PHARMACOGNOSTIC STUDIES ON THE SPINE OF *ZANTHOXYLUM RHETSA* (ROXB.)DC.

Lalitharani S[1], Kalpanadevi V[2] and Mohan V R[2]

[1] Department of Botany, Mount Carmel College, Bangalore, 560052, India.
[2]Ethnopharmacology unit, Botany Research Lab, V.O. Chidambaram College, Tuticorin-628008, Tamil Nadu, India.
vrmohanvoc@gmail.com

ABSTRACT

Zanthoxylum rhetsa (Roxb.) DC. known to the Kanikkars as "Malvapoou" is an important medicinal plant. The Kanikkar tribe, inhabitants of the Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu, applied the paste prepared by rubbing the hard spines on the rock along with water on the breast to give relief from pain and increase lactation in nursing mothers. From the exhaustive literature survey, it is found that so far no proper pharmacognostical and phytochemical studies of spine of *Zanthoxylum rhetsa* have been reported. The present investigation deals with the pharmacognostic studies of the spine of the said plant. Pharmacognostic studies include microscopic, physicochemical constants (ash & extractive values), fluorescence analysis and preliminary phytochemical evaluations.

Key words: *Zanthoxylum rhetsa,* Pharmacognosy, Fluorescence analysis.

INTRODUCTION

Plant materials are used throughout developed and developing countries as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the global drug market. It is therefore essential to establish internationally recognized guidelines for assessor their quality (Rajesh *et al.,* 2010).There is a need for documentation of research work carried out on traditional medicines. With this back drop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies (Ozarkar, 2005). These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy, simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics (Anonymous, 1998).

The *Zanthoxylum rhetsa* (Roxb.) DC. belongs to the family Rutaceae. It is commonly known as "Malvapoou" in Kanikkar tribals of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. Kanikkar tribes applied the paste prepared by rubbing the hard spines on a rock along with water on the breast to give relief from pain and increase lactation in nursing mothers (Lalitharani and Mohan, 2010). However, perusal of literature reveals that, pharmacognostic information on *Zanthoxylum rhetsa* spine totally lacking, hence in the present investigation was undertaken. The objective of the present study is to evaluate various pharmacognostic standards like microscopy, physico-chemical constant, fluorescence analysis and preliminary phytochemical analysis of *Zanthoxylum rhetsa* (Roxb.)DC spines.

MATERIALS AND METHODS

Fresh stem spines of *Zanthoxylum rhetsa* (Plate I) were collected from the well grown plants found in the natural forest of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. Identification and confirmation were done by Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu, India. Voucher specimens were deposited in the ethnopharmacology unit, Reaserch Deparment of Botany, V.O.Chidambaram College, Tuticorin.

For anatomical investigations, standard microtomy techniques (Johansen, 1940) were followed. T.S. of 10 to 12µm thickness was prepared. These microtome sections were stained with 0.25% aqueous Toluidine blue (Meta chromatic stain) adjusted to pH 4.7 (O'Brien *et al*., 1964). Photomicrographs were taken with Nikon trinocular photomicrographic unit. The most accepted descriptive terms were being used to describe the leaf and stem anatomy (Junikka, 1994; Trockenbrodt, 1990).

Physicochemical and fluorescence analyses

These analyses were carried out as per the standard procedure (Lala, 1993). In the present study, the powdered stem spine was treated with various chemical reagents like aqueous 1N Sodium hydroxide, alcoholic 1N sodium hydroxide, 1N hydrochloric acid, 50% sulphuric acid and concentrated nitric acid, picric acid, acetic acid, ferric chloride, conc. HNO_3 +NH_3 and their extracts were subjected to fluorescence analysis in day light and UV light (254 nm and 366 nm). Various ash types and extractive values were determined by following the standard methods Anonymous (1996).

Preliminary phytochemical analysis

Shade dried and powdered stem spines of *Zanthoxylum rhetsa* were successively extracted with petroleum ether, benzene, chloroform and ethanol. The extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedures (Lala, 1993; Brindha *et al*., 1981).

RESULTS AND DISCUSSION

Microscopic features

Stem spine (Plate II 1-7)

The stem spines have broad basal part and abruptly narrow, short, pointed conical upper part. The upper spiny part is 50 µm thick and basal part is 200 µm wide.

