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Research Article

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Pharmacognostic Characterization for Taxonomic Identification of *Bacopa monnieri* (L.) Wettst. for Quality Control

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Abstract: Pharmacognostic studies played a crucial role in checking the possibilities and threat of adulteration among natural drugs by macroscopic and microscopic examination of plant materials. Organoleptic characters, macroscopic and microscopic studies, physicochemical and fluorescence analysis of *Bacopa monnieri* (L.)Wettst. has been carried out to determine the anatomical and pharmacognostic portrayal. The organoleptic studies revealed that *B. monnieri* is green in colour, bears a characteristic smell, leaves have smooth soft texture and bitter in taste. Stomata of anomocytic type and stomatal index was 17.77 and 18.18 in upper and lower epidermis respectively. Glandular trichome was present on the leaf surface and petiole. Study of powder microscopic analysis and fluorescence characteristics reveals round and oval starch granules, sclerenchymatous cells, lignified fibres, vascular bundles, spherical oil globules, raphides, druses, thin fibres and compounds oil droplets, xylem vessels with spiral and reticulates thickenings in the powder of different part of the plant. Observations revealed that the study has a great potential in unraveling the identification keys of plants species by highlighting various pharmacognostic aids.

Keywords: *Bacopa monnieri*; Bramhi; Pharmacognostic studies; Medicinal plants.

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1. INTRODUCTION

Adulteration of crude drugs by the addition of similar appearing plant material has been a common practice nowadays. In order to get the purest form of drug, various methods are available. In Ayurveda (Atharvaveda) and other pharmacopeias, stepwise confirmation is necessary for authentic validation of drugs from plant origin. There are three pilot steps viz. authenticity, purity and assay that are assumed as an integral part of the standardization process.[1] *Bacopa monnieri* [synonym: *Herpestis monniera*], an important medicinal plant, belongs to family Plantaginaceae is a small, fleshy, comestible, creeping herb can be easily cultivated by using its runner. It holds a very prominent place in the indigenous system of medicine due to its remarkable medicinal properties. These properties are attributed to the active principles of the plant specially 'Bacosides' and many others. Bacoside- A, a saponin found in almost all the parts of the plant.[2] Several memory sharpening commercial products are available in the market using this plant powder and liquid extract that has led this plant under high demand and also under the threat of being adulterated. The present study aimed to characterize *B. monnieri* on the basis of morphological, anatomical, phytochemical investigation.

2. MATERIALS AND METHODS

Pharmacognostic study was performed by following standard protocols.[3-6]

2.1 Collection and identification of the experimental plant

Bacopa monnieri was collected in the month of July-August from the medicinal plant garden of Department of Rural Technology and Social development, Guru Ghasidas University, Bilaspur, Chhattisgarh, India and authenticated by Botanist Dr. Ashwini Kumar Dixit, through herbarium voucher number GGV/BOT/PLANT/421 in the Department of Botany, Guru Ghasidas University.

2.2 Organoleptic characters

The organoleptic characteristics viz appearance and color in daylight, smell and their taste of plants were studied.

2.3 Macroscopic studies

The morphological characteristics of the selected plant were done by visual observation and hand lens.

2.4 Microscopic studies

2.4.1 Anatomical study

Fresh plant material was used for the microscopic study. Anatomical study was performed by preparing a double stained permanent slide. The transverse sections of leaf, stem and roots of plant were analysed for epidermal cells, cortical cells, palisade cells, vascular bundle and trichomes.

2.4.2 Quantitative microscopy

For the quantitative microscopy, - stomatal index, vein islet number and vein termination number was determined.[5]

2.4.3 Powder analysis

For powder analysis - physical test, powder microscopy, physico-chemical analysis was conducted. Under physical test - powder was characterized by morphological features like colour, presence or absence of specific characteristic odour and taste. For powder microscopy, fine powder of the whole plant was treated with sudan red III, acetic acid, sulphuric acid, iodine solution and dilute HCl on glass slides and observed under microscope for analysis. For Physico-chemical analysis following parameters have been employed which are ash value determination, total ash per cent, acid-insoluble ash values and water soluble ash values determination was conducted.

