

Research Article**Pharmacognostic and phytochemical investigations of aerial parts of *Nepeta cataria* Linn**Hayat M. Mukhtar¹, Gurvinder Pal Singh^{2*}¹Amritsar Pharmacy College, Amritsar, 143001, Punjab, India²I. K. G Punjab Technical University, Jalandhar, 144001, Punjab, India

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Abstract

Objective: The main objective of the present study pertains to pharmacognostic and phytochemical characteristics of aerial parts of *Nepeta cataria* Linn belonging to family Lamiaceae. This investigation has been done according to guidelines of the World health organization and Ayurvedic Pharmacopeia of India. **Materials and Methods:** The powdered aerial parts of *Nepeta cataria* were used to prepare the extracts of Petroleum ether, chloroform, methanol and aqueous extracts with the help of soxhlet apparatus. After that extracts were concentrated using rotary vacuum evaporator. Morphological evaluation, microscopic evaluation, powder analysis, physiochemical evaluation, preliminary phytochemical screening and TLC profiling of *Nepeta cataria* was performed. The petroleum ether, chloroform, methanol, and water extracts were used to perform the preliminary phytochemical screening. **Results:** The macroscopic studies revealed that leaves are thin with fairly prominent midrib and lateral veins. The presence of epidermis, palisade cells, trichomes (glandular and non-glandular), caryophyllaceous stomata, spongy parenchyma, collenchyma, and various vascular bundles was shown when a transverse section of the leaf was taken. There are two wide air chambers on either side of vascular bundles. Stem showed the presence of wedge-shaped vascular bundles. The bundles have inner thick segments of xylem and outer thin continuous layer of phloem. The parameters like ash value (total ash, water soluble and acid insoluble ash), extractive value (water-soluble and alcohol-soluble), moisture content were as 14.85, 7.13, 2.37, 32.8, 5.76, 7.32%, respectively. The methanolic extract showed the presence of flavonoids, carbohydrates, glycosides and tannins. **Conclusion:** Pharmacognostic and phytochemical investigation of powdered aerial parts of *Nepeta cataria* describing its morphological evaluation, microscopical evaluation, powder analysis, physiochemical evaluation, preliminary phytochemical screening, and TLC profiling has been studied in detail so as to develop a reference for academic and commercial purpose. Further, it can be used for the standardization and pharmacopoeial parameters development.

Keywords: *Nepeta cataria*, pharmacognostic studies, phytochemical analysis, pharmacological guidelines

Introduction

Nepeta cataria L. (Catnip) is a member of the mint family and belongs to the botanical family called Lamiaceae (Labiatae), a perennial herb native of southeast Europe, southwest Asia, and western temperate Himalaya from Dalhousie to Kashmir up to an altitude of 1500 m (Sarkar et al., 1995). This plant can obtain a height of one meter, has pubescent leaves and spikelike

inflorescent with purple-spotted flower (Edewor et al., 2011). In folk medicine, this plant has been used for antispasmodic, carminative, stimulant and tonic properties. Moreover, traditionally, the tea made of its leaves is known as a sedative and also is used to relieve gastrointestinal and respiratory disorders such as colic, diarrhea, cough, asthma, and bronchitis (Zomorodian et al., 2012). This plant is reported various biological activities include antibacterial (Edewor et al., 2011), behavioral effect (Polosomboon et al., 2008), anti-diabetic effects (Aly et al., 2010), spasmolytic and bronchodilatory effect (Gilani et al., 2009), anti-nociceptive and anti-inflammatory effects (Ricci et al., 2010). Various compounds have been identified in the

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essential oil of *Nepeta cataria*, the main constituents so far identified, include β -caryophyllene oxide, 1,8 cineol, citronellol, geraniol, elemol, nerol (Mortuza-Semmani and Saeedi, 2004; Schultz et al., 2004; Sajjadi, 2005). Also, ursolic acid, β -sitosterol, campesterol, α -amyrin, β -amyrin, and sitosterol, β -glucopyranoside have been reported previously (Miceli et al., 2005). In addition, the plant also contains nepetalactone and alkaloids such as actinidine and iridomyecine (Kalputzakis et al., 2005). As is the case with most of the traditional drugs no work has been carried out for standardizing this potentially useful plant. Thus the present investigations were planned to establish pharmacognostic standards for *Nepeta cataria* thereby facilitating authentication of the correct plant material.

