

Original Research Article**PHARMACOGNOSTIC STUDIES AND PHYSICO-CHEMICAL PROFILE OF *MELISSA PARVIFLORA* BENTH.****Kundan Singh Bora*, Aruna Dubey**

Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences & Research, Balawala,
Dehradun, Uttarakhand – 248 161, India

ABSTRACT

Objectives: *Melissa parviflora* (family: Lamiaceae) has been traditionally used as a tranquillizer, relaxants, nervine and sleeping aids throughout the world. The plant is widely valued for its calming properties and has a tonic effect on the heart but no work has ever been carried out for standardizing this potential plant. The present study was designed to establish macroscopic and microscopic determinations, and physico-chemical parameters for *Melissa parviflora* stem.

Methods: WHO recommended methods and other standard procedures were employed for the establishment of pharmacognostic and physico-chemical parameters.

Results: TS of *M. parviflora* stem under the microscope showed an epidermal layer, cortex, continuous thin cylinder of vascular tissues, uniseriate medullary rays and wide pith. The xylem segments possess several wide annular vessels. Glandular sessile trichomes and multicellular covering trichomes were also appeared in the TS of stem. The powdered drug under the microscope showed fragments of parenchymatous tissue with intercellular spaces and strands of vascular bundles. Epidermis appeared double layered elongated cell wall in surface view with papilla present in between the cells. Abundant simple multicellular covering trichomes and occasionally sessile glandular trichomes were found. Abundant annular types of xylem vessels and prismatic calcium oxalate crystals were present in the powdered drug. Physico-chemical parameters help to identify authentic plant material and check adulteration. In the present study different ash values and extractive values were also determined.

Conclusion: The findings of the present study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of *M. parviflora*.

Keywords: *Melissa parviflora*, pharmacognostic standards, physico-chemical parameters, quality control

***Corresponding author: Dr. Kundan Singh Bora, Assoc Prof.** (Pharmacognosy & Natural Products)

Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences & Research Balawala, Dehradun, Uttarakhand – 248 161, India. Tel: +91-135-2686246; Fax: +91-135-2686231; E-mail: kundanresearch1381@gmail.com; Mob.: +91-8126238180

INTRODUCTION

Pharmacognosy has always been a translational or multidisciplinary science, most recently emphasized in the discussion of modern pharmacognosy, as described by Bohlin and co-workers [1]. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [2].

Melissa parviflora (Family - Lamiaceae), commonly called us gentle balm, is an aromatic perennial, pubescent or glabrate herb [3-5]. Traditionally, *M. parviflora* is used as tranquillizer, nervine relaxant and sleeping aids throughout the world. The plant is reported to relieve tension and stress reactions and is widely valued for its calming properties. It also used in migraine associated with

tension, neuralgia, anxiety-induced palpitation and insomnia [4-7]. The major phytoconstituents of the plant *M. parviflora* are alkaloids, tannins, saponins flavonoids and phenolic compounds.

The therapeutic activity of herbs is because of present of various phytoconstituents. The therapeutic effects of herbal products is inconsistent and varies because the chemical constituents vary; they depend on various factors and one of them is the source. In some plants toxic constituents are also present therefore it is essential to evaluate their quality, safety and efficacy. Correct identification and quality assurance of the starting material is, therefore an essential prerequisite to ensure reproducible quality of herbal medicines, which contributes to its safety and efficacy [8].

However, available literature revealed that there is no pharmacognostic study has been carried out so far on this plant; hence the present investigation was under taken to establish various pharmacognostical parameters and physico-chemical analysis studies of the plant *M. parviflora* stem.

MATERIALS AND METHODS

Plant materials

The plant material was purchased from Balkrishna & sons Dawakhana, Dehradun- 248001 (U.K.), India. The identity of the plant was confirmed through NISCAIR, New Delhi, India vide Ref. No. NISCAIR/RHMD/Consult/2014/2426-05.

Pharmacognostic Evaluation

Morphological/orgenoleptic evaluation

In this evaluation the plant material was evaluated by studying color, odor, taste, size, shape, special feature like touch, texture etc.

