**Mazus pumilus** (Burm. f.) Steenis; Pharmacognosy

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*Mazus pumilus* (Burm. f.) Steenis, is a well-known traditional medicinal plant belonging to family Mazaceae. The research work is about the pharmacognostic standardization of *M. pumilus* which includes; macroscopic features and microscopic evaluation of leaf, stem and roots. TS of leaf, stem and root showed the arrangement of the different cells. Histochemistry of TS of leaf, stem and root gave distinctive results with conc. HCl, phloroglucinol, ferric chloride, iodine solution and Sudan III which indicated the presence of Ca²⁺ oxalate crystals, lignin, tannins, starch and oil cells, respectively. Powder study of leaf depicted the presence of fibres, epidermal cells, resinous matter and vessels. The powdered study of stem showed collenchyma, vessels, fibers, cortex cells with tracheids, and helical vessels. While, root powder contained pithed vessels, cork cells, parenchyma and phelloderm. The quantitative analysis of TS of leaf was also performed for the establishment of leaf constants. In fluorescence analysis of herb, different colors were observed under ordinary light, short and high wavelength UV light. Phytochemical analysis of the methanolic extract of whole herb confirmed the presence of alkaloids, glycosides, flavonoids, saponins, sterols, triterpenoids, carbohydrates, proteins and tannins. All these results will help in identification, confirmation and quality characterization besides, laying down the pharmacopoeial standards for *M. pumilus*.

**Keywords:** Mazus pumilus, Mazus japonicas, Histochemistry, Pharmacognostic, Phytochemical, Pharmacognosy

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Plant based medicines are now popularized worldwide and therefore there is a great demand of medicinal plants for herbal medicine system. To meet the need for this purpose the standardization of these plant materials is very important⁹. *Mazus* is a genus of low growing perennial plants consisting of around 30 species. This genus is generally found in damp habitats in lowland or mountain regions of China, Japan, South East Asia, Australia and New Zealand."³. *Mazus pumilus* (Burm. f.) Steens syn. *Mazus japonius* (Thunb.) Kuntze syn. *Mazus rugosus* Lour. is commonly occurring glabrous annual hairy herb in Punjab region of Pakistan³⁴. *M. pumilus* is used as a traditional medicine in different areas of its geographical distribution by the local natives. The plant has been used as a whole as well as by parts, specifically leaves⁵. Ethnmedicinally, the whole plant has been employed as a remedy for epilepsy⁶. The infusion of the whole plant is consumed as tonic²³. Similarly, whole herb is utilized as an antifebrile, emmenagogue and aperitive⁷⁸. Certain species of the genus are edible⁹. In an ethnobotanical survey, it is found to be used as fodder for cattle¹⁰. The herb also possesses antibacterial and antifungal action¹¹. Modern researches have proven that various leaf extracts of *M. pumilus* has high potential of anticancer activity on human cancerous cell lines¹². The juice of the herb is used in the cure of typhoid¹³¹⁴. Due to its good antioxidant potential, the plant material has shown cardioprotective effect. Recent phytochemical investigations of *M. pumilus* have revealed that it is enrich with saponins as a major class of secondary metabolites¹⁵¹⁶.

In spite of a lot of attention to therapeutic effects; there isn’t any detailed pharmacognostic data available on structural morphology and other physicochemical standards, as it is required for the identification and quality standardization of the plant. Therefore, an extensive anatomical, physicochemical and phytochemical screening is required that will be helpful to avoid any ambiguity¹⁷. Morphological results are helpful in explanation of an exclusive drug with a major focus on quantitative and qualitative microscopy¹⁸. Therefore, the present research
comprises of anatomical, structural and histochemical evaluations of the leaf, stem and root, along with the whole herb assessment of physicochemical parameters (ash values, extractive values), fluorescence analysis and preliminary phytochemical properties.

**Methodology**

**Plant material**

The plant was collected from botanical garden of Government College University Lahore Pakistan, and was authenticated by Dr. Uzma Hanif, Assistant Professor, Department of Botany, Government College University Lahore Pakistan. A specimen of plant was deposited in herbarium of Government College University, Lahore Pakistan, under voucher No: GC. Herb. Bot. 2270. All plant parts were dried under shade, were powdered and preserved in brown containers at dry place.

