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Histological and Physico-chemical Evaluation of *Zanthoxylum nitidum* Stem Bark

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Abstract

Zanthoxylum nitidum (Roxb.) DC (Rutaceae), called *Tez-mui* in Assamese, is a large prickly shrub, and its stem bark is ethnomedicinally prescribed in North-East India for treatment of various disease conditions. Scientific parameters are not yet available to identify the exact plant material and to ascertain its quality and purity. The present investigation was therefore undertaken to determine the requisite histological and physico-chemical standards for evaluating the plant material. These studies provided referential pharmaco-botanical information for correct identification and standardization of this plant material. These information will also be helpful to differentiate *Z. nitidum* from the closely related other species of *Zanthoxylum*.

Key words: *Zanthoxylum nitidum*, stem bark, pharmaco-botanical, quality control.

Introduction

The genus *Zanthoxylum* Linn belongs to the family Rutaceae and is a large genus of aromatic prickly trees or shrubs distributed pan-tropically and 13 species of it are found in India. *Zanthoxylum nitidum* (Roxb.) DC (Rutaceae), called *Tez-mui* in Assamese is a morphologically variable plant species occurring in south-east Asian countries and in northen Australia.[1] In India it grows as a large prickly shrub particularly in North-East India (Sikkim, Assam and Nagaland). In India, 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 piscicide. The fruit is used in the treatment of stomachache, cough, colic vomiting, diarrhoea, and paresis and as an aromatic, stimulant and piscicide. The small branches, seeds

and stem bark are prescribed in fever, diarrhoea and cholera.[2 - 4] Recently, Bhattacharya *et al*. (2009) reported that the stem bark has got antibacterial property[5]. However, no scientific standards or pharmaco-botanical parameters are yet available to ascertain the identity and to determine the quality of the plant material. The present work therefore, attempts to report various necessary histological and physico-chemical standards of *Z. nitidum* stem bark growing in upper Assam, India.

Materials and Methods

Plant material

The fully matured entire plants of *Z. nitidum* were collected during the month of November 2006 from Dibrugarh, Assam, India. The species was identified from Botanical Survey of India, Eastern Circle, Shillong, India, and a voucher specimen (No. DUPS-06-003) was deposited in Department of Pharmaceutical Sciences, Dibrugarh University. All the prickles were removed from the stems and branches carefully by using a sharp knife, without harming the bark. Then the barks were peeled off from the shoots. Longitudinal incisions were made by a sharp knife on the shoots and transverse markings were given so as to form the rings which also connect the longitudinal incisions producing the strips which were then peeled off. Then the stem barks were shade dried at temperature 21-24°C for different studies.

Reagents and chemicals

All reagents and chemicals used for testing were analytical grade obtained from Ranbaxy Fine Chemicals Ltd., New Delhi and Loba Chemie, Mumbai, India.

Histological studies

The transverse sections (TS) of freshly collected mature stem bark were obtained by usual techniques.[6] Good sections were collected and observed under compound microscope. A camera lucida was attached with the microscope and the sections were suitably traced out.[7]

Physico-chemical evaluations

Physico-chemical parameters such as the percentage of loss on drying (LOD), total ash, acid insoluble ash and water soluble ash were determined as per the Indian Pharmacopoeia.[8] Water and ethanol soluble extractives were estimated according to the method prescribed by WHO.[9] All determinations were performed in triplicate and the results are presented as mean ± standard error of mean (SEM).

Phytochemical screening [10,11]

The dried and powdered stem bark was subjected to preliminary phytochemical screening for

qualitative detection of phytoconstituents.

Preliminary phytochemical screening of methanol extract of *Z. nitidum* was carried out by using standard procedures described by Kokate (1994) and Khandelwal (2005).

Fluorescence analysis [12]

Fluorescence analysis of dried and powdered stem bark was carried out according to the procedure described by Gupta et al. (2006) by using the reagents as mentioned in Table 4 and viewed in day light, short (254 nm) and long (365 nm) ultraviolet (UV) radiations. The colours and fluorescence (if any) observed by application of different reagents in different radiations were recorded.

Results and Discussion

The TS of stem bark is shown in Fig 1. The TS exhibited a cork consisting of narrow cells. The cortex contained small starch grains, crystals of calcium oxalate, but no sclereids. After cortex there was a narrow band of pericyclic sclerenchyma. The medullary rays were numerous, mainly one cell wide. Calcium oxalate crystals were also found in the phloem. The microscopic or histological features, like presence of pericyclic sclerenchyma, absence of sclereids, etc may be useful diagnostic histological charecters.

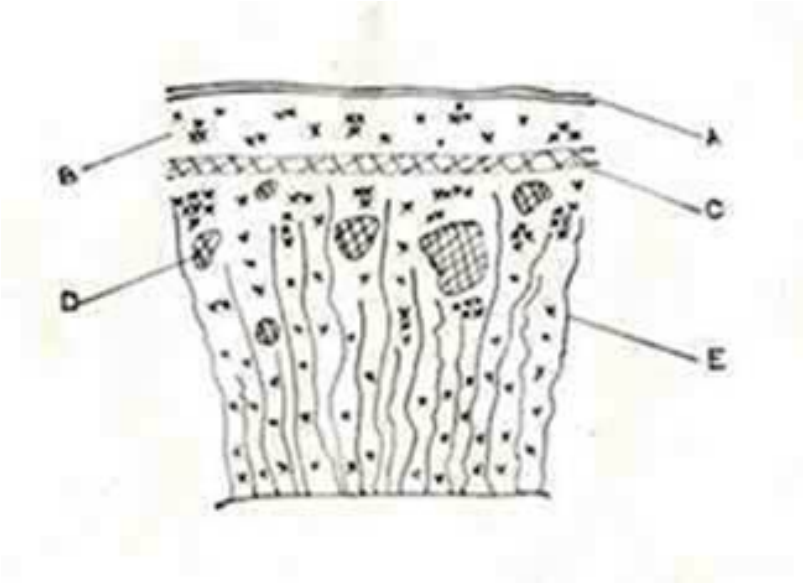

Fig 1. Schematic diagram, TS of the stem bark.
A- cork, B- cortex, C- pericyclic sclerenchyma, D- sclerenchyma
E- medullary ray.

