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PHARMACOGNOSITICAL STUDY OF ZIZYPHUS MAURITIANA

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ABSTRACT

Objective: The present investigation has been carried out to determine preliminary and pharmacognostic characteristics of *Zizyphus mauritiana* Lam. belongs to family Rhamnaceae and commonly known as Indian jujube or ber. Its leaves are used in the treatment of diarrhoea, gastric disorder, fever, liver damage and pulmonary disorders. **Method:** Macroscopic and microscopic study of the fresh and dry drug and determination of physicochemical parameters were performed. **Results:** Leaves are oval or sub-orbicular, alternate and petiolate from 4 mm to 5 mm. The limb, dark green in colour, is polished on the upper side and whitish and then densely tomentose on the lower side. The anatomo-histological cut of the limb showed a median rib slightly curved on the upper surface and strongly bulging on the lower side and a broader limb. Each epidermis consisted of small, visible cells more or less rectangular, is covered with a cuticle, outer lipoidal covering; impermissive and resistant, giving it a protective role. The cross-section of the stem, revealed a quadrangular shape, has two distinct zones: the bark and the central cylinder. The less developed bark comprises of 4 tissues (cuticle, epidermis, collenchyma and cortical parenchyma). The central cylinder, more developed than the bark, is composed of primary tissues (bone, wood, medullary parenchyma, sclerenchyma, and perimedullary fiber). The sclerenchyma occurs in small clusters around the conductive system. The chemical compositions of the leaves are proteins & amino acids, flavonoids, alkaloids, glycosides, terpenoides, saponins, fibers, tannins and phenolic compounds. **Conclusion:** Pharmacognostic analysis and physicochemical characteristics can help in the efficient utilization of this medicinal plant.

KEYWORDS: Pharmacognosy, Phytochemistry, Physiochemical parameters, *Zizyphus mauritiana Lam.*

INTRODUCTION

The immense knowledge of medicinal plants in the developing countries needs to be under a monographic. The World Health Organization (WHO) estimates that more than 80 % of the population in developing countries takes to traditional medicine for their primary health care.[1] These medicinal plants play an important role in the people's socio-cultural life.[2,3] Also these medicinal plants serve as natural resources for research and development of new drugs.[4]

Plants including medicinal plants are also used as an animal's foder for animals. They are indirectly shown by their effects by which animals do not suffer by any types of diseases. Growing plants are one of the cheapest sources of feeding for animals having crude proteins of 14-25% These plants provide vitamins and minerals to the cattle.

In India, like in the other part of the world, several ethno-botanical surveys have been carried out and have shown that *Zizyphus mauritiana* Lam., in traditional medicine, is used to treat many ailments and as laxatives. Also, these plant species have anticonvulsant, anti-inflammatory, anti-cancer properties and are used as cardiac and intestinal stimulants, as central nervous system depressants and analgesics in laboratory animals. Rhamnaceae are also used for dyspepsia, against constipation and treatment of herpes. We also note the toning, astringent, clearing, purgative and emetic aspect of the species of the Rhamnaceae family. Also, they are used as digestive, diuretic, hypotensive and for the treatment of hepatic and dermatological complications. There is also a purgative action. Infusion of leaves is used in the treatment of astringent gargles. The Rhamnaceae are indicated in the treatment of furuncle, abscess, measles, general pains, and dysentery.

Anatomical studies have much significance in different sectors of investigation. This study was aimed to provide valuable anatomical descriptions and Phytochemistry of *Zizyphus mauritiana.* Anatomically, studies on leaves and other parts provide a reliable additional evidence for the taxonomic delimitation. Since the anatomy of this

genus is limited, the present study has been carried out to explore the anatomical features of leaf of *Ziziphus mauritiana.*

MATERIALS AND METHODS

Plant Material

The plant material consisted of the leaf, stem of *Zizyphus mauritiana* Lam. (Rhamnaceae). The leaves of *Zizyphus mauritiana* Lam. were collected in the region of chembarambakkam in Chennai. The drug was formally identified and authenticated by the taxonomist, and a herbarium was constituted then deposited at the Sree Sastha Pharmacy College, Chembaranmbakkam, Chennai.