**Pharmacognostic evaluations**

**Macroscopic evaluation**

All macroscopic evaluations were carried out on 5 samples of each part. The taxonomical description was made according to the related articles and the data given in books.

**Microscopic evaluation**

Fresh leaves, stem and root were immediately fixed in formalin: acetic acid: 70% alcohol (5:5:90) for 24 h. TS of leaf, stem and root were made by commonly used blade and razor method. Sections were stained with safranin and fast green dye.

**Histochemistry**

The sections of leaf, stem and roots were taken and they were treated with various chemicals to note the chemical reaction and color change. Inferences were made to evaluate the particular reaction for histochemical analysis in the plant tissues used, accordingly. Phloroglucinol, concentrated HCl, Iodine solution, Ferric chloride and Sudan III solution were used to locate the presence of Ca oxalate crystals, lignin, starch, tannins and oil globules, respectively. After treatment with particular reagent the sections were observed under light microscope and their photographs were taken using digital camera.

**Powder study**

Chloral hydrate (75 %) solution was used as clearing reagent prior to observation of powder drug using microscope. Slides of powdered leaves, stem and roots were prepared according to the prescribed procedures. Photographs were captured through the microscope with digital camera.

**Quantitative evaluation**

Microscopic examination is employed for the quantitative evaluation and for this purpose some specific histological features including, stomatal index, vein-islet and vein termination number and palisade ratio were noted.

**Fluorescence analysis**

Ultraviolet fluorescence analysis of whole herb was carried out by treating them with different reagents and was observed in ordinary light and UV light (short wavelength of 254 nm and long wavelength of 366 nm).

**Preliminary phytochemical analysis**

The preliminary phytochemical analysis of whole herb was carried out according to the standard procedures.

**Physicochemical analysis**

For the purpose of physicochemical analysis, whole herb was powdered. The powdered samples were subjected to analysis for their extractive values in chloroform, ethyl acetate and n-butanol, along with total ash, water soluble ash and acid insoluble ash values.

**Results**

**Macroscopic evaluation**

*Mazus pumilus* is an annual herb growing to 20 cm. It is a branched herb, average 7 cm tall; stems hispid. Lower leaves are opposite and closely packed, to 2 x 1.1 cm, obovate, obtuse, crenate to serrate, upper ones alternate, smaller.

**Microscopic evaluation**

TS of Leaf showing vascular bundles with xylem and phloem tissue in the central area, which are surrounded by the circular cells of collenchyma, with an outer boundary of epidermal cells (labelled as a, b&d in Fig. 1A). Trichomes were observed on the surface of epidermal cells (labelled as c and a in Figs 1A&B). Abundant stomata were seen in the lower epidermis (Fig. 1C).

Vascular bundles were observed around the central pithy portion of the stem TS. Outer to the vascular
bundles, cortex cells were observed with outer layer of epidermal cells of stem (labelled as a, b, c & d in Fig.2).

The TS of root was almost elliptical in shape and showed marginal epidermal cells, inner cortex, pericycle and rounded pith cells (labelled as a, b, c & d in Fig. 3).

**Histochemical analysis of TS**

Histochemical analysis of the leaf, stem and root was carried out. The transverse sections were exposed to conc. HCl, phlorogucinol, ferric chloride, iodine solution and Sudan III. The histochemistry of leaf showed the presence of Ca$_2^+$ oxalate crystals, tannins, lignins and oil globules. An effervescent response was observed with conc. HCl, megnata coloration when treated with phlorogucinol, greenish black coloration in case of ferric chloride solution. While, the presence of oil globules were confirmed by the treatment with Sudan III, when the globules turned pinkish red. The presence of aleurone and oil cells was also confirmed in powder microscopy of stem. However, it didn’t show any change when exposed to iodine solution which confirms the absence of starch granules in the stem.

On the other hand, transverse sections of root unveiled the presence of Ca$_2^+$ oxalate crystals, tannins, lignins and starch granules. Production of tiny bubbles which were seen on the slide under the microscope, megnata coloration of the section and dark coloration of the cells were clear signs of the presence of Ca$_2^+$ oxalate crystals, tannins and lignins, respectively. In addition to that, on exposure to iodine solution, blue shade appeared within the slide in certain cells. This proved the presence of starch granules in the root. It was further verified in the powder microscopy of root. Unlike stem, the TS of root is devoid of oil cells.