2.4.4 Fluorescence analysis

Fluorescence analysis of plant powder was done by observing under two different range UV (DAPI filter 340nm to 380nm), visible (400-700nm) and (FITC filter 450nm to 490nm) of wavelength by using Fluorescent microscope (trinocular) Primo star Carl ziess Lab.1[7] The colour observed by application of different reagents in different radiations was recorded.

2.4.5 Micrographs and image analysis

Micrographs of different parts of the plant were taken from Olympus binocular microscope attached with MIPS model no. CH20i. Micrographs of different types of cells were measured and analyzed by using Biowizard.ink software.

2.5 Preliminary phytochemical investigation

A series of tests has been conducted for the detection of phytochemicals viz carbohydrates, proteins, fats and oils, glycosides, saponins, flavonoids, alkaloids, tannins and phenolic compounds, acidic compounds found in *Bacopamonnieri* aqueous extract[3].

3. STATISTICAL ANALYSIS

All the results were calculated in triplicates. The data are represented as mean±SE using Microsoft Excel 2007. All the measurement for quantitative microscopic studies of micrographs were analysed by using Biowizard.ink software.

4. RESULTS AND DISCUSSION

4.1 Identification of the Experimental Plant

The whole parts of the plant were studied in fresh as well as dry condition.

4.2 Organoleptic Characters

The organoleptic characteristics of *B. monnieri* namely their appearance and colour in daylight, smell and their taste were studied. This study comprises use of sense organs to identify the nature of herb. *B. monnieri* is green in colour, bears leaves and stem, has a smooth soft texture and possesses a characteristic smell and bitter in taste (Table 1).

Table 1. Organoleptic characteristics of *B. monnieri* plant

S.No.	Features	Observations
1.	Colour	Bright green
2.	Color of leaf at upper side	Dark green
3.	Color of leaf at lower side	Light green
4.	Leaf apex	Rounded
5.	Shape of leaf	Spatulate to obovate
6.	Arrangement of leaf	Opposite
7.	Leaf margin	Entire
8.	Shape	Simple
9.	Taste	Bitter
10.	Surface	Smooth
11.	Odour	Specific/ characteristic

4.3 Macroscopic Studies

The morphological characteristics of the plant were done by visual observation and hand lens. *B. monnieri* is a creeping herb that bears simple, dorsiventral, opposite and fleshy leaves with an entire margin. Leaves protrude from the node provided with adventitious roots. Flowers are white and pentamerous. Stem is soft and smooth with an average diameter 0.5 mm to 2.5 mm. The runner has an average size ranging from 10 cm to 40 cm. Root is generally thin, soft and of off-white colour (Fig.1.A-D).

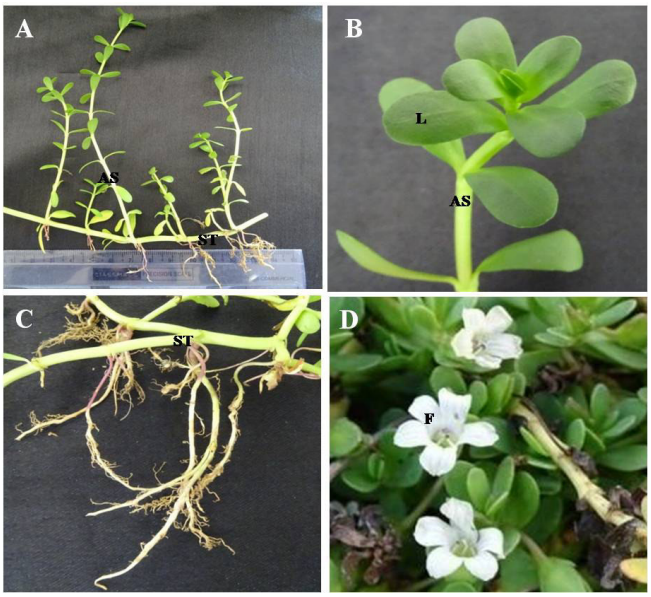

Fig 1. *B. monnieri* (L.) Wettest. (A) Plant showing origin of ascending branches (AS) and roots (R). (B) Ascending branch showing arrangement of leaves (L), (C) Root (D) Flowers.

