

## Antioxidant and HPLC Quantification of Different Parts of *Cichorium intybus L.* Plant

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A research article measuring the antioxidant potential and quantification of bioactive moieties present in different parts of chicory plant.

It's related to food science and nutrition.

### ABSTRACT

Medicinal plants, including chicory (*Cichorium intybus L.*), have been utilized for centuries in traditional medicine due to their bioactive compounds. Chicory contains various phytochemicals such as inulin, tannin, alkaloids, flavonoids, coumarins, and sesquiterpene lactones, alongside essential nutrients like potassium, phosphorus, and vitamins A, K, and B6. It has antioxidant, anti-inflammatory, and anticancer effects. Its roots, rich in inulin, are used as a coffee substitute, while leaves and flowers are consumed in salads. It is a herbaceous shrub named "Kasani" cultivated all around the globe, and in Pakistan. The seeds, roots, stems, and leaves of chicory were procured from the fields manually cleaned and washed to remove dirt. After drying, it was ground into a fine powder and stored in air-tight containers for analysis. Proximate, HPLC quantification, antioxidant, and phytochemical profiling were performed. Results showed the different treatments explicated significant ( $p \geq 0.001$ ) variation among moisture content, crude protein, fat, crude fiber, ash, and nitrogen-free extract. Maximum moisture content was observed in roots, while seeds exhibited higher levels of crude fat, ash, and protein. The antioxidant potential varied across different treatments, with the combination of seeds, leaves, stems, and roots showing the highest total polyphenol content and DPPH scavenging activity. Phytochemical analysis revealed the presence of various bioactive compounds, with chicoric acid being predominant in leaves and caffeoylquinic acids abundant in seeds. This study highlights chicory's diverse bioactive compounds and underscores its potential health benefits. It emphasizes chicory's significance as a valuable source of therapeutic properties.

Keywords: antioxidants, chicory, HPLC, phytochemicals.

## Introduction

Historically, plants have been used to treat various ailments and as a source of therapeutic remedies in traditional medicine systems. These medicinal plants were the major resources that further helped in the discovery and development of antioxidants and bioactive moieties with varied therapeutic potential. There is a wide range of bioactive components that are present including polyphenols, flavonoid contents, alkaloids, terpenoids, and essential oils. The therapeutic potential of these substances was investigated and observed that they possess antioxidant, anticancer, antibacterial, antifungal, and anti-inflammatory properties and are utilized for the treatment of various physiological malfunctions (Zengin et al., 2020).

Chicory, or *Cichorium intybus L.*, is a member of the Asteraceae species and a Mediterranean vegetation. Six different species belonging to the genus *Cichorium* are also grown extensively throughout Asia and Europe. Out of the six varieties, *Cichorium intybus* has been employed as a homemade remedy for many ailments since ancient times (Singh & Chahal, 2018). It is herbaceous shrub generally referred to as "Kasani" cultivated all around the globe, particularly in Pakistan (David & Sears, 2007). Studies on the chicory plants and their pharmaceutical and anti-oxidative properties were conducted and 100 different compounds were characterized and analyzed (Street et al., 2013).

Inulin, tannin, alkaloids, monomeric flavonoids, coumarins, and sesquiterpene lactones are among the key phytochemicals that are predominantly present in the chicory plant (Nwafor et al., 2017; Varotto et al., 2000). Apart from its bioactive constituents, it also has considerable amount of macro and micro nutrients including potassium, phosphorus, zinc, iron, copper, carotenoids, and vitamins A, K, and B6 (Street et al., 2013). Fresh chicory root generally on dry weight comprises of 68% inulin, 5% cellulose, 14% sucrose, 4% ash, 6% proteins, and 3% additional constituents (Singh & Chahal, 2018). It was also observed that chicory root extract yields 98% inulin and 2% of other compounds. The flowers and leaves of the plant are consumed in salad, while the roots are considered as a coffee replacement (Mona et al., 2009). Chicory extracts are further utilized to enhance the flavour of both alcoholic and nonalcoholic beverages through fermentation (Bais & Ravishankar, 2001).

## Research Methodology

### Procurement of Raw Material

The chicory plant including seeds, roots, stems, and leaves were procured from the fields near Faisalabad. Plant was further identified by the taxonomist from Botany Department in G.C University Faisalabad and confirmed that its *Cichorium intybus L.*

All the analytical and HPLC grade standards and reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

### Preparation of Raw Materials

The seeds of the chicory were cleaned manually to remove broken pieces. Then seeds, leaves, stem and roots were washed with tap water to remove particles of dirt and foreign material. After cleaning, all parts of chicory plant were completely dried at room temperature. The dried plant parts were then further grounded to fine powder utilizing a small laboratory grinder (Panasonic, Japan, Model MJ-W176P) and were passed through sieve for refining and were stored separately in air-tight plastic jars till further analysis.

