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# Analysis of Indian mint (*Mentha spicata*) for essential, trace and toxic elements and its antioxidant behaviour

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**Abstract**

*Mint*, belonging to the genus *Mentha* in the family *Labiatae* (*Lamiaceae*) is pungent in taste with post digestive effects and hot potency. Ten samples of mint leaves, collected from four different locations in North-West parts of India (Roorkee, Dehradun, Baghpat and Uttarkashi) were analysed for seven minor (Al, Na, K, Ca, Cl, Mg, and P) and 20 trace (As, Au, Ba, Br, Co, Cr, Cs, Eu, Fe, Hf, Hg, La, Mn, Rb, Sb, Sc, Se, Sn, Th and Zn) elements by instrumental neutron activation analysis (INAA). Also Ni, Cu and Cd were determined by atomic absorption spectrophotometry (AAS). Samples along with reference materials (RMs) and synthetic primary standard were irradiated at $\sim 10^{13}$ n cm$^{-2}$ s$^{-1}$ and its γ-activity was measured using HPGe detector and MCA system. Most elements were found in widely varying amounts depending on the location, e.g. Na (0.21–0.86 mg/g), K (12.4–53.3 mg/g) and Ca (5.82–16.8 mg/g) whereas mean contents of other nutrient elements in mint were: Fe ($108 \pm 22$ μg/g), Mg ($4.83 \pm 0.92$ mg/g), Mn ($53.5 \pm 9.6$ μg/g), P ($3.88 \pm 0.94$ mg/g), Cu ($16.9 \pm 1.8$ μg/g), Zn ($21.0 \pm 4.7$ μg/g) and Se ($0.18 \pm 0.03$ μg/g). Further, DPPH free radical scavenging activity test in diethyl ether extract showed 100% activity at ∼40 μg/L suggesting it to be antioxidant in accordance with literature reports.
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*Keywords:* *Mentha spicata*; Reference materials; INAA; AAS; DPPH; Antioxidant behaviour

## 1. Introduction

*Mint*, commonly known as '*Pudina*' in most Indian languages, belongs to the genus *Mentha* in the family *Labiatae* (*Lamiaceae*) that includes other commonly grown oil yielding plants such as *basil, sage, rosemary, marjoram, lavender and thyme*. There are 25–30 species within the genus *Mentha*, including *spearmint, peppermint, wild mint, corn mint, curled mint, bergamot, American mint, Korean mint*, etc. of which spearmint is the most common of all [1]. It grows abundantly to 1 ft high, in extensive masses in wet places, riverbank, and marshy areas. It is well distinguished by its downy foliage and whorls of lilac flowers, which are crowded into globose heads towards the summit of the stem. *Mint* is pungent in taste with post digestive effect and has hot potency [2]. Various species of mint have many constituents in common and all have been held in high dietary medical repute [3]. Water distillate of spearmint relieves hiccup and flatulence as well as the giddiness of indigestion. The ancients used spearmint to scent their bath and as a restorative [4]. In the fourteenth century, *mint* was used for whitening the teeth. Its distilled oil is still used to flavor toothpaste, confectionery, and chewing gum and also to perfume soaps. Spearmint has antifungal, antiviral, antimicrobial, insecticide, antioxidant, antiamoebic, antihemolytic, allergenic, CNS depressant, antihelmintic and antiancyclostomiasis activity [5,6]. A paste of *mint* leaves with fennel seeds, onion, ginger, tomato, green mango along with some salt and peppers/chilies and other spices makes quick and easy salad dressing often called *chutney*. Chopped *mint* leaves added to tomato soup compliment the sweet acidity of tomatoes. *Mint* leaves crushed with sugarcane forms excellent recipe in hot Indian summers just as dried powdered leaves added to yoghurt also provides relief from heat strokes.

Several workers have separated and identified phenolic acids, flavonoids, terpenoids and other volatile compounds from different extracts of mint. Phenolic acids and their derivatives are widely distributed in plants [7,8]. The role of phenolic acid and flavonoids as natural antioxidants and free radical scavenger has been of interest due to their pharmacological behavior [9,10].

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Some workers have studied its antioxidant properties and compared it with those of common food additives [11,12]. Mattila et al. [13] used HPLC with diode array and electro array detectors to determine flavonoids in plant materials. Only scanty references exist on the elemental contents of mint. Lozak et al. [14] determined twenty elements in *mint* and their infusions by ICP-MS and AAS. Zeinali et al. [15] studied the diversity amongst twelve accessions of Iranian *mint* in relation to yield and mineral contents. Rajput et al. [16] carried out a field experiment in subtropical climate of northern India to study the response of *mint* to application of six micronutrients (Fe, Mn, Zn, Cu, B and Mo). Recently, some Pakistani workers [17,18] have also analysed organic and inorganic constituents in field *mint*. Balaji et al. [19] have analyzed a few essential elements in *mint* from southern parts of India.

In the present study, we have analyzed 10 samples of spearmint collected from four different locations in North-West India for seven essential (Al, Mg, Ca, K, Na, P and Cl) and 23 trace (As, Au, Ba, Br, Cd, Co, Cr, Cs, Eu, Fe, Hf, Hg, La, Mn, Ni, Pb, Rb, Sb, Sc, Se, Sn, Th and Zn) elements using thermal neutron activation analysis (TNAA) and atomic absorption spectrophotometry (AAS). Several reference materials (RMs) obtained from the National Institute of Standards and Technology (NIST, USA), Institute of Nuclear Chemistry and Technology (INCT, Poland) and International Atomic Energy Agency (IAEA, Vienna) were also analysed for quality assurance and data validation. Further, different extracts of *mint* were assayed for DPPH free radical scavenging activity and thereby antioxidant property.

