ORIGINAL ARTICLE

Pharmacognostical and physico-chemical standardization on the leaves of *Ziziphus oenoplia*

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Background: *Ziziphus oenoplia* is a shrub belonging to the family of Rhamnaceae. It is commonly known as Jackal jujube in English, Surai izlanthai in Tamil and Izlanthai, Makkai in Hindi. In the present era, adulteration and substitution has become a major problem due to unavailability of standards relating to the genuineness of the herbal drugs. The WHO has emphasised the need to ensure quality control of herbal products by using modern techniques. **Aim:** Therefore, an establishment of pharmacognostical and physicochemical standards on identification, quality and purity of the *Ziziphus oenoplia* is required. **Materials and Methods:** Microscopical studies were done by using trinocular microscope. Physicochemical properties including total ash, water-soluble ash, acid insoluble ash, sulphated ash and extractive value using various solvents, loss on drying, swelling and foaming index were determined. **Results:** In the microscopic study the leaf is dorsiventral in shape and shows fibre sheath, druses of calcium oxalate crystals, uniseriate multicellular covering trichomes and anisocytic stomata. The stomatal number, stomatal index, vein islet and veinlet termination number were found to be 130/sqmm, 70/sqmm, 75/sqmm and 41/sqmm, respectively. Linear measurements of trichomes were also determined. Total ash value, acid-insoluble ash, water-soluble ash, sulphated ash, alcohol-soluble extractive value, water-soluble extractive value, loss on drying, swelling index and foaming index were found to be 6.68% w/w, 2.34% w/w, 1.25% w/w, 10.57% w/w, 11.8% w/w, 6.2% w/w, 6% w/w, 8 ml/gm and less than 100, respectively. **Conclusion:** The information generated by this study will establish a proper identification and authentication of the leaf of *Ziziphus oenoplia* by future investigators.

Key words: Pharmacognostical, physico-chemical, *Ziziphus oenoplia*

INTRODUCTION

Ziziphus oenoplia is a spreading, climbing, thorny shrub growing up to 1.5 m in height. It is distributed from the Indian subcontinent through southern China and South-east Asia to northern Australia.[1] It grows along the roadside forests and thickets. *Ziziphus oenoplia* plant is widely used in Ayurveda for the treatment of various diseases, such as ulcer, stomach ache, obesity, asthma and it has an astringent, digestive, antiseptic, hepatoprotective, wound healing and diuretic property.[2-4] The present work aims to contribute towards solving the problem of controversial drugs prevalent in Ayurveda besides helping in laying down standards. Pharmacognostical studies include macroscopy, microscopy, powder microscopy, leaf constants, linear measurements and physicochemical evaluation which help to determine the quality and purity of the *Ziziphus oenoplia*. Literature review reveals that, such work is not reported and hence the present study was carried out.

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MATERIALS AND METHODS

Collection and Authentication of Drug

The fresh leaves of *Ziziphus oenoplia* were collected from Muthulapuram village, Theni district, Tamil Nadu. The collected plant materials were authenticated by Botanist Dr. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Tambaram, Chennai (PARC/2012/1306).

Macroscopic Evaluation

The macroscopic features of the fresh leaves of *Ziziphus oenoplia* were studied according to the methods of Evans.[5]

Microscopic Evaluation

Leaves were cut and removed from the plant and fixed in FAA (Formalin 5 ml + Acetic acid 5 ml + 70% Ethanol 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol. Infilteration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until tertiary butyl alcohol attained supersaturation. The specimens were cased into paraffin blocks. The paraffin-embedded specimen was sectioned with the help of rotary microtome. The thickness of the section was 10-12 µm. After dewaxing, the sections were stained with suitable reagents.[6-10]

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Received: 17-07-2013; **Accepted:** 03-12-2013

Powder Microscopy

The shade dried leaves of *Ziziphus oenoplia* were powdered well and then the powder was passed through sieve no 60. The prepared powder was examined for specific microscopic characters.

Leaf Constants

For studying the stomatal morphology, venation pattern, trichome distribution, paradermal sections as well as clearing of leaf with 5% Sodium hydroxide solution and epidermal peeling by partial maceration employing Jeffrey's maceration were prepared. Glycerin mounted on temporary preparations were made for macerated materials and used for the study of leaf constants.

Linear Measurements

Linear measurements were done on coarse powder of 1-mm size and used for the linear measurement of trichomes.[11]

Physicochemical Evaluation

Total ash, water-soluble ash, acid-insoluble ash, sulphated ash and extractive value using various solvents, loss on drying, swelling and foaming index were determined according to the standard procedure given in Indian Pharmacopoeia.[11,12]

RESULTS AND DISCUSSION

Macroscopical Evaluation

Ziziphus oenoplia leaf is pale green in colour and has a characteristic odour, mucilaginous taste, leaflet appeared as alternate compound leaves. Ovate to ovate lanceolate in shape, entire or crenate, glabrous margin, symmetrical base and it has a three prominent nerves with numerous transverse nervules. Flowers appear yellowish green in sub-sessile axillary cymes [Figure 1].