Materials and methods

Collection and authentication of plant material

Dried aerial parts of *Nepeta cataria* were procured from the JK Medicinal plants introduction center, a unit of Govt of Jammu & Kashmir in September 2016 and dried in the shade. The identity of the plant was confirmed through Head Dr. Sheikh Gulzaar at JK medicinal plant introduction center vide seller no. JKMPIC/R&D 20119-AS dated 22-09-2016.

Morphological evaluation

Fresh aerial parts of *Nepeta cataria* were collected and different organoleptic features viz. shape, size, color, odor, taste were observed for morphology. These parameters were evaluated as per WHO guidelines.

Microscopic evaluation

Microscopic examination was undertaken with a thin section of fresh leaves and stems of *Nepeta cataria*. The tissues were described with the help of photomicrograph. The Nikon lab photo 2 microscopic unit was used to get photographs. The Bright fields were used for normal observations (Wallis et al., 1985).

For staining of sections, toluidine blue was used according to methods published by (O'Brien et al., 1964). As toluidine blue is a polychromatic stain. The staining results were remarkably good. The dye rendered pink color to the cellulose walls, blue to lignified cells, dark green to suberin, violet to mucilage, blue to protein bodies etc.

Quantitative microscopy

The determination of leaf constants viz., stomatal index, vein islet number, and veinlet termination number were made by using camera lucida and stage micrometer following the process which has been elaborated by Evans. The binocular photomicroscopic apparatus (LEICA, 'Italy') was used for taking photomicrograph attached with Nikon digital camera.

Powder microscopy

The shade dried aerial parts of the plant were powdered and passed through 100# sieve. A small quantity of dried powder was placed on the glass slide. It was cleared from chlorophyll by heating with chloral hydrate solution and was suspended in 50% v/v glycerol in water. The powder was observed under the microscope to study different cell content viz. parenchyma cell, xylem, trichomes (glandular and non-glandular), annular, spiral element and recorded with the help of a digital camera (Akbar et al., 2014).

Physiochemical evaluation

For physiochemical evaluation, foreign organic matter, ash value (total ash, water soluble and acid insoluble ash), extractive value (water-soluble and alcohol-soluble), moisture content etc. were determined three times and average values were calculated (Kokate et al., 2003).

Foreign organic matter

The determination of Foreign organic matter of *Nepeta cataria* was made by spreading 100g aerial parts on clear smooth background according to the standard procedure given in the Indian Pharmacopoeia.

Moisture content

The determination of moisture content of *Nepeta cataria* was done by the following procedure given in the Indian Pharmacopoeia.

Determination of total ash value

Powdered plant material (2 g) was taken in a tared silica crucible and then incinerated at a temperature not exceeding 450°C until free from carbon. The resultant ash was cooled and weighed. The reference to the air-dried drug was considered while calculating the percentage of ash.

Acid-insoluble ash value

Accurately weighed powder (5 g) of the plant material was taken and macerated with 100 ml of 95% alcohol for 24 h. During the first 6 hours, contents were continuously shaken and put to remain for 18 hours. After 24 hours, the extract was made and 25 ml of the filtrate was evaporated. The extract was dried at 105°C to a constant weight.

Determination of water-soluble extractive value

The determination of water-soluble extractive value was obtained by using the procedure described for alcohol-soluble extractive.

Preparation of extract

Soxhlet apparatus was used for the extraction of powdered aerial parts of the plant (1kg) in increasing order of polarity of the solvents like petroleum ether, chloroform, methanol,

and water. These solvents were used for exhaustive extraction. Buchi 461 rotary vacuum evaporator was employed for the recovery of the solvents from four extracts. The dried extracts were kept in vacuum desiccators to avoid moisture. Dried extracts were used for phytochemical screening and establishment of thin layer chromatography (TLC) profile.

