ORIGINAL ARTICLE

# Microscopic and pharmacognostic standardization of *Mollugo cerviana* (L.) Ser.: A comprehensive study on morphological and anatomical characteristics for quality assessment in herbal medicine

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## Abstract

**Objectives:** The aim of the presented work is to evaluate standardization parameters and identifies the microscopic structure of the medicinal plant *Mollugo cerviana* (L.) ser., which is therapeutically important plant belonging to the family *Molluginaceae* and used widely in ethnomedicine with its important therapeutic significance. This article also aims to assess lupeol in plants using high performance thin-layer chromatography (HPTLC). **Materials and Methods:** Macro and microscopic, physicochemical, and phytochemical analyses to establish standard parameters. Standardization of plant material including ash value, extractive value, foaming index swelling index, and pH was performed using the appropriate method. HPTLC was done for the detection of lupeol. **Results:** The vascular bundle, collenchyma, sclerenchyma, palisade cells, and wavy and straight-walled epidermal cells are visible under a microscope in the leaf, stem, and root. The anomocytic stomata were found in the leaf. In powdered microscopy of the sample, the prismatic calcium oxalate crystals, cork cells, fibers, and lignified vessels were also observed. Phytochemical screening found the presence of phenolics, glycosides, saponins, tannins, flavonoids, triterpenes, and sterols. The moisture content, ash content, swelling index, foaming index, pH of edible extracts, and solvent-soluble extractives were among the physiochemical characteristics that were measured. Fluorescence microscopy was conducted to investigate the luminous chemicals in both visible and ultraviolet light. Thin layer chromatography was carried out for the determination of the maximum number of spots in various solvent systems followed by HPTLC analysis was found in the concentration of 730.8 µg/g min ethanolic extract of *M. cerviana*. **Conclusion:** The current findings offer the details required for accurately identifying and evaluating the quality of crude drugs made from *M. cerviana*.

**Key words:** Macroscopy, microscopy, *Mollugo cerviana,* pharmacognostic, physicochemical phytochemical

## INTRODUCTION

For many years, herbal medicines have played an important role as a natural source to treat many illnesses, safety measures, and diseases in people of all ages, particularly in underdeveloped nations. Plant research has increased tremendously in the past few decades, and numerous sources of evidence demonstrate the enormous scope of medicinal herbs utilized in diverse traditional systems.[1] As a consequence, phytoconstituents derived from traditional medicines must be evaluated using varied phytochemical screening, pharmacological, and analytical approaches.[2] The World Health Organization (WHO) promotes, recommends, and encourages the use of herbal treatments in national healthcare programs because they are inexpensive and safe.[3] The absence of standardization is the major downside to the use of plant

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medicine. This paves the opportunity for misidentification, the inadvertent substitution of species that are closely analogous species, and purposeful adulteration of real herbs with inferior ones to meet market-raising demands.[4]

Pharmacognostic standardization is an essential process in the field of herbal medicine. It involves determining the individuality, superiority and purity of natural products, such as plant-based medicine or crude drugs. The goal of pharmacognostic standardization is to ensure that the products are safe and effective for use in various medicinal and therapeutic applications. To ensure repeatable quality and efficacy, standardization criteria for medicinal product identification, extraction, and purification must be specified.[5] According to the WHO, the first stage in confirming the authenticity and purity of a medicinal plant is a macroscopic and microscopic examination.[3] Plant secondary metabolites such as flavonoids and phenolics have achieved major recognition for their antioxidant and antiradical activities. The main active substances derived from higher vegetation are phenolics and flavonoids. These polyphenols exhibit vital biological actions, including anti-inflammatory, antioxidant, anticancer, and antibacterial characteristics, among others.[6]

*Mollugo cerviana* (L.) Ser. of family *Molluginaceae* commonly known as thread stem carpet weed and pita bhaji as a local name is widely used for its medicinal properties. Entire plant parts are edible and consumed by human beings. It grows as a weed in many distinct kinds of dry, sandy habitats on most continents. The plant is well-known for its traditional medicine commonly harvested for both local use and sale in the local markets.[7] Regardless of having inordinate medicinal importance, there are only limited details available on the standardized characteristics of *M. cerviana* plant. Hence, the proposed work can attempt for providing a complete report on quality control and standardization of this plant. In our recent report, we studied the pharmacognostic characterization (macroscopy, microscopy, powder microscopy, and fluorescent microscopy) and standardization parameters (Moisture content, ash value, extractive value, swelling index, foaming index, and pH) and chromatographic technique thin layer chromatography (TLC) and high performance TLC (HPTLC) for quality control of whole plant of *M. cerviana* (L.) ser. The outcomes of this trial may serve as guidelines for future pharmacological and nutritional product development.