### Proximate analysis

The seeds, leaves, roots and stems of the chicory plant were divided in to separate combinations T<sub>1</sub> Chicory seeds (CSD), T<sub>2</sub> Chicory leaves (CLV), T<sub>3</sub> Chicory stems (CST), T<sub>4</sub> Chicory roots (CRT), T<sub>5</sub> CSD+CLV (Chicory seed + Chicory leaves), T<sub>6</sub> CSD + CST (Chicory seed + Chicory stem), T<sub>7</sub> CSD + CRT (Chicory seed + Chicory root), T<sub>8</sub> CLV + CST (Chicory leaves + Chicory stem), T<sub>9</sub> CLV + CRT (Chicory leaves + Chicory roots), T<sub>10</sub> CST + CRT (Chicory stem+ Chicory root), T<sub>11</sub> CSD + CLV + CST + CRT (Chicory seed + Chicory leaves + Chicory stem + Chicory roots).

Moisture content of all the combinations was measured following AACC Method No. 44-01. AACC Method No. 46-10 was used to measure the crude protein in different parts of chicory plant using Kjeldahl's equipment (Model: Technik GmbH D-40599, Behr Labor, Germany). Employing the Soxhlet equipment (Model: HT2 1045 Extraction unit, Hoganas, Sweden), specimens of chicory were examined for crude fat according to AACC Method No. 30-10. Crude Fiber was measured using (Model: Labconco corporation, Kansas, USA), following the procedure of AACC Method No. 32-10. The ash percentage of chicory isolates was assessed as indicated in AACC Method No. 08-01 (AACC, 2000).

## Preparation of Extract

Bioactive compounds from all the eleven treatments with different combinations of roots, stems, leaves and seeds were extracted with methanol:water (70:30 v/v) following a 1:10 sample-to-solvent ratio and water, Whatman No. 1 filter paper was used to separate the extract from the residue. Later, extracts were concentrated utilizing rotary evaporator and were stored at 4°C until tested and analyzed.

## Antioxidant and phytochemical profiling of chicory

Following Singleton *et al.* (1999)'s methodology, the total polyphenols (TP) were estimated using the Folin-Ciocalteu procedure. The 1,1-diphenyl-2-picrylhydrazyl (1,1-diphenyl-2-picrylhydrazyl) free radicals capturing capability of chicory isolates were determined using Muller *et al.* (2011) technique. Whereas, Total flavonoids were estimated following the procedure of Ordon-ez *et al.* (2006).

## Quantification through HPLC

Implementing the method of Bocchini *et al.*, (2001) several chicory isolate preparations HPLC quantitative measurement method for determination. The HPLC Shim-Pack CLC-ODS C18 column (15 cm x 4.6 mm, size distribution at 5.0 µm) with ultra violet to visible monitor was utilized to carry out the operation. The 20-L specimen was submitted using an autosampler (WISP Model 710). The interior column's temperatures being kept constant at 25 ° c. A mobile phase made up of DDHO2: Methanol (1:1) was used to estimate the amount of allicin in experimental samples at 254 nm although the rate of circulation was fixed at 1.0 mL per minute.

## Statistical Analysis

The information was collected utilizing a completely randomised methodology and then statistical evaluation was completed applying the Statistical Package for the Social Sciences (SPSS) (Costat-2003, Co-Hort, v 6.1.). According to the guidelines provided by Steel *et al.*, (1997) the level of significance was established (ANOVA) using 2-factor factorial CRD where feasible.

## Results and Discussion

### Proximate Analysis

The results of the different treatments explicated significant ( $p \geq 0.001$ ) variation among moisture content, crude protein, fat, crude fiber, ash and nitrogen free extract. The results varying proximate composition of different part of *C. intybus* illustrated in (Table 1) showed maximum moisture in the T<sub>4</sub> (CRT) 9.75±0.56% followed by T<sub>9</sub> (CLV + CRT)