As seen in transectional view, the spine has an epidermal layer of squarish cells with heavily thick walls. The remaining portion is sclerenchymatous; the cells are thick walled sclereids. The epidermal and subepidermal cells have dark cell inclusions.

The ground tissue at the basal part of the spine consists of polygonal or rectangular, thick walled cells mixed with wider hyaline cells. The cells other than the colourless cells have dense wide circular simple pits. The hyaline cells have no pits and their walls are comparatively thin.

The terminal part of the spine has elongated brachysclereids. The sclereids have thick lignified walls, narrow lumen and simple canal-like pits. In transectional view, the sclereids appear polygonal with thick walls and dense pits.

POWDER ANALYSIS OF THE DRUG

Ash and extractive values

The results of the ash and extractive values of spine of *Zanthoxylum rhetsa* drug powder are depicted in Table - 1. The total ash content of the powdered spine is 4.41% and extractive value of water is more than in other solvents.

Fluorescent analysis

The results of fluorescent analysis of spine powder of *Zanthoxylum rhetsa* are shown in Table -2. The spine powder shows the characteristic fluorescent green colour treated with 1N aqueous Na OH under short UV light.

Preliminary Phytochemical screening

The results of preliminary phytochemical screening of spine extracts of *Zanthoxylum rhetsa* are presented in Table -3. The ethanol extracts of the spine shows the presence of alkaloid, terpenoid, catechin, coumarin, tannin, flavonoid, phenol, xanthoprotein, sugar and fixed oil.

DISCUSSION

The present study attempts a modest comprehensive investigation of the stem spines of *Zanthoxylum rhetsa*. Since the whole plant of *Zanthoxylum rhetsa* as the folklore claims has therapeutic qualities the, present investigations has laid down a set of anatomical features of the stem spines which can be employed for its botanical diagnosis. The salient features of identification of the fragmentary sample are;

- The stem spines are extremely hard, conical in shape with pointed tip and wide basal part.
- The terminal part of the spine consists of compact, vertically oblong brachy-sclereids with thick walls and canal-like pits.
- The basal wide part has circular, compact parenchyma cells. Some of the cells are wider and hyaline; major portion has thick walled cells with dense simple pits.
- The epidermal and subepidermal layers of the lower part of the spine have dense accumulation of dark contents.

PHYSICOCHEMICAL CONSTANTS

Ash values

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs African Pharmacopoeia (1986). Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica (Ali, 1994).

Table 1: Ash and extractive values of the powdered stem spine of *Zanthoxylum rhetsa*[a]

Ash Values

Sr. No	Type of Ash	% of Ash
1	Total ash value of powder	4.41 ± 0.03
2	Water soluble ash	1.83 ± 0.01
3	Acid insoluble ash	0.94 ± 0.03
4	Sulphated ash	3.98 ± 0.12

Extractive Values

Sr. No	Nature of extract	Extractive value (%)
1	Petroleum ether	1.39 ± 0.06
2	Benzene	1.49 ± 0.01
3	Chloroform	2.19 ± 0.03
4	Acetone	2.48 ± 0.11
5	Methanol	2.95 ± 0.07
6	Ethanol	2.88 ± 0.03
7	Water	3.99 ± 0.05

Table 2: Fluorescence analysis of the powdered stem spine of *Zanthoxylum rhetsa*

Experiments	Visible / Day light	UV Light	
		254nm	365nm
Drug powder as such	Pale Brown	Brown	Dark Brown
Powder + 1N NaOH (aqueous)	Yellowish brown	Fluorescent green	Fluorescent green
Powder + 1N NaOH (alcohol)	Yellow	Yellow	Fluorescent yellow
Powder + 1N HCL	Pale yellow	Pale yellow	Fluorescent yellow
Powder + 50% H_2So_4	Pale yellow	Pale yellow	Pale yellow
Drug powder + Nitric acid	Pale yellow	Pale yellow	Pale yellow
Drug Powder + Picric acid	Yellow	Yellow	Yellowish brown
Drug Powder + Acetic acid	Pale yellow	Yellow	Fluorescent yellow
Drug Powder + Ferric chloride	Brownish yellow	Greenish yellow	Brown
Drug Powder + HNO_3 + NH_3	Light brown	Pale yellow	Yellow

[a] All values are mean of triplicate determinations.
± Standard error.