## MATERIALS AND METHODS

### Sample Collection and Preparation for Pharmacognostic Studies

Fresh, healthy, and disease-free samples of the whole plant of *M. cerviana* (L.) Ser. belonging to the family *Molluginaceae* were collected from the Arpa River side of Bilaspur, Chhattisgarh, India. The collected plant was identified and verified (authentication no. Bot/GGV/2022/10) in the Department of Botany, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur, Chhattisgarh. The plant material was shade-dried at room temperature for 7 days. Followed by drying, the plant material was crushed to a coarse powder with a grinder and stored properly for future uses.

### Organoleptic Evaluation

Several sensory factors including color, odor, taste, shape, and texture of fresh samples were investigated for organoleptic estimation.

### Macroscopic Evaluation

For macroscopic studies fresh and healthy plants of *M. cerviana* including the type of leaf, leaf base, presence or absence of petiole, and lamina surface characteristics were recorded. Lamina surface characteristics to be observed included the leaf shape, venation, apex, base, incision, and margin. Thirty fresh leaves were selected and used in the determination of the average length and width of the leaf. Flowering, fruiting, and inflorescence of plants are also observed.

### Physicochemical Analysis

Various physiochemical parameters including ash content (total ash, water-soluble ash, and acid-insoluble ash), moisture content, pH, swelling index, and foaming index were analyzed according to the standard methods. The extractive value of the powdered plant material was determined using the technique described in Evans.[8]

### Qualitative Microscopic Evaluation

The model of microscope used in microscopic studies was the XSZ-N107T light microscope. Sharp blades were used to cut transverse sections (TS) of fresh plant components (leaf, stem, and root). A fine thin section was mounted on a slide and stained with phloroglucinol/safranin solution and a drop of concentrated hydrochloric acid (HCl) for the observation of the types and distribution of several plant cells and observed under microscope at different magnifications and photographed.

### Quantitative Microscopy

A small piece of the cleaned leaf was taken and then put in a 5% glycerine solution at a different magnification of examination. The number of epidermal cells, stomatal index, veinlet terminations, and vein-islets per square millimeter was determined and recorded using a camera lucida and stage micrometer.

### Phytochemical Analysis

Primary phytochemical screening to detect classes of plant secondary metabolites present in the ethanolic and ethyl acetate extracts of the whole plant of M. cerviana was done according to established methods mentioned in Evans.[8]

### Chromatographic Studies

TLC of the petroleum ether, ethyl acetate, and ethanolic extract of *M. cerviana* was done in different solvent system. HPTLC was performed in ethanolic extract for determination of lupeol.

#### *TLC studies*

A stock solution was prepared by dissolving about 1 mg of different solvent extracts in their respective solvents. Using a capillary tube, tiny spots (not wider than 3–4 mm) were transferred onto the beginning lines of TLC plates that had already been activated. After that, the TLC plates were transferred into the selected solvent system (Toluene: Ethyl acetate; 3:1 and 4:1, Chloroform: methanol; 90:10, Hexane: ethyl acetate; 7:3). The spots were allowed to dry and placed into developing chamber. The chromatogram was developed at room temperature. The produced spots were observed in daylight and under ultraviolet (UV) light. Spots were also developed in an iodine chamber and risk factor values of spots were calculated.

#### *HPTLC studies*

Using an HPTLC analyzer, the ethanolic extract is further examined for lupeol. The separation was performed on an HPTLC system equipped with a CAMAG TLC scanner, integration software, and a TLC scanner (Win CATS). Under both regular and UV light, the formed bands were examined. The mobile phase was toluene: methanol (9:1), and their stationary phase was pre-washed HPTLC silica gel plates 60 F254. Both standard and UV lighting were used to analyze the produced bands. The maxima of plant extracts and conventional lupeol were contrasted. The plates were photographed with the use of white and UV light.[9]

### Powder Microscopy

Powder microscopy of different plant parts was determined using XSZ-N107T light microscope using the procedure mentioned in Wallis[10] at the pharmacognosy lab, Department of Pharmacy, Guru Ghasidas Vishwavidyalaya, Chhattisgarh. A nip of fine powder was put in a test tube and heated briefly in a KOH solution. On the glass slide, a few drops of powder were smeared and mounted with phloroglucinol followed by a few drops of concentrated HCl. The developed slides were then examined and captured using a microscope.

### Fluorescence Analysis

In test tubes, a small quantity of the powdered plant sample was added. A few ml of various solvents, some acidic or basic chemical reagents were added and allowed to stand for a few minutes. The characteristic fluorescence colors emitted by the various solutions were observed in daylight as well as under UV light.