## 2. Materials and methods

### 2.1. Sample collection and preparation

Ten different batches of *mint* samples were from home gardens or purchased from the local vegetable shops within a time interval of 6 months during summers of 2004: Roorkee (4), Dehradun (2), Uttar Kashi (2) and Baghpat (2). All the sampling locations were at least 100 km away from each other. Dehradun and Uttar Kashi are hilly areas though Dehradun is the capital city of Uttaranchal state. However, Roorkee and Baghpat are from plains. Leaves were separated from the stems and soaked in water to remove any dirt. Further, its surface contamination was wiped with tissue paper and left for air drying and then in an oven at ~80 °C. The samples were powdered in agate mortar and passed through 100-mesh sieve. Various RMs such as Mixed Polished Herbs (MPH-2) from the INCT, Poland [20], Apple Leaves (SRM-1515) from the NIST, USA [21] and Cabbage (IAEA-359) from IAEA, Vienna [22] were also dried as per recommended procedure before use. A synthetic multielemental standard of As, Au, Co, Cr Fe, Hg Sb, Se and Zn was prepared by depositing 1–5 μg/g amounts of aqueous solutions of AR/HP grade salts on a Whatman 42 paper strip. Moisture content was determined by heating the leaves at ~80 °C for 24 h and found to be 81.6 ± 0.4% which compares well with 84.9% reported in literature [3].

### 2.2. Irradiation and counting

Fifty milligrams aliquots each of powdered samples and RMs were accurately weighed and packed in alkathene/aluminum foil (Superwrap) for short (1 min)/long (1 d) irradiation, respectively at $10^{13}$ n cm$^{-2}$ s$^{-1}$ in Dhruva reactor at the Bhabha Atomic Research Centre (BARC), Mumbai, India. Short-lived activities were measured using an 80 cm$^3$ coaxial HPGe detector (EG & G ORTEC) and 4 k MCA at the reactor site and later at the Radiochemistry Laboratories of BARC, Mumbai. A typical spectrum illustrating photo peaks due to various short lived nuclides is shown in Fig. 1. Long irradiated samples were air lifted to our laboratories in Roorkee and γ-activity was measured using a HPGe detector with 1.8 keV resolution at 1332 keV of $^{60}$Co with 20% relative efficiency and 8 k MCA with GENIE-2000 software (Canberra, USA). Counting was followed for 1, 2, 6 and 12 h at different intervals up to 3 months. Care was taken to obtain maximum elemental information from more than one counting and the reproducibility of data was checked. Elemental contents were calculated by comparator method using synthetic multielemental standard and RMs as comparators. Phosphorus was determined by measuring $\beta^-$ activity due to $^{32}$P on an end window G.M. counter (Nucleonix, Hyderabad) using 27 mg cm$^{-2}$ aluminium filter after a delay of ~3 weeks [23].

### 2.3. AAS measurements

For the analysis of Cd, Cu and Ni, AAS method was followed using atomic absorption spectrophotometer (GBC Avanta, Australia). About 2 g each of sample was accurately weighed and dissolved in a (5:1) mixture of nitric and perchloric acids [24] and repeated heating. After a clear solution was obtained, few drops of HCl were added and the solution was made up to 25 mL. Prior to the analysis, instrument was calibrated using standard solutions of Ni, Cu, Cd and Pb salts of AR/HP grade. Pb, however, could not be detected because of it being below detection limit (<60 ng/g).

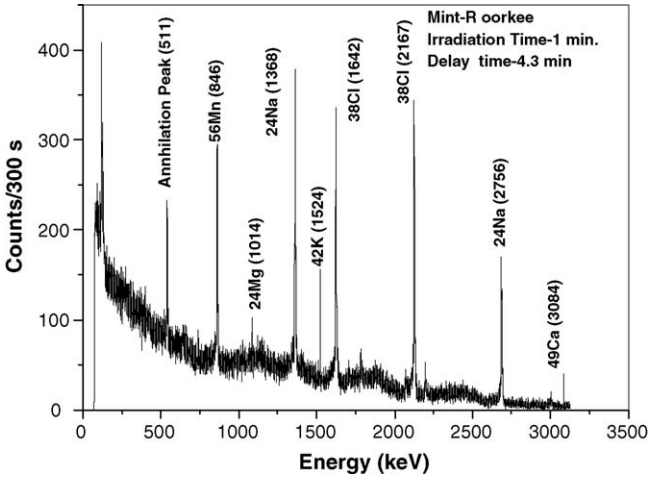


Fig. 1. A typical γ-ray spectrum for short lived nuclides in mint.

### 2.4. DPPH assay

One-hundred grams dried leaves were grounded in an agate mortar and passed through 100-mesh sieve and Soxhlet extracted with 500 mL methanol for 12 h. The extract was evaporated in a rotavapor at a temperature below the b.p. (∼100 °C) till about 25 mL of extract remains. To this was added, three times its volume of water and conc. HCl drop wise until pH 2 was attained. At this stage, resins are precipitated which are filtered off and the aqueous filtrate was transferred to a separating funnel with 50 mL diethyl ether (twice). To the 100 mL diethyl ether extract, ∼5 g anhydrous sodium sulfate was added and decanted in a petridish whence all the ether got evaporated. The spectrophotometric assay was carried out using purple colored ethanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, which got bleached by the H atom or electron donation ability of the extract. Various concentrations of the ethanol extract were added to 100 μM ethanol solution of DPPH. After 30 min incubation period, absorbance was recorded at $\lambda_{max} = 517$ nm [25]. Inhibition of free radical in percent was calculated using the relationship: $I(\%) = (A_{blank} - A_{sample}/A_{blank}) \times 100$, where $A_{blank}$ is the absorbance of the control reaction containing all reagents except the test compound, and $A_{sample}$ is the absorbance of the test compound.

