AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Pharmacognostic Profiling and Pharmacological Screening of *Zanthoxylum Acanthopodium* DC – A Sub-Himalayan Shrub of Ethnomedicinal Value

Supriya Datta*[1], Debarupa D.Chakraborty[1], Nilanjan Sarkar[1], Prithviraj Chakraborty[1], Jyotirmoy Deb[1], Amitava Ghosh[1]

1.Bengal College of Pharmaceutical Sciences and Research, B.R.B. Sarani, Durgapur, West Bengal, 713212, India

ABSTRACT

The world is blessed with so many closely related plant species belonging to the same family and genus that their correct identification, organoleptic, physical, pharmacological evaluation and detection of adulterants, is of paramount importance. The objective of the proposed work is to carry out the Pharmacognostic investigation, volatile oil isolation, preliminary analysis and pharmacological study of the isolated volatile oil from the plant *Zanthoxylum acanthopodium* DC. Organoleptic studies, determination of stomatal number, and stomatal index, powder microscopy of the fruits, proximate analysis, essential oil isolation by hydro distillation technique, preliminary analysis and anti inflammatory activity of the essential oil. The fruit powder exhibited high values of total ash and sulphated ash content indicating high quantity of carbonates and oxides whereas lower acid insoluble ash indicating less siliceous materials like earth or sand. The essential oils exhibited significant anti-inflammatory activity in experimental animals compared to the standard drug Diclofenac Sodium. These values are helpful in determining the quality and purity of crude drugs in powdered form. As proper information regarding this plant was not available this study was carried out with an effort for correct identification of the plant and evaluation of pharmacological activity of its extracted oils. The encouraging finding of the study prompts for further research on the subject.

Keywords: Fluorescence analysis, Essential oil, Physical constants evaluation, Powder characteristics, *Zanthoxylum acanthopodium*.

*Corresponding Author Email: sup.d1976@gmail.com

Received 7 May 2013, Accepted 20 May 2013

Please cite this article in press as: Datta S. *et al.*, Pharmacognostic Profiling and Pharmacological Screening of *Zanthoxylum Acanthopodium* DC – A Sub-Himalayan Shrub of Ethnomedicinal Value. American Journal of PharmTech Research 2013.

INTRODUCTION

The highlands of Eastern and Southeast Asia of upto 3000 m and above are the habitat for the evergreen medicinal shrub *Zanthoxylum acanthopodium* DC. belonging to the family rutaceae.[1] India houses almost 11 different species like; *Z. budrunga, Z. oxyphyllum, Z. ovalifolium, Z. acanthopodium, Z. planispinum, Z. armatum, Z. nitidium Z. rhesta, Z. simulans, Z. avicennae* and *Z. limonella*. [2] The Khampti tribes of Arunachal Pradesh utilize the aqueous leaf and stem barks extract of *Z. acanthopodium* DC for the management of diabetes mellitus. [3] The fruits of this plant are also used to remove tumors. [4]

The present study was focused on analyzing the important parameters to facilitate authentication of the plant material, and to evaluate the anti inflammatory potential of the essential oils obtained from the fruits of *Z. acanthopodium* DC.

MATERIALS AND METHODS

Collection, and processing of the plant materials:

The plant *Zanthoxylum acanthopodium* DC was collected from Pandam Zone (East Sikkim) in the month of September to November and authenticated at BSI Gangtok India. After collection the collected plant materials were dried at room temperature for 3-4 days. The fruits of the plants were separated and stored in a well closed container. The picture of the plant and its fruits were displayed in figure 1.

Figure 1: Plant and Fruits of *Zanthoxylum acanthopodium* DC.

Pharmacognostical study:

Pharmacognostical study was performed by evaluating both microscopic and macroscopic parameters. The dried fruits were taken for the study and characterization of the materials with respect to its colour, odour, taste, texture was performed. For microscopic studies the thinnest

possible transverse sections of the leaf was treated with appropriate staining reagents and were mounted on glass slides and observed under the microscope.

