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Proximate, mineral and chemical composition of different parts of chicory (*Cichorium intybus* L.)

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Abstract
Chicory (*Cichorium intybus* L.) is a medicinally important plant which belongs to the family Asteraceae. The present study was undertaken to investigate the proximate, mineral and chemical composition of different parts *viz*. seeds, aerial parts and roots of *C. intybus* cultivated in Hisar region. Results revealed that all parts of *C. intybus* are good source of crude fibre, crude protein, fat and carbohydrate. On the basis of calorific value, all parts of *C. intybus* were found to be very rich source of energy. Seeds, aerial parts and roots of Chicory contained significant amount of minerals *viz*. Fe, Cu, Zn and Mn. Different parts of Chicory were also found to contain ascorbic acid, starch, tannins, total sugars, reducing sugars and non-reducing sugars in varying amounts.

Keywords: *Cichorium intybus*, proximate composition, mineral composition, chemical composition

1. Introduction
In the present scenario, traditional medicines of plant origin have become a major area of scientific research due to the widespread use of traditional herbs, spices and medicinal plants. A multitude of plant species are still widely used for the traditional as well as modern systems of medicine. The knowledge pertaining to the medical significance of plant species have passed from previous to the present generation through oral communication and folklore [1]. About 80% population of the developing world is still dependent on the traditional medicines [2]. Most of the expensive lifesaving drugs manufactured by Western pharmaceutical companies use medicinal and aromatic plants either as intact plant or in the form of crude extract [3].

Cichorium intybus L., commonly known as chicory in English and kasni in Hindi is an important medicinal herb. It belongs to family Asteraceae. Aerial parts, flowers, seeds and roots are the commonly used parts of this plant. All parts of this plant possess medicinal importance due to the presence of a number of medicinally important compounds such as volatile oil, fatty acids, unsaturated sterols, alkaloids, triterpenes, vitamins, flavonoids, tannins, coumarins, saponins, terpenoids, cardiac glycosides, sesquiterpene lactones, anthocyanins and phenols [4-6]. Chicory roots contain inulin which has negligible impact on blood sugar and hence is suitable for diabetics. *C. intybus* has been traditionally used for the treatment of fever, diarrhoea, jaundice and gallstones. *C. intybus* possess various pharmacological activities like antimicrobial, anthelmintic, antimalarial, hepatoprotective, antidiabetic, gastroprotective, anti-inflammatory, analgesis, antioxidant, tumor-inhibitory, antiallergic [7,8]. It is used as a coffee substitute and grown as a crop for livestock [9], but very little information is known about its nutritional value. Therefore, the objective of the present study was to analyze the proximate, mineral and chemical composition of different parts viz. seeds, aerial parts and roots of chicory (*Cichorium intybus* L.) cultivated in Hisar region.

2. Materials and methods
2.1 Plant material
Seeds, aerial parts and roots of Chicory (*Cichorium intybus* L.) were procured from the experimental area of Medicinal, Aromatic & Potential Crops Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana.

2.2 Proximate analysis
2.2.1 Estimation of moisture content
Two gram of the powdered samples of seeds, aerial parts and roots of Chicory were taken in three replications and dried initially at 80-90°C and finally at 100-102°C. Weights of dried samples were noted until constant weights were obtained. The percentage of moisture content was calculated as follows:-

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$$\text{Moisture content (\%)} = \frac{\text{Wt. of powder (before drying) - Wt. of powder (after drying)}}{\text{Wt. of powder (before drying)}} \times 100$$

2.2.2 Estimation of fat

Two gram of the dried powdered samples of seeds, aerial parts and roots of Chicory were taken in a thimble and placed in a soxhlet extractor. A dried and pre-weighed round-bottomed flask (250 mL) was connected to the soxhlet assembly. Then petroleum ether was added up to one and a half siphons i.e. approximately 150-175 mL. The assembly was heated and extraction was carried out for 8 h. After extraction, petroleum ether was evaporated from the round-bottomed flask and weight of the round bottomed flask along with the extract was determined again. The crude fat (%) contents were calculated using the following formula:

$$\text{Fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

2.2.3 Estimation of ash

Two gram of the powdered samples of seeds, aerial parts and roots of Chicory were weighed and transferred into previously ignited and weighed crucible and placed in a muffle furnace (preheated at 600°C) for 2 h. The crucibles with the samples were transferred directly from the furnace into a desiccator, allowed to cool and weight was taken. The ash contents (%) were calculated using the following formula:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.2.4 Estimation of protein