## 3. Results and discussion

Elemental concentrations were calculated by using synthetic primary standard and RMs as comparators and the data so obtained for three RMs along with their certified/information values [20–22] are listed in Table 1. A comparison of the ranges and mean elemental concentrations in ten *mint* leaves samples collected from four different locations in North-West India is given in Table 2. AAS data for Cu, Cd and Ni are also included in the same table. Pb content was below detection limit of 60 ng/g. It is observed that our data in Table 1 match well within ±10% of the certified/information values for most elements with some exceptions such as Co, Cs Sc and Th and SDs are <10% suggesting a high order of precision. On the basis of this comparison, it is presumed that elemental concentrations in *mint* leaves reported here should be accurate and precise within ±10%.

### 3.1. Elemental contents in mint

A perusal of elemental data in Table 2 shows that mean elemental contents in mint leaves from one location to another vary in a small range for most elements (Ca, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, P, K, Se and Zn) whereas for others these are in a wide range. A perusal of data in Table 2 shows that mean values of four different locations do not vary significantly and their corresponding S.D. values are very small suggesting not much variation within a small area where soil characteristics do not change significantly. However, S.D. values for $n = 10$ are large suggesting significant variation due to difference in soil characteristics from four different regions which are >100 km away from each other. It is observed that *mint* is enriched in several essential elements such as Ca (12.4 ± 3.49 mg/g), Mg (4.83 ± 0.92 mg/g), K (23.4 ± 12.1 mg/g), P (3.88 ± 0.94 mg/g) and to a lesser extent in Na (0.64 ± 0.20 mg/g) and Fe (108 ± 22 μg/g). Mint leaves from Roorkee are especially enriched in K (28.5 ± 14.6 mg/g), Mg (5.51 ± 0.58 mg/g), and Se (162 ± 35 ng/g) whereas those from Dehradun are rich in Ca (16.2 ± 0.7 mg/g), Fe (130 ± 10 μg/g)

Table 1
Elemental concentrations in reference materials for data validation

| Element | Mixed polish herbs (INCT-MPH-2)[18] | Apple leaves (NIST, SRM-1515)[19] | Cabbage leaves (IAEA-359)[20] |
|---|---|---|---|
| Al (mg/g) | 0.64 ± 0.04 (0.67 ± 0.11) | 0.26 ± 0.02 (0.29 ± 0.01) | ND {−} |
| As (μg/g) | 0.47 ± 0.09 {−} | 0.41 ± 0.05 (0.38 ± 0.006) | ND {−} |
| Ba (μg/g) | 32.6 ± 0.5 (32.5 ± 2.5) | 55.0 ± 5.0 (49.0 ± 2.0) | 10.0 ± 1.5 [11] |
| Br (μg/g) | 8.10 ± 0.45 (7.71 ± 0.61) | 1.66 ± 0.05 [1.8] | 7.10 ± 0.65 [6.72] |
| Ca (mg/g) | 11.2 ± 0.8 (10.8 ± 0.7) | 15.5 ± 1.0 (15.3 ± 0.2) | 19.6 ± 1.2 [18.5] |
| Cl (mg/g) | 2.96 ± 0.32 (2.84 ± 0.20) | 0.580 ± 0.060 (0.579 ± 0.023) | 0.150 ± 0.011 {−} |
| Co (ng/g) | 346 ± 24 (210 ± 25) | 85.5 ± 7.5 [90] | 105 ± 15 [120] |
| Cr (μg/g) | 1.78 ± 0.45 (1.69 ± 0.13) | 0.35 ± 0.02 (0.30) | 1.57 ± 0.15 (1.30 ± 0.19) |
| Cs (ng/g) | 64.5 ± 5.4 (76.0 ± 7.0) | 201 [177] | 37.5 ± 1.2 {−} |
| Cu (μg/g) | 14.2 ± 1.8 {−} | 5.58 ± 0.28 (5.64 ± 0.24) | ND {−} |
| Fe (μg/g) | 515 ± 48 [460] | 73 ± 7 (83 ± 5) | 153 ± 23 [148] |
| Hg (ng/g) | 116 ± 15 {−} | 47.7 ± 2.7 (44 ± 4) | ND {−} |
| K (mg/g) | 18.7 ± 0.6 (19.1 ± 1.2) | 15.8 ± 1.3 (16.1 ± 0.2) | 35.7 ± 2.3 (32.5 ± 3.0) |
| La (μg/g) | 0.580 ± 0.02 (0.571 ± 0.046) | 18.2 ± 0.05 [20] | 1.00 ± 0.09 {−} |
| Mg (mg/g) | 2.88 ± 0.18 (2.92 ± 0.18) | 2.75 ± 0.30 (2.71 ± 0.08) | ND {−} |
| Mn (μg/g) | 185 ± 5 (191 ± 12) | 49.2 ± 2.2 (54 ± 3) | 30.9 ± 1.0 [31.9] |
| Na (mg/g) | 401 ± 20 [350] | 26.0 ± 2.4 (24.4 ± 1.2) | 630 ± 40 (580 ± 70) |
| P (mg/g) | 2.39 ± 0.03 [2.5] | 1.60 ± 0.07 (1.59 ± 0.11) | ND {−} |
| Rb (μg/g) | 10.6 ± 0.7 (10.7 ± 0.7) | 10.2 ± 0.2 (10.2 ± 1.5) | 6.10 ± 0.40 [6.04] |
| Sb (ng/g) | 62.8 ± 4.7 (65.5 ± 9.1) | 15.0 ± 2.0 [13] | 30.0 ± 4.0 [26] |
| Sc (ng/g) | 186 ± 14 (123 ± 9) | 35.0 ± 4.0 [30] | 220 ± 20 [24.6] |
| Th (ng/g) | 221 ± 12 (154 ± 13) | 33.0 ± 3.0 [30] | 208/ ± 10 {−} |
| Zn (μg/g) | 32.7 ± 8.3 (33.5 ± 2.1) | 12.8 ± 0.1 (12.5 ± 0.3) | 39.2 ± 4.1 [38.6] |

