IJPSR (2021), Volume 12, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 October 2020; received in revised form, 08 January 2021; accepted, 12 January 2021; published 01 February 2021

PHARMACOGNOSTICAL STUDY OF THE AUTHENTICITY OF *MORINGA OLEIFERA* LEAVES

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Keywords:

Moringa oleifera, Standardization, Medicinal plant raw materials, Leaves, Microscopic analysis, Preliminary phytochemistry analysis

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ABSTRACT: *Moringa oleifera* is widely used in Asia and Africa as an anti-inflammatory, anti-diabetic, and cold agent. However, there are no Moringa medicines on the Russian pharmaceutical market; therefore, it is of interest for research as a promising source of raw materials and introduction to the State Pharmacopoeia of Russian Federation. This study was conducted to compare the characteristics of the authenticity of leaves from different regions. Moringa whole leaf from India and powder from Namibia were studied using macroscopic, microscopic, and qualitative chemical analysis. The results showed that there are both similarities and differences of characters depending on the area of collection of raw materials. A quantitative assessment of diagnostic signs was carried out; for the first time, trichomes and druses, as well as stomata. Qualitative phytochemical analysis revealed the presence of tannins, flavonoids, and saponins in both samples. It was established that the studied samples corresponded to pharmacopoeial requirements for authenticity indicators. A relatively larger number of calcium oxalate druses was found in the Indian raw materials. The obtained new data on the quantitative assessment of micro diagnostic signs will be used to standardize the authentic *Moringa oleifera* raw materials to improve regulatory documentation, ensure the quality and safe use of drugs on the pharmaceutical market.

INTRODUCTION: *Moringa oleifera* Lam. (Syn. Moringa pterygosperma) Gaertn. Moringaceae family) [16] - is a small, fast-growing evergreen or deciduous tree that usually reaches 10-12 m in height. It has a spreading, open crown of drooping, fragile branches, feathery foliage of tripinnate leaves, and thick, corky, whitish bark [9].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(2).1272-76

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(2).1272-76

This species is native to India and is cultivated all over the world, especially in Asia, Latin America, Florida, the Caribbean, the Pacific Islands, and Africa [3, 8]. Due to its edible leaves, roots, fruits, flowers, and nutritious foods, it can be used as food, nutraceuticals, and medicine [10]. The most used parts of the plant are leaves containing a large number of biologically active compounds [7, 13], including vitamins, calcium, iron, protein, [4] carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins [6]. A large number of biologically active substances can explain its therapeutic properties, which include antibiotic, antioxidant,

anti-inflammatory, hypercholesterolemia, pain-killers, wound healing, antitumor, antidepressant, antiviral hypotensive, antiulcer effects, cardiac stimulants, antimalarial, antiparasitic, and others [1, 2, 11, 14, 15, 19].

MATERIALS AND METHODS:

Plant Material: Leaves of *Moringa oleifera* were collected in November 2019 (Hassan, India) CH/DP/UT/2019, leaf powder was purchased from private procurers in 2019 (Windhoek, Namibia).

Methods:

Macroscopy: Macroscopic studies of leaves and powder, including organoleptic characteristics (appearance, color, smell, taste, shape, and size) were performed in accordance with the requirements of the State Pharmacopoeia of the Russian Federation of the XIV edition [17].

Microscopy: Whole dried leaves of *Moringa oleifera* were boiled in the ratio (1: 1) of a 5% solution of NaOH and purified water in a flask until enlightened. After cooling, the leaves were washed with purified water. The leaves were placed in a Petri dish with water, cut into small pieces, and then placed in a diluted solution of glycerol or chloral hydrate on a glass slide, covered with a coverslip, and examined using a «Mikmed-6» microscope (Russia) at magnifications of X40 and x100, x400 [17]. The powder was boiled in 2.5% NaOH until clear. A small amount of powder was placed on a glass slide in a drop of glycerol, covered with a coverslip, and examined with a «Mikmed-6» microscope (Russia) at magnifications of x40, x100, x400 [17].