## RESULTS AND DISCUSSION

Proper identification, quality assurance, and establishment of pharmacognostic standards are salient aspects in the evaluation of therapeutic plants.[3] Over the past few years, physicochemical and phytochemical analyses have been used to identify many plant species. By these methods, adulteration and substitution have been detected.[4] Present investigation revealed the standardization of the whole plant of *M. cerviana* based on its pharmacognostic, physicochemical, and phytochemical properties.

### Morphological/Macroscopic Study

Using sensory organs, a specific species can be verified through organoleptic and macroscopic evaluation. The basic characteristics of the leaf, such as form, border, apex, base, and kind of venation, are primarily assessed for the quick ability to recognize the plant in its natural habitat [Figure 1]. Changes in these features result in alterations to quality grade. To distinguish between various plant species based on their internal architecture and cellular organization, microscopic studies of plant cells are crucial. This will be helpful for easily distinguishing among plant species that share similarities and have identical micromorphology.[12] The results of morphological and macroscopic features are given in [Tables 1 and 2]. A similar study was observed by Nagannawar and Jayaraj, Pratap *et al*.[13,14] The WHO advises that the identification and purity of such materials should be established by macroscopical and microscopic investigation of a medicinal plant before undertaking any testing. Changes in these features result in alterations to quality grade.

### Physicochemical Analysis

Physical and chemical characteristics, including loss on drying, ash content, pH, swelling, and foaming index, can be used to identify and detect the quality of crude drugs. The loss on drying method used for measuring how much moisture is in a powdered sample. The moisture content is a crucial factor for estimating the efficacy of a drying method as well as the chance of damage due to excessive moisture content during storage and processing. During storage, the moisture content of powdered drug should be kept low to inhibit the growth of bacteria, yeast, or fungus.[15] The

moisture content determined by loss on drying method was found to be 18.66 ± 1.32 [Table 3].[16]

## Microscopic/Anatomical Study

A concept of plant's microscopic attributes, such as epidermal cells, cork cells, cortex, secondary phloem, and fibers, which serve as key components for the quality control and standardization of herbal products, is provided by microscopy.

**Table 1:** Organoleptic properties of *Mollugo cerviana* plant

| Characteristics | Leaves | Stem | Root |
|---|---|---|---|
| Color | Dark green | Reddish brown | Off-white |
| Odor | Pungent | Indistinct | Indistinct |
| Taste | Bitter | Bitter | Characteristic |
| Texture | Smooth | Soft | Rough |

**Table 2:** Macroscopic attribute of *Mollugo cerviana* plant

| Habit | Herbaceous perennial |
|---|---|
| Root | Taproot |
| Stem | Creeper week stem |
| Leave | Compound leaf |
| Leaf margin | Dentate |
| Leaf base | Symmetrical |
| Leaf apices | Acute |
| Leaf shape | Lanceolate |
| Leaf surface | Glabrous |
| Leaf venation | Unicostate parallel |
| Length | 1.50–2.0 cm |
| Width | 0.5–1 cm |
| Flower sepal arrangement | Radial |
| Inflorescence | Umbel |

### *Leaf*

At the midrib region, the upper epidermis was composed of a single layer of cells that had almost equal width and length. The lower epidermis was single-layered, thick-walled, with compactly arranged cells. There were numerous unicellular covering trichomes on both surfaces of the leaves. A thin cuticle layer was interrupted by anomocytic stomata with wavy walled epidermal cells and was present on [Figure 2]. Mesophyll was composed of upper (palisade) and lower (spongy) parenchymatous layers. The palisade consisted of 1–2 layered compact and radially arranged wide and long cylindrical cells. Spongy parenchyma cells were toward the lower epidermis and were loosely arranged. One prominent arc-shaped vascular bundle, more toward the lower side, was a small, open collateral vascular bundle, and was surrounded by spongy parenchymatous cells [Figure 3].

### *Stem*

The TS of the stem revealed numerous unicellular covering trichomes. The epidermis consists of single or double-layered rounded and compactly arranged cells with well-defined cuticles. Hypodermis was collenchymatous. The cortex was composed of thin-walled parenchymatous cells. A single layered endodermis was also present at the outer side of the bundles. The pericycle consisted of 2–3 layered polygonal lignified parenchyma cells. In the vascular bundle, the phloem was toward the inside. The xylem was toward the center and consisted of thick-walled and radially arranged Xylem fibers and vessels. Large central pith made of polygonal, thick-walled parenchyma cells [Figure 4].