Microscopical evaluation

T. S of Leaf

The leaf is dorsiventral in shape. The midrib is boat-shaped with single layer of epidermis. The vascular bundle is of collateral type surrounded by fibrous sheath. Mucilage cells, druses of calcium oxalate crystal and starch grains seen in mesophyll cortex. The lower epidermal cells have papillose type of thickening. Uniseriate multicellular covering trichomes and anisocytic stomata with irregular epidermal cells present [Figures 2 and 3].

T. S of Lamina

The lamina is smooth and even on both sides. The adaxial and abaxial epidermis is made up of single layer of cells. The adaxial epidermis consists of rectangular cells; some of the cells are semicircular and wider oblong cells. Beneath the epidermis a chain of enlarged cells with mucilage and starch grains are present. In adaxial zone it consists of a double layer of vertical cylindrical palisade cells; the remaining zone includes four or five layers of large-lobed spongy parenchyma cells. The epidermal cells are squarish, thick-walled and have heavy cuticular layer. The submarginal mesophyll tissue includes undifferentiated mass of thick-walled cells. Druses of calcium oxalate are found scattered in the mesophyll region. Compound starch grains are also scattered in the lamina region [Figure 4].

T.S of Petiole

The petiole is ovoid in outline with thin layer of epidermis. The epidermis is extended as uniseriate 2-3-celled covering trichome. The epidermis is surrounded by mesophyll cortex scattered with solitary cortical fibres. The central portion is occupied by vascular bundles arranged in tetrads (three are united and one is separated). The bundle is of collateral type capped with fibres. Idioblast with mucilage is found scattered in the cortical region. Cells with simple starch grains are scattered [Figures 5 and 6].

Powder Microscopy

The leaf powder of *Ziziphus oenoplia* was pale green colour, with characteristic odour and mucilaginous taste.

Powder of the leaf shows the following features.

Trichomes - uniseriate multicellular covering trichome; Stomata - anisocytic stomata; calcium oxalate - druses of calcium oxalate crystals are seen in the powder drug [Figures 7 and 8].

Quantitative Microscopy

Leaf Constants

Anisocytic stomata are seen on the adaxial and abaxial epidermis. The quantitative measurements of stomatal number, stomatal index and vein islet and veinlet termination numbers were determined and reported in the Table 1.

Linear Measurement of Trichomes

Linear measurements of length and width of uniseriate multicellular covering trichomes were reported in Table 2.

Physicochemical Analysis

The results of physicochemical parameters are given in Table 3.

Table 1: Leaf constants of *Ziziphus oenoplia*

Parameters	Values in sq mm
Stomatal number (upper epidermis)	130-134
Stomatal index (upper epidermis)	70-73
Vein islet number	75-77
Veinlet termination number	41-45

Figure 1: Macroscopy

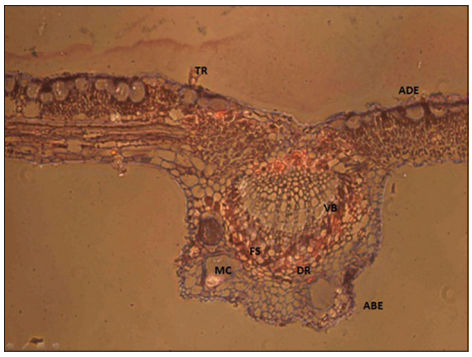

Figure 2: T. S. of leaf

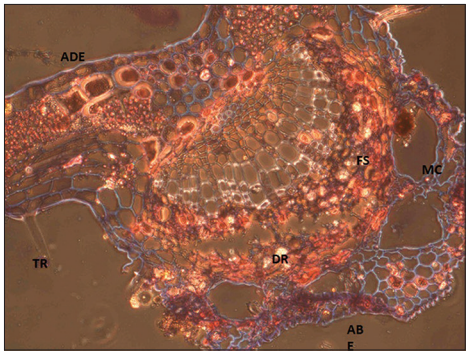

Figure 3: T. S. of midrib entire view. ABE - Abaxial epidermis, ADE - Adaxial epidermis, DR - Druses of calcium oxalate crystal, FS - Fibre sheath, MC - Mucilage cell, TR - Trichome, ST - Stomata, VB - Vascular bundle

