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Phytochemical analysis of methanolic and chloroform extract of *Nepeta leucophylla* from Uttarakhand, India

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Abstract
This paper summarizes the presence of phytochemicals in methanolic and chloroform extract of the flower of *Nepeta leucophylla*. For this, solvents such as methanol, water, chloroform were used to extract a wide number of chemicals. The presence of the highest percentage of carbohydrates, sugars and amino acids was identified in chloroform and methanol extracts. The results of this study provide a good resource for the development of food, agriculture, and pharmaceutical products.

Keywords: Methanol extract, chloroform, lamiaceae, *Nepeta*

1. Introduction
The state of Uttarakhand is a part of northwestern Himalaya and still maintains a dense vegetation cover (65%). The maximum species of medicinal plants have been reported from Uttarakhand [1, 2] followed by Sikkim and North Bengal. The trans-Himalaya, in contrast, sustains about 337 species of medicinal plants, which is low compared to other areas of the Himalayas due to the distinct geography and ecological marginal ecological conditions. The Himalayan range in the northern part of India harbors a great diversity of medicinal plants of the approximately 8000 species of angiosperms, 44 species of gymnosperms, and 600 species of pteridophytes that have been reported in the Indian Himalaya, 1748 species are known for their medicinal properties [3-5]. The genus *Nepeta*, one of the largest genera of the Lamiaceae family, belongs to the subfamily Nepetoideae and tribe Mentheae. It comprises ca. 300 herbaceous perennial, rarely annual species, most of which are spread out over the larger part of central and southern Europe, the Near East, central and southern Asia, and some areas of Africa. The plants of this genus have beautiful flowers with a pleasant odor. The pollen grains are hexacolpate [6]. *Nepeta* is the second largest genus of the Indian labiates, with 41 species in all, 37 of which occur in the Western Himalaya [7]. A literature survey revealed that several *Nepeta* spp. are used in folk medicine as diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge, and sedative agents and for the antiseptic and astringent properties as topical remedy in children with cutaneous eruptions, and for snake and scorpion bites. Some species are used as medicinal herbs in Iran, for example, *N. ispahanica*, *N. binaloudensis*, *N. bracteata*, *N. pogonosperma*, and *N. pungens*, while *N. crispa* is used as a culinary herb. *N. caesarea*, an endemic species in Turkey, has folkloric uses in southern Anatolia and is used as a herbal tea to treat gastric disorders [8]. Among the various medicinal properties, *Nepeta* species are famous for treating cardiovascular complaints, such as angina pectoris, cardiac thrombosis tachycardia, and heart muscle weakness [9]. A literature survey shows *Nepeta* members are rich with fatty acids, flavones, flavone glycosides, coumarins, steroids, monoterpenic lactones and eudesmane diterpenoids [10]. The present study reveals the presence of phytochemicals in methanol and chloroform extract of *N. leucphylaa* from Uttarakhand region.

2. Materials and Methods
2.1 Plant collection: Fresh leaves and flowers of *N. leucophylla* were collected from Pangot Road of Nainital in Uttarakhand at a height of 2600 meters (Latitude 29.380304°N and Longitude of 79.463570°E) in the month of November 2024. The flowers were dried and the plant was further identified and authenticated from the BSI Dehradun and deposited in laboratory as a voucher specimen HAMPRL/2024/01.

2.2 Extract preparation and filtration: The leaves of *N. leucophylla* were collected from Pangot Road, Nainital and then cleaned and shade-dried to remove moisture while preserving their active constituents.

The dried leaves were then powdered using a mechanical grinder to obtain a fine powder. The fine powder was soaked separately in methanol, and chloroform using 1:10 ratio for 24-48 h at room temperature, to increase the maximum solubility. Filtrations and extraction were done using Whatman's filter paper. The resulting extract was collected for further analysis (Figure 1. A-D).