In parenthesis round brackets are certified values, in square brackets are information values, in {−} no data available, ND: not detected.

Table 2
Range and mean elemental concentrations in *mint* leaves from different locations

| Element | Roorkee ($n=4$) | | Dehradun ($n=2$) | | Baghpat ($n=2$) | | Uttarkashi ($n=2$) | | Total ($n=10$) |
|---|---|---|---|---|---|---|---|---|---|
| | Range | Mean ± S.D. | Range | Mean ± S.D. | Range | Mean ± S.D. | Range | Mean ± S.D. | Mean ± S.D. |
| Al (mg/g) | 0.80–0.94 | 0.65 ± 0.22 | 0.22–0.24 | 0.23 ± 0.01 | 0.98–1.12 | 1.05 ± 0.07 | 0.25–0.29 | 0.27 ± 0.02 | 0.57 ± 0.35 |
| As (ng/g) | 306–320 | 313 ± 7 | 98–250 | 174 ± 76 | – | <20 | 144–167 | 156 ± 12 | 196 ± 89 |
| Au (ng/g) | 13.9–15.7 | 14.8 ± 0.9 | 1.5–3.7 | 2.6 ± 1.1 | – | <0.5 | 1.7–1.9 | 1.8 ± 0.1 | 4.9 ± 6.1 |
| Ba (μg/g) | 30.2–42.9 | 37.3 ± 4.6 | 25.9–30.5 | 28.2 ± 2.3 | 50.7–55.3 | 53.0 ± 2.3 | 18.4–20.2 | 19.3 ± 0.9 | 35.0 ± 12.3 |
| Br (μg/g) | 3.26–3.50 | 3.38 ± 0.12 | 4.74–5.24 | 4.99 ± 0.25 | – | <0.10 | 1.41–1.43 | 1.42 ± 0.01 | 3.26 ± 1.80 |
| Ca (mg/g) | 11.0–15.3 | 12.8 ± 1.7 | 15.5–16.8 | 16.2 ± 0.7 | 5.82–8.95 | 7.39 ± 1.57 | 11.1–14.7 | 12.9 ± 1.8 | 12.4 ± 3.5 |
| Cd (ng/g) | 199 | 199 | 15–275 | 213 ± 63 | – | <15 | 450–772 | 611 ± 11 | 369 ± 252 |
| Cl (mg/g) | 4.77–8.14 | 6.10 ± 1.36 | 6.67–10.0 | 8.34 ± 1.67 | 7.55–7.91 | 7.73 ± 0.18 | 9.88–10.5 | 10.2 ± 0.3 | 7.69 ± 2.02 |
| Co (ng/g) | 69–151 | 114 ± 32 | 85–99 | 92 ± 7 | 86–93 | 90 ± 4 | 70–92 | 81 ± 11 | 97.9 ± 26.2 |
| Cr (μg/g) | 1.27–1.60 | 1.42 ± 0.15 | 1.10–1.30 | 1.20 ± 0.10 | 1.70–1.71 | 1.71 ± 0.05 | 0.99–1.21 | 1.10 ± 0.11 | 1.37 ± 0.25 |
| Cs (ng/g) | 131–215 | 168 ± 37 | 129–152 | 141 ± 12 | 252–282 | 267 ± 15 | 44.7–44.8 | 44.8 ± 0.1 | 157.1 ± 77.9 |
| Cu (μg/g) | 15.7–18.0 | 16.5 ± 1.1 | 17.2–20.2 | 18.7 ± 1.5 | 16.8–18.5 | 17.7 ± 0.9 | 13.8–16.7 | 15.3 ± 1.5 | 16.9 ± 1.8 |
| Eu (ng/g) | 14.7–48.5 | 28.6 ± 12.3 | 40.8–64.8 | 52.8 ± 12.0 | 25.2–27.9 | 26.6 ± 1.4 | 44.1–45.5 | 4.8 ± 0.1 | 36.3 ± 14.9 |