Preliminary phytochemical screening of extracts

Petroleum ether, chloroform, methanol and aqueous extract of *Nepeta cataria* were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as carbohydrates, glycoside, proteins, saponin, tannin, sterol, alkaloids, and flavonoids. This examination was done using the standard procedure.

Results

Morphological evaluation

Leaves are thin with fairly prominent midrib and lateral veins. Leaves are narrow to broadly deltoid, ovate and pale green in color, petiole as long as the blade. The stem is sturdy, branched and light green in color.

Microscopical evaluation

From the microscopical study, it was observed that leaf contain epidermis, palisade cells, trichomes, (glandular and non-glandular), caryophyllaceous stomata, spongy parenchyma, collenchyma, and various vascular bundles. There are two wide air chambers on either side of vascular bundles. Stem contains the presence of epidermis, ground parenchyma, cortex, pith, phloem, wedge-shaped vascular bundles, trichomes. The bundles have inner thick segments of xylem and outer thin continuous layer of phloem.

Quantitative microscopy

Various leaf constants viz. palisade ratio, stomatal number, stomatal index, vein- islet number, veinlet termination number were determined as per standard methods and recorded in table 1.

Powder microscopy

Powdered plant material showed the presence of fragments of parenchyma cell, xylem element, epidermal trichome, non-glandular trichome, annular and spiral elements.

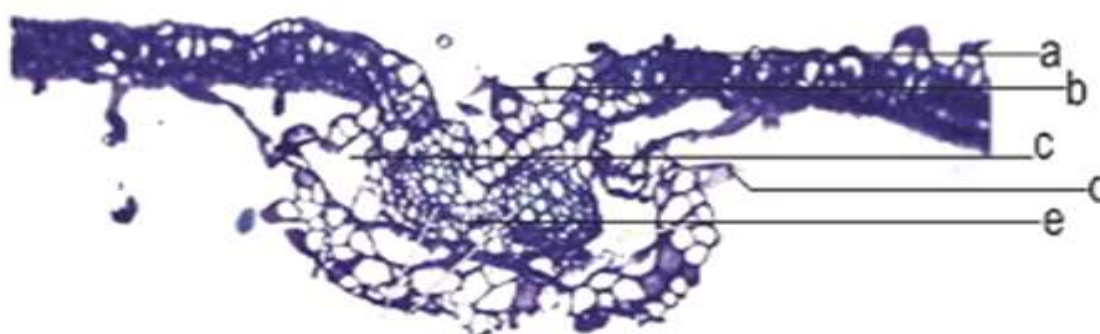


Figure 1. Transverse section leaf of *Nepeta cataria* (10x): (a) Lamina (b) Adaxial cavity (c) Air chamber (d) Trichome (e) Vascular bundle