**Powder microscopy**

The powder microscopy of leaf shows; reticulate vessels with inter costal region in which Ca$_2^+$ oxalate crystals were distributed, thin asymmetric elongated fibers with pointed edges, boarder pitted grouped vessels, vein islets with brownish resinous matter associated with it and brick shaped rectangular epidermis cells (Fig. 4).
The powder of stem highlighted the thin polygonal cortex cells, group of pithed vessels, collenchyma, irregular shaped Ca$^{+2}$ oxalate crystals, collenchyma, elongated fibers of xylem, i.e. tracheids, aleurone and oil cells with round to circular dispersed oil globules, flexible and twisted helical vessels and thin fibers with visible lumen and pointed edges (Fig. 5).

The powder microscopy of root revealed the presence boarded pitted vessels that were fused into each other, cells of phelloderm—a part of periderm with crisscross network, brick shaped quadrilateral secondary phloem tissues with Ca$^{+2}$ oxalate crystals, dense walled either penta or hexagonal cork cells (phellem), small irregular shaped parenchyma cells with starch granules in small aggregates, and thick walled polygonal pith cells (Fig. 6).

**Quantitative evaluation**

The quantitative evaluation helped in establishing leaf constants including; palisade ratio (7.0 ± 0.31), stomatal index (11.35 ± 0.41), vein-islet number (11.0 ± 0.5 5) and vein termination number (15.5 ± 0.76) (Table 1).

**Fluorescence analysis**

The fluorescence analysis of whole herb revealed various colors of the extracts under ordinary light, Short wavelength (254 nm) UV light, and Long wavelength (366 nm) UV light indicating the presence of fluorescent compounds in the methanolic extract (Table 2).

**Preliminary phytochemical analysis**

The phytochemical screening of whole herb mainly revealed the presence of terpenoids, sterols, glycosides, flavonoids, alkaloids, carbohydrates, tannins, saponins, and proteins (Table 3).

**Physicochemical constants**

The extractive value of whole herb in ethyl acetate was high, followed by n-butanol. The chloroform extractive value was the lowest as compare to other solvents (Table 4). The ash values of whole herb showed high content of total ash and water-soluble ash followed by acid insoluble ash (Table 5).

**Discussion**

Herbal drug’s purity, safety, identity, and quality can be maintained by means of standardization which
involve both qualitative and quantitative parameters. Among qualitative parameters microscopy is simple and economical measure to identify source material. On the other hand, the quantitative parameters involve determination of stomatal index, vein islet, vein termination number and palisade ratio. By these values the purity of drug can be illustrated. Literature revealed that both qualitative and quantitative studies are important for identification of raw material during drug manufacturing\textsuperscript{30,31}.

The transverse section of leaf showed the basic profile of botanical anatomy showing the arrangement of vascular bundles, collenchyma tissues, trichome and epidermis. The abundant stomata were also observed in the lower epidermis (Fig. 1). The TS of stem vascular bundles arranged in circular form along with pith cells, cortex and epidermis (Fig. 2). The TS of root also disclosed the presence of cortex, pith, epidermis and pericycle in the middle (Fig. 3).

Histochemical assessment of transverse sections were carried out to acquire a realistic image on preparatory scale at cellular level utilizing conc. HCl, phloroglucinol, ferric chloride solution, iodine solution, and Sudan III, which demonstrated the existence of tannins, lignin and Ca\textsuperscript{2+} oxalate crystals in the leaf, stem and root. Effervescent response of all parts to conc. HCl indicted the presence of Ca\textsuperscript{2+} oxalate crystals. Ferric chloride solution determined the existence of tannins in all parts by showing blackish coloration on exposure, and megnata coloration due to the application of phlorogucinol demonstrated presence of lignins. Occurrence of oil globules in the pith cells of stem was confirmed, as they turned pinkish red in color on exposure of Sudan III dye, though results were negative with leaf and root indicating they are devoid of oil cells. Similarly, the iodine solution gave positive result for starch granules in root, when its exposure turned them blue. While, it gave negative results for leaf and stem.