Methods

The collected drug was cleaned and then dried at laboratory temperature (24-26 °C). The dry plant materials was coarsely pulverized, using a grinder. The powdered drug was used for pharmacognostic studies and physicochemical examination.

Macroscopic studies

The macroscopic study is a morphological study. This study allowed us to describe the plant and determine the shape, texture and colour of the drug.

Organoleptic character studies

The study of the organoleptic characteristics focused on the pulverized drug and related to the taste, appearance, colour and odour of the drug. The smell test was performed with 1 mg of pulverized drug taken between the thumb and index finger. The odoriferous constituents released were tested slowly and repeatedly. The intensity of the odour was first tested by the following parameters: "None, Weak, Marked, and Strong". Next, was determined the type of odour: "Odourless, aromatic, fruity, characteristic". For the taste, Five gram of drugs were placed and kept in the mouth, without swallowing, for 10 to 30 sec. After having spit out the sample, the mouth was rinsed, then the taste appreciated: "Peppery, fade, sour, bitter, sweet, salty, hot." This important study allows for the identification of the drug and its normalization. The appearance and colour required observation.

Microscopic studies

The fresh organs were preserved for macroscopic and anatomo-histochemistry study. It was then fixed in FAA (formalin – 5 ml, acetic acid – 5 ml and 70% ethyl alcohol – 90 ml) for 24 hour. After fixation, they were washed thoroughly in distilled water, dehydrated and embedded in paraffin wax. Then the paraffin wax embedded specimens were sectioned using rotary microtome. De-waxing was done by customary procedure (Johansen, 1940). The sections were stained with toluidine blue as per the method published by O'Brien et al. (1964).Where ever necessary, certain sections were also stained with safranin and fast green. Photographs of different magnifications were taken using Nikon digital camera 12 mega pixel and for normal observations, bright field was used. Descriptive terms of the anatomical features are as given in the standard anatomy books (Esau, 1964). Anatomic – histological-chemical studies allowed the identification of tissues and localization of secondary metabolite sites, respectively. These studies were carried out on leaf and stem cuts, as well as plant powder.

Anatomo-Histological Sections

It consisted mainly of observations under an optical microscope. Thin transverse sections were made using a microtome on vegetative organ fragments. Then, the sections were treated according to the technique recommended by GABE.[5] The colorant used was the mirror carmine-green. The best cuts were mounted and preserved between blade and slide in glycerine for observation and taking images using a digital camera incorporated in the microscope.

Micrographic study

A small amount of fine powder was mixed with a few drops of 5% KOH on a slide and then covered with a slide. The observation was carried out under the optical microscope with the objective 10x. The characteristic features of the drug powder were noted and photographed.

Physicochemical study

The study of the physicochemical parameters including the determination of the water and ash content was carried out according to the following protocol:

Moisture Content

The determination of the water content was carried out by the gravimetric method according to the protocol carried out by Linden and Lorient and Mukherjee: 5 test samples of 5 g of powder were introduced into 5 calibrated crucibles.[6,7] The samples were dried in an oven at a temperature of 105 °C for 24 h. The crucibles were cooled in a desiccator and weighed. The masses obtained made it possible to calculate the loss of mass and to calculate the water content of the powder expressed as %.

Ash Contents

Total Ashes

The powders dried during the determination of the water content were reduced toash in an oven at 600 °C for 6 h. After cooling in a desiccator, the ash was weighed. The masses obtained made it possible to calculate the ash masses and to calculate the total ash content, expressed as a percentage.

Ashes Insoluble in 10% Hydrochloric Acid

The total ash obtained was taken up with 20 ml of 10% Hydrochloric acid. The whole was brought to a boil in a bath for 15 min. The resulting solution was filtered on Whatman paper. The residue was collected in a tempered

crucible and calcined in an oven at 600 °C for 6 h. The crucible was cooled in a desiccator. The mass of the ash insoluble in hydrochloric acid was expressed as a percentage.