4.4 Microscopic Studies

4.4.1 *Anatomy of Leaf*

Transverse section of the leaf shows the presence of a single layer of upper and lower epidermis, surrounded by cuticle with ridges; more prominent striations were observed in the lower epidermis. In between the upper and lower epidermis lies the mesophyll region (isobilateral), consisting of undifferentiated palisade parenchyma and spongy parenchyma. Size of the mesophyll cell (41.420 X 23.146 µ - 58.275 X 28.881µ) was observed (Fig.2.A). The lower epidermis seems to possess a single layer of palisade parenchyma cell placed at 90° with the mesophyll region. The epidermal cells located near the midrib at the ventral part of the leaf bears dumbbell shaped cells (186.443 X 47.063 µ - 94.006 X 54.902 µ). Conjoint collateral type of vascular bundle surrounded by achlorophyllous bundle sheath cells was present in the midrib region. Vascular bundles contain metaxylem, proto xylem and phloem along with connective tissues (Fig.2.B).

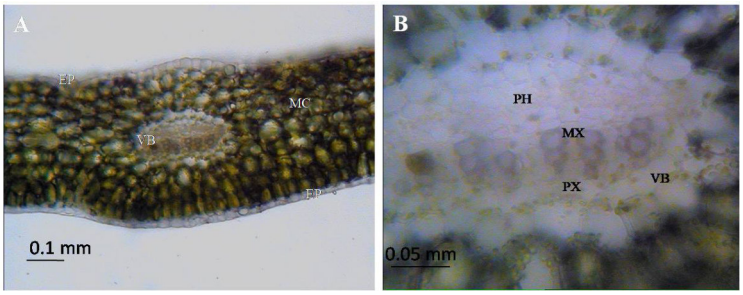

Fig 2. *B monnieri* (L.)Wettest.(A) T.S. of leaf through mid-rib, (B) Vascular bundle. EP- Epidermis; MC- Mesophyll cells; VB- Vascular bundle; PH- Phloem;PX- protoxylem; MX- Metaxylem.

4.4.2 *Anatomy of Stem*

Transverse section of stem clearly depicts distinct cells comprising different layers. The upper most layer is epidermis which bears barrel shaped cells that are arranged in a chain like manner in a single row. Beneath this, lies two to three layers of hypodermis composed of chlorophyll containing parenchymatous cells and cortex region consisting of parenchyma cells with large air spaces. (Fig.3.A). Starch grains and prismatic crystals of calcium oxalate are seen in some cells of this region. Followed by endodermis, the encircled portion just below is pericycle which consists of barrel-shaped, thin walled cells and the next place below to this is held by radially arranged vascular bundles. Xylem consists of proto-xylem and metaxylem. Proto-xylem (19.014 X 33.568 µ to 37.097 X 24.458 µ) located towards pith whereas; meta-xylem (22.686 X 46.383 µ to 50.054 X 64.357 µ) lies towards the pericycle (Fig.3.B). Phloem is placed on the upper side of xylem. The conducting tissues and centrally placed large pith along with parenchymatous cells bears some prismatic crystals.

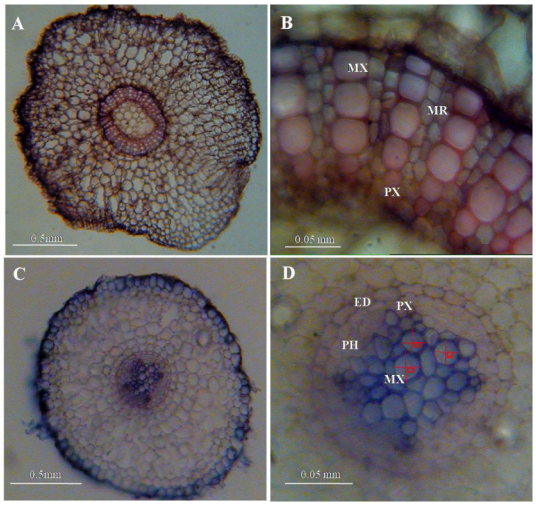

Fig 3. *B monnieri* (L.) Wettest. (A) T.S. of stem. (B) Vascular tissues (C) T. S. of root,(D) Vascular tissues , ED- Endodermis; MR- Medullary rays; PH- Phloem; PX- Protoxylem; MX- Metaxylem.