Table 3: Preliminary phytochemical screening of stem spine extracts of *Zanthoxylum rhetsa*

Test	Petroleum ether	Benzene	Chloroform	Ethanol
Alkaloid	-	-	-	+
Anthraquinone	-	-	-	-
Catechin	+	-	+	+
Coumarin	+	+	+	+
Flavonoid	-	+	-	+
Phenol	-	-	-	+
Quinone	-	-	+	-
Saponin	-	-	-	-
Steroid	+	+	+	-
Tannin	-	-	+	+
Terpenoid	+	-	-	+
Sugar	-	+	-	+
Glycoside	-	-	+	-
Xanthoprotein	+	-	+	+
Fixed oil	+	+	-	+

The ash values of stem spine of *Zanthoxylum rhetsa* is 4.41%. This ash value is indicative of the impurities present in the drug. Since the ash values are constant for a given drug, these values are also one of the diagnostic parameters of the drug. Samples have more water soluble ash than acid insoluble ash. The ash values are generally the index of the purity as well as identity of the drug.

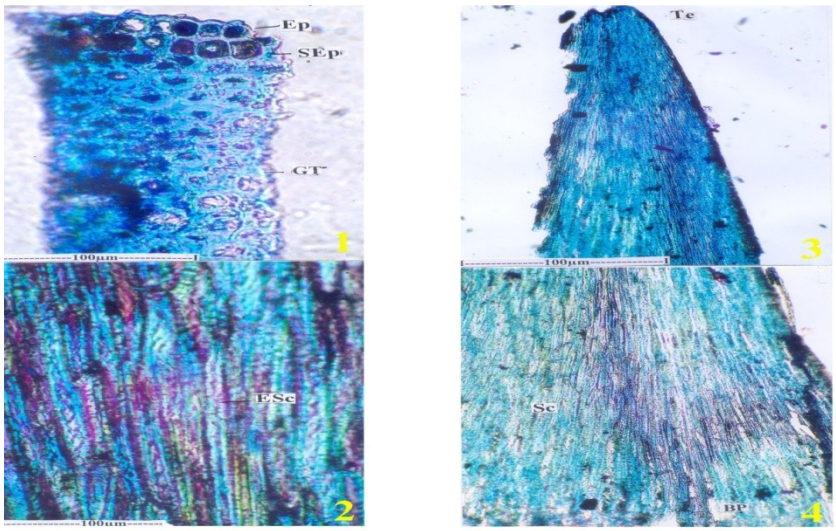
Fluorescent analysis

The powder from the stem spine of *Zanthoxylum rhetsa* fluoresced pale brown under day light, brown under short UV and dark brown in long UV light. Many phytocompounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyte over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples (Chakaravarthy *et al*., 1980).

Preliminary phytochemical analysis

Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Different chemical compounds such as alkaloid, terpenoid, catechin, coumarin, tannin, saponin, flavonoid, phenol and glycoside were detected in *Zanthoxylum rhetsa* which could made the plant useful for treating different ailments as having a potential of providing useful drugs for human use. This is because the, pharmacological activity of any plant is usually traced to a particular compound. Therapeutically, terpenoids exert wide spectrum of activities such as antiseptic, stimulant, diuretic, antihelmintic, analgesic and counter-irritant (Chaltopudhyay *et al*., 2006). Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering (Claus, 1956). They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and antidote (Evans, 1996). Saponins, a group of natural products occur in stem spine of *Zanthoxylum rhetsa*. In plants, the presence of steroidal saponins like, cardiac glycosides appear to be confined to many families and these saponins have great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D etc.,(Evans, 2001).