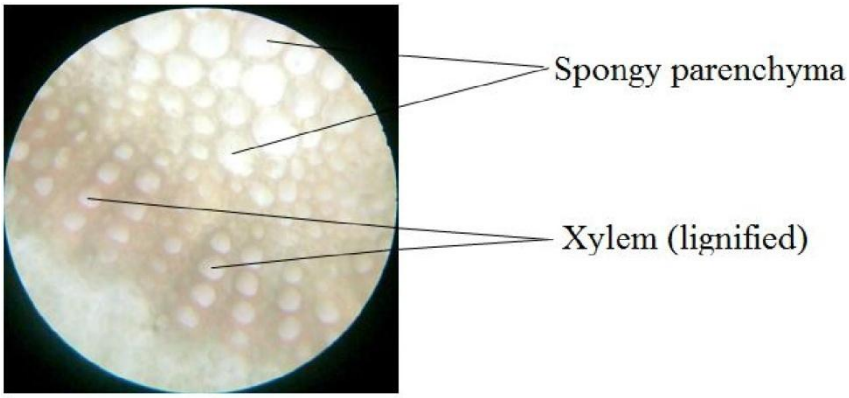

Figure 2: Transverse section of the leaf of *Zanthoxylum acanthopodium* (DC) 450X

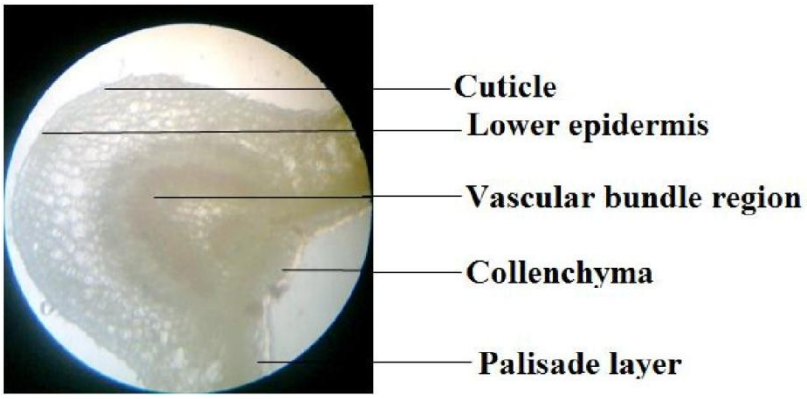

Figure 3: Transverse section of the leaf of *Zanthoxylum acanthopodium* (DC) 100X

Stomatal number and Stomatal index:

The important identifying characteristics of leaf constants were carried out by performing both stomatal index and stomatal number according to the standard method.

Powder microscopy of fruits:

The behaviour of the powder after treating with different chemical reagents was studied by following the standard methods [5-9].

Proximate analysis:

Physical evaluation of the powdered drug including Fluorescence analysis and the percentage of ash values were carried out according to the official methods prescribed in Indian Pharmacopoeia. [10-14]

Isolation of volatile oil by hydro-distillation technique:

Extraction of essential oil from the fruits of *Zanthoxylum acanthopodium* was carried out by Hydro-distillation technique according to the British Pharmacopoeia vol. IV. [14]

Organoleptic properties of oil:

2ml of essential oil obtained from *Zanthoxylum acanthopodium* DC was taken in a 10 ml

stoppered borosilicate glass tube and its organoleptic characters were observed. These are recorded on table 4.

Relative density of oil:

The relative density of the isolated essential oil was performed as per the procedure given in Indian pharmacopoeia 1996 vol-II. The relative density of the volatile oil was recorded in table 4.

Refractive index of oil:

The refractive index of the essential oil was done as per the procedure given in Indian pharmacopoeia 1996 vol-II. [10] The value was recorded in table 4.

Anti inflammatory activity of essential oil:

The anti-inflammatory activity of the essential oil obtained from the fruits of *Zanthoxylum acanthopodium* was evaluated by using carrageenan induced rat paw oedema model as per the methodology described by Bose A *et al.*, 2007 and Pank Y M *et al.*, 2005. [15-16]

The results were evaluated statistically by one way ANOVA followed by Dunnet's t test and was displayed in figure 9.

RESULTS AND DISCUSSION

Macroscopic characters of Leaves:

Thick glabrous sclerophyllous leaves, deep green (upper surface) and light green (lower surface) with characteristics odour, pungent taste, 5.0-7.6 cm length, 2.1-2.4 cm width, acute apex, sub-entire margin, symmetrical base, trifoliolate /imparipinnate venation and leaflets upto 6 pairs.

Macroscopic characters of fruits:

Light green (at the time of collection) and greyish black (after shade drying) with characteristics odour and taste, 4-5.8 mm diameter and surface rough and wavy like appearance.

Histological studies:

The transverse sections of the leaf were carried and observed under the microscope which shows the presence of spongy parenchyma, upper epidermis, lower epidermis, xylem (lignified), phloem (none lignified), cuticle, collenchymas, palisade layer and vascular bundle region were seen under microscope. The results were displayed in Fig.no.2 and 3. Stomatal number and stomatal index of the leaf was determined with the help of camera lucida. The results were recorded in Table 1. Powder microscopy of the fruits showed the presence of lignified xylem vessels, endosperm, and clusters of starch grain and oil globules. The results were displayed in Figure 4,5,6,7 and 8. Fluorescence analysis of the powdered fruits was also carried out. The

powder drug showed brown, black color in visible rays with different reagents whereas it was found to be brown, deep brown, dark brown color in short UV rays and black under long wave. The results were displayed in Table 2. The physicochemical constants like, ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash of the fruit powder was determined and recorded in Table 3.