Nitrogen and crude protein content in the powdered samples of Chicory were estimated by following conventional micro-kjeldahl's method. 100 mg powdered samples of seeds, aerial parts and roots of Chicory were weighed and transferred to 100 mL micro-kjeldahl's digestion flasks. About 1 g of K_2SO_4: $CuSO_4$ (9:1) was added to it followed by 10 mL conc. H_2SO_4. The flasks were then kept in an inclined position on the hot plate in the digestion chamber and heated gently till the solution became transparent giving a bluish green colour. After cooling, the contents of the flask were mixed with distilled water, cooled, transferred to 100 mL volumetric flask and volume was made up to the mark with distilled water. 10 mL of N/100 H_2SO_4 was taken in a conical flask which acts as a receiving flask. This flask was placed in such a way that outlet of the condenser of micro-kjeldahl's distillation apparatus dips into the acid solution. Then, 10 mL of acid digested sample was transferred to the steam chamber of micro-kjeldahl's apparatus followed by 10 mL of 40% NaOH. Immediately, the stopcock was closed, steam was passed through the steam chamber and ammonia was distilled till 30-40 mL of distillate was collected in the receiving flask. Receiving flask was removed and the contents were titrated against N/100 NaOH and volume of NaOH used was noted. The end-point was reached when colour changed from pink to yellow. A blank was also run simultaneously which has been digested and distilled in similar manner.

Calculations

Amount of nitrogen (%) = (A − B) x 1.4

Where, A = Volume of N/100 NaOH used for blank (mL)

B = Volume of N/100 NaOH used for sample (mL)

Protein content (%) in sample = Nitrogen content in sample x 6.25

2.2.5 Estimation of crude fibre

Crude fibre was estimated by the modified method of Maynard [10]. One gram of moisture and fat free powdered samples of seeds, aerial parts and roots of Chicory were weighed and transferred to the spoutless one litre beaker and added 200 mL of 1.25% (w/v) sulphuric acid. The beaker was then placed on hot plate and allowed to reflux for 30 min timed from onset of boiling and the contents were shaked after every 5 min. After boiling for 30 min beaker was removed from hot plate and filtered through a muslin cloth using suction. The residue was washed with hot water till it became free from acid, then the material was transferred to the same beaker and added 200 mL of 1.25% NaOH solution and the contents were again refluxed for 30 min. It was filtered again through muslin cloth with the help of vacuum or suction pump and the residue was washed with hot water till it became free from alkali. The residue was then transferred to a crucible and placed in hot air oven, allowed to dry to constant weight at 80-110°C and recorded its weight. The residue was ignited in muffle furnace at 550-660°C for 2-3 h, then cooled and weighed again. The loss of weight due to ignition is weight of crude fibre. The crude fibre contents (%) were calculated using the following formula:

$$\text{Crude fibre content(\%)} = \frac{\text{Weight of crude fibre}}{\text{Original weight of sample}} \times 100$$

2.2.6 Estimation of total carbohydrates

Total carbohydrates content was calculated by difference as follows:

Total carbohydrates content (%) = 100 − [Moisture (%) + Fat (%) + Ash (%) + Protein (%) + Crude fibre (%)]

2.2.7 Estimation of calorific value

The calorific value in kilocalories (kcal) was calculated according to the Atwater system as follows:
Calorific value (kcal) = (4 x Protein content) + (9 x Fat content) + (4 x Total carbohydrates content)

2.3 Estimation of minerals

0.5 g of powdered samples of seeds, aerial parts and roots of Chicory were weighed and transferred to 100 mL conical flask. To this, 10 mL of diacid mixture of HNO_3 and $HClO_4$ in a ratio of 4:1 was added and the samples were allowed to stand overnight. The samples were heated on a hot plate gently at first and then vigorously until a clear colourless solution results or till white fumes ceased to come out. Samples were not heated to dryness. Heating was discontinued when the volume reduced to 2 - 3 mL. The samples were cooled, transferred to 50 mL volumetric flask, made up to the mark by adding distilled water, filtered through Whatman no. 1 filter paper and used for the estimation of Fe, Cu, Zn and Mn using Varian AA240FS Fast Sequential Atomic Absorption Spectrophotometer (Agilent Technologies).

