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Antioxidant activity of essential oil of *Nepeta laevigata* (D. Don) Hand.-Mazz from Himalayan region of Uttrakhand

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Abstract
Antioxidants are agents that scavenge the free radicals and stop the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA. The chemical profile of the hydro distilled essential oil obtained from the aerial parts of *Nepeta laevigata* from Kumaun Himalaya was analyzed by capillary GC-FID and GC-MS. The essential oils tested for antioxidant activity.

Keywords: *Nepeta laevigata*, essential oil, antioxidant, GC-FID, GC-MS.

1. Introduction
The use of medicinal plants by humans dates back thousands of years due to their medicinal and nutritional properties. Many natural compounds extracted from plants have important biological activities. Among these compounds, we highlight the essential oils, which are increasingly attracting the attention of various segments of industry due to their multiple functions, especially antioxidant and antimicrobial activities [1]. Essential oils are marketed by various companies as raw material for various products with applications in perfumery, cosmetics, foods, and as adjuncts in medicines, among others. There are approximately 300 essential oils of commercial importance in the world. In the food industry, essential oils, besides imparting aroma and flavor to food, have important antioxidant activity, a property that further encourages its use [2]. In foods, lipid peroxidation is responsible for the development of unpleasant flavors and odors, making them unfit for consumption, and causes other changes that may affect the nutritional quality due to degradation of fat soluble vitamins and essential fatty acids, as well affecting the integrity and safety of food. In biological systems, lipid peroxidation on cell membrane unsaturated lipids causes membrane damage, disrupting the metabolite exchange mechanisms, and may cause cell death, becoming largely responsible for early aging and cardiovascular disease, cataracts, immune system decline and brain dysfunction, among others [3, 4].
Nepeta (Lamiaceae) is a genus of about 250 species of flowering herbaceous, small shrub, rarely tree, often with quadrangular stems, glandular and aromatic with opposite leaves placed successively at right angles to each other [5]. *N. laevigata* is an erect, 30-90 cm, flowers are blue-purple, in dense whorls crowded into long terminal spikes and leaves usually stalked ovate, acute to triangular-lanceolate, found at the height of 3000-3500 m near glaciers [6]. Among 31 species reported in the Himalayan region six vials, *N. connata* Royle ex Benth, *N. leucophylla* Benth, *N. ciliaris* Benth, *N. distants* Royle ex Benth, *N. elliptica* Royle ex Benth, *N. spicata* Benth, are found in the Kumaun region [7]. *Nepeta* species are used as antispasmodic, diuretic, febrifuge, diaphoretic, antimicrobial and antiseptic agents and also in the treatment of dysentery, tooth trouble and kidney and liver diseases. Diverse biological activities viz. feline attractant, canine attractant, insect repellant and arthropod defense are accredited to the presence of biologically active iridoids/monoterpene nepetalactones in *Nepeta* species [8-11]. *Nepeta* species are widely used because of their antispasmodic, expectorant, diuretic, antiseptic, febrifuge, antitussive and antiasthmatic effects [12, 13]. Aydin *et al.* (1998) investigated the anti-nociceptive effects of essential oils from *Nepeta* species, including *N. phyllochlamys, N. nuda* L. ssp. nuda, and *N. caesarea Boiss*, using a tail flick and tail immersion tests. These authors detected central and peripheral anti-nociceptive effects in

these plants. *Nepeta* species are used in the traditional medicine of many countries and have a large ethnobotanical effect: diuretic, diaphoretic, vulnerary, antitussive, antispasmodic, antiasthmatic, tonic, febrifuge, emmenagogue and carminative [14, 15]. Iridodial β-monoenolacetate isolated from essential oil of *N. leucophylla* Benth., and actinidine isolated from essential oil of *N. clarkei* Hook f. were shown to have significant antibacterial and antifungal activities [16, 17]. The essential oils from six Himalayan *Nepeta* species, *viz*. *Nepeta leucophylla* Benth., *Nepeta discolor* Royle ex Benth., *Nepeta govaniana* Benth., *Nepeta clarkei* Hook f., *Nepeta elliptica* Royle ex Benth., and *Nepeta erecta* Benth., were tested for their *in vitro* antimicrobial activity against six pathogenic bacteria and two fungal strains with some significant results [18]. The essential oil of *N. laevigata* revealed the presence of 1, 8-cineole (9.08%), caryophyllene oxide (11.16%), mannol (7.91%) and pimaradiene (4.60%) were the principle components [19]. The aim of this investigation was to evaluate the antioxidant activity of leaf essential oils of *N. laevigata*.

2. Materials and methods

2.1 Plant materials

The fresh aerial parts of *Nepeta laevigata* were collected from Milam glacier (3600 m) in September 2008. Plant identification was done from BSI, Dehradun and voucher specimen No. CHEM DST/NL-08 has been deposited in the Phytochemistry Lab, Chemistry Department, Kumaun University, Nainital.

2.2 Extraction of oil

The fresh plant materials (leaves 1 kg.) were subjected to steam distillation. The distillates were saturated with NaCl and extracted with *n*-hexane and dichloromethane. The organic phase was dried over anhydrous sodium sulfate and the solvents were distilled off at 30 ºC. The oil yield was 0.3% (v/w) respectively.