Table 1. Stomatal number and Stomatal index of *Zanthoxylum acanthopodium* (DC).

Parts of plant	Stomatal number	Stomatal index
Upper surface	88- 133	27.25- 33.27
Lower surface	133- 177	22.05- 27.25

Table 2. Fluorescence analysis of powdered seeds of *Zanthoxylum acanthopodium* DC in different solvents.

Chemical reagents	Ordinary light	λ_{max} (nm) in UV spectrophotometer	
		UV-254 nm	UV-365 nm
Powder as such	Brown	Brown	Black
50% sulphuric acid	Brown	Green	Black
50% nitric acid	Light brown	Brown	Dark brown
1N Hydrochloric acid	Brown	Black	Deep black
Methanolic sodium hydroxide	Dark brown	Dark green	Black
5% potassium hydroxide	Brown	Green	Black
Methanol	Black	Black	Deep black
Cold water	Brown	Deep brown	Black
Hot water	Brown	Green	Black
Picric acid	Light green	Dark green	Black
Ammonia solution	Brown	Dar green	Black
Chloroform	Brown	Very light green	Dark brown
Glacial acetic acid	Dark brown	Black	Deep black
5% iodine solution	Yellowish red	Green	Deep black
Ferric chloride solution	Yellowish red	Light green	Deep black

Table 3. Different ash values (%w/w) of *Zanthoxylum acanthopodium* (DC).

Total ash	Acid insoluble ash	Water soluble ash	Sulphated ash
7.84	2.10	3.95	4.05

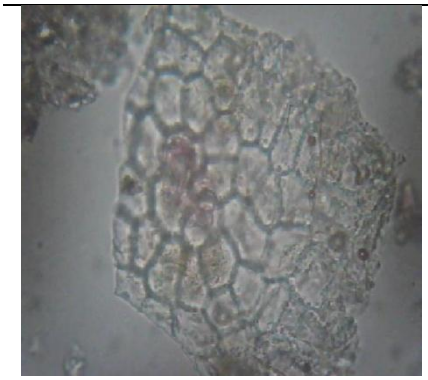

Figure 4: Endosperm (1000X)

Figure 5: Lignified xylem vessels (1000X)

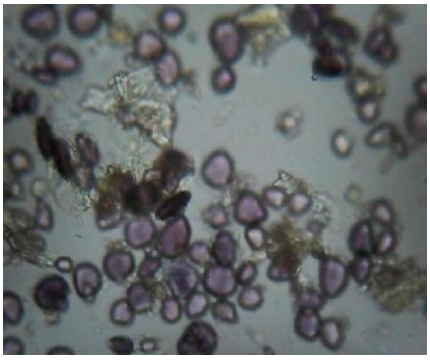

Figure 6: Starch grains (1000X)

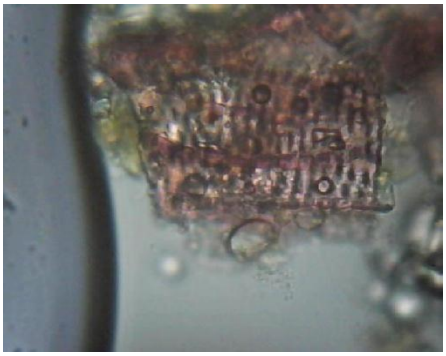

Figure 7: Lignified scalar form xylem vessels (1000X)

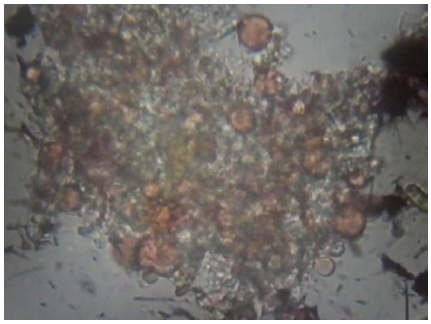

Figure 8: Volatile oil glands in the endosperm (1000X)

Isolation of volatile oil resulted in obtaining 4.56 ml of volatile oil and the rate of isolation of volatile oil was 0.019ml/min. and the percentage yield of volatile oil was found to be 0.912% v/w. The isolated essential oil was clear colourless, tasteless and imparts aromatic odour like citronella. The organoleptic properties of essential oil were tabulated in Table 4. Relative density of this volatile oil was found to be 0.876, Refractive index was found to be 1.4535. The results were displayed in Table 4. The isolated essential oil of *Z. acanthopodium* DC revealed maximum inhibition of paw edema volume (27.55%) as compared with standard diclofenac sodium (31.2%) at dose of 450 mg/kg b.w. The anti inflammatory potential of oil was displayed in Figure 9.