8.97±0.75%, T<sub>10</sub> (CST + CRT) 8.49±0.67%, T<sub>7</sub> (CSD + CRT) 8.31±0.78%, T<sub>2</sub> (CLV) 8.25±0.62%, T<sub>11</sub> (CSD + CLV + CST + CRT) 8.02±0.72%, T<sub>8</sub> (CLV + CST) 7.74±0.63%, T<sub>5</sub> (CSD + CLV) 7.56±0.52%, T<sub>3</sub> (CST) 7.25±0.67%, T<sub>6</sub> (CSD + CST) 7.02±0.34 and minimum was recorded in T<sub>1</sub> (CSD) 6.88±0.56% respectively. Moreover, crude fat contents were observed as 12.52±1.12%, 1.35±0.11%, 1.39±0.12%, 1.49±0.13%, 6.93±0.54, 6.95±0.56, 7.01±0.67, 1.36±0.09, 1.41±0.12, 1.43±0.12 and 4.17±0.38 in T<sub>1</sub> (CSD), T<sub>2</sub> (CLV), T<sub>3</sub> (CST), T<sub>4</sub> (CRT), T<sub>5</sub> (CSD + CLV), T<sub>6</sub> (CSD + CST), T<sub>7</sub> (CSD + CRT), T<sub>8</sub>(CLV + CST), T<sub>9</sub> (CLV + CRT), T<sub>10</sub> (CST + CRT) and T<sub>11</sub> (CSD + CLV + CST + CRT) respectively.

Similarly, protein and fibre contents in T<sub>1</sub> (CSD), T<sub>2</sub> (CLV), T<sub>3</sub> (CST), T<sub>4</sub> (CRT), T<sub>5</sub> (CSD + CLV), T<sub>6</sub> (CSD + CST), T<sub>7</sub> (CSD + CRT), T<sub>8</sub>(CLV + CST), T<sub>9</sub>(CLV + CRT), T<sub>10</sub> (CST + CRT) and T<sub>11</sub> (CSD + CLV + CST + CRT) were 16.92±1.32 & 37.48±2.45, 11.48±1.12 & 17.58±1.65, 7.89±0.67 & 28.65±1.26%, 5.35±0.48 & 29.56±1.67, 14.2±0.11 & 27.53±1.54, 12.4±0.11 & 33.06±2.43, 11.13±0.09 & 33.52±3.24, 9.68±0.89 & 23.11±2.12, 8.41±0.76 & 23.57±2.32, 6.61±0.54 & 29.1±2.86 and 10.4±0.08 & 28.31±2.32 correspondingly. The ash contents were recorded as 14.67±1.32% in T<sub>1</sub> (CSD), 8.75±0.65, 11.03±1.32, 4.08±0.34, 11.7±1.12, 12.84±1.21, 9.37±0.87, 9.88±0.76, 6.41±0.56, 7.55±0.62 and 9.61±0.87% in T<sub>2</sub> (CLV), T<sub>3</sub> (CST), T<sub>4</sub> (CRT), T<sub>5</sub> (CSD + CLV), T<sub>6</sub> (CSD + CST), T<sub>7</sub> (CSD + CRT), T<sub>8</sub> (CLV + CST), T<sub>9</sub> (CLV + CRT), T<sub>10</sub> (CST + CRT) and T<sub>11</sub> (CSD + CLV + CST + CRT), substantially. Moreover, the individual specimens' reported NFE levels as 11.53±1.12, 52.59±4.32, 43.79±3.23, 49.77±3.46, 32.08±2.56, 27.73±2.67, 30.67±2.54, 48.23±3.34, 51.23±4.32, 46.82±3.43 and 39.49±2.87 % in T<sub>1</sub> (CSD), T<sub>2</sub> (CLV), T<sub>3</sub> (CST), T<sub>4</sub> (CRT), T<sub>5</sub> (CSD + CLV), T<sub>6</sub> (CSD + CST), T<sub>7</sub> (CSD + CRT), T<sub>8</sub>(CLV + CST), T<sub>9</sub> (CLV + CRT), T<sub>10</sub> (CST + CRT) and T<sub>11</sub> (CSD + CLV + CST + CRT), respectively.

The findings of the current research work is in accordance with Jangra and Madan (2018) estimated three different parts of *C. intybus* seeds, aerial parts and roots and observed maximum amount of moisture, fiber and total carbohydrates in roots whereas, fat, ash, and protein were higher in seeds. Khalaf et al. (2018) observed that carbohydrates content were observed maximum in root followed by leaf and seed. The plant defense system is mainly dependent on the carbohydrate content. The variation in the nutritional content is due to environmental conditions like weather as well as on species of plant and the cultivation method adopted (Petropoulos et al., 2017). The nine

different varieties of *Cichorium intybus L.* including seven wild and two cultivated were estimated by Jančić et al. (2016) for the nutritional composition and found ash in range of (1.05-1.58%), fat content (0.22-0.49%), dietary fiber (2.90-6.16%) and protein as (1.63-2.78%).