| Fe (μg/g) | 86.9–138 | 103 ± 21 | 120–139 | 130 ± 10 | 88.9–100 | 94.4 ± 5.5 | 101–129 | 115 ± 14 | 108 ± 22 |
| Hf (ng/g) | 125–192 | 167 ± 32 | 143–183 | 163 ± 20 | 110–210 | 160 ± 50 | 173–201 | 187 ± 14 | 178 ± 22 |
| Hg (ng/g) | 131–983 | 410 ± 338 | 93–115 | 104 ± 11 | 101–186 | 144 ± 43 | 97–114 | 106 ± 9 | 235 ± 272 |
| K (mg/g) | 16.1–53.3 | 28.5 ± 14.6 | 12.4–14.0 | 13.2 ± 0.8 | 25.9–29.1 | 27.5 ± 1.6 | 13.2–25.5 | 19.4 ± 6.2 | 23.4 ± 12.1 |
| La (μg/g) | 1.32–1.71 | 1.51 ± 0.14 | 1.38–1.84 | 1.61 ± 0.23 | 1.47–1.65 | 1.56 ± 0.10 | 1.13–1.41 | 1.27 ± 0.14 | 1.49 ± 0.20 |
| Mg (mg/g) | 4.63–6.10 | 5.51 ± 0.58 | 3.90–5.15 | 4.53 ± 0.63 | 4.53–5.43 | 4.98 ± 0.45 | 3.49–3.75 | 3.62 ± .1 | 4.83 ± 0.92 |
| Mn (μg/g) | 46.1–69.6 | 57.5 ± 8.7 | 37.0–57.4 | 47.2 ± 10.2 | 48.0–63.7 | 55.1 ± 7.9 | 48.0–51.2 | 49.6 ± 1.6 | 53.5 ± 9.6 |
| Na (mg/g) | 0.41–0.86 | 0.64 ± 0.17 | 0.36–0.43 | 0.40 ± 0.04 | 0.51–0.53 | 0.52 ± 0.01 | 0.21–0.25 | 0.23 ± 0.02 | 0.48 ± 0.20 |
| Ni (ng/g) | 1.17 | 1.17 | 3.22 | 3.22 | <0.2 | <0.2 | 0.37–2.82 | 1.90 ± 1.17 | 1.90 ± 1.35 |
| P (mg/g) | 3.20–3.42 | 3.31 ± 0.11 | 4.46–4.61 | 4.54 ± 0.04 | 2.48–2.88 | 2.68 ± 0.20 | 4.86–5.12 | 4.99 ± 0.13 | 3.88 ± 0.94 |
| Rb (μg/g) | 11.1–30.4 | 23.1 ± 7.4 | 21.9–29.3 | 25.6 ± 3.7 | 16.0–16.4 | 30.7 ± 1.15 | 29.5–31.8 | 16.2 ± 0.2 | 23.7 ± 7.2 |
| Sb (ng/g) | 23.5–315 | 138 ± 110 | 15.8–27.2 | 21.5 ± 5.7 | 178–282 | 240 ± 52 | 12.1–19.6 | 15.9 ± 3.8 | 109 ± 115 |
| Sc (ng/g) | 42–139 | 69.3 ± 40.4 | 34–42 | 38 ± 4 | 55–62 | 59 ± 4 | 29–36 | 33 ± 4 | 54.0 ± 32.0 |
| Se (ng/g) | 127–197 | 162 ± 35 | – | <100 | 187–195 | 191 ± 4 | – | <100 | 177 ± 33 |
| Sn (ng/g) | 153–184 | 168 ± 15 | 172–191 | 182 ± 10 | – | <30 | 147–172 | 160 ± 13 | 173 ± 17 |
| Th (ng/g) | 44–74 | 58.3 ± 11.1 | 39–50 | 44.5 ± 5.5 | 110–193 | 152 ± 42 | 31–37 | 34 ± 3 | 69.3 ± 49.1 |
| Zn (μg/g) | 16.0–24.3 | 19.9 ± 3.5 | 22.5–28.4 | 25.5 ± 3.0 | 14.8–15.8 | 15.3 ± 0.5 | 23.2–25.4 | 24.3 ± 1.1 | 21.0 ± 4.7 |