### *Root*

Epiblema consisted of thin-walled cells. The cortex was composed of 2–3 layered, loosely arranged parenchyma

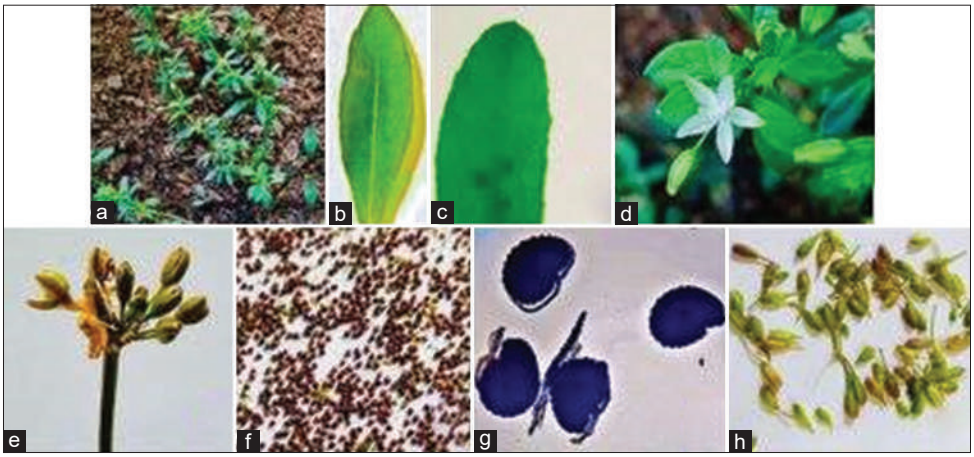


**Figure 1:** Morphological attribute of *Mollugo cerviana*. (a) Herbaceous perennial plant, (b) Leaf with venation, (c) Leaf section enlarge for dentate leaf margin (×10), (d) Actinomorphic flower showing radial corolla arrangement, (e) Inflorescence umbel, (f) Seed semi-spheroid shape, (g) Seed sector enlarge with hilum (×10), (h) Flower buds

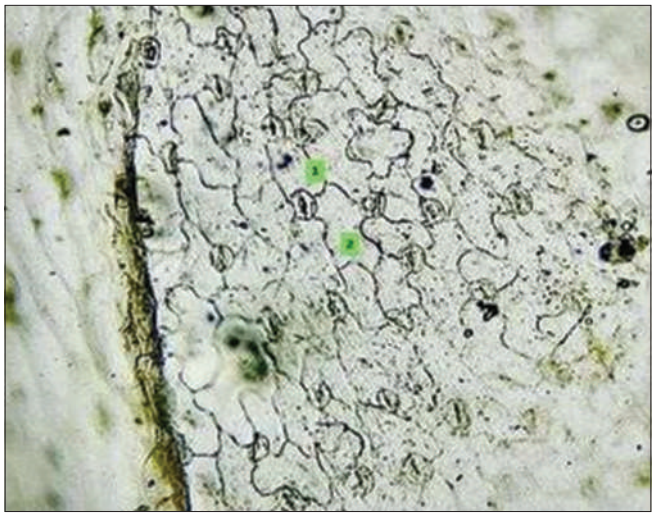

**Figure 2:** Anomocytic stomata showing wavy walled epidermal cells (×10)

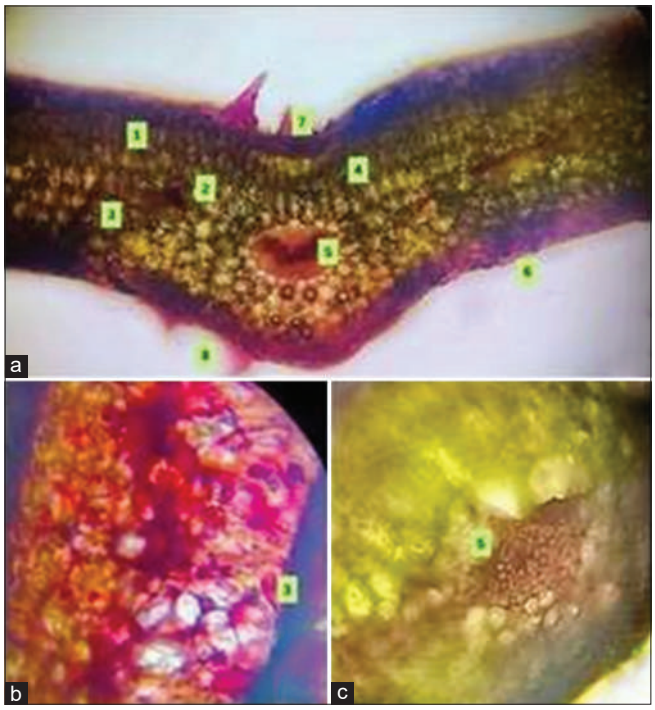

**Figure 3:** (a) (×40), (b) (×10), and (c) (×10) showing transverse section of a leaf where 1. Upper epidermis, 2. Double layered palisade parenchyma, 3. Spongy parenchyma, 4. Cholenchyma, 5. Vascular bundle, 6. Lower epidermis, 7. Upper trichomes, and 8. Lower trichomes