### Antioxidant Indices of Spices Extracts

The means regarding TPC of solvent extracts (Table 2) showed that the highest TPC  $332.89 \pm 31.52$  mg/100g was observed in T<sub>11</sub> (CSD + CLV + CST + CRT) followed by T<sub>1</sub> (CSD)  $310 \pm 28.23$  mg/100g, T<sub>7</sub> (CSD + CRT)  $294.50 \pm 26.97$  mg/100g, T<sub>4</sub> (CSD + CLV)  $279 \pm 26.81$  mg/100g, T<sub>5</sub> (CSD + CLV)  $188.62 \pm 17.65$  mg/100g, T<sub>9</sub> (CLV + CRT)  $173.11 \pm 15.23$  mg/100g, T<sub>6</sub> (CSD + CST)  $159.78 \pm 14.23$  mg/100g, T<sub>10</sub> (CST + CRT)  $144.27 \pm 12.17$  mg/100g, T<sub>2</sub> (CLV)  $67.23 \pm 5.23$  mg/100g, T<sub>8</sub> (CLV + CST)  $38.39 \pm 2.52$  mg/100g and minimum output in T<sub>3</sub> (CST)  $9.59 \pm 0.64$  mg/100g.

The mean values indicate that the maximum TFC was noticed in T<sub>1</sub> 135 mg/100g trailed by T<sub>11</sub>, T<sub>5</sub>, T<sub>6</sub>, T<sub>7</sub>, T<sub>2</sub>, T<sub>8</sub>, T<sub>9</sub>, T<sub>3</sub>, T<sub>10</sub> and T<sub>4</sub> as 98.29, 88.63, 77.42, 67.24, 42.86, 31.05, 21.47, 19.25, 9.66 and 0.09 mg/100g, respectively (Table 2).

DPPH activity in (Table 2) illustrate the mean values in T<sub>11</sub> 36.42 IC<sub>50</sub> µg/ml, T<sub>1</sub>  $29 \pm 1.35$  IC<sub>50</sub> µg/ml, T<sub>5</sub>  $26.81 \pm 1.78$  IC<sub>50</sub> µg/ml, T<sub>2</sub>  $24.62 \pm 1.46$  IC<sub>50</sub> µg/ml, T<sub>7</sub>  $23.31 \pm 2.12$  IC<sub>50</sub> µg/ml, T<sub>9</sub>  $21.31 \pm 1.78$  IC<sub>50</sub> µg/ml, T<sub>4</sub>  $18 \pm 1.23$  IC<sub>50</sub> µg/ml, T<sub>6</sub> (CSD + CST)  $15.11 \pm 1.12$  IC<sub>50</sub> µg/ml, T<sub>8</sub>  $12.92 \pm 1.23$  IC<sub>50</sub> µg/ml, T<sub>10</sub>  $9.61 \pm 0.89$  IC<sub>50</sub> µg/ml and T<sub>3</sub>  $1.23 \pm 0.56$  IC<sub>50</sub> µg/ml.

Polyphenols can be extracted from plant materials by various methods using different solvents and extraction techniques. The extraction procedure is of prime importance in the analysis of polyphenols (Aires, 2017). The polyphenols and antioxidant activity of chicory aerial plant (stem and leaves) ethanolic extract were estimated by Iqbal et al. (2021) and observed TPC as 0.44 mg GAE/g, TFC as 0.77 mgQE/g and DPPH as 0.12 mg AAE/g. The phenolic contents of the plants are highly associated with its antioxidant potential. Similarly, El-Mehy (2018) conducted a research to evaluate the TPC and TFC content in Chicory leaf and root with different concentrations and solvents and observed maximum content in leaf. The addition of water in alcohol enhance the efficiency and extractability of alcohol, providing assistance in the formation of hydroxyl bond between phenolic compound and water (Dai & Mumper, 2010). Later, Choudhary et al. (2021) investigated different parts including roots, leaves and seeds of *Cichorium intybus L.* and observed total flavonoid varied from  $1.60 \pm 1.69$  to  $1.37 \pm 0.796$  mg/gdw (CA) and total

phenolic content as  $1.94 \pm 0.895$  to  $1.66 \pm 0.506$  mg/gdw (QE) of methanolic extract. Similarly Eray et al. (2020) observed maximum TPC and TFC in roots in comparison with leaves and shoots.