2.4 Chemical analysis
2.4.1 Estimation of ascorbic acid

Ascorbic acid was estimated by titrimetric method [11]. 5 mL of the working standard solution was pipetted out into a 100

mL conical flask, added 10 mL of 4% oxalic acid and titrated against the dye (V_1 mL). End point was the appearance of pink colour which persisted for a few minutes. One gram of the powdered samples of seeds, aerial parts and roots of Chicory were extracted in 4% oxalic acid by using centrifuge and made up to a known volume i.e. 100 mL. 5 mL of the plant extracts was pipetted out into a 100 mL conical flask, added 10 mL of 4% oxalic acid and titrated against the dye (V_2 mL).

Calculation

$$\text{Amount of ascorbic acid mg/100 g sample} = \frac{0.5\,\text{mg} \times V_2\,\text{mL} \times 100\,\text{mL}}{V_1\,\text{mL} \times 5\,\text{mL} \times \text{Wt. of the sample}} \times 100$$

2.4.2 Estimation of starch
Starch was estimated by following the method of Sadasivam and Manickam [11]. 0.2 g of finely powdered samples of seeds, aerial parts and roots of Chicory were weighed and placed in 60 mL centrifuge tubes and added 20 mL of hot 80% alcohol to remove sugars. The tubes were then shaked for 5-10 min, centrifuged at 3000 rpm for 10 min and supernatant was decanted. The residue was again extracted repeatedly with hot 80% alcohol until the supernatant was free of sugars as judged by negative test with anthrone reagent. The residue was cooled in ice water and added 5.0 mL of water and 6.5 mL of 52% perchloric acid while stirring the contents with a glass rod. It was allowed to stand for 15 min with occassional stirring, centrifuged and supernatant fractions were collected. The extraction step using perchloric acid was repeated 2-3 times. All the supernatants were collected; pooled and final volume was made up to 100 mL with water. Then 0.2 mL aliquot of the extract was taken and made up to 1 mL with water. After that, 4 mL freshly prepared anthrone reagent was added, mixed properly and the tubes were transferred to boiling water bath and heated for 8 min. Then, the tubes were cooled rapidly under running tap water and the intensity of green to dark green colour was read at 630 nm using UV-Vis Double beam spectrophotometer Model 2203 (Systronics Co.) against a blank prepared similarly but containing respective solvent instead of extracts. A standard curve was prepared using 0 to 100 µg glucose as per the procedure described above. Amount of glucose was calculated in the sample aliquots from the standard curve and multiplied by a factor 0.9 to arrive at the starch content.

2.4.3 Estimation of tannins
Tannins content was estimated as catechin equivalent by vanillin-hydrochloric acid method [12]. Five hundred mg of powdered samples of seeds, aerial parts and roots of Chicory was taken in a 50 mL test tube and 10 mL of methanol was added to it. The tubes were closed with pith corks. The contents of the tubes were shaken occasionally and allowed to stand overnight at 25 to 32ºC. One mL of clear supernatant was then pipetted in a test tube and 5 mL of vanillin-HCl reagent was added to it. The absorbance of brownish red colour so produced was measured at 525 nm after 25 min on a Spectronic 20 colorimeter. A blank containing methanol was also run simultaneously. A standard curve of catechin was prepared simultaneously in order to calculate amount of tannin.

2.4.4 Estimation of total sugars
Total sugars were estimated by the modified phenol sulphuric acid method [13]. For estimation of total sugars in aqueous extracts of seeds, aerial parts and roots of Chicory, 1.0 mL of each extract was diluted with respective solvent to adjust the absorbance within calibration limits. Then, 2.0 mL of phenol solution (2%, w/v) was added followed by 5.0 mL concentrated sulphuric acid. Acid was added in such a way that it directly pours on the solution. The test tubes were allowed to cool for 30 min and absorbance of the solution was measured at 490 nm using UV-Vis Double beam spectrophotometer Model 2203 (Systronics Co.) against a blank prepared similarly but containing respective solvent instead of extracts. The amount of total sugars present in the extracts was calculated from the standard curve of glucose and the results are expressed as milligrams per gram.

