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# Pharmacognostical and physicochemical investigation of the leaf of *Calamintha officinalis* Moench

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## ABSTRACT

**Objective:** To study salient diagnostic pharmacognostical characters of the leaves of *Calamintha officinalis* Moench along with their phytochemical analysis, physicochemical parameters, fluorescence analysis of leaf powder and HPTLC study. **Methods:** Fresh leaf and dried powder sample of leaf were studied macro-morphologically and microscopically. Preliminary phytochemical investigation of leaf for the standardization was performed. HPTLC analysis of ethanolic extract was performed using solvent system benzene: methanol in the ratio (9:1). **Result:** Epidermis of leaf was found to be formed of a single row of cells, those of the upper epidermis being larger than those of the lower epidermis and uniseriate non-glandular and glandular trichomes were observed. Mesophyll was heterogeneous and asymmetric; two types of parenchyma was clearly differentiated; palisade and spongy. The upper one (palisade parenchyma) was formed of a single row of elongated cells. Abundant chloroplasts were observed. The lower, spongy parenchyma was formed of irregularly shaped cells with large intercellular spaces. Vascular tissue was found at the level of the spongy parenchyma. Prismatic shape of calcium oxalate crystals has been found. The preliminary phytochemical screening shows the presence of carbohydrate, flavonoids, steroid and triterpenes. HPTLC analysis of ethanolic extract showed eight peaks confirming the presence of eight compounds in the ethanolic extract of the leaves. **Conclusions:** The pharmacognostical and phytochemical parameters are major reliable and inexpensive criteria for confirmation of the crude drugs. The present work therefore attempts to report various necessary standards for the leaf of *Calamintha officinalis* Moench.

## 1. Introduction

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1].

*Calamintha officinalis* (Moench) is a plant species belonging to the family Lamiaceae, similar to the common mints not only regarding its morphology but also in regard to its scent characteristics. This has led to it being used popularly as a substitute for the official mints in various beverages. Although similar pharmacological properties between these species could be speculated, in so far as we know the literature cites only chemical data regarding C.

*officinalis* and other related species [2]. A genus of aromatic herb or shrubs, native to north temperate regions, a few species extending into the tropical mountains [3].

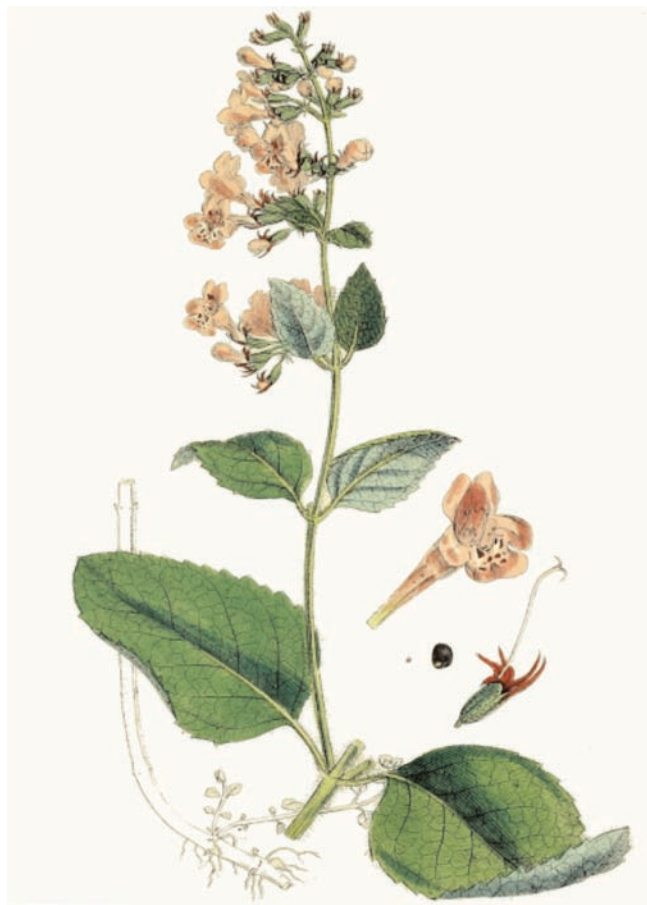
It is aromatic, diaphoretic and expectorant. The infusion is good for fevers, flatulent colic and debility of the stomach, and is also used for depression, insomnia and painful menstruation. It has expectorant action and is good for colds and respiratory infections [4].

## 2. Materials and methods

### 2.1 Material

*Calamintha officinalis* Linn. was collected from hilly region of Uttaranchal state of India in May and June 2009 and air-dried at 40 °C. The plant sample was identified and authenticated by taxonomist Dr. Gaurav Nigam, B.U. (India). A voucher specimen (No. BIT/6574) has been deposited in the herbarium of BIT Mesra Ranchi, India. (Fig.–1)

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**Fig .1** *Calamintha officinalis* Moench.