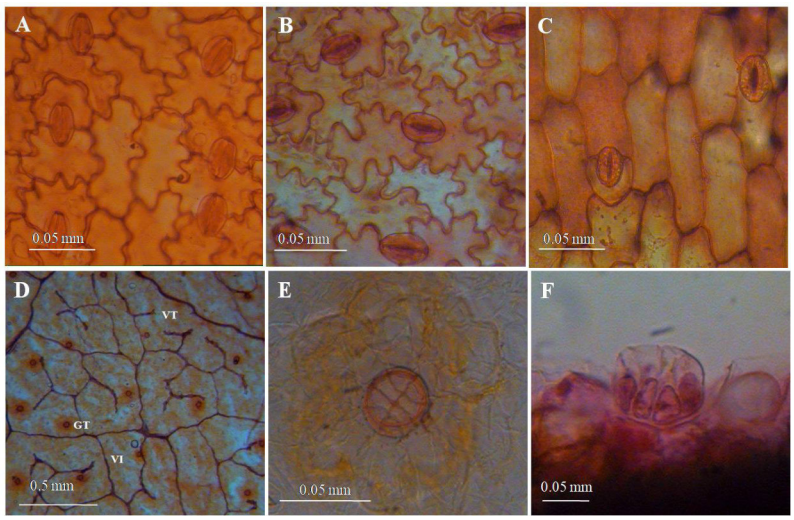

Fig 4. *B. monnieri* (L.) Wettest. (A) Upper epidermal cells, (B) Lower epidermal cells, (C) Lower epidermal cells near midrib, (D) A portion of leaf showing vein islet patterns and glandular trichomes (E) Arrangement of cells in glandular trichome (F) Lateral view of glandular trichome.

4.4.3 *Anatomy of Root*

T.S. of root depicts a layer of epiblema bearing unicellular root hairs. Hypodermis is absent. Cortex is very well developed and is not differentiated into exodermis. Endodermis lies below the cortex region, which is less thickened and lacks passage cells meant for absorption of water. Below this present a single layer of pericycle composed of parenchyma (Fig.3.C). Vascular bundles are radial, exarch and open usually 2- 6 in numbers. Size of the proto and metaxylem of vascular bundle were observed between (7.403µ X 6.348 µ - 10.217µ X 9.182 µ) and (19.478 µ X 10.505 µ - 15.849 µ X 15.861 µ) respectively. Pith is very less developed with the presence of conjunctive tissues. Pith rays are absent. (Fig.3.D)

4.4.4 Quantitative microscopy

Under this section stomatal index, vein Islet number, vein termination number has been determined. There is a little variation in the values of stomatal index of upper epidermis and lower epidermis. Stomatal index was 17.77 and 18.18 in upper and lower epidermis respectively. Anomocytic type of stomata (64.297 X 42.215 µ - 58.552 X 31.442 µ) was observed more in the lower epidermal layer as compared to upper. Kidney shaped guard cells were seen (Fig.3.A, B and C). Vein- islet number was 21 and vein termination number was 46 (Table 3; Fig.3.D).Eight celled glandular trichomes (Curved area 246.203µ) were also seen on both the surface of the leaf. Glandular trichome consists of eight cells and the size of the cells ranged from 22.021 X 23.537 µ to 13.008 µ X 31.519 µ) (Table 2; Fig.3 E & F).

Table 2. Different attributes of quantitative microscopic values of *Bacopa monnieri* leaf.

S. No.	Cell type	Size (µm)
1.	Mesophyll Cells	41.42 X 23.14 - 58.27 X 28.88
2.	Epidermal cell	94.00 X 54.90 - 186.44 X 47.06
3.	Stomata	58.55 X 31.44 - 64.29 X 42.21
4.	Trichome	13.00 X 31.51 - 22.53 X 31.70

Table 3. Quantitative microscopic values of *Bacopa monnieri* leaf

S.No.	Parameters	Value (Mean±SE)
1.	Stomatal index (Lower epidermis)	18.18±.62
2.	Stomatal index (upper epidermis)	17.77±.52
3.	Glandular trichome	1.6 ±.08
4.	Vein -Islet number	21±.91
5.	Vein-termination number	46±.33

n=3, SE= Standard error

4.4.5 Powder Analysis

Powder was characterized by morphological features like colour and presence or absence of specific characteristic and taste (Table 4). Microscopic study of fine powder of the whole plant was done by different treatments i.e. sudan red III, acetic acid, sulphuric acid, iodine solution and dilute HCl. In *B. monnieri* round and oval starch granules, sclerenchymatous cells, lignified fibres, vascular bundles, spherical oil globules, raphides, druses, thin fibres and compound oil droplets, pectins, xylem vessels with spiral and reticulate thickenings were seen (Table 5; Fig.5).