Quantitative Microscopy: A microscopic evaluation of leaf constants, such as stomatal number, trichome number, and druse number, was investigated. The number of stomata, trichomes, and druses on the upper and lower surface of the leaf of 1 mm^2 at magnifications of x40 and x100 in the field of view was calculated. The diameter and length of the trichomes, the diameter of the druse was determined using an ocular micrometer.

Preparation of Plant Extract: 5 g of Moringa powder was obtained after grinding and sieving through a 2 mm sieve. 50 ml of water was added to the powder in the flasks. The ratio of powder to water is 1:10. Extraction-infusion was prepared after heating for 15 min, cooled for 45 min at room temperature. Filtration - after cooling the extract, it was filtered through a filter paper. The filtrate was obtained and used to identify various phyto-chemicals [17].

Qualitative Analysis:

Tannins Detection: A few drops of 1% iron-ammonium alum were added to 2 ml of the aqueous extract [17]. To 2 ml of the test solution, a few drops of 5% iron chloride were added to observe a brownish-green or blue-black color, indicating the presence of tannins [12].

Flavonoids Detection: A few drops of a 2% alcohol solution of aluminium chloride were added to 1ml of the aqueous extract [17].

Saponins Detection: 3ml of the aqueous extract was vigorously shaken in a test tube until a stable foam formed [17].

Data Analysis: All leaf constant parameters were determined, and the results were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION:

The Study of External Signs of Raw Materials: Determination of the morphological features of the studied images showed that: Whole leaves were a mixture of whole leaves and petioles. The leaves are complex, ovate, obtuse to the apex, the margin of the leaves -whole, venation-reticulate. They are up to 2.1 cm long and 0.6 cm wide. The color of the leaves is dark green to green above, pale green below. Taste and smell are characteristic. The powder was green and consisted of pieces of leaves of various shapes, passing through a sieve with a diameter of 2 mm. Taste and smell are characteristic **Fig. 1**.

Microscopic Signs of Whole Leaves: Examination of the leaf surface showed a slightly sinuous cuticle in both epidermises. Upper epidermis with straight polygonal cells, simple unicellular hairs, short and long, often some curved and slightly curved, with thick walls and a rough, warty surface. The lower epidermis had wavy cells, a random distribution of stomata of the anomocytic type, more on the lower surface. Mesophyll contains many druse of calcium oxalate. Spiral and annular vessels, cells with mucus, places of attachment of hairs, reddish-

brown cells with resinous substances are visible **Fig. 2**.

Microscopic Analysis of Powder: The powder is characterized by the presence of fragments, the lower epidermis and upper epidermis, consisting of polygonal and rectangular cells, a spiral and annular vessel, simple hairs, stomata of the anomocytic type, cells with resin, druse of calcium oxalate and fiber **Fig. 3**.

FIG. 1: EXTERNAL SIGNS OF MORINGA LEAVES WHOLE (1) AND POWDER (2)

