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Pharmacognostical studies on the leaves of *Ziziphus nummularia* (Burm. F.)

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ABSTRACT

Ziziphus nummularia (Burm. f.), a member of the family Rhamnaceae, commonly known as Jharber, is used traditionally in treatment of mental retardation, preventing frequent attacks of influenza, colds, treating dysentery, diarrhoea and colic. It is also used in ulcers, fevers, wound healing, pharyngitis, bronchitis, burns, anaemia, irritability, hysteria and as a nervine tonic. In this present work, this plant has been subjected to the pharmacognostic standardization. In the microscopical studies, some cell structures and parameters were studied and in physical evaluation the ash values, extractive values and moisture content were studied. This paper also aims to characterize the extract(s) of Z. nummularia by preliminary phytochemistry as quality control parameter.

Key words: *Ziziphus nummularia*, leaf constants, microscopy, fluorescence analysis, Phytochemical screening.

INTRODUCTION

Ziziphus nummularia (Burm. f.) belonging to the family Rhamnaceae, commonly known as Jharber in Hindi is a most commonly occourring branched thorny shrub species in the Indian desert with a height of 1-2 m and light coloured bark [1]. The leaves are antipyretic and reduce obesity. The fruit is cooling, tonic, digestible, laxative aphrodisiac and removes biliousness, thirst, vomiting and burning sensations [2]. The dried fruits contain alkaloids, triterpenoids and saponins. They are anticancer, anodyne, refrigerant, sedative, pectorial, styptic, stomachic and tonic. They are used to purify the blood and aid digestion. They are also used interally in the

treatment of a range of conditions including loss of appetite, chronic fatigue, diarrhea, pharyngitis, bronchitis, burns, anaemia, irritability, hysteria [3]. The drug has been scientifically validated for certain pharmacological effects viz. antitomor [4], anthelmintic [5], antibacterial [6][7], and antifertility effects[8]. Phytochemical reports on *Ziziphus nummularia* have revealed the presence of polysaccharides, a pectin composed of l-rhamnose, d-galacturonic acid, d-galactose, l-arabinose, peptide alkaloids, cyclopeptides, flavonoides, saponins, triterpenoides, fatty acides, Ziziphin N, O, P & Q and dodecaacetylprodelphinidin B_3 [5]. The compounds nummularogenin [9], zizynummin [10] and lapachol [4] have been isolated from the plant.

However, there are no reports on the pharmacognostical studies of the plant. Hence, the present work is an attempt in this direction and includes morphological and physical evaluation, determination of physico-chemical constants and preliminary phytochemical screening of different extracts of *Z. nummularia.*

MATERIALS AND METHODS

Plant material

The leaves of the plant *Ziziphus nummularia* (Burm. f.), Rhamnaceae, was collected in the month October 2010 from the surrounding areas of MIET, Meerut, U.P, India. It was identified and authenticated by NBPGR, Pusa Camphus, New Delhi under the Ref. No. NHCP/NBPGR/2010/43. The shade dried leaves were further size reduced and stored until further use in an air tight container. Fresh plant material was obtained for the microscopical evaluation.

Chemicals and instruments

All the chemicals used were of laboratory grade. Compound microscope, watch glass, glass slides, cover slips, and other common glasswares were used in this experiment. Photographs were taken with using Nikon Labphot 2 Microscopic Unit, and trinocular microscope. Various solvents used mainly petroleum ether, ethanol (95%),and reagents used for staining different sections like phluoroglucinol, iodine solution, safranin, and acetic acid were procured from CDH, Mumbai, India.

Macroscopic and microscopic analysis

The Macroscopic and microscopic of the plant was studied according to the methods of Evans , the cross sections were prepared and stained with phluoroglucinol, iodine solution, safranin, and acetic acid. The microscopic analysis of powder and the leaf constants namely stomatal number and stomatal index were studied [11].

Physico- chemical analysis

Air dried plant material was used for the quantitative determination of ash and extractive values according to standard procedure of Indian Pharmacopoeia,1996 [12] and WHO/QCMMPM ,1992 [13]. Fluorescence analysis of the extract(s) was carried out according to standard procedure [14][15]. Powdered leaf was subjected to analysis under ultra violet light after treatment with various reagents and chemicals like sulphuric acid, ethanol, dilute hydrochloric acid and sodium hydroxide.

Preliminary phytochemical screening

Preliminary phytochemical evaluation was carried out by using standard procedure. Total alkaloidal and glycosidal content were determined according to standard procedure [16][17].

RESULTS AND DISCUSSION

Macroscopic characters of leaves

Leaves are green variable 2.2-2.6 by 1.4-2.2 cm, obovate, ovate round, margin is serrate, apex is obtuse, base is symmetrical and surface is glabrous.

Microscopic characters of leaves

The Microscopy of upper surface of the leaf showed the presence of epidermal cells, anomocytic stomata (Fig. A) and uniseriate trichome (Fig. B).