Table 4. Powder analysis of *B. monnieri*

S.No	Test	Observation	Inference
1.	Colour	Greyish green	Leaf drug
2.	Odour	Specific	Aromatic crude drug
3.	Taste	Bitter	Drug contains triterpenoid and alkaloid

Table 5. Powder analysis of *B. monnieri*

S.No.	Reagent	Observation	Characteristics
1.	Powder + Sudan red III	Pink colour	Cuticle present
2.	Powder + Acetic acid	colourless	Raphides and druses present
3.	Powder + Dilute HCl	Soluble	Calcium oxalate crystals present
4.	Powder + Conc. Sulphuric acid	Brown colour	Stone cells absent
5.	Powder + Iodine solution	Blue black colour	Starch present in endodermis

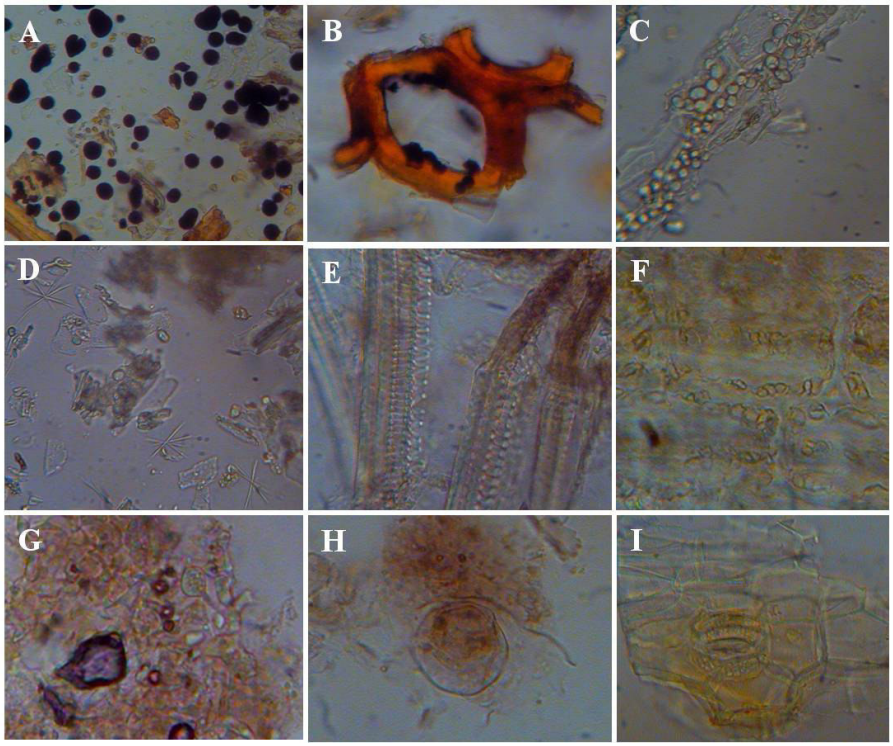

Fig 5. *B. monnieri* (L.) Wettest. (A) Starch granules (B) A portion of sclerenchymatous cells (C) Series of oil globules (D) Calcium crystals, raphides and druses (E) Spirally appearing xylem vessels for water and mineral conduction (F) Calcium and magnesium deposits in the lining of wall (G) Starch and other grains, (H) Upper view of trichome (I) A portion of stomata along with guard cells

4.4.6 Ash value analysis

Total ash value was 9.63%, acid insoluble ash was 1.2% and water soluble ash was 1.3%. Low ash value of *B. monnieri* indicates less content of inorganic salts *i.e.* carbonates, phosphates, silicates of potassium, calcium, magnesium etc. Employed parameters have been mentioned in tabular form (Table 6).