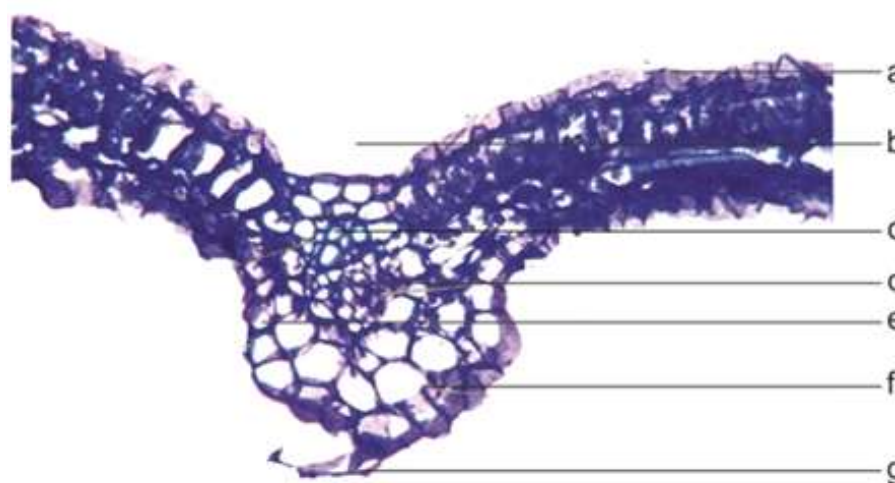


Figure 2. Transverse section leaf of *Nepeta cataria* (40x): (a) Lamina (b) Adaxial cavity (c) Xylem (d) Phloem (e) Epidermis (f) Ground parenchyma (g) Trichome

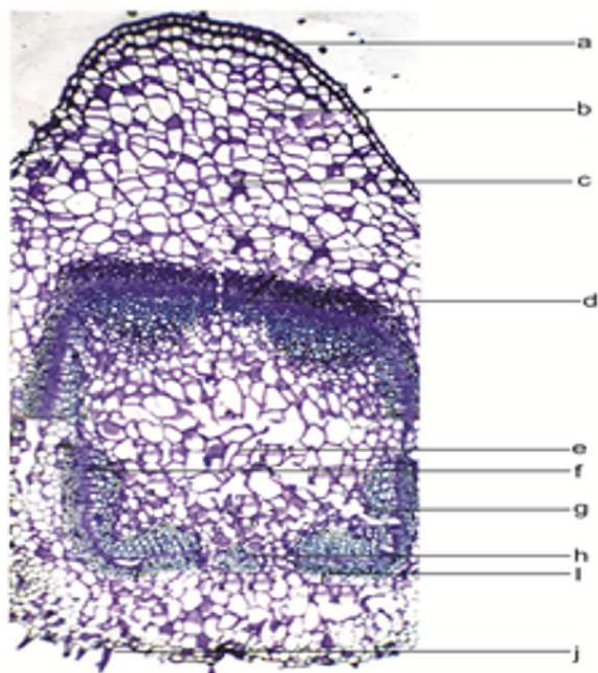


Figure 3. Transverse section of the stem of *Nepeta cataria* (40x): (a) Epidermis, (b) Ground parenchyma, (c) Cortex, (d) Phloem, (e) Pith, (f) Xylem, (g) Lateral vascular strand, (h) Median vascular strand, (i) End part of vascular strand, (j) Trichome

Physiochemical evaluation

Results of various physiochemical parameter viz ash, foreign organic matter, moisture content, ash value, extractive values are summarized in table 2.

Table 1. Mean values of stomatal number, stomatal index, vein-islet number and veinlet termination number of *Nepeta*

Leaf constant parameters		Range
Palisade Ratio		2
Stomatal number	Upper surface	3 mm/sq
	Lower surface	1 mm/sq
Stomatal index		33
Vein-islet number		5-7 mm/sq
Veinlet termination number		2-3 mm/sq

Table 2. Physiochemical evaluation of aerial parts of *Nepeta cataria*

S. No	Parameters	Observations(% w/w)
1	Foreign organic matter	0.26
2	Moisture content	7.32
3	Total ash	14.85
4	Water-soluble ash	7.13
5	Acid-insoluble ash	2.37
7	Water-soluble extractive values	32.88
8	Alcohol-soluble extractive value	5.76

Phytochemical screening

The various extracts were subjected to preliminary phytochemical screening. It has shown the presence of carbohydrates, glycosides, proteins, saponin, phytosterol, alkaloids, flavonoids, phenolic compounds and tannins. The results of all four extracts were recorded in table 3.