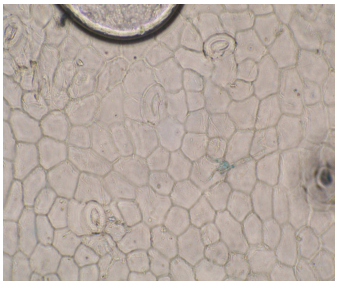

Fig.A: Epidermis showing Stomata

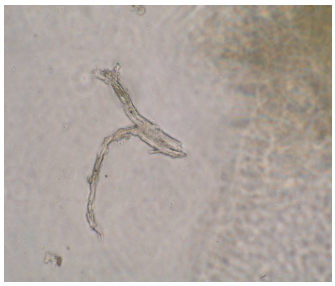

Fig.B: Trichome

Powder microscopy

The powder microscopy of leaf was done and data mentioned below (Fig. C-G).

a) Stomata: Anomocytic type of stomata.
b) Trichomes: Uniseriate glandular with unicellular head.
c) Epidermis: normally single layer of flattened cells with straight walled.
d) Fibers: are seen in large numbers.
e) Calcium oxalate crystals: are of prism type.
f) Starch grains : are not seen.

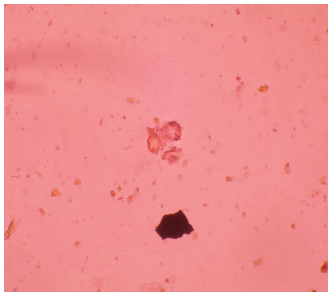

Fig.C: Anomocytic Stomata

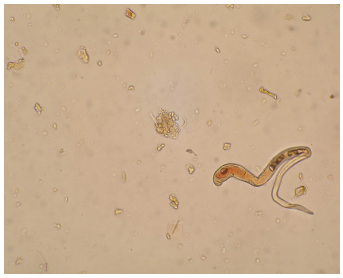

Fig.D: Trichome

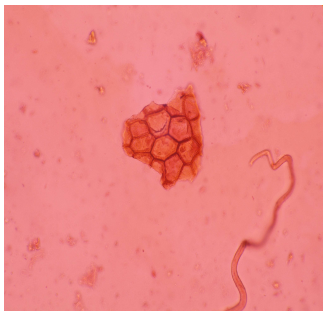

Fig.E: Epidermis cork cell

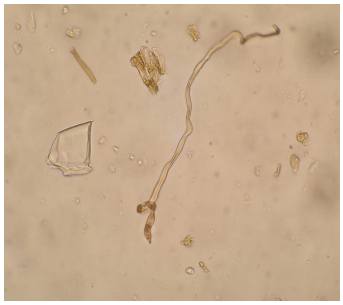

Fig.F: Fiber

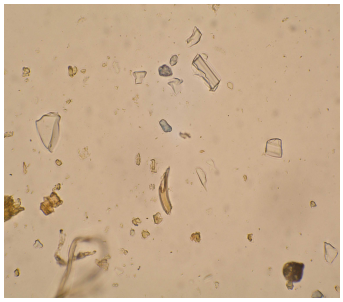

Fig.G: calcium oxalate crystals

Determination of Leaf constants

Stomatal indices and palisade ratio of upper epidermis in leaf surface were determined and recorded as per standard procedure (Table 1).

Table 1: Quantitative microscopy of leaf of *Ziziphus nummularia*.

S.No.	Parameters	Measurement
1.	Stomatal index upper epidermis	8.8 /Sq.mm
2.	Stomatal number upper epidermis	1.2 /Sq.mm
3.	Palisade ratio upper epidermis	4.5 /Sq.mm

Physiochemical analysis

Air dried material was used for quantitative determination of phytochemical values. Total, Water soluble ash, acid insoluble ash, water soluble and alcohol soluble extractive were determined for five times as per WHO recommendations. Water soluble extractive value was found to be very high when compared to other extractable matter in the drug (Table 2).

Table 2: Physical evaluation of leaf of *Ziziphus nummularia.*

S.No.	Parameters	Results
1.	Moisture content (LOD)	10.4%
2.	Total ash	7.4%
3.	Acid insoluble ash	1.0%
4.	Water soluble	3.2%
5.	Alcohol-soluble extractive	12%
6.	Water soluble extractive	15%

Preliminary phytochemical screening

The preliminary phytochemical test was performed on the extracts of plant of *Ziziphus nummularia.* They show the presence of the alkaloids, glycosides, saponin, steroids triterpinoids, flavonoids, fixed oil and protein starch (Table 3).The fluorescence analysis is a tool for the determination of constituents in the plant that gives a definite idea of the chemical nature. Thus the fluorescence analysis of the powder was carried out and data is presented in the Table 4.

Table 3: Preliminary phytochemical screening of two extracts of the leaves *Ziziphus nummularia*
.(+ positive test, - negative test).

S.No.	Test	Pet. Ether	Ethanol
1.	Alkaloids		
a)	Mayer's reagent	-	+
b)	Dragendroff's reagent	-	+
c)	Hager's reagent	-	+
d)	Wagner's reagent	-	+
2.	Glycosides		
a)	Liebermann-Burchard's test	+	+
b)	Legal's test	+	+
c)	Borntrager test	+	+
3.	Saponins		
a)	Foam test	+	+
b)	Haemolysis test	+	+

Table 4: Fluorescence analysis of leaf powder of *Ziziphus nummularia.*

S.No.	Treatment	Day light	U.V light
1.	Drug powder	Yellowish green	Green
2.	Powder + 1N HCl	Dark yellowish green	Dark green
3.	Powder + 1N H_2SO_4	Light green	Dark green
4.	Powder + 1N HNO_3	Pale green	Green
5.	Powder + 1N NaOH	Brown	Blackish brown

CONCLUSION

The study of Pharmacognostic features of *Ziziphus nummularia* had shown the standards which will be useful the detection of its identity and authenticity. The other study *viz.* physical evaluation, preliminary phytochemical test and Fluorescence analysis add to its quality control and quality assurance for proper identification.

Acknowledgement
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