Table 6: Physicochemical analysis of *Bacopa monnieri*

S.No.	Parameters	Value (%)
1.	Total Ash value	9.63%
2.	Acid Insoluble Ash	1.2%
3.	Water Soluble Ash	1.3%

4.4.7 Fluorescence analysis

Plant metabolites possess some unique properties to respond in the electromagnetic spectrum. Some constituents fluoresce in day light whereas, some phyto-chemicals show colour change only in ultra violet radiation. In addition, some phyto-constituents get converted into fluorescent derivatives or decomposition products by applying different reagents. This property of plants is used by the researchers in correct identification of the plant material by the fluorescence analysis. Fluorescence analysis of plant powder showed remarkable difference in the colour (Table 7).

Table 7. Fluorescence analysis of *Bacopa monnieri*

S.No.	Sample	FITC (450nm-490nm)	Visible light (400nm -700nm)	DAPI (340nm-380nm)
1.	Powder alone	White	Yellow	White
2.	Powder + water	Light green fluorescence	Dark brown	White
3.	Powder + Conc. H_2SO_4	Light green fluorescence	Dark brown	Brown
4.	Powder + Ethanol	Light green fluorescence	Dark green	White Fluorescence
5.	Powder + Ammonia	Light green fluorescence	Brown	White
6.	Powder + KOH	Light green fluorescence	Greyish brown	Red
7.	Powder + NaOH	Dark green fluorescence	Brick red	Brick red
8.	Powder + Iodine	Light green fluorescence	White	White

4.5 *Phytochemical analysis*

Preliminary phytochemical screening of *B. monnieri* revealed the presence of primary and secondary metabolites. Aqueous extract of the *B. monnieri* confers the presence of alkaloids, glycosides, reducing sugar (carbohydrates) and phenolic compounds. Presence of peptides was symbolized by the positive test of proteins and ninhydrin in the plant's aqueous extract. The findings of preliminary phytochemical investigation of *B. monnieri* are illustrated in (Table 8).

Table 8. Preliminary phytochemical investigation of aqueous extract of *Bacopa monnieri* (L.)Wettst.

S.No.	Phytochemicals	Name of Tests	Present (+)/ Absent (-)	Inference
1.	Carbohydrate: General test	Molisch's test	+	Violet ring is formed at the junction of two liquids confirms the presence of carbohydrates
2.	Test for Reducing sugar	Fehling's test	+++	First yellow then brick red ppt. was formed which showed the strong presence of reducing sugar in the sample
		Benedict's test	++	Yellow ppt. was observed which reflected moderate presence of reducing sugar in the plant extract.
3.	Mucilage test		-	Powdered drugs do not swell in water. Mucilage absent
4.	Protein: General test	Biuret test	+	Violet colour appeared which confirms the presence of protein
5.	Amino acids: General test	Ninhydrin test	+++	Bluish purple colour confirmed the strong presence of amino acids in the sample
6.	Fats and Oils	Solubility test-Benzene	-	The test sample was not soluble in benzene and showed the absence of fats and oil.
		Solubility test-Chloroform	-	The test sample was not soluble in chloroform showed the absence of fats and oil.
7.	Steroids	Salkowski test	+	Chloroform layer reflected orangish red colour against the yellow fluorescence of the test sample, showed the presence of steroid.
8.	Glycosides	Cardiac Glycosides	-	No significant colour change was observed in the test sample
	Deoxysugar	Keller Killiani test	-	No significant colour change was observed in the test sample
9.	Saponin	Foam test	+++	showed stronger saponin content
10.	Flavonoids	Shinoda test	++	Occurrence of purple colour after treatment reflected moderate presence of flavonoids
11.	Alkaloids	Dragendorff's test	+++	Orangish brown ppt. confers the strong presence of alkaloid
		Mayer's test	++	Appearance of ppt. commemorated moderate presence of alkaloids in the test samples.
12.	Tannins & Phenolic compounds	Ferric Chloride test	++	Occurrence of deep blue colour in *B. monnieri* aqueous extract showed moderate presence of tannins.
		Acetic acid test	++	Colour change in the solution from yellow to red reflected the moderate presence of tannin in the test samples.
		Iodine test	+	After treatment, transient red colour of the test samples showed the presence of tannins
13.	Acidic compounds	Sodium carbonate test	++	Effervescence was produced in the test samples which confirmed the moderate presence of acidic compounds in the aqueous extract