Sulphuric Ash at 50%

Five test samples of 5 g of powder were introduced into calibrated crucibles. It was added to the contents of each crucible 5 ml of 50% H_2SO_4. The whole was placed in the oven at a temperature of 600 °C for 6 h. After calcination and cooling in a desiccator, the ash was weighed, and the mass of the sulphur ash was expressed as a percentage.

RESULTS

Macroscopic study

This study allowed us to make an identification of the plant material, and is the first step in the characterization of the crude drug. *Zizyphus mauritiana.*

Morphological characteristics

Tree, shrub or bush, 16 m long, 5 m or 4 m, *Zizyphus mauritiana* Lam. has falling branches with rounded tops. Its little cracked bark is grey to brown, then pale red. The oval or sub-orbicular leaves are alternate and petiolate from 4 mm to 5 mm. The limb, dark green in colour, is varnished on the upper side and whitish and densely tomentose on the underside.

The inflorescences are sessile tomentose or woolly fascicles, from 3 to 8 flowers. The flowers are numerous, small and yellowish. The fruits are drums of 1 cm to 2 cm and containing a large nucleus coated with a whitish pulp, which is more or less floury.

Figure 1: Leaf and Fruits of *Zizyphus mauritiana.*

Figure 2: Habitat of *Zizyphus mauritiana.*

Organoleptic study

All the organoleptic characteristics revealed during this study are recorded as odourless, peppery taste, Light green colour, woolly texture.

Microscopic Studies

The microscopic study of the cross section of the leaf and stem plays an important role in the diagnosis of the identification and differentiation of the drug studied.

Anatomo-histological Sections

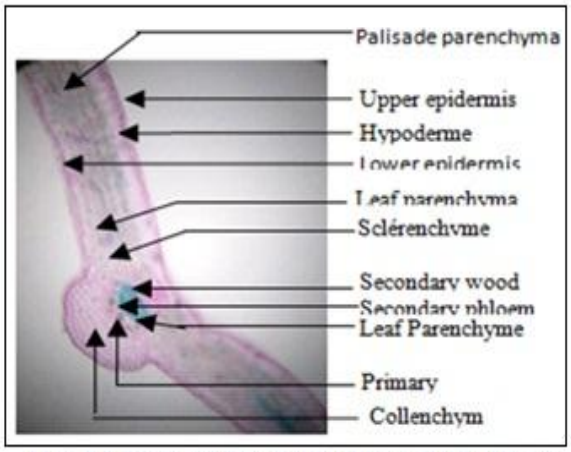

FIG. 3: CROSS-SECTION OF PORTION OF YOUNG BLADE OF *ZIZIPHUS MAURITIANA* LAM.

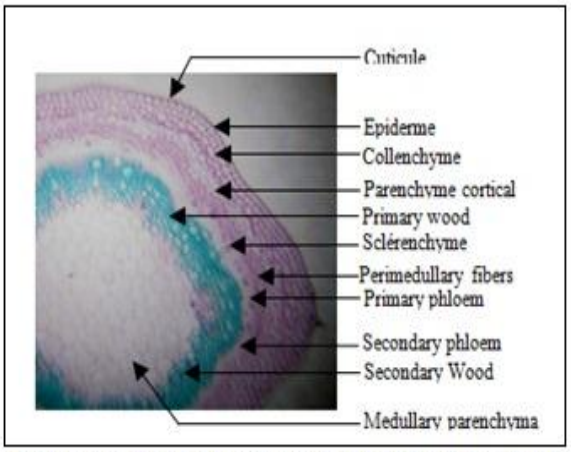

FIG. 4: CROSS-SECTION OF PORTION OF STEM OF *Z. MAURITIANA* LAM. (SOURCE FOFIE YVETTE)