The results of preliminary phytochemical screening are shown in Table 1. The results demonstrated

presence of true alkaloids, carbohydrates and flavonoids in the stem bark extract of *Z. nitidum.*

Table 1. Results of phytochemical screening of methanol extract of stem bark of *Z. nitidum.*

Constituents	Methanol extract
Alkaloids	+
Purine alkaloids	-
Carbohydrates	+
Glycosides	-
Steroids	-
Flavonoids	+
Saponins	-
Fixed oils and fats	-
Tannins	-
Proteins and amino acids	-
Mucilage	-

+ = Present, – = Absent.

The values of all physico-chemical determinations are summarized in Table 2 and 3.Water soluble ash was found to be quite greater than acid insoluble ash value. The results showed that ethanol yielded higher extractive value. These all are important quantitative parameters for quality control of plant material.

Table 2. Loss on drying (LOD) and ash values of powdered stem bark of *Z. nitidum.*

Parameters	Values of three replicates (% w/w)	Mean (% w/w) ± SEM
Loss on drying (LOD)	4.17 6.20 7.19	5.85 ± 0.89
Ash values: 1) Total ash	5.80 6.00 5.62	5.81 ± 0.11
2) Acid insoluble ash	0.90 0.75 0.79	0.81 ± 0.05
3) Water soluble ash	3.79 3.94 3.72	3.82 ± 0.06

SEM = Standard Error of Mean

Table 3. Extractive values of stem bark of *Z. nitidum.*

Method of extraction	Values of three replicates (% w/ w)	Mean (% w/w) ± SEM
1) Water soluble	2.32 2.96 2.52	2.62 ± 0.18
2) Alcohol soluble	3.73 3.84 3.36	3.73 ± 0.10

SEM = Standard Error of Mean

The results of fluorescence analysis are summarized in Table 4. Different colours on application of different reagents on powdered stem bark were found under day light and UV light. However, no detectable fluorescence was observed.

Table 4. Fluorescence analysis of powdered stem bark of *Z. nitidum.*

Powdered drug	Visible/Day light	UV 254 nm (short)	UV 365 nm (long)
Powder as such	Light yellowish brown	Brown	Blackish brown
Powder + 1M NaOH	Yellowish brown	Dark yellowish brown	Dark brown
Powder +1% Picric acid	Yellowish brown	Brown	Black
Powder + Acetic acid	Brown	Dark brown	Blackish brown
Powder + 1M HCl	Brownish yellow	Brown	Dark brown
Powder + Dil HNO_3	Brownish yellow	Brown	Dark brown
Powder + 5% Iodine	Yellowish brown	Dark brown	Black
Powder + 5% $FeCl_3$	Yellowish brown	Brown	Black

Conclusion

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there have been an emphasis in standardization of medicinal plants of therapeutic potential. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken. [8]

After present investigation it can be concluded that the histological and physico-chemical studies of *Z. nitidum* stem bark yielded a set of qualitative and quantitative pharmaco-botanical parameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. As previously mentioned, *Z. nitidum* being a morphologically variable species, these information will also be helpful to differentiate *Z. nitidum* from the closely related other species and varieties of *Zanthoxylum.*

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References

1. Hu J, Zhang WD, Shen YH, Zhang C, Xu L, Liu RH, *et al*. 2007. Alkaloids from *Zanthoxylum nitidum* (Roxb.) DC. *Biochemical Systematics and Ecology* 35:114-117.
2. Kanjilal UN. 1997. *The Flora of Assam.* Vol. I (Part I). New Delhi: Omsons Publications.
3. Kirtikar KR, Basu BD. 1933. *Indian Medicinal Plants*. Vol. I. 2nd ed. Bishen Singh Mahendra Pal Singh, New Delhi.
4. Anonymous. 1976. *The Wealth of India, Raw Meterials*. Vol. XI: X-Z., Council of Scientific and Industrial Research, New Delhi.
5. Bhattacharya S. Zaman MK, Haldar PK. 2009. Antibacterial activity of Indian *Zanthoxylum nitidum. Asian Journal of Pharmaceutical and Clinical Research* 2 (1): 30-34.
6. Dwivedi JN, Singh RB. 1985. *Essentials of Plant Technique*.1st ed. Jodhpur: Scientific Publishers.
7. Evans WC. Trease and Evans 2002. *Pharmacognosy.* 15th ed. Edinburgh: W.B. Saunders.

8. Anonymous. 1996. *Indian Pharmacopoeia*. Vol II. 4th ed. New Delhi: Controller of Publications, Government of India.

9. Anonymous. 2002. *Quality Control Methods for Medicinal Plant Materials* (An authorized publication of World Health Organisation, Geneva). A.I.T.B.S. Publishers & Distributors (Regd.). New Delhi.

10. Kokate CK. 1994. Practical Pharmacognosy. 3rd ed. New Delhi: Vallabh Prakashan.

11. Khandelwal KR. 2005, *Practical Pharmacognosy: Techniques and Experiments*. 13th ed. Pune : Nirali Prakashan.

12. Gupta MK, Sharma PK, Ansari SH, Lagarkha R. 2006. Pharmacognostical evaluation of *Grewia asiatica* fruits. *International Journal of Plant Sciences* 1 (2): 249-251.