and Zn (25.5 ± 3.0 μg/g). Other micronutrients viz. Mn (53.5 ± 9.6 μg/g), Zn (21.0 ± 4.7 μg/g), Cu (16.9 ± 1.8 μg/g) and Cr (1.37 ± 0.25 μg/g) from various locations vary in a small range as evident from S.D. values suggesting independence from the soil characteristics and geo-environmental conditions. Another structural element phosphorus was also found in a comparable range, 2.48–5.12 mg/g in all the samples. Bar plots showing comparison of essential and trace element concentrations in *mint* leaves from different locations are illustrated in Figs. 2 and 3, respectively. It is observed that most elemental contents in *mint* leaves exhibit small variations with very little effect of geo-environmental factors though Mn, Fe, Cu, and Zn are found at μg/g level and Co at ng/g level only. In view of the antioxidant properties of Mn(II) and Zn(II) [26,27], their elemental contents are of special importance as these may remain complexed with organic molecules such as carveol identified in methanolic extract [28].

The toxic heavy metals like Hg (97–983 ng/g), Sb (1.8–315 ng/g), Ni (0.37–3.22 ng/g), Cd (15–772 ng/g) and As (98–320 ng/g) are all found at ng/g level only but vary in a wide range. Leaves from Roorkee show much higher amounts of As (313 ± 7 ng/g), Hg (410 ± 338 ng/g) and Cd (199 ng/g) whereas Ni content (3.22 ng/g) is higher in Dehradun. This may possibly be attributed to the fact that both Roorkee and Dehradun (capital city of Uttaranchal state) are relatively urbanized townships where these pollutant elements could originate from industrial emissions and anthropogenic activities. Not surprisingly, Uttarkashi, a hilly town having no industrial activity shows much lower concentrations of Br (1.42 ± 0.01 μg/g) and Ni (1.90 ± 1.17 ng/g). On the other hand leaves from Baghpat, a town near Delhi the capital and a mega city of India show higher amounts of Cs (267 ± 15 ng/g), Hf (160 ± 50 ng/g) and

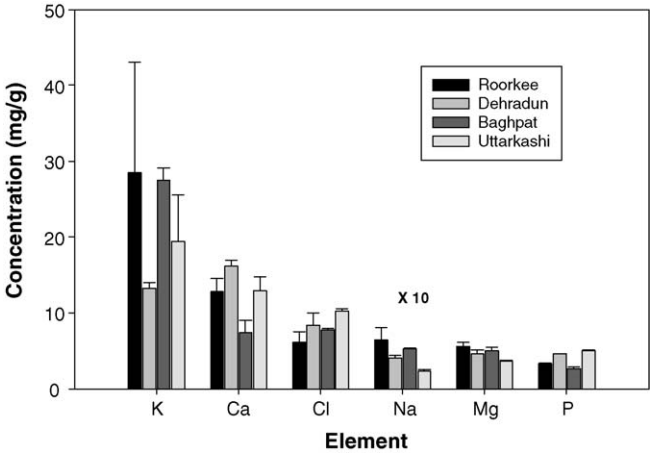


Fig. 2. Comparison of essential element concentration of mint from different areas.

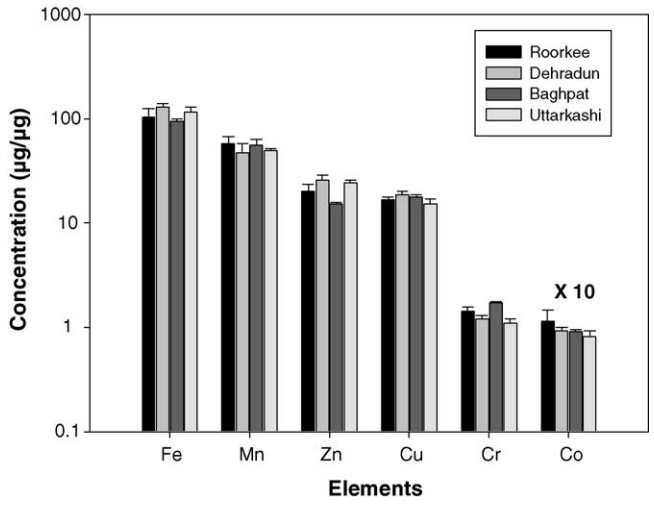


Fig. 3. Comparison of trace element concentration of mint from different areas.

Th ($152 \pm 42$ ng/g). However, Pb was found below the detection limit of 60 ng/g. On the basis of these it is suggested that *mint* leaves are safe to eat as far as toxic heavy elements are concerned.

There exists a strong inverse correlation between Mg & Na with Cl as shown in Fig. 4a and b, respectively. Similarly Cr and Zn, the two transition elements well known for their role in biochemical processes, are also inversely correlated (Fig. 4c), all having $r = 0.91$–$0.97$. This is interesting because the availability of Zn in the range of 14.8–28.4 μg/g may be beneficial for diabetic patients as its deficiency has been correlated with acute and chronic mal absorption states [29,30]. Cr (III) may be bound with glycine, cysteine and glutamic acids to form a complex molecule called glucose tolerance factor [31]. Similarly, small amounts of Se ($177 \pm 33.2$ ng/g) may be responsible for its anticancer properties because Se as glutathione peroxidase inhibits the replication of tumor viruses and prevents the malignant transformation of cells [32]. Promising antimutagenic/anticarcinogenic potential of this herb may possibly be due to potential bioavailability of these elements. K/Na and K/P ratios in *mint* leaves collected from four different locations are bar plotted in Fig. 5. K/Na in four different locations varies by a factor of 3 while K/P varies in a much wider range of 2–10. In both cases, *mint* leaves from Dehradun show the lowest ratio.

### 3.2. Comparison with the literature data

Since several workers [3,18,19] have analysed mint leaves we compared our data in Table 3. Our values for Na, Co,