## 2.2 Morphology (Macroscopy)

The freshly leaves of the plant were collected and investigated in different organoleptic features by repeated observations. Morphological studies, such as shape, size, apex, surface, base, margin, venation, taste and odour of leaves, were carried out. [5, 6, 7]

## 2.3 Microscopy

Microscopic studies were carried out by preparing of thin hand section of leaf. The sections were cleared with alcohol and stained as per the protocol [8, 9, 10, and 11].

## 2.4 Quantitative microscopy

In this microscopy leaf constant parameters like stomatal no, stomatal index, vein termination no, vein islet no, palisade ratio were determined.

## 2.5 Powder microscopy

The dried leaf was powdered and studied under microscope. Different staining reagents (such as iodine for detection of starch grains and phloroglucinol for detection of lignified components) were used. A little quantity of leaf powder was taken onto a microscopic slide, 1–2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated

hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. The presence of starch grain and calcium oxalate crystal was detected by the formation of blue colour on addition of 2–3 drops of 0.01 M iodine solution. The characteristic structures and cell components were observed and their photographs were taken using photomicrography. [12]

## 2.5 Physicochemical parameters

Extractive value determine the amount of active constituents extracted with solvent from given amount of medicinal plant material. In leaves the alcohol soluble extractive value was greater than other. (Table–3). The ash remaining following ignition of medicinal plant materials was determined by three different methods which measure total ash, acid–insoluble ash and water–soluble ash. The total ash method is designed to measure the total amount of material remaining after ignition. This includes both “physiological ash”, which is derived from the plant tissue itself, and “non–physiological” ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. Acid–insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water–soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water. [13 and 14]

## 2.7 Fluorescence analysis

Many drugs fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents. [15, 16]

## 2.8 Preliminary phytochemical evaluation

The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, steroid, alkaloids and glycosides [17, 18, 19, 20 and 21].

## 2.9 HPTLC of oil *Calamintha officinalis*

HPTLC: It was carried out by National Botanical Research Institute, Lucknow, Using solvent system Benzene: Ethyl acetate in the ratio 95: 5.[22]

## 3. Result

### 3.1 Morphology (Macroscopy)

The leaves are opposite, 20 – 40 x 10 – 35 mm, shaped ovate to orbicular – ovate, with or slightly crenate margin,

with between 5 and 8 teeth on each side. Venation of leaf is reticulate, Color of the leaves is blackish green, and appearance, odour, and taste of leaves is rough and scabrous, aromatic, acrid respectively. (Fig.1, 2 &3)

Figure: Measurement of mature leaf length & width in cm.

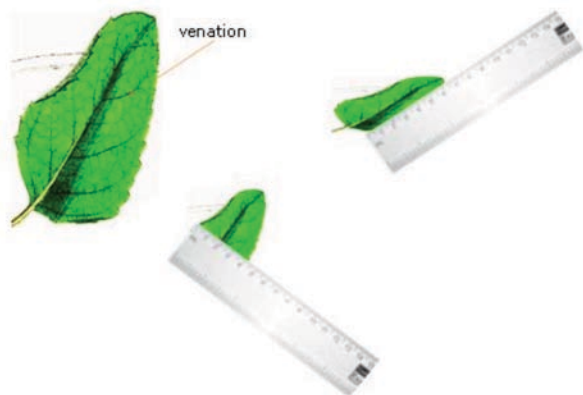


Fig 2.



Fig.3

3.2 Microscopy

The leaves of *Calamintha officinalis* were boiled with saturated chloral hydrate solution for microscopical observation. Leaves were sectioned using microtome and free hand sectioning. Numerous temporary and permanent mount of microscopical section of leaf specimen were made and examined. (Fig.4 A and B) Epidermis formed of a single row of cells, those of the upper epidermis being larger than those of the lower epidermis. Pluricellular uniseriate non-glandular trichomes and glandular trichomes were observed. Mesophyll heterogeneous and asymmetric; two types of parenchyma can be clearly differentiated palisade and spongy. The upper one (palisade parenchyma) is formed of a single row of elongated cells. Abundant chloroplasts were observed. The lower, spongy parenchyma is formed of irregularly shaped cells with large intercellular spaces. Vascular tissue found at the level of the spongy parenchyma. Pericycle ring formed of lignified fibers in layers one or two

cells thick; they are especially characteristic at the four corners where the vascular bundles are more developed.