The powdered microscopy of the leaf of the herb depicted the reticulate vessels, with inter costal region among them, along with that thick walled vessels, epidermal cells, thin irregular shaped fibers (Fig. 4). The powdered micrographs of stem showed the helical vessels, crystals of Ca\textsuperscript{2+} oxalate, aleurone with oil forming cells. The compound and single vessels with piths were also visible. Besides that, the basic structures including cortex, collenchyma tissues, thin fibers pointing at the edges, and tracheids (Fig. 5). The powder study of root revealed bordered pithed vessels, pith cells, secondary phloem tissues with Ca\textsuperscript{2+} crystals. In addition to them, thick and thin cork cells were also observed. Phelloderm (modified cork cells) was also seen (Fig. 6).

The quantitative evaluation of TS of leaf helped in the establishment of leaf constants that proved to be a helpful tool to aid in the authentication and confirmation process of this therapeutically important drug (Table 1). The fluorescence analysis is an appreciated, easy and direct method for the identification of fluorescent compounds. Different compounds give fluorescence when they are exposed to short and long wavelength UV light\textsuperscript{32}. The powder of whole herb gave various colors when observed under ordinary daylight, in short and long wavelength of UV light Table 2. The preliminary phytochemical screening of methanolic extract of \textit{M. pumilus} whole

| Table 1 — Leaf constants of leaves of \textit{M. pumilus} |
|----------------|----------------|----------------|
| Leaf constants | Range           | Mean ± SEM     |
| Palisade ratio | 6 – 8           | 7.0 ± 0.31     |
| Stomatal index of lower epidermis | 10.4 – 12.3 | 11.35 ± 0.41 |
| Vein-islet number | 9.5 – 12.5  | 11.0 ± 0.55   |
| Vein termination number | 13.2 – 17.8 | 15.5 ± 0.76  |

| Table 2 — Fluorescence analysis of whole herb, \textit{M. pumilus} |
|----------------|----------------|----------------|
| S. No. | Chemicals | Ordinary light | Short wave length | Long wave length |
|       |           |               | 245nm            | 366nm            |
| 1     | Water     | Light green   | Purplish blue    | Dark green       |
| 2     | Chloroform| Dark green    | Reddish          | Yellowish green  |
| 3     | Aniline   | Dark red      | Brick red        | Dark green       |
| 4     | Powder    | Light green   | Bluish green     | Light green      |
| 5     | 66% H\textsubscript{2}SO\textsubscript{4} | Brownish green | Light green      | Dark green       |
| 6     | 50% H\textsubscript{2}SO\textsubscript{4} | Pale yellow   | Brownish black   | Dark green       |
| 7     | 50% HNO\textsubscript{3} | Lemon yellow | Brownish green   | Dark green       |
| 8     | 5% NaOH   | Light green   | Brownish black   | Dark green       |
| 9     | 5% FeCl\textsubscript{3} | Brown     | Dark grey        | Dark green       |
| 10    | 10% KOH   | Yellowish green| Reddish green    | Dark green       |
The herb, revealed the presence of various important active constituents: carbohydrates, proteins, saponins, alkaloids, glycosides, flavonoids, lipids, and terpinoids (Table 3).

The extractive values are an integral part of physicochemical analysis and these quantitative values are determined for further standardization of the plant powder. These values give a clear idea about the yields of the extract in different solvents. The evaluation was performed on whole herb with chloroform, ethyl acetate and n-butanol and the results are tabulated in Table 4. The ash values for the powdered plant are calculated to figure out the amount of siliceous material left over in the residue and to determine the extraneous material adhered to the plant while its collection. The ash values of whole herb were carried out and the results are presented in Table 5.

### Traditional significance of study

The herb has been used as a traditional medicine. This standardization approach will invoke the scientists to work further for the assessment of the distinctive chemical constituents present in its extract that are rendered responsible for its biological activities and traditional uses. Their chemistry and structural elucidation will assist in describing the mechanism of their actions. Besides that, these constraints will help in the identification of the correct herb while collection and minimize the chances of adulteration, substitution or sophistication.

### Conclusion

The study of pharmacognostical features had shown the standards, which will be useful for the recognition of its distinctiveness and genuineness. Physicochemical parameters like ash values, and extractive values are all indicators of the quality of material. These anatomical perspectives, pharmacognostical evaluations and physicochemical characterization, carried out in this research work can be utilized as a reference for authenticity of this plant material.

### Declaration of interest

We declare that we have no conflict of interest.

### Acknowledgement

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