC leaf was determine as total ash, water soluble ash and acid insoluble ash was found to be 10.27%, 6.53% and 12.5% respectively. The Extractive Value of *Z. armatum* was found to be 14.66% and 10.33% in Ethanolic and Aqueous extract. These data can provide valuable information on standardisation and evaluation of the leaves of *Zanthoxylum armatum* DC.

Table 1: Percentage loss in weight on drying

S.No.	Weight of the powder taken (gms)	% Loss in weight (w/w)	Mean value
1	1.5	0.263	
2	1.5	0.161	
3	1.5	0.102	0.15675
4	1.5	0.101	

Table 2: Ash value

Weight of the drug powder taken (gm)	Weight of ash obtained (gm)	Percentage w/w total ash	Mean value
3.0	0.286	9.53	
3.0	0.349	11.63	10.27
3.0	0.29	9.66	

Table 3: Percentage water soluble ash values

Weight of total ash (gms)	Weight of water soluble ash (gms)	Percentage w/w water soluble ash	Mean value
2.0 2.0 2.0	0.132 0.130 0.130	6.6 6.5 6.5	6.53

Table 4: Percentage acid insoluble ash values

Weight of the drug powder taken (gms)	Weight of acid insoluble ash(gms)	Percentage w/ w acid insoluble ash	Mean value
2.0 2.0 2.0	0.266 0.235 0.249	13.3 11.75 12.45	12.5

Table 5: Extractive value

S.No.	Solvent	Initial Weight of Sample	Amount of Solvent	Final Weight of Sample	Extractive Value
1.	Ethanol	15gm	150ml	12.80gm	14.66%
2.	Water	15gm	150ml	13.45gm	10.33%

Table 6: Phytochemical screening of Leaf extracts of *Zanthoxylum armatum* DC-

Phytoconstituents	***Test performed***	***Result***	
		Aqueous	***Ethanol***
Carbohydrates	Molish's test	+	+
	Benedict's test	-	-
	Fehling's test	-	-
Glycosides	Legal test	+	-
	Modified Borntrager's test	+	-
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
Alkaloids	Dragendorff's test	+	+
	Wagner's test	+	+
	Hager's test	+	+
	Mayer's test	+	+
Sterols and Terpenoids	Libermann-burchard test	+	+
	Salkowski test	+	+
Protein and Amino acid	Biuret test	-	-

	Ninhydrin test	+	+
Flavonoids	Zinc Hydrochloride reduction test	-	-
	Alkaline reagent test	-	-
Gum and Mucilages	Ruthenium red test	-	-

6. Acknowledgement

Authers are thankful to Mrs. Neetu Kainth (M.D) and Prof. Kumud Upadhyaya, Director; for providing laboratory facilities for this research work.

7. References

1. Des Pande VH, Shastri RK, Indian J. Chem., 1977, 15B, 95-96.
2. Singh B, Uniyal AK, Todaria NP. Studies on Allopathic Influence of *Zanthoxylum armatum* D.C. on Important Field Crops Seeking its Sustainable Domestication in Existing Agroforestry Systems of Garhwal Himalaya, India. J sustainable agriculture 2007; 30(3): 51-56.
3. Upadhyaya Kumud, Kumar AP. Concentration dependent antioxidant activity of *Zanthoxylum armatum*. J Pharm Res, 2010; 3(7): 1581-1582.
4. Dongmo PMJ, Tchoumbougnang F, Tchinda SE, Manedong KS, Amvam ZPH, Menut C, Antioxidant and anti-inflammatory potential of essential oils of some *Zanthoxylum* (Rutaceae) of Cameroon. Int. J. Essent. Oil Therap., 2008; 2: 82-88.
5. Dube S, Kumar A, Tripathi SC. Antifungal and Insect-Repellent Activity of Essential Oil of *Zanthoxylum alatum*. Annals of Botany. 1990; 65: 457-459.
6. Gilani Syed Naeemuddin, Khan Arif-ullah, Gilani Anwarul Hassan. Pharmacological basis for the medicinal use of *Zanthoxylum armatum* in gut, airways and cardiovascular disorders, Phytotherapy Res, 2010; 24(4): 553–558
7. Kokate SD, Venkatachalam SR, Hassarajani SA, *Zanthoxylum armatum* extract as mosquito larvicide. *Proceedings of the National Academy of Sciences, India.Section B—Biological Sciences*. Allahabad: National Academy of Sciences, India 2001; 71B (3&4): 229
8. Kokashi CJ, Kokashi RJ, Sharma M. Fluorescence of powdered vegetable drugs in ultra-violet radiation. J American Pharm Assoc. 1958; 47:715-717.
9. Mukherjee PK. Quality Control of Herbal Drugs: An approach to evaluation of botanicals. 1st ed. New Delhi: Business Horizons; 2002.
10. Chopra RM,Nayer SL, Chopra IC, Glossary of Indian medicinal plants. Council of Scientificand Industrial Research, New Delhi, India, 1956.
11. Bisht D, Chanotiya CS, 2-Undecanone rich leaf essential oil from *Zanthoxylum armatum* , Nat. Prod. Commun, 2011; 6: 111-114.
12. Akhtar N, Ali Mohammad, Alam Mohammad Sarwar. Chemical constituents from the seeds of *Zanthoxylum alatum*, J Asian Natural Products Res, 2009; 11(1): 91 – 95.