PLATE-I: Morphology of *zanthoxylum rhetsa* (Roxb.) DC spine



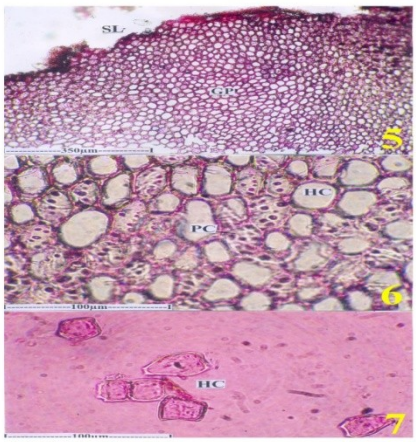


PLATE II: *zanthoxyllum rhesta* (Roxb.) DC

1. T.S. of the spine – a sector showing dark contents in the epidermis and subepidermal cells and sclereids in the ground tissue
2. L.S. of spine showing vertically elongated, narrow, compact-sclereids
3. L.S. of spiny portion – upper pointed part
4. L.S. of spiny portion – winder part
5. T.S. basal part of the spine showing the ground parenchyma
6. A portion of the cells enlarged showing densely pitted thick walled cells and non pitted hyaline wider cells
7. Hyaline cells – separated to show the variation in shape of the cells

Bp – Basal part; Ep – Epidermis; Esc – Elongated sclereids; GT – (Sclerotic) ground tissue; Gp – Ground parenchyma; HC – Hyaline cells; Pc – Pitted cells; SL – Superficial layer; Sep – Subepidermal layer.

From plant sapogenins a synthetic steroid is prepared to treat a wide variety of diseases such as rheumatoid arthritis, collagen disorders, allergic and asthmatic conditions (Geetha, 1994).

Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles (Gokhale *et al*., 2003; Handa and Kapoor, 1992). Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats (Haydon, 1975). Flavonoids act as insulin secretagogues (Horborne, 1976). Most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc, which are frequently implicated as having anti diabetic effects (Loew and Kaszkin, 2002). Saponin reduce the uptake of certain nutrients including glucose and cholesterol at the got through intra-luminal physicochemical interactions. Hence, it has been reported to have hypocholesterolemic effect and thus may aid lessening metabolic burden that would have been placed in the liver (Musa *et al*., 2006).

To understand the nature of the fluorescence emission from these crude preparations under different conditions, the preliminary phytochemical analysis of these crude preparations was compared. The comparative analysis clearly showed a correlation between a compound present in it and their fluorescent behaviour under different conditions. The major bioactive compounds present in these crude preparations are the coumarins, flavones, tannins, alkaloids and saponins. Coumarin especially hydroxyl amino acid derivatives like o-coumaric acid appears yellowish green in alkaline condition under short UV radiation. Flavones which are light yellow in aqueous condition under UV light turns to bright yellow under alkaline conditions. Similarly the phytosterols when treated with 50% H_2SO_4 show green fluorescence under UV light. Terpenoids especially sapogenins exhibit yellow green fluorescence under short UV light (Oliver-Bever, 1986). Quinine, aconitin, berberin and emetin show specific colour of fluorescence (Aconitin - light blue; berberin - light yellow; emetin - orange). Fixed oils and fats fluoresce least, waxes more strongly and mineral salts most of all (Pimenta *et al*., 2006; Price *et al*., 1987) studied the photophysical characters of coumarins. Hydroxy methyl coumarin fluoresced in 420-440nm when observed in different solvents with increasing polarity (Rhemann and Zaman, 1989). The fluorescence analysis of the crude drug of *Zanthoxylum rhetsa* exhibited clear fluorescence behaviour at different radiations which can be taken as standard fluorescence pattern.

Since the stem spine of *Zanthoxylum rhetsa* is useful in traditional medicine for the treatment of various ailments, it is important to standardize to use it as a drug. The pharmacognostic study of the *Zanthoxylum rhetsa* has been carried out for the first time. The pharmacognostic constant of the various parts of above said plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

LITERATURE CITED

African Pharmacopoeia, 1986. General Methods for Analysis Ist ed. 2: (OAU/STRC) Lagos. p. 123.

Ali M, 1994. Text book of Pharmacognosy. CBS Publishers and Distributors, New Delhi, pp.405.

Anonymous, 1996. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, The Controller of Publications, Civil Lines, Delhi – 110054.Vol. I & II.

Anonymous, 1998. Macroscopic and microscopic examination: Quality Control Methods for Medicinal Plant Materials, WHO, Geneva.

Brindha P, Sasikala P, Purushothaman KK, 1981. Pharmacognostic studies on merugan kizhangu. *Bull. Med. Eth. Bot. Res*. **3**: 84-96.