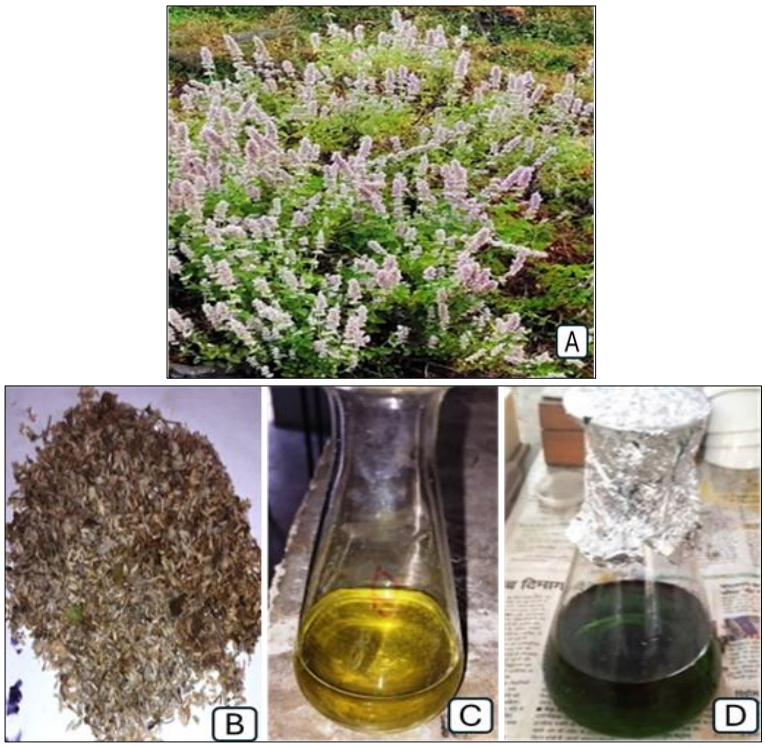

Fig 1: A-Plant in field with flowers, B-Dry flowers C-Methanol extract D-chloroform extract

2.3 Phytochemical analysis

2.3.1 Qualitative analysis: Qualitative tests of both the aqueous and methanol extracts were performed to confirm the presence of bioactive compounds having pharmacological importance through the following test.

2.3.2 Test for reducing sugars (Benedict reagent): The Benedict's test is a chemical test used to detect the presence of reducing sugars in a sample. When a reducing sugar is added to Benedict's reagent and heated, the blue solution turns a color ranging from green to red, indicating a positive result for reducing sugars. The test is based on the reduction of copper (II) ions (Cu^{2+}) in Benedict's reagent to copper (I) ions (Cu^{+}) by the reducing sugar, which then forms a reddish-brown precipitate of copper (I) oxide. - About 2 ml of both filtrate was taken to which 5 drops of Benedict reagent is added. This mixture was heated for about 3 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugar (Fig. B) [11-12].

2.3.3 Test for carbohydrates (Molish's reagent): Molisch's test is a chemical test used to detect the presence of carbohydrates. It involves adding a mixture of α-naphthol in ethanol (Molisch's reagent) and concentrated sulfuric acid to a sample. A positive result, indicating the presence of carbohydrates, is a purple or violet ring forming at the interface between the solution and the acid. About 2 ml of the filtrate was taken to which 4 to 5 drops of Molish reagent was added to both extracts and results are shown in the Figure 2 [11-12].

2.3.4 Test for amino acid: The Ninhydrin test is chemical test used to detect the presence of amino acids, peptides, and proteins. It is based on a reaction between ninhydrin and these compounds, resulting in a characteristic purple or blue color. To 2 ml of both extracts, a few drops of ninhydrin reagent (10 mg of ninhydrin in 200 ml of acetone) were added. The appearance of purple colour indicates the presence of amino acid (Fig B) [12, 13].