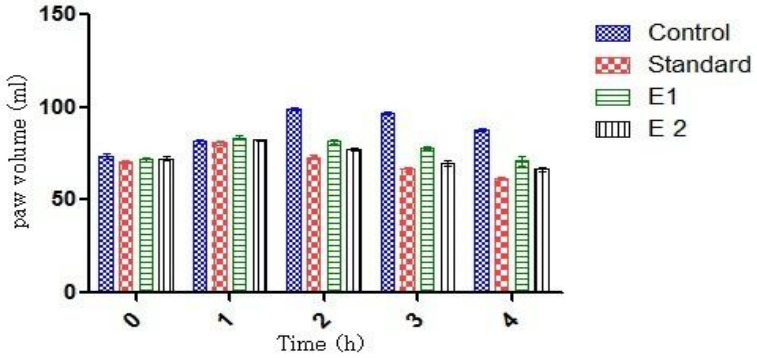

Figure 9: Anti inflammatory activity of essential oils on carrageenan-induced paw edema in rats.

Table 4. Organoleptic properties and physical parameters of oil obtained from the fruit of *Zanthoxylum acanthopodium* (DC).

Organoleptic properties				**Physical parameters**	
Color	Odor	Taste	Clarity	Relative density	Refractive index
Colorless	Aromatic	Characteristic	Clear liquid	0.876 gm/cc	1.4535

Table 5 The results were evaluated statistically by one way ANOVA

ANOVA Table	SS	df	MS		
Treatment (between columns)	832.0	3	277.3		
Residual (within columns)	933.6	16	58.35		
Total	1766	19			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Control vs Standard	17.14	3.548	Yes	**	4.618 to 29.67
Control vs E1 1	10.54	2.182	No	ns	-1.984 to 23.06
Control vs E 2 2	13.97	2.892	Yes	*	1.450 to 26.50

The microscopic and macroscopic observations characterize the genuinity of any plant drugs. The histological parameters were performed for proper standardization and better identification of different constituents present in the drug to prevent adulteration. Stomata are the important parameters for identification of different plant species and also provide immunity during bacterial attack. Variation in stomatal number of plant species is due to the long term environmental adaptation of the same. Leaf areas with a greater stomatal density have a greater rate of transpiration and vice versa. The stomatal density varies from 100 to 1000 depending on the plant species and environmental condition during development. In *Z. acanthopodium* DC, the availability of the stomata on the lower side of the leaf is more as compared to the upper epidermis for maximum exposure to sunlight and to prevent excess water loss.[17-21] Ash values are helpful in determining the quality and purity of powdered crude drugs. A high ash value is indicative of contamination, substitution or adulteration. The acid-insoluble ash value particularly indicates contamination with siliceous materials like earth or sand. [12] The results for ash values of *Z. acanthopodium* fruit powder had shown higher values of total ash (7.84%w/w) and sulphated ash (4.05 %w/w) indicating presence of high quantity of carbonates and oxides. Lower acid insoluble ash indicates less siliceous materials like earth or sand. Fluorescence behaviour of powder drug reveals a lot, some natural compounds like flavonoids having π electrons have tendency to form a charge transfer complex and binds with heavy metal ions e.g. Hg^{2+} and showing fluorescence. [22] Hence some crude drugs are often assessed qualitatively by

this way and thus it is an important parameter for evaluation. The volatile oils isolated from the fruits of *Z. acanthopodium* DC exerts significant anti-inflammatory activity in experimental animals at the dose of 450 mg/kg b.w. as compared with the standard drug Diclofenac sodium at dose level of 50mg/kg b. w. as well as control group.

CONCLUSION:

Adulteration of crude drugs is one of the prime hindrances in effective production of quality herbaceuticals. The principal focus of this study was to ensure proper profiling of *Z. acanthopodium* DC encompassing a detailed assessment of macroscopic, microscopic and physical parameters of the plant to prevent contamination with cheaper and inferior alternatives. In addendum thereto, the isolated essential oil from the fruits of *Z. acanthopodium* DC exhibited promising anti-inflammatory potential as evidenced from the experimental observations on rat paw edema model. The encouraging finding of the study prompts for further research on the subject to reveal the unexplored therapeutic potential of Z. acanthopodium DC and its optimal utilization for the mankind.

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