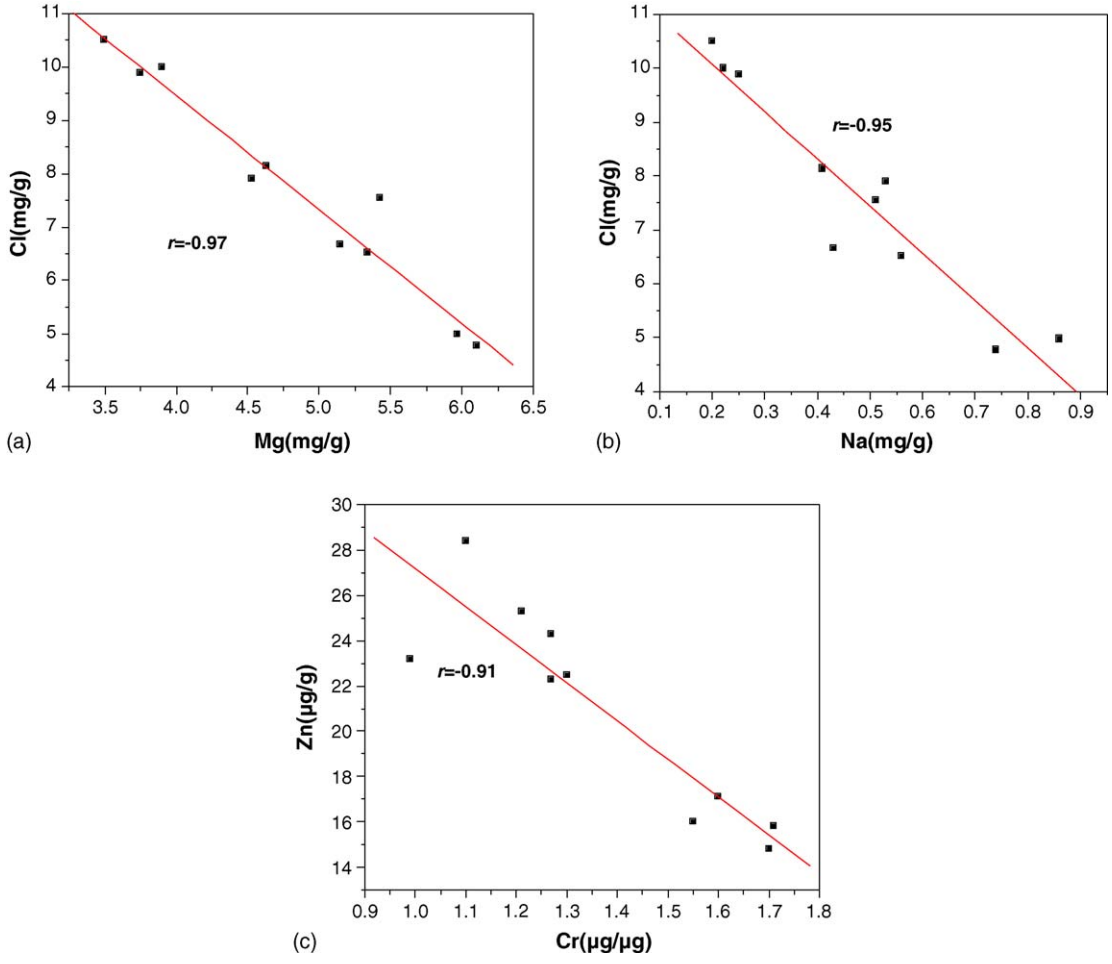


Fig. 4. Elemental correlations in mint leaves.

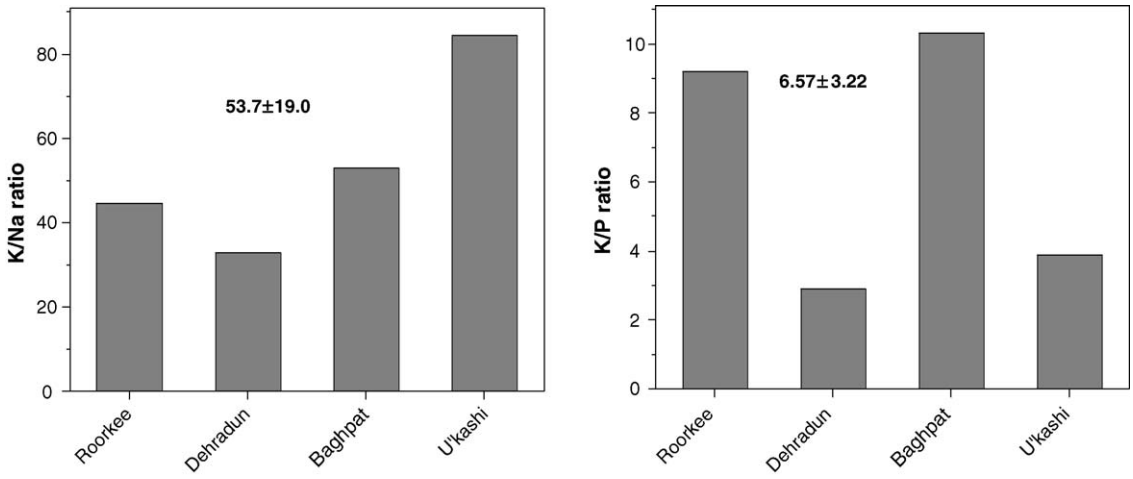


Fig. 5. K/Na and K/P ratios in mint samples from different locations.

Cr, Cs and Eu are in excellent agreement with those reported by Zaidi et al. [18] whereas Al, K and Mn match with those of Balaji et al. [19]. Concentration of Fe ($108 \pm 22$ μg/g) matches well with that reported in the compilation by Gopalan et al. [3] (156 μg/g) though Zaidi et al. [18] reported a substantially higher value ($861 \pm 44$ μg/g). It should be noted that most other analyses are on the basis of one or two samples whereas we have analyzed 10 samples collected from four different locations. On the basis of this comparison, it can be inferred that our data for other elements should be reliable though others have not reported it.