Microscopical evaluation [9]

In microscopic studies, transverse sections (TS) and powdered plant material were evaluated for internal structure and cells of plant like type of vascular bundles, epidermis, calcium oxalate crystals, trichomes etc.

TS of *M. parviflora* stem

Healthy and suitable pieces of *M. parviflora* stem were taken and soaked in water for 2 hr. Free hand transverse sections were cut with a razor blade. The clear sections were selected and decolorized & mounted on a clean glass slide and covered with cover slip using glycerin. Routine staining with safranin was done. Stem section were cut by free hand sectioning and numerous sections were examined microscopically [9].

Powder microscopy of *M. parviflora* stem

Powder microscopy was done by the Dutch process. Firstly 2 g of powder was taken from the sample and added 10% nitric acid solution (50 ml) and warm for 2 min. Then filtered the solution and residue was obtained, it was washed with hot water and then filtered. Again residue was taken and adds 10% sodium hydroxide solution (50 ml), warm for 2 min. Again filtered the solution, residue washed with hot water and again filtered. Finally taken residue for powder microscopy [9].

Physico-Chemical Evaluation [10,11]

Ash values

The determination of ash is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter. The determination of ash value is more applicable to powdered drugs. Following types of ash values are given below:

Determination of total ash

A total ash figure is useful to exclude drugs which have been coated with chalk, lime or calcium sulphate to improve their appearance, as is done with nutmegs and ginger. Weigh accurately 2 to 3g of the air dried crude drug in a tarred platinum or silica crucible and incinerate, gently at first gradually increase the temperature to $675\pm 25^{\circ}\text{C}$ until free from carbon, cool, and weigh. If a carbon free ash cannot be obtained in this way, extract the charred mass with hot water, collect the insoluble residue on an ash less filter paper, incinerate the residue and filter paper until the ash is white or nearly so, then add the filtrate, evaporate it to dryness and heat the whole to a temperature of $675\pm 25^{\circ}\text{C}$. If a carbon free ash cannot be obtained in this way, cool the crucible, add 15ml of ethanol (95%), break up the ash with a glass rod, burn off the ethanol and again heat the whole to a temperature $675\pm 25^{\circ}\text{C}$. Cool in a desiccator, weigh the ash and calculate the percentage of ash, with reference to the air dried crude drug.

The %w/w of total ash was calculated as follows –

$$\text{Total ash (\%w/w)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of acid insoluble ash

The determination of acid insoluble ash is more valuable than total ash. The majority of drugs contain calcium oxalate, sometimes in large amount and the amount is often very variable. Due to the variation the total ash is therefore useless to detect earthy matter, adherent to such drug. The addition of hydrochloric acid in total ash can remove all the variable constituents of the ash and gives constant results. Boil the ash (used total ash method) with 25 ml of 2M HCl for 5 min, collect the insoluble matter in a Goach crucible or on an ash less filter paper, wash with hot water, ignite, cool in a desiccator and weigh calculate the percentage of acid insoluble ash on the dried drug basis.

The % w/w of acid insoluble ash was calculated as follows –

$$\text{Acid insoluble ash (\%w/w)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of water soluble ash

Boil the ash (by total ash method) for 5 min with 25 ml of water, collect the insoluble matter in a Goach crucible or an ash less filter paper, wash with hot water, and ignite for 15min at a temperature not exceeding 450°C . Subtract the weight of the insoluble matter from the weight of ash: the difference in weight represents the water soluble ash. Calculate the percentage of water soluble ash on the dried basis.

The % w/w of water soluble ash was calculated as follows –

$$\text{Weight of water soluble ash (\%w/w)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of sulphated ash

Heat a silica or platinum crucible to redness for 10 min, allow to cool in a desiccator and weigh. Unless otherwise specified in the individual monograph, transfer to the crucible 1g of substance under examination and weigh the crucible and the contents accurately. Ignite, gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1ml of H_2SO_4 , heat gently until the white fumes are no longer evolved and ignite at $800\pm 25^{\circ}\text{C}$ until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible cool; add a few drops of H_2SO_4 and heat. Ignite as before, allow to cool and weigh. Repeat the operation until two successive weighings do not differ by more than 0.5 mg.