### 1.4.3. Phytochemical or Bioactive Molecules in chicory plant

Mean values in Chicoric acid illustrated in treatments *i.e* T<sub>2</sub> (CLV), T<sub>5</sub> (CSD + CLV), T<sub>6</sub> T<sub>8</sub> (CLV + CST) and T<sub>11</sub> (CSD + CLV + CST + CRT) showed  $2.97 \pm 1.13 \mu\text{g/g}$ ,  $1.43 \pm 0.09 \mu\text{g/g}$ ,  $1.42 \pm 0.12 \mu\text{g/g}$ ,  $1.46 \pm 0.11 \mu\text{g/g}$  and  $0.73 \pm 0.03 \mu\text{g/g}$  whereas it was not detected in other treatments, respectively (Table 4). The mean values in (Table 4) illustrate caffeoylquinic acids in T<sub>1</sub> (CSD), T<sub>2</sub> (CLV), T<sub>4</sub> (CRT), T<sub>5</sub> (CSD + CLV), T<sub>6</sub> (CSD + CST), T<sub>7</sub> (CSD + CRT), T<sub>8</sub> (CLV + CST), T<sub>9</sub> (CLV + CRT), T<sub>10</sub> (CST + CRT) and T<sub>11</sub> (CSD + CLV + CST + CRT) as  $9.6 \pm 8.54$ ,  $1.35 \pm 0.32$ ,  $0.5 \pm 0.02$ ,  $5.47 \pm 0.21$ ,  $4.8 \pm 0.32$ ,  $5.05 \pm 0.43$ ,  $0.67 \pm 0.04$ ,  $0.92 \pm 0.03$ ,  $0.25 \pm 0.02$  and  $2.87 \pm 0.01 \mu\text{g/g}$ , whereas in T<sub>3</sub> (CST) it was not detected, respectively.

The mean values indicate that the maximum Chlorogenic acid was noticed in T<sub>2</sub> (CLV)  $26.66 \pm 2.32 \mu\text{g/g}$  trailed by T<sub>9</sub> (CLV + CRT)  $23.67 \pm 2.12$ , T<sub>4</sub> (CRT)  $20.68 \pm 2.14$ , T<sub>8</sub> (CLV + CST)  $14.18 \pm 1.12$ , T<sub>5</sub> (CSD + CLV)  $13.33 \pm 1.12$ , T<sub>11</sub> (CSD + CLV + CST + CRT)  $12.26 \pm 1.09$ , T<sub>10</sub> (CST + CRT)  $11.19 \pm 1.11$ , T<sub>3</sub> (CST)  $1.71 \pm 0.12$  and T<sub>6</sub> (CSD + CST)  $0.85 \pm 0.02 \mu\text{g/g}$ , whereas it was not-detected in T<sub>1</sub> (CSD), respectively. The means regarding p-Coumaric acid showed that the highest p-Coumaric acid in  $27.81 \pm 2.13 \mu\text{g/g}$  was observed in T<sub>4</sub> (CRT) followed by CRT  $4.74 \pm 0.32 \mu\text{g/g}$ , T<sub>9</sub> (CLV + CRT)  $19.3 \pm 1.12$ , T<sub>7</sub> (CSD + CRT)  $13.91 \pm 1.12$ , T<sub>2</sub> (CLV)  $10.77 \pm 1.05$ , T<sub>11</sub> (CSD + CLV + CST + CRT)  $9.8 \pm 0.89$ , T<sub>8</sub> (CLV + CST)  $5.69 \pm 0.43$ , T<sub>5</sub> (CSD + CLV)  $5.39 \pm 0.45$ , T<sub>3</sub> (CST)  $0.61 \pm 0.02$  and minimum values were recorded in T<sub>6</sub> (CSD + CST)  $0.3 \pm 0.02$ , whereas not detected in T<sub>1</sub> (CSD), respectively.

Similarly, Caffeic acid and gallic acid contents in T<sub>2</sub> (CLV), T<sub>3</sub> (CST), T<sub>4</sub> (CRT), T<sub>5</sub> (CSD + CLV), T<sub>6</sub> (CSD + CST), T<sub>7</sub> (CSD + CRT), T<sub>8</sub> (CLV + CST), T<sub>9</sub> (CLV + CRT), T<sub>10</sub> (CST + CRT) and T<sub>11</sub> (CSD + CLV + CST + CRT) were  $17.07 \pm 1.13$  &  $3.52 \pm 0.31$ ,  $1.24 \pm 0.11$  &  $10.73 \pm 1.67$ ,  $13.93 \pm 1.11$  &  $4.74 \pm 0.34$ ,  $8.53 \pm 0.78$  &  $1.76 \pm 0.12$ ,  $0.62 \pm 0.04$  &  $5.36 \pm 0.43$ ,  $6.96 \pm 0.65$  &  $2.37 \pm 0.21$ ,  $9.15 \pm 0.89$  &  $7.12 \pm 0.67$ ,  $9.49 \pm 0.87$  &  $4.13 \pm 0.32$ ,  $1.58 \pm 0.12$  &  $7.73 \pm 0.66$ ,  $8.06 \pm 0.78$  &  $4.74 \pm 0.43$  and in T<sub>1</sub> (CSD) it was not detected, correspondingly.