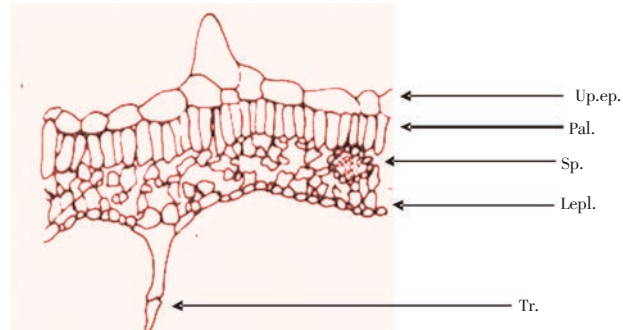


Fig.4.A. Leaf in trans-section

U.ep; upper epidermis. Pal; palisade parenchyma. Sp; spongy parenchyma. L.ep; lower epidermis. Cot; collenchyma. Tr; trichome, Xyl; xylem. Phl; phloem.

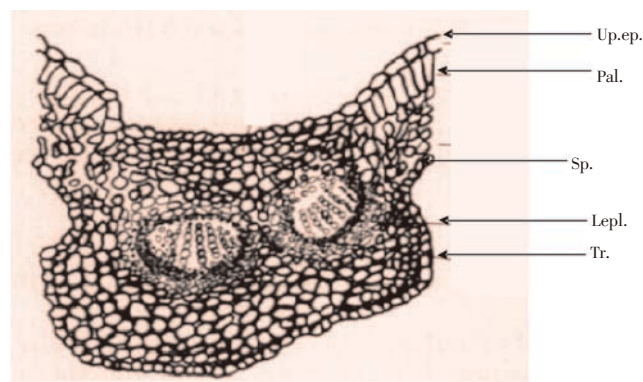


Fig.4B. A) leaf, B) leaf at the level of the median vein.

U.ep; upper epidermis. Pal; palisade parenchyma. Sp; spongy parenchyma. L.ep; lower epidermis. Tr; trichome, Xyl; xylem. Phl; phloem.

3.4 Quantitative microscopy

Parameters like stomatal no, stomatal index, vein termination no, vein islet no. palisade ratio are shown in table 1.

Table1

Quantitative Microscopy: Leaf constant of Calmintha officinalis

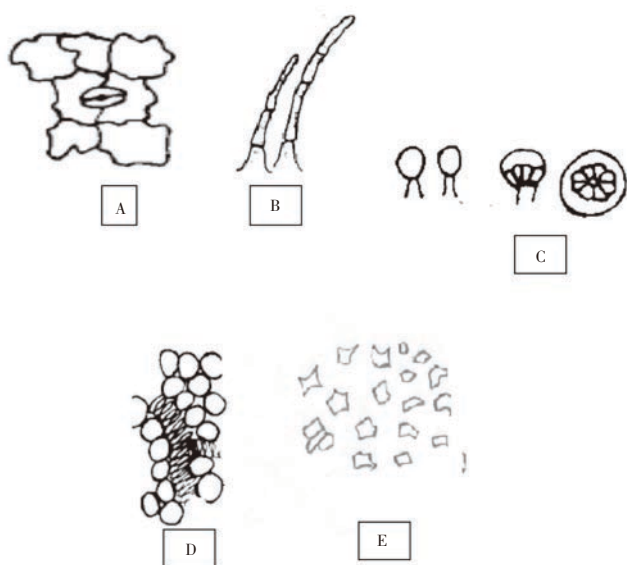
Parameter	Range	Mean
Palisade ratio	6.00–8.00	6.85 + 0.3841
Stomata number Upper surface	8.00–10.00	8.80 + 0.1864
Stomata number Lower surface	5.00–9.00	6.95 + 0.2348
Stomata index Upper surface	17.02–23.26	20.2785 + 0.4150
Stomata index Lower surface	13.95–17.65	16.0275 + 0.2617
Vein islet number	3.00–4.50	3.35 + 0.2915
Veinlet termination number	2.75–3.00	2.85 + 0.0612

3.5 Powder microscopy

The powder microscopy of leaf was done and the data mentioned below is correlated

a) Stomata: Diacytic type of stomata with two subsidiary cells around it. b) Trichomes: glandular, non glandular trichome uniseriate, multi-cellular, 3–4 celled with pointed tips

slightly bent at apex. c) Spongy parenchyma. e) Calcium oxalate crystals are of prism type (Fig.-5).



**Fig.5.** Powder microscopy of leaf

### 3.6 Physicochemical parameters

Physicochemical parameters such as extractive value, ash value and fluorescence analysis were observed. Extractive Value are shown in the table 2. and Ash value is as follow In this whole process total ash, Acid – insoluble ash and Water – soluble ash values are  $10.3167 \pm 0.2565$ ,  $5.1667 \pm 0.1351$ , and  $2.1667 \pm 0.0630$  were found respectively.