cells. The cortex ended with a single-layered parenchymatous endodermis. A growth ring is present. Vascular bundles were radially arranged. The phloem was situated beneath the pericyclic region followed by the xylem. The xylem consisted of thick-walled xylem parenchyma cells, pitted xylem vessels, and lignified fibers. The vessels were solitary, circular to oval. Medullary rays were rectangular to oblong shaped, arising from the central region and extending up to the inner layers of the phloem region [Figure 5]. *Mollugo nudicaulis* leaves and roots revealed the presence of similar

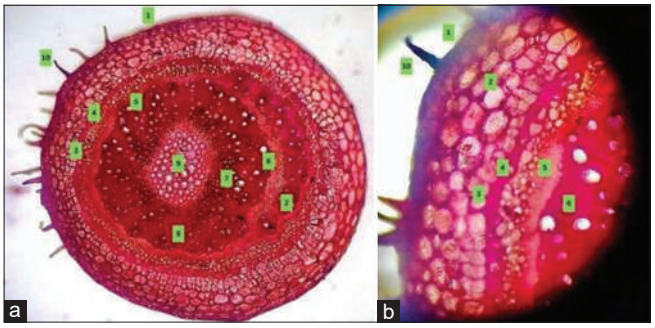

**Figure 4:** (a) (×40) and (b) (×10) showing a transverse section of stem where 1. Epidermis, 2. Hypodermis, 3. Cholenchyma, 4. Endodermis, 5. Schlerenchyma, 6. Phloem, 7. Metaxylem, 8. Protoxylem, 9. Wood parenchyma, and 10. Pith cavity

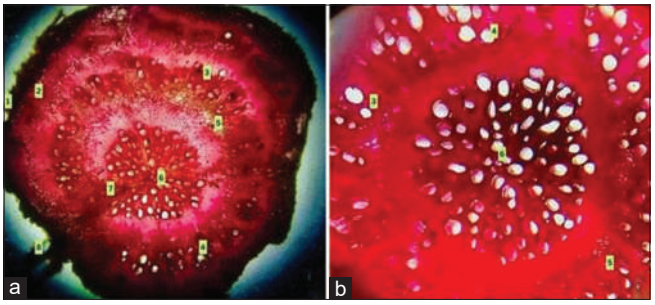

**Figure 5:** (a) (×40) and (b) (×10) showing a transverse section of root where 1. Single layer epiblema, 2. Two to three layered cortex, 3. Growth ring, 4. Vessels, 5. Xylem fiber, 6. Vascular bundle, 7. Medullary rays, and 8. Trichome

**Table 3:** Physicochemical analysis of whole plant of *Mollugo cerviana*

| Physicochemical parameter | Observation |
|---|---|
| Total ash | 7.5±1.2 g |
| Acid insoluble ash | 1.19±0.6 g |
| Water soluble ash | 3.15±1.2 g |
| Swelling index | 2±0.2 cm |
| Foaming index | 125±0.25 |
| pH | 6–7±0.1 |
| Moisture content | 18.66±1.32 |

**Table 4:** Quantitative leaf microscopy of the whole plant of *Mollugo cerviana*

| Leaf surface parameter | Result | Mean±standard deviation |
|---|---|---|
| Stomatal number/mm$^2$ | 17–30 | 23.5±9.19 |
| Stomatal index (%) | 20–25 | 22.5±3.5 |
| Vein-islet number/mm$^2$ | 25–28 | 26.5±2.12 |
| Vein termination number/mm$^2$ | 14–18 | 16±2.82 |

tissues and are in accordance with the present work.[14] Rashid *et al*. claimed that microscopy is obligatory to detect adulteration and contamination of the phytomedicines and

thus renders means for evaluating the genuineness and quality assets of crude drugs.[17] According to Velvizhi *et al*. the microscopic attribute is the first and rudimentary step toward standardization of the crude drug.[18]

## Leaf Constants Study

The results of the leaf constants are given in [Table 4]. Anomocytic stomata were found on the surface and were slightly sunken related to the epidermal cells [Figure 2]. The stomatal number was 17–30 with (23.5 ± 9.19) average and standard deviation, respectively. The range of stomatal index was 20–25 with (22.5 ± 3.5) mean and standard deviation, respectively. Vein islet and vein termination number of leaves were 25–28 (26.5 ± 2.12) and 14–18 (16 ± 2.82) mean and standard deviation, respectively. Many investigators studied the leaves surface features of medicinal plants. Birendra *et al*. stated that leaf constants serve as a requisite tool to identification and play a vital role in plant systematics.[19] Bashir *et al*. claimed that it is