The % w/w of sulphated ash was calculated as follows –

$$\text{Sulphated ash (\%w/w)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Extractive values

These values are helpful in evaluating the constituents of crude drug, which cannot be determined by any other means. It also indicates the nature of the constituent present in the drug. There are different types of extractive values, such as ethanol soluble and water soluble. Extractive values were determined by following the procedures given in World Health Organization.

Ethanol soluble extractive

Macerate the 5 g of air dried drug, coarsely powdered with 100 ml of ethanol of the specified strength in a closed flask for 24 hrs, shaking frequently during the first 6 hrs and allowing stand for the 18 hrs. Thereafter, filter rapidly taking precautions against loss of ethanol, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, dry at 105°C & weigh. Calculate the percentage of ethanol soluble extractive with reference to the air dried drug.

The % w/w of alcohol soluble extractive value (EV) was calculated as follows –

$$\text{Alcohol soluble EV (\%w/w)} = \frac{\text{Weight of residue} \times 100}{\text{Volume of evaporated extract} \times \text{Weight of sample}}$$

Water soluble extractive

Add 5 g to 50 ml of water at 80°C in a stoppered flask. Shake well & allow to stand for 10 min, cool, add 2 g of Kieselguhr & filter. Transfer 5ml of the filtrate to a tarred evaporating dish, 7.5 cm in diameter, evaporate the solvent on a water bath, continue drying for 30min, finally dry in a steam oven for 2hrs & weigh the residue. Calculate the percentage of water soluble extractive with reference to the air dried drug.

The % w/w of water soluble extractive value (EV) was calculated as follows –

$$\text{Water soluble EV (\%w/w)} = \frac{\text{Weight of residue} \times 100}{\text{Volume of evaporated extract} \times \text{Weight of sample}}$$

Loss on drying

Weigh a glass stoppered shallow weighing bottle that has been dried under the same conditions to be employed in the determination. Transfer to the bottle the quantity of the sample specified in the individual monograph, cover it & accurately weigh the bottle & the contents. Distribute the sample as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10 mm. Dry the substance by placing the loaded bottle in the drying chamber as directed in the monograph, remove the stopper and leave it also in the chamber. Dry the sample to constant weight on for the specified time & at the temperature indicated in the monograph. Dry by one of the following procedures. After drying is completed, open the drying chamber, close the bottle promptly & allow it to cool to room temperature (where applicable) in a desiccator before weighing. Weigh the bottle and the contents.

RESULTS

Pharmacognostic Evaluation of *M. parviflora*

Results of macroscopic and microscopic characters of TS and powdered stems of *M. parviflora* are shown in Table 1 and Figures 1-6 respectively.

Table 1. Macroscopic characters of *M. parviflora* stem

Color	Brownish to greyish green
Odor	Slight
Taste	Bitter
Shape	Cylindrical
Size	5-6 cm in length



Figure 1. Stems of *M. parviflora*.

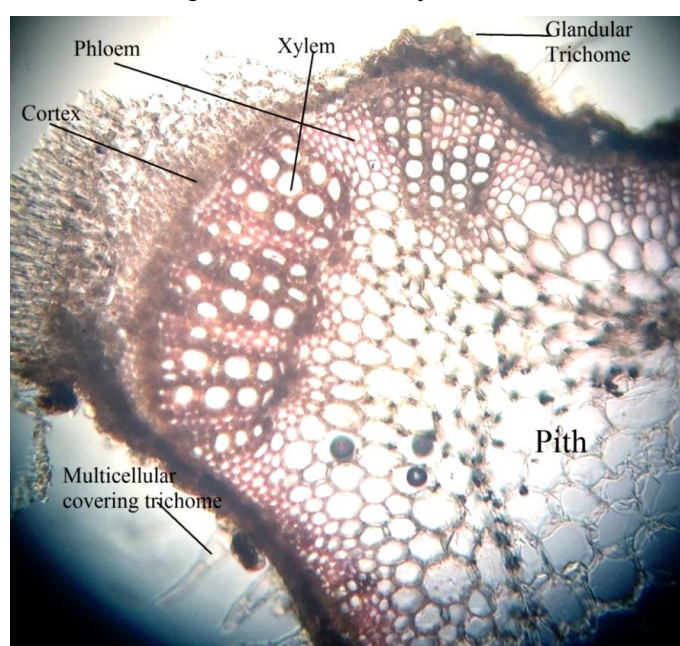


Figure 2. TS of *M. parviflora* Stem (10X).