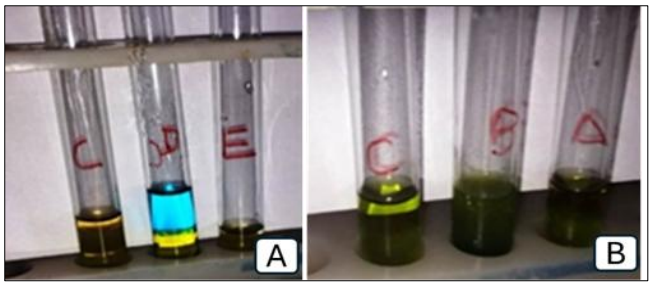

Fig 2: B Showing color of different reagent with extract

3. Results and Discussion

The results of the phytochemical screening likely revealed the presence of various bioactive compounds, such as carbohydrates, reducing sugars, and amino acids, in *N leucophylla* as summarized in Table 1. Methanolic and chloroform extracts show the presence of, carbohydrates, reducing sugars and amino acids, in both types of extract major presence of sugar is present in a high percentage in compared to other phytochemicals.

Table 1: Qualitative phytochemical screening of methanol and chloroform extract of *N. leucophylla*

Solvent used for extraction	Carbohydrates	Reducing sugars	Amino acids
Methanol	+	+++	+
Chloroform	+	+++	+

A literature survey revealed that a number of *Nepeta* species are studied for essential oils composition rather than extract studies. Previous study showed that *Nepeta* species contained different isomers of *Nepeta*lactone and some species contained compounds other than isomers of *Nepeta*lactone like 1, 8-cineole, ß-caryophyllene, caryophyllene oxide, ß-farnesene, a-citral, ß-citronellol etc as their major constituents [14]. Maximum species of contains 4aa, 7a, 7aa-*Nepeta*lactone, 4aa, 7a, 7aß-*Nepeta*lactone, 4aß, 7a, 7aß-*Nepeta*lactone, 4aß, 7a, 7aa-*Nepeta*lactone as their key compounds. These species also contained isomers 4aa, 7ß, 7aß-*Nepeta*lactone, 4aa, 7ß, 7aa-*Nepeta*lactone, 4a dihydro*Nepeta*lactone, 4ß-dihydro*Nepeta*lactone and 5, 9-dihydro*Nepeta*lactone. 4aa, 7a, 7aa-*Nepeta*lactone has found as major ingredient in species like *N. govaniana* (Benth.) [15], *N. royleana* [16] *N. laevigata* (D.Don) Hand.Mazz [17] 4aa, 7a, 7aß-*Nepeta*lactone was reported in *N. elliptica* (Royle ex Benth.) [18]. Aerial parts essential oil of *N. laevigata* from Kumaun, Uttarakhand: 1, 8-cineole (11.1%), caryophyllene (5.7%), caryophyllene oxide (15.2%), manool (7.9%) were reported [19]. Previous extracts studies in *Nepeta cataria* showed that the most abundant phytochemicals in methanol extract were 1-isopropylcyclohex-1-ene (concentration = 27.376) and bicyclo [2.2.1] heptan-2-one (concentration=20.437), whereas in ethanol extract, it was 9, 12, 15-octadecatrienoic acid (concentration=27.308) and 1-isopropylcyclohex-1-ene (concentration=25.854). An abundance of 2 methyl indoles, conhydrin, and coumarin was found in water extracts; a good concentration of eucalyptol was found in acetone extract; and 7, 9-di-tert-butyl-1-oxaspiro is the most abundant phytochemicals in hexane extracts. The highest concentration of flavonoids and phenols were identified in hexane and methanol extracts, respectively [20]. A number of important useful compounds and biological activities have been reported from *Nepeta* species [21].

4. Conclusions: Various types of chemical constituents within the genus *Nepeta* have been reported, such as monoterpene derivatives, sesquiterpenes, diterpenes, triterpenes, flavonoids, phenolic compounds, and essential oils. The present investigation reveals that carbohydrates, sugars and amino-acids were present in methanol and chloroform extract of *Nepeta leucophylla.* In addition, the phytochemicals present can be used in food and other materials in the future.

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Conflict of interest: There is no conflict of interest.

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