2.4.5 Estimation of reducing sugars
Reducing sugars were estimated by the method of Nelson [14] as modified by Somogyi [15]. For estimation of reducing sugars in aqueous extracts of seeds, aerial parts and roots of Chicory, 1.0 mL of each extract was diluted with respective solvent to adjust the absorbance within calibration limits. Then, 1.0 mL distilled water was added, followed by addition of 1.0 mL alkaline copper reagent, solution was mixed, covered with aluminum foil and heated in boiling water bath for 20 min. The tubes were cooled to room temperature and 1.0 mL of arsenomolybdate reagent was added. The contents were mixed thoroughly and volume was made up to 10.0 mL with distilled water. The absorbance of the solution was measured at 520 nm using UV-Vis Double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank prepared similarly but containing respective solvents instead of extracts. The amount of reducing sugars present in the extracts was calculated from the standard curve and the results are expressed as milligrams per gram.

2.4.6 Estimation of non-reducing sugars
The non-reducing sugars were calculated from the difference between the content of total sugars and that of reducing sugars.
Non-reducing sugars = Total sugars – Reducing sugars

3. Results and Discussion
3.1 Proximate composition
The data of proximate composition of seeds, aerial parts and roots of Chicory is given in Table 1. Amongst different parts of Chicory, moisture content was highest in roots (9.88%) followed by 7.74% in aerial parts and 6.72% in seeds; fat content was highest in seeds (21.18%) followed by 6.54% in roots and 4.97% in aerial parts; ash content was highest in seeds (11.39%) followed by 8.00% in aerial parts and 3.87% in roots; protein content was highest in seeds (18.61%) followed by 7.50% in aerial parts and 5.06% in roots; crude fibre content was highest in roots (28.50%) followed by 25.80% in aerial parts and 23.70% in seeds; total carbohydrates content was highest in roots (46.15%) followed by 45.99% in aerial parts and 18.40% in seeds and calorific value was highest of seeds (338.63 kcal) followed by roots (263.72 kcal) and aerial parts (258.69 kcal).
Similar findings have also been reported by other research workers. Moisture content, fat content, ash content and protein content in two varieties of seeds of *C. intybus* grown in China ranged from 6.40 to 6.65, from 22.56 to 22.89, from 6.80 to 6.91 and from 19.20 to 19.57 g/100g, respectively [16]. Jan and co-workers [17] reported that ash content in roots, leaves and seeds of *C. intybus* was 8.12, 18.65 and 11.55%, respectively; protein content in seeds, leaves and roots of *C.*

intybus was 18.55, 14.10 and 5.54%, respectively; crude fibre content in leaves, seeds and roots of *C. intybus* was 17.61, 36.63 and 27.32%, respectively and carbohydrate content in leaves, seeds and roots of *C. intybus* was 49.31, 19.69 and 57.98%, respectively.

Table 1: Proximate composition of seeds, aerial parts and roots of Chicory

Parameter	Seeds	Aerial Parts	Roots
Moisture (%)	6.72 ± 0.04	7.74 ± 0.05	9.88 ± 0.02
Fat (%)	21.18 ± 0.48	4.97 ± 0.04	6.54 ± 0.03
Ash (%)	11.39 ± 0.06	8.00 ± 0.03	3.87 ± 0.04
Protein (%)	18.61 ± 0.13	7.50 ± 0.26	5.06 ± 0.13
Crude fibre (%)	23.70 ± 1.12	25.80 ± 0.15	28.50 ± 0.21
Total carbohydrates (%)	18.40 ± 0.62	45.99 ± 0.38	46.15 ± 0.37
Calorific value (kcal)	338.63 ± 7.12	258.69 ± 0.88	263.72 ± 0.84