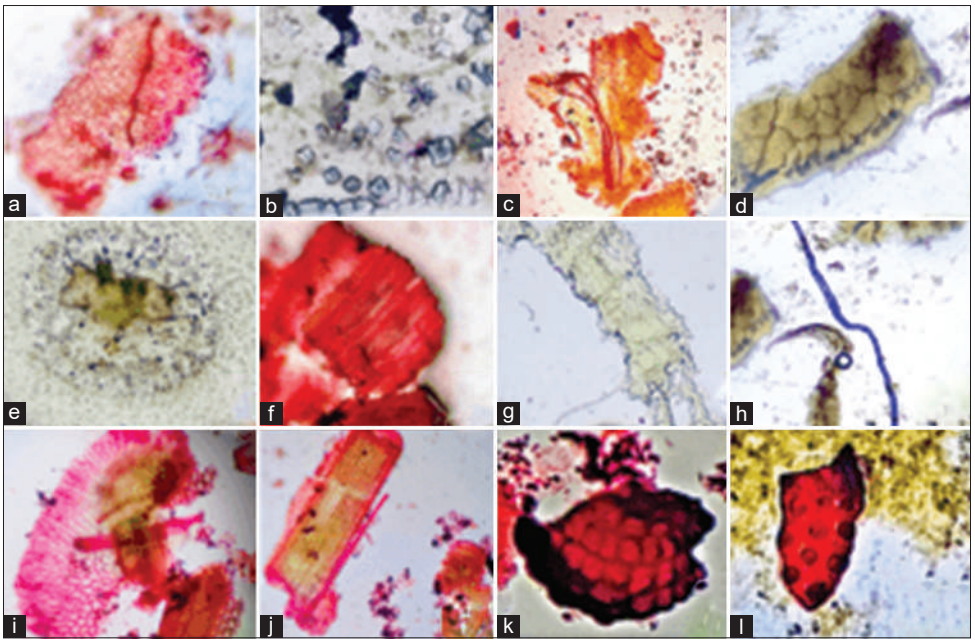


**Figure 6:** Powder microscopy of a leaf where (a) Epidermal cells (×10), (b) Calcium oxalate crystals (×40), (c) Leaf fibers (×10), (d) Cork cell (×10). Powder microscopy of the root where (e) Vascular bundle (×40), (f) Lignified fiber (×10), (g) Root vessels (×10), (h) Phloem fiber (×40). Powder microscopy of the stem where (i) Cortex (×10), (j) Lignified fiber (×10), (k) Stem cork cells (×10), (l) Vascular bundle (×10)

**Table 5:** Fluorescent analysis of different parts of *Mollugo cerviana* whole plant

| Treatment | Plant parts | | | | | |
|---|---|---|---|---|---|---|
| | Leaves | | Stem | | Root | |
| | Daylight | UV light | Daylight | UV light | Daylight | UV light |
| Powder+Water | Green | Neon green | Light green | Neon green | Pale white | Neon green |
| Powder+Ethanol | Green | Dark olive green | Pale green | Dark green | Off white | Light fluorescent |
| Powder+Methanol | Pale green | NF | Dark green | Neon green | Off white | Dark fluorescent |
| Powder+Ethyl acetate | Dark green | Neon green | Olive green | Dark green | Light yellow | NF |
| Powder+petroleum ether | Pale green | Black | Pale green | Black | White | NF |
| Powder+Chloroform | Pale green | Black | Pale green | Dark green | Off white | Light fluorescent |
| Powder+KOH | Green | NF | Dark green | NF | White | Dark fluorescent |
| Powder+$HNO_3$ | Light green | Light fluorescent | Green | Black | Light yellow | Black |
| Powder+HCL | Olive green | Black | Dark Brown | NF | Brown | Black |
| Powder+$FeCl_3$ | Pale green | Light fluorescent | Green | Light fluorescent | Yellow | NF |
| Powder+$H_2SO_4$ | Light green | Black | Dark Brown | Black | Dark olive green | Black |

NF: No fluorescence, UV: Ultraviolet

**Table 6:** Extractive value in different solvents of *Mollugo cerviana*

| Solvents | Extractive values (%) |
|---|---|
| Petroleum ether | 0.47±0.55 g |
| Water | 2.15±0.81 g |
| Chloroform | 2.34±0.90 g |
| Ethyl acetate | 3.50±1.25 g |
| Ethanol | 4.11±1.90 g |

one of the important features of microscopic assessment. It supports in tracing the origin and proper identification of herbal drugs.[20]