HPLC is a technique that is frequently utilized for the most plant based sources for the quantification of polyphenols. The pharmaceutical properties of phenolic acids with

bioactive moieties provide them the ability to reduce physiological malfunctioning. These are the main constituents that are accountable for the high antioxidant potential of plant extracts. Iqbal et al. (2021) studied chicory plant for its phenolic compounds and observed chicoric acid was maximum in comparison with other. Similarly Mona et al. (2009) investigated the roots and leaves of chicory and found caffeic acid was maximum in leaves in comparison with roots whereas, p-coumaric acid was maximum in roots. Later Saybel et al. (2020) observed 8.3 % of chicoric acid in methanolic extract of chicory ariel part. They also observed that dominant substances were esculetin, chicoryin, and chicoryic acid. Jurgonński et al (2011) investigated roots, seeds and leaves of chicory and explicated that chicoric acid was only found in leaves and not identified in roots and seeds.

### **Conclusion**

It is inferred as chicory plant showed promising nutritional and antioxidant potential in different parts of the plants as well as in there combination. The Total phenolic content were maximum in the combination of stem, roots, seeds and leaves. However, Chicoric acid is an active compound present in leaves only whereas, chlorogenic acid, p-coumaric acid, gallic acid and caffeic acid were not identified in seeds and caffeoylquinic acid was not identified in stems. However, it is recommended that in future effect of supercritical fluid extraction and ultra sound extraction techniques should be evaluated.



Table 1 Means for proximate analysis of chicory plant

Treatments	Moisture (%)	Crude fat (%)	Crude protein (%)	Crude fiber (%)	Ash (%)	NFE (%)
T <sub>1</sub>	6.88±0.56k	12.52±1.12a	16.92±1.32a	37.48±2.45a	14.67±1.32a	11.53±1.12k
T <sub>2</sub>	8.25±0.62e	1.35±0.11k	11.48±1.12d	17.58±1.65dk	8.75±0.65h	52.59±4.32a
T <sub>3</sub>	7.25±0.67i	1.39±0.12i	7.89±0.67g	28.65±1.26f	11.03±1.32d	43.79±3.23f
T <sub>4</sub>	9.75±0.56a	1.49±0.13f	5.35±0.48i	29.56±1.67	4.08±0.34k	49.77±3.46c
T <sub>5</sub>	7.56±0.52h	6.93±0.54d	14.20±0.11b	27.53±1.54h	11.70±1.12c	32.08±2.56h
T <sub>6</sub>	7.02±0.34j	6.95±0.56c	12.40±0.11c	33.06±2.43c	12.84±1.21b	27.73±2.67j
T <sub>7</sub>	8.31±0.78d	7.01±0.67b	11.13±0.09de	33.52±3.24bj	9.37±0.87g	30.67±2.54i
T <sub>8</sub>	7.74±0.63g	1.36±0.09j	9.68±0.89f	23.11±2.12i	9.88±0.76e	48.23±3.34d
T <sub>9</sub>	8.97±0.75b	1.41±0.12h	8.41±0.76g	23.57±2.32	6.41±0.56j	51.23±4.32b
T <sub>10</sub>	8.49±0.67c	1.43±0.12g	6.61±0.54h	29.10±2.86e	7.55±0.62i	46.82±3.43e
T <sub>11</sub>	8.02±0.72f	4.17±0.38e	10.4±0.08ef	28.31±2.32g	9.61±0.87f	39.49±2.87g

T<sub>1</sub> = Chicory seeds (CSD) powderT<sub>2</sub> = Chicory leaves (CLV) powderT<sub>3</sub> = Chicory stems (CST) powderT<sub>4</sub> = Chicory roots (CRT) powderT<sub>5</sub> = CSD + CLV powderT<sub>6</sub> = CSD + CST powderT<sub>7</sub> = CSD + CRT powderT<sub>8</sub> = CLV + CST powderT<sub>9</sub> = CLV + CRT powderT<sub>10</sub> = CST + CRT powderT<sub>11</sub> = CSD + CLV + CST + CRT powder