Chakaravarthy BK, Gupta S, Gambir SS Gode KD, 1980. Pancreatic beta cell regeneration.A novel antidiabetic mechanism of *Pterocarpus marsupium* Roxb. *Indian J. Pharmacol*. **12:** 123-127.

Chaltopudhyay N, Mallick A, Sengupt S, 2006. Photophysical studies of hydroxyl methyl coumarin: A new fluorescent chemosensor for Zinc and Nickel ions in water. *J. Photochem. Photobiol.* **177**: 55-60.

Claus EP, 1956. Pharmacognosy. (Ed.). Lea and Febiger, Philadelphia. pp.1- 697.

Evans WC Saunders WB,(Eds.) 2001. Trease and Evan's Pharmacognosy Tokyo. pp. 1- 579.

Evans WC, 1996. Trease and Evans' Pharmacognosy. Singapore, Harcourt Baraco and Company Asia Pvt. Ltd pp. 1-437.

Geetha BS, Mathew BC, Augusti KT, 1994. Hypoglycemic effects of leucodelphinindin derivative isolated from *Ficus bengalensis* Linn. *Indian J. Ohysiol. Pharmacol*. **38**: 220-222.

Gokhale SG, Kokate CK Purohit AP, 2003. A text Book of Pharmacognosy, Nirali Prakashan, Pune.

Handa SS, Kapoor VK (Eds.) 1992. Pharmacognosy Vallabah Prakashan Publications, New Delhi. pp. 51.

Haydon SC, 1975. Spectroscopic letters. 8: 815.

Horborne JB,1976. Phytochemical methods. Chapman & Hall, New York. pp. 1- 288.

Johansen DA, 1940. Plant Microtechnique . MCGraw-Hill Boook Company, Inc. New York and London, pp.1-523.

Junikka L, 1994. Survey of English Macroscopic bark terminology, *IAWA Bull*, **15**: 3- 45.

Lala PK, 1993. Lab Manuals of Pharmacognosy, CSI Publishers and Distributors, Calcutta, 5 th (Ed).

Lalitharani S, Mohan VR, 2010. GC-MS analysis of ethanolic extract of *Zanthoxylum rhetsa* Roxb. DC spines. *J. Herbal. Med.Toxi*. **4:** 191-192.

Loew D, Kaszkin M, 2002. Approaching the problem of bioequivalence of herbal medicinal products. *Phytpther. Res.* **16:** 705-711.

Musa KY, Katsayal AU, Ahmed A, Mohammed Z, Danmalam UH, 2006. Pharmacognostic investigation of the leaves of *Gisekia pharmacioides*. *African. J. Biotech.* **5**: 956-957.

O'Brien TP, Feder N, dMc Cull ME, 1964. Polychromatic staining of plant cell walls by toluidine blue – O.Protoplasma. **59**: 364-373.

Oliver-Bever B, 1986. Medicinal plants in tropical West Africa, Cambridge University Press, London. pp. 245-267.

Ozarkar KR, 2005. Studies on anti-inflammatory effects of two herbs *Cissus quadrangularis* Linn. and *Valerina wallichi* DC using mouse model Ph.D.Thesis, University of Mumbai, Mumbai.

Pimenta AM, Montenegro MC, Ara Ujo AN, Mart'inez JC, 2006. Application of sequential injections analysis to pharmaceutical analysis. *J. Pharm. Biomed. Anul.* **40:** 16-34.

Price KR, Johnson LI, Ferewick H, 1987. The chemical and biological significant of saponins in food and feeding staffs. *CRC Critical Rev. Food. Sci. Nut.* **26**: 127-135.

Rajesh P, Latha S, Selvamani P, Rajesh kannan V, 2010. *Capparies sepiaria* Linn. Pharmacogonstical standardization and toxicity profile with chemical compounds identification (GC-MS). *Inter. J. Phytomed*. 271-72.

Rhemann AV, Zaman K, 1989. Medicinal plants with hypoglycemic activity. *J.Ethnopharmacol.***26**:1-55.

Trockenbrodt M, 1990. Survey and discussion of the terminology used in bark anatomy. *IAWA Bull*. **11**: 141-166.

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