## Extractive Values Determination

The quantity of the active ingredients in a given amount of plant material after extraction with a certain solvent is determined by the estimation of extractive values. Any raw drugs can be extracted with a specific solvent to get a mixture of phytoconstituents.[16] We succeeded in determining the quantity and kind of active phytoconstituents in the extract by conducting the extraction using multiple solvents and comparing the extractive percentage in each solvent. Using alcohol (ethanol) as a solvent tends to extract polar compounds, such as phenols, glycosides, and flavonoids. These compounds are often associated with antioxidant, anti-inflammatory, and other medicinal properties. A non-polar solvent (n-hexane, petroleum ether, and Toluene) is employed to extract non-polar substances, such as lipids and steroids. These compounds are usually associated with structural components of cells and may have effects on cell membranes and hormone regulation while water is a polar solvent and is used to extract water-soluble compounds, such as sugars, acids, and inorganic compounds. The results revealed that the highest extractive value was shown by plant in ethanol followed by ethyl acetate, chloroform, water, and petroleum ether [Table 6]. In addition, the result indicated that petroleum ether (0.47 ± 0.55 g), water (2.15 ± 0.81 g), chloroform (2.34 ± 0.90 g), ethyl acetate (3.50 ± 1.25 g), ethanol (4.11 ± 1.90 g). It was supported by Baidoo *et al*. who reported similar findings. Nagannawar and Jayaraj reported similar results for *Mollugo oppositifolia*, that is, confirmed that the plant parts revealed the highest extraction in water (11.21 w/w) followed by ethanol (12.73 w/w), and ethyl acetate (6.9 w/w).[13] The extractive values are an essential part of the physiochemical profile and they are measured to strengthen the standardization procedure.[12] Upadhyay *et al*.[21] stated that the extractive values are efficiently used for standardization and quality assurance of herbal drugs.

## Preliminary Qualitative Phytochemical Analysis

The present study revealed that the alcoholic and ethyl acetate extracts of the whole plant of M. cerviana contained alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, saponins, and tannins. Ethanol extract and ethyl acetate extracts showed the presence of a rich variety of secondary metabolites depicted in Table 7.

**Table 7:** Preliminary phytochemical screening of *Mollugo cerviana* extract

| Phytoconstituents | Ethyl acetate | Ethanol |
|---|---|---|
| Tannin | | |
| 10% NAOH test | + | + |
| Saponin | | |
| Foam test | + | + |
| Reducing sugar | | |
| Fehling test | - | - |
| Glycosides | | |
| Conc. $H_2SO_4$ test | + | + |
| 10% NAOH | - | + |
| Cardiac glycoside | - | - |
| Keller–killani test | | |
| Flavonoid | | |
| Coc. $H_2SO_4$ test | + | + |
| Ammonia test | + | + |
| Shinoda test | - | - |
| Carbohydrate | | |
| Molisch test | + | - |
| Test for Pentose | - | + |
| Protein and amino acid | | |
| Ninhydrin test | - | - |
| Phytosterol | | |
| Sulphur powder test | + | + |
| Phenolic compound | | |
| Iodine test | + | + |
| FeCl3 test | - | - |
| Quinone | | |
| Conc. HCL | + | + |
| Alkaloid | | |
| Dragendorff test | + | - |
| Wagner test | - | - |
| Hager test | - | + |

+ = Present, - = Absent

## Chromatographic Studies

### *TLC analysis of plant extract*

For the separation, identification, and quantification of several kinds of natural products, TLC is a crucial analytical technique. The TLC plates evaluation revealed the presence of tannin derivatives, flavonoids, steroids, and saponins in the spots. Table 8 summarizes the findings from the TLC experiments.

**Table 8:** Observation of TLC of different solvent extracts of *Mollugo cerviana*

| S. No. | Plant extract | Solvent system | No. of spots | Rf values | Visualization normal light | UV light |
|---|---|---|---|---|---|---|
| 1. | Petroleum ether extract | Toluene: Ethyl acetate (3:1) | 4 | 0.35, 0.62, 0.60, 0.71 |  |  |
| 2. | Ethyl acetate extract | Toluene: Ethyl acetate (4:1) | 4 | 0.89, 083, 0.38 |  |  |
| 3. | Ethyl acetate extract | Chloroform: Methanol (90:10) | 5 | 0.33, 0.41, 0.5, 0.75, 0.83 |  |  |
| 4. | Ethanolic extract | Toluene: Methanol (9:1) | 3 | 0.4, 0.70, 0.8 |  |  |