Table 2. Mean values for TPC, TFC &amp; DPPH

Treatments	TPC (mg GAE/100g)	TFC (mg QE/100g)	DPPH (IC50)
T <sub>1</sub>	310±28.2 b	135±12.54 a	29±1.35 b
T <sub>2</sub>	67.23±5.23 i	42.86±3.26 f	24.62±1.46 d
T <sub>3</sub>	9.59±0.64 k	19.25±1.54 i	1.23±0.56 k
T <sub>4</sub>	279±26.81 d	0.09±0.004 k	18±1.23 g
T <sub>5</sub>	188.62±17.65 e	88.63±7.54 c	26.81±1.78 c
T <sub>6</sub>	159.78±14.23 g	77.42±6.53 d	15.11±1.12 h
T <sub>7</sub>	294.50±26.97 c	67.24±4.24 e	23.31±2.12 f
T <sub>8</sub>	38.39±2.52 j	31.05±2.12 g	12.92±1.23 i
T <sub>9</sub>	173.11±15.23 f	21.47±1.57 h	21.31±1.78 f
T <sub>10</sub>	144.27±12.17 h	9.66±0.53 j	9.61±0.89 j
T <sub>11</sub>	332.89±31.52 a	98.29±8.65 b	36.42±2.65 a

T<sub>1</sub> = Chicory seeds (CSD) extract

T<sub>2</sub> = Chicory leaves (CLV) extract

T<sub>3</sub> = Chicory stems (CST) extract

T<sub>4</sub> = Chicory roots (CRT) extract

T<sub>5</sub> = CSD + CLV extract

T<sub>6</sub> = CSD + CST extract

T<sub>7</sub> = CSD + CRT extract

T<sub>8</sub> = CLV + CST extract

T<sub>9</sub> = CLV + CRT extract

T<sub>10</sub> = CST + CRT extract

T<sub>11</sub> = CSD + CLV + CST + CRT extract

Table 3. Mean values for HPLC Quantification

Treatments	Chicoric acid ( $\mu\text{g/g}$ )	Caffeoylquinic acids ( $\mu\text{g/g}$ )	Chlorogenic acid ( $\mu\text{g/g}$ )	p-Coumaric acid ( $\mu\text{g/g}$ )	Caffeic acid ( $\mu\text{g/g}$ )	Gallic acid ( $\mu\text{g/g}$ )
T <sub>1</sub>	ND	9.6 $\pm$ 8.54a	ND	ND	ND	ND
T <sub>2</sub>	2.97 $\pm$ 1.13a	1.35 $\pm$ 0.32f	26.66 $\pm$ 2.32a	10.77 $\pm$ 1.05e	17.07 $\pm$ 1.13a	3.52 $\pm$ 0.31g
T <sub>3</sub>	ND	ND	1.71 $\pm$ 0.12i	0.61 $\pm$ 0.02i	1.24 $\pm$ 0.11i	10.73 $\pm$ 1.67a
T <sub>4</sub>	ND	0.5 $\pm$ 0.02i	20.68 $\pm$ 2.14c	27.81 $\pm$ 2.13a	13.93 $\pm$ 1.11b	4.74 $\pm$ 0.34e
T <sub>5</sub>	1.43 $\pm$ 0.09b	5.47 $\pm$ 0.21b	13.33 $\pm$ 1.12e	5.39 $\pm$ 0.45h	8.53 $\pm$ 0.78e	1.76 $\pm$ 0.12i
T <sub>6</sub>	ND	4.8 $\pm$ 0.32d	0.85 $\pm$ 0.02j	0.3 $\pm$ 0.02j	0.62 $\pm$ 0.04j	5.36 $\pm$ 0.43d
T <sub>7</sub>	ND	5.05 $\pm$ 0.43c	10.34 $\pm$ 0.12h	13.91 $\pm$ 1.12d	6.96 $\pm$ 0.65h	2.37 $\pm$ 0.21h
T <sub>8</sub>	1.42 $\pm$ 0.12b	0.67 $\pm$ 0.04h	14.18 $\pm$ 1.12d	5.69 $\pm$ 0.43g	9.15 $\pm$ 0.89d	7.12 $\pm$ 0.67c
T <sub>9</sub>	1.46 $\pm$ 0.11bc	0.92 $\pm$ 0.03g	23.67 $\pm$ 2.12b	19.3 $\pm$ 1.12b	9.49 $\pm$ 0.87c	4.13 $\pm$ 0.32f
T <sub>10</sub>	ND	0.25 $\pm$ 0.02j	11.19 $\pm$ 1.11g	14.21 $\pm$ 1.12c	1.58 $\pm$ 0.12h	7.73 $\pm$ 0.66b
T <sub>11</sub>	0.73 $\pm$ 0.03c	2.87 $\pm$ 0.01e	12.26 $\pm$ 1.09f	9.8 $\pm$ 0.89f	8.06 $\pm$ 0.78f	4.74 $\pm$ 0.43e