+++ = Strongly present; ++ = moderately present; + = present; - = absent

5. DISCUSSION

Pharmacognosy is the investigation of crude drugs from natural sources, principally from plants. It essentially manages standardization, validation, investigation and authentication of herbal drugs. Most of the pharmacognostic research concentrated in recognizing doubtful identification of plants, authentication of usually utilized conventional medicinal plants through morphological, phytochemical and physicochemical investigation.[8] *B. monnieri* is an important medicinal plant and widely used in many traditional systems of medicine and also an important ingredient in many commercial memory enhancing drugs, therefore proper identification of crude drugs becomes imperative. In present investigation morphological, anatomical and micro chemical characterization was attempted and found that the leaves

and stem has a smooth soft texture. Similar findings with slight differences were reported by researchers that may vary due to the habitat and seasonal variation.[9,10] According to World Health Organization (WHO) microscopic inspection of herbal material is indispensible for identification of broken or powdered materials[11] Cell measurement, an unique analysis of the sections (anatomical studies) of *B. monnieri* plant was attempted for the first time. The epidermal cells located near the midrib at the ventral part of leaf bears dumbbell shaped cells and this was in agreement with the findings.[12] The presence of large air cavity spaces is seen in the T.S. of leaf of *B. monnieri,* which is in agreement with the findings.[13,14] Starch grains and prismatic crystals of calcium oxalate are seen in some cells of the cortex region of the stem. Followed by endodermis which consists of barrel-shaped cells, below to the epidermis pericycle is present. Vascular bundles are radially arranged. Similar to the present study, the presence of aerenchyma, and prismatic crystals was also observed.[9] The presence of large air cavity spaces is seen in the T.S. of stem of *B. monnieri*(contains uniform structural features); similar observation was also reported.[13] Study of anatomy of root is also an important parameter for identification and no previous report has been documented by earlier workers. In powder form it becomes very difficult to identify the actual source of natural drug. In the quest of competition among various drug industries, quality of the drug is compromised by adding drugs of similar resemblance exhibiting different properties which leads to reduction and alteration in drug value. Another problem is the wrong identification of natural drugs that may be attributed to assigning one common vernacular name to two or more varied plant species.[15] Powder analysis plays a significant role in identification of crude drugs. These characters will help in the identification of the right variety and search for adulterants. In *B. monnieri* round and oval starch granules, lignified fibres, spherical oil globules, thin fibres and compound oil droplets were seen. In another study similar findings were noticed, who have reported the presence of single fibre, simple and compound starch grains, oil globule and lignified fibres in the powder samples of *B. monnieri.*[9] Preliminary phytochemical investigation of *B. monnieri* aqueous extract reveals the presence of carbohydrates which was in agreement of the findings of *B.monnieri* in the aqueous extract.[16] Reducing sugar was present in the plant aqueous extract which is further supported by the findings.[17]Protein was detected in the plant extract. Similar findings match with the observations reported.[17] In addition to these several types of secondary metabolites i.e. steroids, alkaloids, tannins and saponins have also been detected in the aqueous extract of *B. monnieri* that was found to be in agreement with the findings. [18-21][17] To the contrary, glycosides were absent in the aqueous extract of *B. monnieri.*

6. CONCLUSION

Pharmacognostic studies guarantee plant identity sets down standardization parameters to avoid adulteration. Such examinations will help in validation of the plants and guarantee reproducible nature of herbal drugs which will prompt safety, viability and efficacy of natural medications. The pharmacognostic standardization parameters such as organoleptic characters, macroscopic study, microscopic study, powder study, physicochemical investigation, fluorescence analysis etc were investigated to confer safe and valid drugs. To overcome these problems pharmacognostic studies play a crucial role in checking the possibilities and threat of adulteration among natural drugs by macroscopic and microscopic examination of plant material. Thus, this study helped in identification and authentication of the plant material. The present study can be considered as a reference material that may throw light upon the correct identification of the medicinal plant. This may also be useful for the preparation of monograph. Further, it will act as a tool to detect adulterants and substitutes and will help in maintaining the quality of herbal products.

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9. AUTHOR CONTRIBUTION STATEMENT

Dr. Naureen Shaba Khan has conceptualized and gathered the data with regard to this work. Dr. Bhaskar Chaurasia analyzed the anatomical and microscopic study of the plant. Dr. A.K. Dixit gave the necessary inputs for identification of plant material and phytochemical analysis of plant. All authors contributed equally to finalize the manuscript.

10. CONFLICT OF INTEREST

Conflict of interest declared none.

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