### 3.3. Antioxidant behaviour

Further, the diethyl ether, methanol and dichloromethane extracts of mint were subjected to free radical scavenging activity test. Reactive oxygen species (ROS) including superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$) and non-free radical species such as $H_2O_2$ are likely to induce oxidative damage to biomolecules such as lipids, nucleicacids, proteins and carbohydrates. Their damage causes malaria, immunodeficiency syndrome, heart disease, stroke, diabetes and cancer [33–35]. It is observed from Fig. 6 that diethyl ether extract shows almost 100% activity at ~40 μg/L whereas the other two

Table 3
Comparison of our data with those of other references

| Elements | Present work | Zaidi et al. [18] | Gopalan et al. [3] | Balaji et al. [19] |
|---|---|---|---|---|
| Al (mg/g) | 0.57 ± 0.35 | – | – | 0.40 ± 0.03 |
| Ba (μg/g) | 35.0 ± 12.3 | 61.4 ± 5.2 | – | – |
| Br (μg/g) | 3.26 ± 1.80 | 6.20 ± 0.41 | – | 6.5 ± 0.4 |
| Ca (mg/g) | 12.4 ± 3.5 | – | – | 16.5 ± 0.3 |
| Cl (mg/g) | 7.69 ± 2.02 | 2.18 ± 0.10 | 0.34 | 14.2 ± 0.1 |
| Co (ng/g) | 97.9 ± 26.2 | 110 ± 70 | – | – |
| Cr (μg/g) | 1.37 ± 0.25 | 1.68 ± 0.08 | 0.8 | – |
| Cs (ng/g) | 157.1 ± 77.9 | 130 ± 10 | – | – |
| Eu (ng/g) | 36.3 ± 14.9 | 52 ± 4 | – | – |
| Fe (μg/g) | 108 ± 22 | 861 ± 44 | 156 | – |
| Hf (ng/g) | 178 ± 22 | 62 ± 3 | – | – |
| Hg (ng/g) | 235 ± 272 | 12.0 ± 2.0 | – | – |
| K (mg/g) | 23.4 ± 12.1 | 1.97 ± 0.07 | 57.0 | 24.9 ± 3.2 |
| Mg (mg/g) | 4.83 ± 0.92 | – | – | 8.5 ± 0.6 |
| Mn (μg/g) | 53.5 ± 9.6 | 51.1 ± 2.6 | – | 50.2 ± 4.4 |
| Na (mg/g) | 0.48 ± 0.20 | 0.67 ± 0.03 | – | 1.11 ± 0.08 |
| Rb (μg/g) | 23.7 ± 7.18 | 3.15 ± 0.24 | – | – |
| Sb (ng/g) | 109 ± 115 | 60.0 ± 4.0 | – | – |
| Sc (ng/g) | 54.0 ± 32.0 | 280 ± 30 | – | – |
| Se (ng/g) | 177 ± 33.2 | 4580 ± 300 | – | – |
| Th (ng/g) | 69.3 ± 49.1 | 260 ± 30 | – | – |
| Zn (μg/g) | 21.0 ± 4.70 | 169 ± 8.6 | 44.0 | – |

–: means not reported.

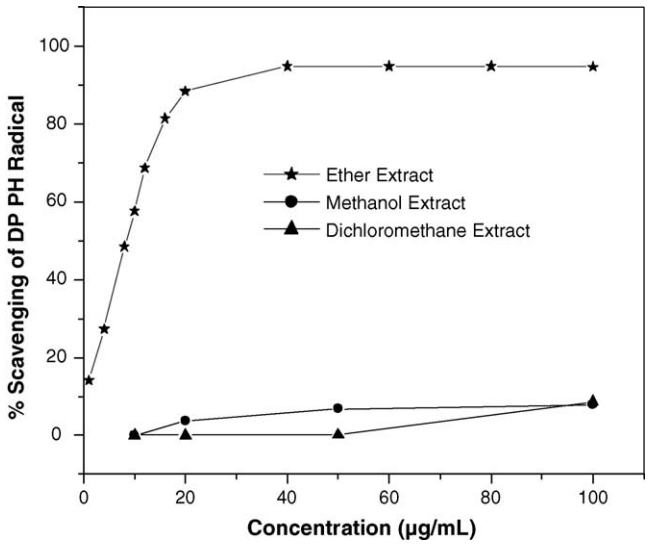


Fig. 6. DPPH radical scavenging activity of mint leaves.

extracts show very little activity. All aerobic organisms including human beings have antioxidant defense against oxidative damages. However, this natural antioxidant can be insufficient, and hence intake of antioxidant compounds is important. Thus, it is proved that mint contains active organic constituents (such as carveol) in complex form with transition elements such as Mn, Zn and Co and exhibit beneficial properties by the presence of antioxidants. Thus compounds containing alcoholic groups and essential transition elements in mint are likely to be responsible for the free radical scavenging activity of the extract [36].

## 4. Conclusion

Ten mint leaves samples collected from four different locations in North-West India were analyzed for seven minor and 23 trace elements including heavy toxic metals by NAA and AAS. Wide variations were observed in Na ($0.48 \pm 0.20$ mg/g), K ($23.4 \pm 12.1$ mg/g), Ca ($12.4 \pm 3.9$ mg/g) whereas mean contents of other nutrient elements in mint leaves were; Fe ($108 \pm 22$ μg/g), Mg ($4.83 \pm 0.92$ mg/g), Mn ($53.5 \pm 9.6$ μg/g), P ($3.88 \pm 0.94$ mg/g), Cu ($16.9 \pm 1.8$ μg/g) and Zn ($21.0 \pm 4.70$ μg/g) were in a narrow range. Variations in elemental contents could be attributed to local soil characteristics, climatic conditions and anthropogenic activities. Inverse linear correlations were observed for Zn versus Cr ($r = -0.91$), Mg versus Cl ($r = -0.97$), and Na versus Cl ($r = -0.95$). DPPH free radical scavenging of diethyl ether extract showed 100% activity at ~40 μg/L suggesting strong antioxidant property of mint leaves. It is suggested that alcoholic compounds such as carveol may remain complexed with transition metals like Mn, Zn, etc.

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