2.3 GC and GC-MS analysis

The oils were analyzed by using a Nucon 5765 gas chromatograph (Rtx-5 column, 30 m × 0.32 mm, FID), split ratio 1: 48, N_2 flow of 4 kg/cm^2 and on Thermo Quest Trace GC 2000 interfaced with MAT Polaris Q Ion Trap Mass spectrometer fitted with a Rtx-5 (Restek Corp.) fused silica capillary column (30 m × 0.25 mm; 0.25 µm film coating). The column temperature was programmed 60-210 ºC at 3 ºC/min using helium as carrier gas at 1.0 mL/min. The injector temperature was 210 ºC, injection size 0.1 µL prepared in hexane, split ratio 1:40. MS were taken at 70 eV with a mass range of 40-450 amu.

3. Antioxidant activity

3.1 Free radical scavenging activities

The free radical scavenging activity of the extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the standard method [20]. 0.1 mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of oil solution in water at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was measured at 517 nm in a spectrophotometer. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The DPPH radical concentration was calculated using the following equation: DPPH scavenging activity (%) = (Ac-As) /Ac × 100, where Ac was the control and As was absorbance of the sample.

3.2 Reducing power activity

The reduction potential of the essential oils was determined according to the method of Oyaizu [21] taking its different concentrations (5, 10, 15, 20 mg/ml) in 1 ml of distilled water and mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 ^{0}C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifugation for 10 min at 1000 rpm (MSE Mistral 2000, UK). The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. The higher absorbance of the reaction mixture indicated greater reductive potential.

3.3 Chelating activity

The chelating of ferrous ions by the essential oils and standards was estimated by the method of Dinis, Madeira and Almeida [22]. Essential oils were added to a solution of 2mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. The experiments were run in triplicate and averaged. The percentage of inhibition of ferrozine- ferrous ion complex formation was given as: % Inhibition = $(A_0-A_1)/A_1 \times 100$, where A_0 was the absorbance of the control and A1 was the absorbance in the presence of the sample of the essential oil and standards. The control contains $FeCl_2$ and ferrozine complex formation molecules.

3.4 Calculation of 50% Inhibitory Concentration (IC50)

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions. Percentage inhibition (I %) was calculated using the formula, I % = (Ac-As)/Ac x 100 where Ac is the absorbance of the control and As is the absorbance of the sample.

4. Results and discussion

The essential oils were tested for their antioxidant activity by DPPH scavenging, reducing power and cheating power activities (Table 1-3). The leaf essential oil was found to possess the highest DPPH free radical scavenging power (84.67%) at the lowest concentration which is increased by increasing concentration of oil. This can be seen by comparing IC50 values for leaf essential oil is 5.54 mg/ml and for BHT, Catechin and Gallic acid it is 3.31, 5.60 and 1.61 respectively. It is comparable to Catechin and significant to BHT and Gallic acid. In case of reducing power activity, it is also comparable to standard and highest is 0.384 at higher concentration. The highest chelating power was shown by the leaf essential oil (81.32%) which is quite comparable to the standard Gallic acid (84.48%) at its lowest concentration. The IC50 values from for oil and standard are as 0.33 for leaf oil 1.61 for Gallic acid, 6.27 for EDTA and 5.06 for Citric acid. These are quite comparable and significant.

IC 50 (mg/ml)

Oil/Standard	DPPH	Chelating Power
Leaf Oil	5.54	0.33
BHT	3.31	-
Catechin	5.60	-
Gallic acid	1.61	1.61
EDTA	-	6.27
CA	-	5.06

Table 1: DPPH scavenging activity shown by leaf essential oil of *N. laevigata*

Oil/Standard	5 mg	10 mg	15 mg	20 mg
Leaf Oil	84.67%±0.09	93.14%±0.00	97.55%±0.02	98.16%±0.02
BHT	93.60%±0.00	94.50%±0.00	97.30%±0.00	97.70%±0.01
Catechin	91.90%±0.00	92.46%±0.01	94.10%±0.00	94.70%±0.00
Gallic acid	84.48%±0.00	85.65%±0.05	85.93%±0.05	85.95%±0.08

Table 2: Reducing power activity shown by leaf essential oil of *N. laevigata*

Oil/Standard	5 mg	10 mg	15 mg	20 mg
Leaf Oil	0.161	0.238	0.273	0.384
BHT	2.303	2.413	2.456	2.497
Gallic acid	4.000	4.000	4.000	4.000
Linoleic acid	0.373	0.379	0.395	0.411
Catechin	4.000	4.000	4.000	4.000

Table 3: Chelating power activity shown by leaf essential oil of *N. laevigata*

Oil/Standard	5 mg	10 mg	15 mg	20 mg
Leaf Oil	81.32±0.24	98.62±0.06	99.20±0.00	99.35±0.02
EDTA	89.32±0.06	87.63±0.90	88.04±0.00	88.11±0.87
Citric acid	86.18±0.66	87.04±0.02	89.36±0.00	89.56±0.02
Gallic acid	84.48±0.00	85.65±0.05	85.93±0.08	85.95±0.05

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