The anatomo-histological sections are shown in Figures 3 and 4. The transverse section of the leaf blade Figure 3 presents a median rib slightly curved on the upper surface and strongly bulging at the lower surface and the blade itself larger. Each epidermis, formed of small, visible cells more or less rectangular, are covered with a cuticle, outer lipoidal covering; Waterproof and resistant, giving it a protective role. The mesophyll, comprised between the two epidermises, contains various tissues (hypodermis, palisade parenchyma, lacunous parenchyma, collenchyma, sclerenchyma, wood, and liber). The collagenase placed beneath the epidermis is formed of cells with thick, cellulosic walls; it is more abundant on the underside. This lower face is covered with filamentary, translucent, entangled and folded structures. The vascular apparatus forms a closed arc with phloem (liber) on the periphery and xylem (wood) on the inside. The cross-section of the stem Figure 4, of quadrangular shape, has two distinct zones: the bark and the central cylinder. The less developed bark comprises 4 tissues (cuticle, epidermis collenchyma and cortical parenchyma). The central cylinder, more developed than the bark, is composed of primary tissues (bone, wood, medullary parenchyma, sclerenchyma, and perimedullary fiber). The sclerenchyma occurs in small clusters around the conductive system. The conductive device has a centripetal and a centrifugal wood. The medullary parenchyma, formed of large polygonal cells.

Physicochemical study

This study is essential in that it helps identify poor handling practices and assess the quality of the proposed drug being studied. Various parameters have been defined and recorded in Table 1

Table 1: Physicochemical study.

Parameter	Humidity %	Total ash %	Sulphated ash%	HCl insolouble ash %
Rate	7.54	7.41	10.8	5.32

DISCUSSION

My study, which was carried out in this context, focused on the pharmacognostic examination of the leaves of *Zizyphus mauritiana*. (Rhamnaceae). The scientific literature allowed us to review a large number of bibliographic data on drugs, including plant systematics, various domestic uses and traditional medicine. The normalization of the macroscopic and microscopic characteristics of the drug of *Zizyphus mauritiana* remains essential to avoid and to identify the falsification.

Thus the comparative anatomy of the cross-section of the leaf and stem showed structural similarities. The two sections present medullary parenchyma and a collenchyma. A thin cuticle is observed on the leaf on the upper and lower epidermis. Also, palisadic tissues above lacunous parenchyma. At the level of the stem, secretory pockets are visible on the surface of the medullary parenchyma, as well as sclerenchymatous cells supporting primary tissue (primary lib). The distinct cortical parenchyma can be seen towards the cutting periphery. Organoleptic characteristics are important elements in the distinction of drugs, as they play a role in the detection of falsified or substituted drugs 16. The micrograph on the powder revealed several characteristic elements, namely: epidermal cells, stomate type, spiral bundles, cystoliths, hair, spiral wood bundles, and cells are diagnostic substances of crude drug of vegetable origin. These diagnostic elements are consistent with the botanical standard and WHO guidelines.[8,9]

The study of physicochemical parameters such as moisture content and ash values is useful for determining the physiological and nonphysiological state of the ash, detecting the possibility of microbial growth or contaminant and finally the presence of impurities. The moisture content of the drug studied has a rate of 7.54 ± 0.00, which is less than 10%. This result respects the standards established by the international pharmacopeia because this water content would prevent oxidation, fermentation reactions and would give less possibility for microbial growth and contamination in the drug.[10] Therefore, for good preservation of medicines made with the leaves of *Zizyphus mauritiana*, it would be desirable to use those whose water content is less than or equal to 10%.

The total ash dosage gave us a rate of 7.41 ± 0.02. This value informs us about the mineral content of the drug . Sulfuric ash has a rate of 10.08 ± 0.01. They result from the conversion of organic salts into sulfates.[11] This value, approximately equal to the average of 10.80% found during the determination of sulfur ash in the various samples of *Sclerocarya birrea* (A. Rich) Hoscht.[12] The ash insoluble in hydrochloric acid gave a rate of 5.32 ± 0.01. Indeed, the ash insoluble in hydrochloric acid informs us about the contamination of the drug by the siliceous elements.[11]

CONCLUSION

This study allowed us to demonstrate the presence of different pharmacognosic parameters in the drug of *Zizyphus mauritiana* Lam., by standard botanical observations and WHO guidelines. The results of the water content are considered satisfactory insofar as the content presented in the test drug allows good preservation and prevents oxidation, fermentation, and microbial growth reactions. In the light of these results, pharmacognostic analysis andphysicochemical characteristics can help the pharmacopoeia to use this plant efficiently, within the framework of a policy of standardization, identification and research on the drug of *Zizyphus Mauritiana* Lam.

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