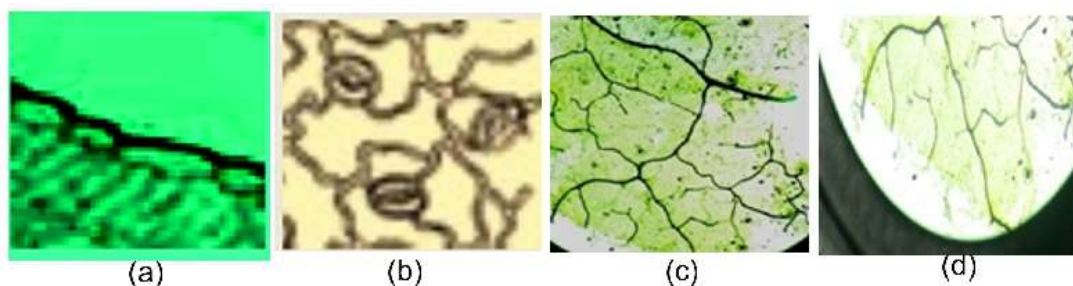


Figure 4. Quantitative microscopy: (a) Palisade cells, (b) Stomatal cell, (c) Veinlets, (d) Veinlet terminations

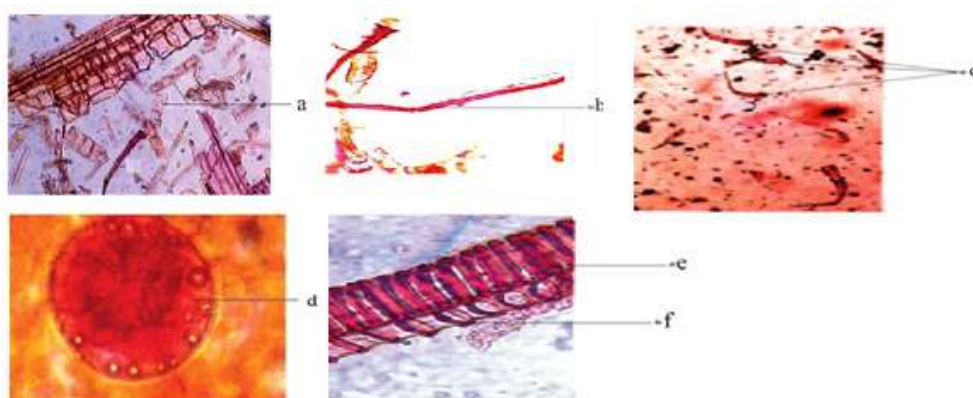


Figure 5. Powder microscopy: (a) Parenchyma cell, (b) Xylem element, (c) Epidermal trichome, (d) Non-glandular trichome, (e) Spiral element, (f) Annular element

Table 3. Phytochemical screening aerial parts of *Nepeta cataria*

Constituents	Tests	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Carbohydrate	Molish's test	+	-	+	+
	Fehling's test	+	-	+	+
Glycoside	Legal's test	+	-	+	-
	Keller killanis test	+	-	+	-
Proteins and amino acids	Million's test	-	-	-	+
	Ninhydrin test	-	-	-	+
Saponins	Foam test	-	-	-	+
Tannins	FeCl ₃ test	-	-	+	+
	Lead acetate test	-	-	+	+
Phytosterol	Salkowski test	-	+	-	-
	Liebermannburchard test	-	+	-	-
Alkaloids	Dragendroff's test	-	+	-	-
	Mayer's test	-	+	-	-
	Hager's test	-	+	-	-
Flavonoids	Aq. NaOH test	-	-	+	+
	Conc. H ₂ SO ₄ test	-	-	+	+
	Shinoda's test	-	-	+	+

Conclusion

Newsday's, counterfeit herbal materials are often found in the market, that's the reason why standardization of herbal medicines is necessary so as to assure the quality of the drug. Through this study, the identity, purity, safety as well as the quality of the drug will be confirmed for sake of mankind. The important detecting features that might be useful in determining genuineness and identifying adulteration of the crude drug are observed through this analysis. Consequently, the conclusion obtained from this analysis would be helpful in identification as well as standardization of this plant. Therefore this research, therefore, will prove to be useful for the manufacturer to determine the purity of raw material and to establish the development of pharmacopeial parameters for identification and authentication of *Nepeta cataria*.

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Conflicts of interest

Authors declare no conflict of interest.

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