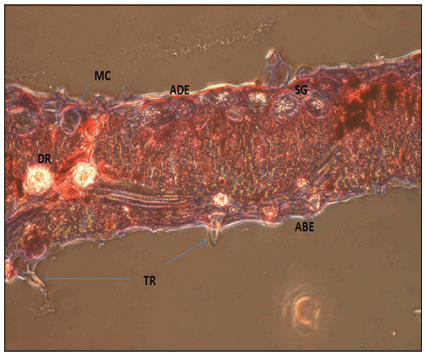

Figure 4: T.S. of Lamina. ABE - Abaxial epidermis, ADE - Adaxial epidermis, DR - Druses of calcium oxalate crystal, MC- Mucilage cell, TR - Trichome, SG - Starch grain, ST - Stomata

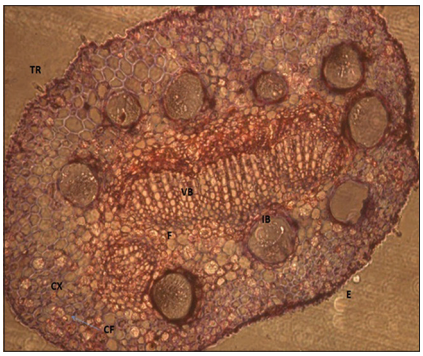

Figure 5: T.S. of Petiole

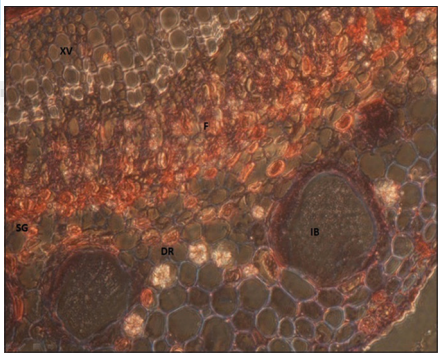

Figure 6: Petiole enlarged. CF - Cortex fibre, CX - Cortex, DR - Druses of calcium oxalate crystal, E - Epidermis, F - Fibre, IB - Idioblast, SG - Starch grain, TR - Trichome, VB - Vascular bundle, XV - Xylem vessel

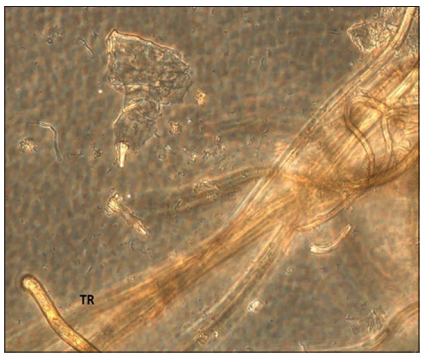

Figure 7: Powder microscopy. DR - Druses of calcium oxalate crystal, TR - Trichome

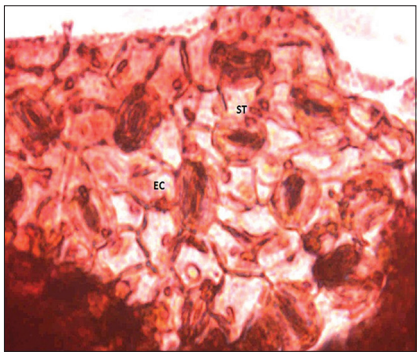

Figure 8: Stomata. EC - Epidermal cell, ST - Stomata

Table 2: Linear measurements of trichomes of *Ziziphus oenoplia*

Parameters	Minimum (µm)	Average (µm)	Maximum (µm)
Length	130	321	619
Width	11.9	16.66	23.8

Table 3: Physicochemical constants of leaf powder of *Ziziphus oenoplia*

Parameters	Percentage (W/W)
Ash values	
Total ash	6.687±0.01
Acid-insoluble ash	2.34±0.15
Water-soluble ash	1.25±0.1
Sulphated ash	10.57±0.32
Extractive values	
Water-soluble extractive	6.2±0.15
Alcohol-soluble extractive	11.8±0.3
Loss on drying	6±0.1
Swelling index	8ml/gm
Foaming index	>100

CONCLUSIONS

Standardisation of herbal drugs is a matter of great concern. Standardisation is very much essential for assessment of purity and identification of any sample. Pharmacognostical evaluation such as macroscopic, microscopic analysis, powder microscopy, leaf constants, linear measurements and physicochemical parameters were carried out on plant samples in order to establish appropriate data that can be used in identifying crude drugs particularly those supplied in powder form. These are standard pharmacognostical parameters that can be used to differentiate closely related plant species or varieties. The present work can be used as one of the tool for standardisation of this crude drug to identify and to decide the authenticity of this drug in herbal industry/trade.

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How to cite this article: ***.

Source of Support: Madras Medical College, Chennai, **Conflict of Interest:** None declared.