TLC: Thin layer chromatography, Rf: Risk factor, UV: Ultraviolet

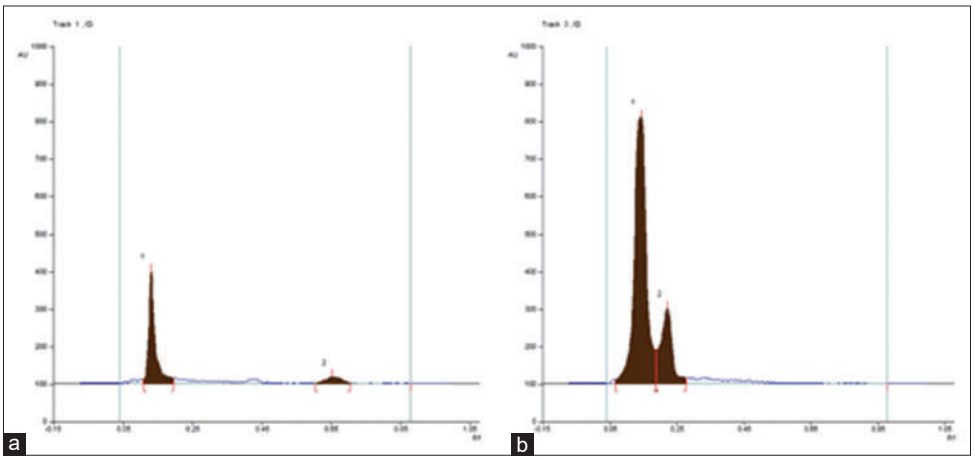


**Figure 7:** Represent chromatogram of lupeol (a) Standard, (b) Ethanolic extract of *Mollugo cerviana* by using toluene: Methanol (9:1) as a solvent system

### *HPTLC for lupeol*

HPTLC is a simple, rapid, and efficient procedure for analyzing plant material. The HPTLC fingerprint has a greater resolution and can predict active components more quickly and accurately. In the current investigation, HPTLC on the ethanolic extract of *M. cerviana* revealed the presence

of lupeol under chromatographic conditions (Toluene: Methanol; 9:1 as a solvent system) and the concentration was 730.8 µg/g of extract. Figure 7 shows the chromatogram for both tracks (for standard and plant extract).

### Powder Drug Study

Before being utilized or sold, the majority of crude drugs are processed into powder form. Setting guidelines for the identification of adulteration in samples of powdered drugs is therefore important. The cytomorphological criteria, such as collenchyma, epidermal cells, vascular bundle, vessels, and calcium oxalate crystals, are utilized for analyzing herbal material after it is powdered. Powder microscopy of the leaf showed the presence of a xylem vessel, epidermal cells, calcium oxalate crystals, leaf fibers, and cork cells. After that, powder microscopy of the root revealed the existence of a vascular bundle, lignified fiber, root veins, and phloem fiber. Other findings included the presence of lignified fiber. Powder microscopy of the stem showed the presence of cortex, lignified fiber, stem cork cells, and vascular bundle [Figure 6]. According to the WHO, powder drug microscopy is useful to know the quality and purity of the drug.[22]

### Fluorescence Study

The fluorescence analysis of the selected plant parts was carried out under visible light and UV light of 366 nm wavelengths. Various shades of color were observed [Table 5].

Rather and Jain investigated that the fluorescence character of the drug is due to the presence of various functional groups in the drug.[23] It is a valuable characteristic feature for the identification of genuine drugs. As reported by Kedar *et al*. (2018) it is an implied and simple method for the determination of adulterants.[24]

## CONCLUSIONANDRECOMMENDATIONS

In the above-mentioned study, the pharmacognostic analyses of the whole *M. cerviana* (L.) Ser. plant was presented. The attained results include comprehensive research on pharmacognostic methods, including macro and micro-morphology, extraction, ash values, and fluorescence examination of whole, fragmented, and powdered drug materials, which have been beneficial in drug identification. Monitoring the quality of the product from collection through processing to the complete packaged product is necessary to ensure the safety and efficacy of herbal drugs. To identify and standardize this plant that can produce an effective drug for the organization, the information that emerged from the current study may be used as markers. Conclusively, it is suggested that *M. cerviana* whole plant are rich sources of minerals and nutritional supplements, and may be more therapeutically helpful as well.

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## DECLARATION

Not applicable.

## AUTHOR'S CONTRIBUTION

Nirupama Rani Dewangan: Investigation, Methodology, Writing - original draft, Writing - review and editing. Aditi Soni: Formal analysis and Investigation. Sonali Bhujabal: Formal analysis and Investigation. Bharti Ahirwar: Conceptualization, Methodology, Supervision.

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