Figure 3. Simple multicellular covering trichome (10X).

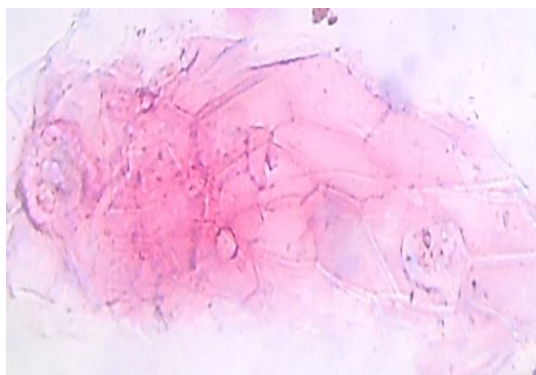


Figure 4. Epidermis in surface view showing Papilla (10X).

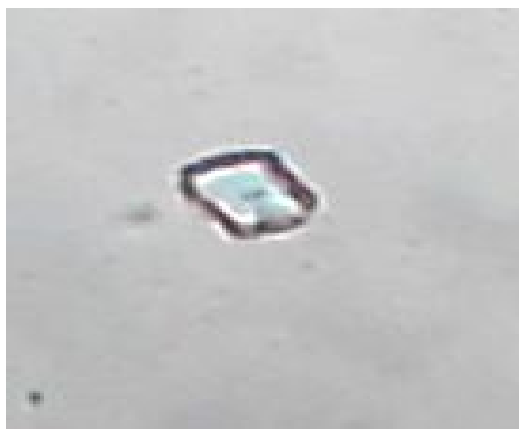


Figure 5. Calcium Oxalate Crystal (10X).



Figure 6. Annular Types of Vessels (10X).

Physico-chemical Parameters of *M. parviflora*

Results of physico-chemical parameters (moisture content, various ash value and extractive values) of *M. parviflora* are shown in Table 2 & 3. Each parameter evaluated in triplicate and mean results.

Table 2. Physico-chemical parameters of *M. parviflora*.

S. No.	Parameters	% (w/w)
1.	Ash value	
	➤ Total ash	19.2%
	➤ Acid insoluble ash	18.5%
	➤ Water soluble ash	16.5%
	➤ Sulphated ash	13.5%
2.	Loss on drying	8.45%

Table 3. Extractive values of *M. parviflora*.

S. No.	Extractive values	Methods	%(w/w)
1.	Water soluble	Hot maceration	51.2
2.	Ethanol soluble	Cold maceration	48.0
3.	Methanol soluble	Cold maceration	50.0
4.	Chloroform soluble	Cold maceration	14.4

DISCUSSION

The plant *Melissa parviflora* Benth. (Family: Lamiaceae) is an aromatic perennial, pubescent or glabrate herb, has been traditionally used as a tranquillizer, relaxants, nervine and sleeping aids throughout the world. An exhausted literature survey on *M. parviflora* revealed that sporadic phytochemical, pharmacognostic and pharmacological reports are available on this plant. As *M. parviflora* has been used traditionally for the treatment of various ailments, this plant holds great potential for in depth phytochemical, pharmacognostic and pharmacological evaluation. The present study is emphasized on macroscopic and microscopic studies, and physico-chemical analysis for *M. parviflora*.

The plant material was evaluated morphologically/organoleptically by studying color, odor, taste, size, shape, special feature like touch, texture etc. In this study, TS and powdered plant material was evaluated for internal structure and cells of plant like type of vascular bundles, epidermis, calcium oxalate crystals, trichomes etc. Such descriptions form the basis for the identification of drugs.