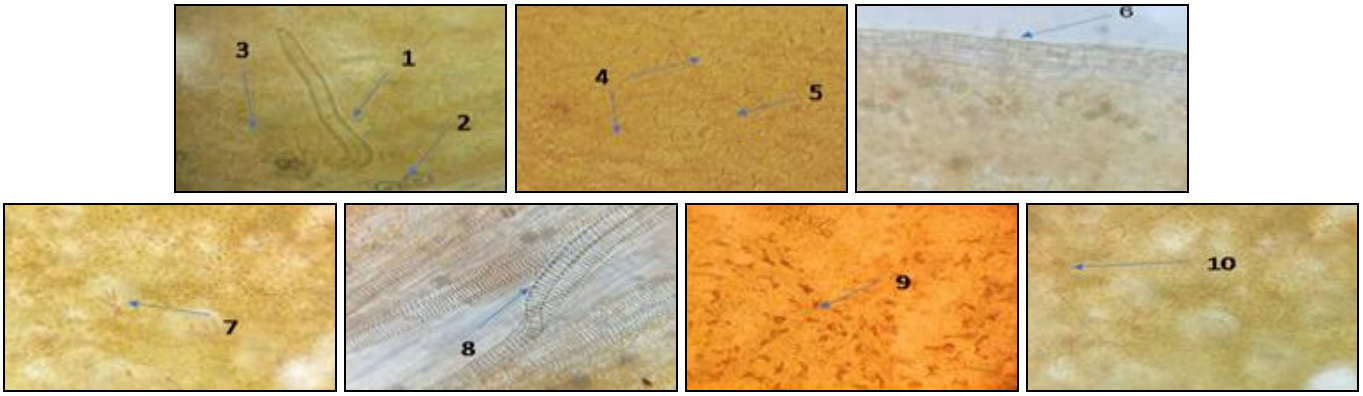

FIG. 2: MICROSCOPIC SIGNS OF THE LEAF: SIMPLE UNICELLULAR HAIR (1), DRUSE (2), CELLS OF THE UPPER EPIDEMIC (3), STOMATA OF THE ANOMOCYTES TYPE (4), CELLS OF THE LOWER EPIDEMIC (5), CUTICLE (6), CELLS WITH MUCUS (7), VESSELS (8), A CELL WITH A RESINOUS SUBSTANCE (9), THE PLACE OF ATTACHMENT OF THE HAIR (10)

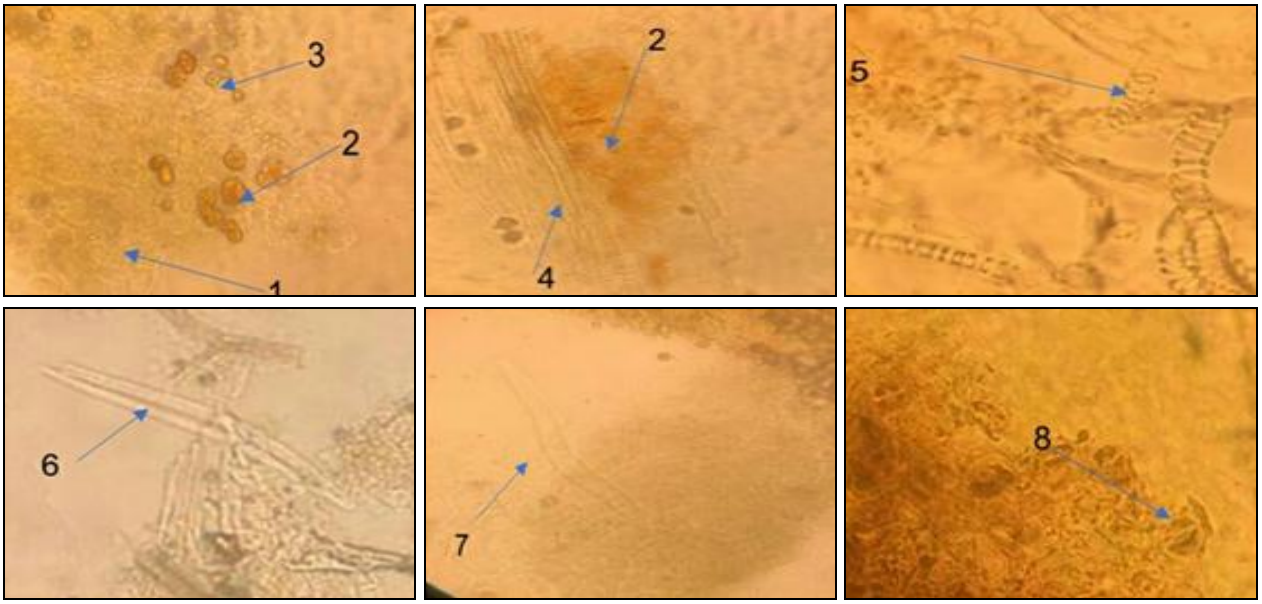

FIG. 3: MICROSCOPIC SIGNS OF POWDER: FRAGMENTS OF CELLS OF THE UPPER EPIDEMIC (1), RESIN (2), DRUSE (3), FRAGMENTS OF SPIRAL(4), ANNULAR (5), VESSES, FRAGMENT OF MECHANICAL FIBERS (6), SIMPLE HAIR (7), STOMATA ANOMOCYTIC TYPE (8)