**Table 2**

Extractive value with different solvents

Parameter	Observed value. Mean (% w/w)
Ethyl acetate soluble extractive value	$0.1583 \pm 0.0022$
Ether soluble extractive value	$0.1673 \pm 0.0012$
Chloroform soluble extractive value	$0.4783 \pm 0.0032$
Alcohol soluble extractive value	$0.5873 \pm 0.0027$
Water soluble extractive	$0.3440 \pm 0.0017$

### 3.7 Fluorescence analysis

When powder is examined as such in UV light it appears green color, then powder of *C. officinalis* is treated with Concentrated HCL amber green colour were observed while in Concentrated  $\text{HNO}_3$  it seems to be yellow in colour, whereas with Concentrated  $\text{H}_2\text{SO}_4$  and Glacial acetic acid colour observed were brownish black brownish green respectively.

### 3.8 Preliminary phytochemical evaluation

Preliminary phytochemical screening revealed the presence of Alkaloids, Flavonoids, and Triterpenes as shown in table 3.

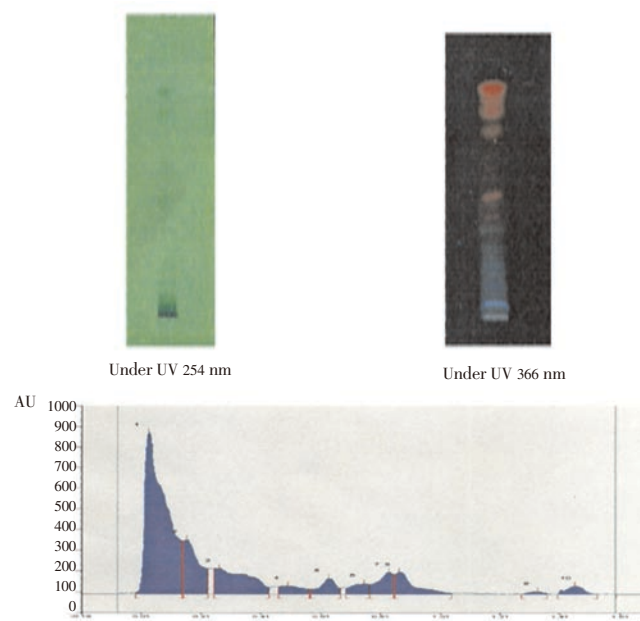
**Table 3**

Preliminary phytochemical screening of leaves of *C. Officinalis*

Phytoconstituents	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	–	+	–	–
Carbohydrates	–	–	–	–
Flavonoids	–	–	++	++
Saponins	–	–	–	+
Cardiac glycosides	–	–	–	–
Anthraquinones	–	–	–	–
Tannins	–	–	–	–
Proteins	–	–	–	–
Steroids	–	–	–	–
Triterpenes	+	++	++	++

### 3.8 HPTLC of *Calamintha officinalis* oil.

HPTLC of oil show 10 peaks confirming that 10 compound may be present in the oil of the *Calamintha officinalis* Moench. Fig.6.



**Fig. 6.** HPTLC Chromatogram of oil of *Calamintha officinalis*.

## 4. Discussion

The present study establishes macro and microscopic characteristics, physicochemical values, fluorescence analysis of powder and phytochemical screening of *Calamintha officinalis*. In recent years, there has been a rapid increase in the standardization of selected medicinal plants with significant potential as therapeutics due to their specific healing properties and potential actions. In this view, pharmacognostical standardization of *Calamintha officinalis* is necessary. As the most cost effective aid in identification of a medicinal herb, microscopic characteristics have been the mainstay of

classical pharmacognosy and remain a vital component of the modern monograph. The salient pharmacognostical characteristic of the leaves of *Calamintha officinalis* are, as seen in the transectional views of the leaves; two epidermal layers and the hypodermal regions of both surfaces are collenchymatous, the palisade parenchyma is interrupted, and the central zone contains the phloem and xylem, disposed bicollaterally. Pericycle ring formed of lignified fibers they are especially characteristic at the four corners where the vascular bundles are more developed. Stomata of leaves are diacytic in nature; trichomes are glandular, non glandular trichome uniseriate, multi-cellular, 3–4 celled with pointed tips, parenchyma is spongy, and calcium oxalate crystals are of prism type.

The extractive values (w/w) of *Calamintha officinalis* leaves in chloroform, ethanol and water were about 0.4783, 0.5873 and 0.3440 respectively. Phenolic compounds and triterpenoids, were present in the extracts.

## Conclusion

After the present investigation it can be concluded that the Pharmacognostical studies of the leaves from *Calamintha officinalis* yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant materials for future studies. These parameters also will serve as standard data for quality control studies of pharmaceutical preparations from the leaves of *Calamintha officinalis*.

## Conflict of interest statement

We declare that we have no conflict of interest.

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