In the current study the color of *M. parviflora* stems observed brownish to grayish green, odor was slight and taste was bitter (Table 1). TS of *M. parviflora* stem under the microscope showed an epidermal layer, cortex, continuous thin cylinder of vascular tissues (xylem and phloem), uniseriate medullary rays and wide pith. The xylem segments posses several wide annular vessels. Glandular sessile trichomes and multicellular covering trichomes were also appeared in the TS of stem (Figure 2).

The powdered drug under the microscope showed fragments of parenchymatous tissue with intercellular spaces and strands of vascular bundles. Epidermis appeared double layered elongated cell wall in surface view with papilla present in between the cells. Abundant simple multicellular covering trichomes were found. Occasional sessile glandular trichomes with large multicellular spherical head, encircled by thick cuticle were also appeared in the powdered drug. Abundant annular types of xylem vessels and prismatic calcium oxalate crystals were present in the powdered drug (Figures 3-6).

Physico-chemical parameters help to identify authentic plant material and check adulteration. The extracts obtained by exhausting drugs are indicative of approximate measures of the chemical constituents. Taking into consideration the diversity in chemical nature and properties content of drugs, various solvent are used for determination of extractives. In the present study, ethanol and water were used to evaluate the extractable constituents in the stem of *M. parviflora*. Ethanol, chloroform, methanol and water soluble extractive value were estimated 48.0%, 50.0, 14.4% and 51.2% respectively (Table 2). Presence of excess moisture in plant acts as an adulterant and may lead to deterioration of plant material and its phytoconstituents by promoting microbial growth. So it was essential to study the

moisture content, helpful for determination of moisture present in plant and also controlled to maintain quality. Moisture content (LOD) of air dried stem of *M. parviflora* was found to be 8.45%. The determination of ash is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter. The total ash was estimated 19.2%. The addition of dilute hydrochloric acid in total ash can removes all the variable constituents of the ash. The acid insoluble ash was estimated 18.5%. The water soluble ash is used to detect the presence of material exhausted by water. The water soluble ash was found 16.5%. The determination of sulphated ash is widely used to control the extent of contamination by non volatile inorganic impurities in organic substances. The sulphated ash was found to be 13.5% (Table 3).

The micro-morphological investigations have given useful pharmacognostic elements for the correct identification of the drugs both in scraped and powdered forms and this is of great interest for quality control in basic research and drug production, especially for imported items and for raw material sold by traditional herbalists.

CONCLUSION

In the present study all the pharmacognostic and physico-chemical parameters which are being reported for the first time may be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs. Additionally, the present findings could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant *M. parviflora*.

REFERENCES

1. Bohlin L, Goransson U, Backlund A. Modern pharmacognosy: connecting biology and chemistry. Pure Appl Chem. 2007; 79: 763–74.
2. Quality Control Methods for Medicinal Plant Materials (An authorized publication of World Health Organisation, Geneva). New Delhi: A.I.T.B.S. Publishers & Distributors (Regd.); 2002.
3. Chopra RN, Chopra IC. Indigenous drugs of India: Academic publishers; 1958.
4. Kirtikar KR, Basu BD, Blatter E. Indian medicinal plants, 1975.
5. Scartezini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. J ethnopharmacol. 2000; 71: 23-43.
6. Kennedy DO, Little W, Scholey AB. Attenuation of laboratory-induced stress in humans after acute administration of *Melissa officinalis* (Lemon Balm). Psychosomat med. 2004; 66: 607-613.
7. Moradkhani H, Sargsyan E, Bibak H. *Melissa officinalis* L., a valuable medicine plant: J Med Plants Res. 2010; 4(25): 2753-2759.
8. Joshi K, Chavan P, Warude D, Patwardhan B. Molecular markers in herbal drug technology. Curr Sci. 2004; 87: 159–65.
9. Wallis T.E , Textbook of Pharmacognosy, Fifth edition, CBS Publishers & Distributors Pvt. Ltd. ,New Delhi,2005.
10. WHO (World Health Organization). Quality control methods for medicinal plant materials. World Health Organization, Geneva, 1998.
11. Indian Pharmacopoeia. IP, Vol I. Ministry of Health and Family Welfare, Government of India, Published by the Indian Pharmacopoeia Commission, Ghaziabad, India, 2010; 82-83 & 139 & 201.