3.2 Mineral composition
The data of mineral (Fe, Cu, Zn and Mn) composition of seeds, aerial parts and roots of Chicory is given in Table 2. Amongst different parts of Chicory, Fe content was highest in seeds (641.00 ppm) followed by in aerial parts (543.87 ppm) and roots (493.70 ppm); Cu content was highest in seeds (18.93 ppm) followed by in roots (8.60 ppm) and aerial parts (8.47 ppm); Zn content (Table 4) was highest in seeds (92.61 ppm) followed by in aerial parts (31.61 ppm) and roots (24.33 ppm) and Mn content was highest in seeds (43.20 ppm) followed by in aerial parts (42.67 ppm) and roots (27.13 ppm). Fe, Cu, Zn and Mn content in two varieties of seeds of *C. intybus* L. cv. Puna (Puna Chicory) and Commander (Commander Chicory) ranged from 0.33 to 0.50 mg/g, from 22.33 to 23.00 μg/g, from 55.50 to 60.83 μg/g and from 32.83

to 37.66 μg/g, respectively [16]. Fe and Cu content in flowers of chicory (*C. intybus*) cultivated in Southeast Serbia was 402 and 20.5 mg/kg, respectively [18]. Hence, our findings are in accordance with findings of other research workers.

Table 2: Minerals content (ppm) in seeds, aerial parts and roots of Chicory

Minerals ▼ Plant Part ➤	Mineral content (ppm)		
	Seeds	Aerial Parts	Roots
Fe	641.00 ± 3.75	543.87 ± 11.26	493.70 ± 8.58
Cu	18.93 ± 0.20	8.47 ± 0.03	8.60 ± 0.46
Zn	92.61 ± 0.39	31.61 ± 0.59	24.33 ± 1.46
Mn	43.20 ± 0.26	42.67 ± 1.39	27.13 ± 3.18

3.3 Chemical composition
The data of chemical composition of seeds, aerial parts and roots of Chicory is given in Table 3. Amongst different parts of Chicory, ascorbic acid content (mg/100g) was highest in aerial parts (43.11) followed by in seeds (23.85) and roots (9.34). Helaly and Abdullah [19] reported that ascorbic acid content in Chicory landraces collected from eight geographical regions in Egypt varied from 15.33 to 33.92 mg/100g.
Amongst different parts of Chicory, starch content (mg/g) was highest in aerial parts (17.28) followed by in seeds (5.57) and roots (3.22) and tannins content (mg/g) was highest in aerial parts (6.14) followed by in roots (2.21) and seeds (1.72). The findings of present studies are in accordance with Shad and co-workers [20] who reported that tannins content in root, stem, leaves and seeds of *C. intybus* was 0.607, 0.529, 0.260 and 0.431 g/100 g dry weight, respectively.

Table 3: Chemical composition of seeds, aerial parts and roots of Chicory

Parameter	Seeds	Aerial Parts	Roots
Ascorbic acid (mg/100g)	23.85 ± 0.28	43.11 ± 0.52	9.34 ± 0.29
Starch (mg/g)	5.57 ± 0.12	17.28 ± 0.31	3.22 ± 0.31
Tannins (mg/g)	1.72 ± 0.04	6.14 ± 0.08	2.21 ± 0.07
Total sugars (mg/g)	12.30 ± 0.12	19.48 ± 0.19	10.82 ± 0.09
Reducing sugars (mg/g)	2.15 ± 0.12	5.40 ± 0.10	3.50 ± 0.06
Non-reducing sugars (mg/g)	10.15 ±0.12	14.08 ± 0.29	7.32 ± 0.12

Amongst different parts of Chicory, total sugars content (mg/g) was highest in aerial parts (19.48) followed by in seeds (12.30) and roots (10.82); reducing sugars content (mg/g) was highest in aerial parts (5.40) followed by in roots (3.50) and seeds (2.15) and non-reducing sugars content (mg/g) was highest in aerial parts (14.08) followed by in seeds (10.15) and roots (7.32). Total sugars content, reducing sugars content and non-reducing sugars content in different parts of *C. intybus* cultivated in Pakistan ranged from 2.02 to 4.50 g/100g, from 0.13 to 0.44 g/100g and from 1.89 to 4.27 g/100g, respectively [5]. Hence, the findings of present study are in agreement with reported literature.

4. Conclusion
All parts of *C. intybus* possess good proximate and mineral composition. All parts of *C. intybus* were found to be very rich source of energy, on the basis of their calorific values. Different parts of Chicory contained significant amounts of ascorbic acid, starch, tannins, total sugars, reducing sugars and non-reducing sugars. Due to good proximate, mineral and chemical composition, chicory plant as a whole including seeds, aerial parts and roots would be of significant importance in pharmaceutical formulations.

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