Quantification of Microdiagnostic Signs: The estimated stomata density is 126 on the lower epidermis and 60.6 on the upper epidermis. The leaf has more crystals of calcium oxalate on the upper epidermis 330.7 and fewer hairs on the upper surface than on the lower **Table 1**.

TABLE 1: QUANTIFICATION OF *MORINGA OLEIFERA LEAF* CONSTANTS PER 1mm^2

Features/Signs	Bottom surface	Top surface
Stomata	126 ± 1.2	60.6 ± 1.0
Druse	60 ± 1.3	330.7 ± 4.4
Simple hairs	26.3 ± 0.6	10.9 ± 0.3

mean ± SD, n = 10

Measurement of Diagnostic Signs: Measurements show that *Moringa oleifera* leaf has an average hair length of 6.6 and an average diameter of 1.69 µm. In this case, the average crystal diameter is 17.5 µm **Table 2**.

TABLE 2: DIMENSIONS OF DRUSE AND TRICHOME LEAVES OF *MORINGA OLEIFERA*

Features /signs	Length, µm	Diameter, µm
Simple hairs	6.6 ± 1.3	1.69 ± 0.1
Druses	-	17.5 ± 0.9

mean ± SD, n = 10 for trichomes, n = 5 for druses

Qualitative Analysis: The results obtained indicate the presence of tannins, flavonoids, and saponins in the aqueous extract of *Moringa oleifera* leaves **Table 3**. All reactions carried out with various reagents were detected and strongly positive. The reaction to tannins with iron-ammonium alum, received a black-green color, and flavonoids detected with aluminium chloride received a yellow-green color, indicates the presence of condensed tannins in the Moringa leaves. Saponins detected after vigorous shaking of the water extract formed persistent foam not disappearing for an hour. According to past studies, the foam has stood for 30 min [20, 21].

TABLE 3: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF MORINGA LEAF WATER EXTRACT

S. no.	Biological active substance group	Water extract	Results
1	**Tannins**		
	- Iron Ammonium Alum	++	Black-green staining
	-$FeCl_3$	++	brown-black staining
2	**Flavonoids**		
	- $AlCl_3$	++	Yellow green staining
3	**Saponins**		
	- Shaking	++	Foam formation

Note: ++ is present in high concentration

CONCLUSION: External signs of the studied samples corresponded to Pharmacopoeial requirements. Microscopic examination of both samples had similar characteristics, but in the Indian sample of raw materials, a greater number of druse was noted. Using qualitative reactions, flavonoids, tannins and saponins were found in the resulting aqueous extract

For the first time, the data obtained on the quantitative assessment of microdiagnostic signs of trichome and druse, are absent in the pharmacopeia and will be used to standardize the medicinal plant materials *Moringa oleifera* to determine the authenticity of authentic raw materials.

Financial Support and Sponsorship: No

ACKNOWLEDGEMENT: We express our deep gratitude to Dr. Prakash L. Hegda, Professor, Head of the Department of Dravyaguna SDM Ayurvedic College (Hassan, India) for help in collecting samples for research.

CONFLICTS OF INTEREST: Nil

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How to cite this article:
Gravel IV, Amutenya HN and Upadhyaya UK: Pharmacognostical study of the authenticity of *Moringa oleifera* leaves. Int J Pharm Sci & Res 2021; 12(2): 1272-76. doi: 10.13040/IJPSR.0975-8232.12(2).1272-76.