T<sub>1</sub> = Chicory seeds (CSD) extractT<sub>2</sub> = Chicory leaves (CLV) extractT<sub>3</sub> = Chicory stems (CST) extractT<sub>4</sub> = Chicory roots (CRT) extractT<sub>5</sub> = CSD + CLV extractT<sub>6</sub> = CSD + CST extractT<sub>7</sub> = CSD + CRT extractT<sub>8</sub> = CLV + CST extractT<sub>9</sub> = CLV + CRT extractT<sub>10</sub> = CST + CRT extractT<sub>11</sub> = CSD + CLV + CST + CRT extract

## References

- AACC. (2000). Approved methods of the American Association of Cereal Chemists, 10<sup>th</sup> Ed. AACC, St. Paul, MN, USA.
- Aires, A. (2017). Phenolics in foods: extraction, analysis, and measurements. *Phenolic Compounds*, 61-88.
- Bais, H. P., & Ravishankar, G. A. (2001). Cichorium intybus L–cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects. *Journal of the Science of Food and Agriculture*, 81(5), 467-484.
- Bocchini, P., Andalo, C., Pozzi, R., Galletti, G. C., & Antonelli, A. (2001). Determination of diallyl thiosulfinate (allicin) in garlic (*Allium sativum* L.) by high-performance liquid chromatography with a post-column photochemical reactor. *Analytica Chimica Acta*, 441(1), 37-43.
- Choudhary, M., Rajput, P., Sharma A. & Sharma, R. A. (2021). Assessment of total flavonoid and polyphenol content of different parts of *cichorium intybus* L. *Internation Journal of Pharmaceutical Science and Research*, 12(7): 3769-3773.
- Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313-7352.
- Dar, R. A., Shahnawaz, M., Ahanger, M. A., & Majid, I. (2023). Exploring the diverse bioactive compounds from medicinal plants: a review. *The journal of Phytopharmacology*, 12, 189-195.
- David D. C., & Sears, B. (2007). Chicory: An Alternative Livestock Forage. *Agric. Nat. Resour.* 4: 190-191.
- EL-Mehy, M. E. M. (2018). Studies on using extracts of chicory (*cichorium intybus*) herb treated by gamma irradiation in some food products (*Doctoral dissertation, MSc. Thesis, Fac. of Agric., Moshtohor, Benha Univ., Egypt*).
- Eray, N., Kartal, D. İ., & Çelik, İ. (2020). Antioxidant properties of Cichorium intybus L.(chicory) extracts and their cytotoxic effects on HepG2 cells. *Yuzuncu Yil University Journal of Agricultural Sciences*, 30(3), 444-453.
- Iqbal, Y., Ponnambalam, E. N., Suleria, H. A., Cottrell, J. J., & Dunshea, F. R. (2021). LC-ESI/QTOF-MS profiling of chicory and lucerne polyphenols and their antioxidant activities. *Antioxidants*, 10(6), 932.
- Jančić, D., Todorović, V., Basić, Z., & Šobajić, S. (2016). Chemical composition and nutritive potential of Cichorium intybus L. leaves from Montenegro. *Journal of the Serbian Chemical Society*, 81(10), 1141-1149.

- Jangra, S. S., & Madan, V. K. (2018). Proximate, mineral and chemical composition of different parts of chicory (*Cichorium intybus* L.). *Journal of Pharmacognosy and Phytochemistry*, 7(5), 3311-3315.
- Jurgoński, A., Milala, J., Juśkiewicz, J., Zduńczyk, Z., & Król, B. (2011). Composition of chicory root, peel, seed and leaf ethanol extracts and biological properties of their non-inulin fractions. *Food Technology and Biotechnology*, 49(1), 40-47.
- Khalaf, H. H. A., El Saadani, R. M. A., EL Desouky, A., Abdeldaiem, M. H. & El Mehy, M. E. (2018). Antioxidant and antimicrobial activity of gamma irradiated chicory (*Cichorium intybus* L.) leaves and roots. *Annals of Agricultural Science, Moshtohor*, 56(1), 51-60.
- Mona, I. M., Wafaa, A. A., & Elgindy, A. A. (2009). Chemical and technological studies on chicory (*Cichorium intybus* L.) and its applications in some functional food. *Journal of Advanced Agricultural Research*, 14(3), 735-42.
- Nwafor, I. C., Shale, K., & Achilonu, M. C. (2017). Chemical composition and nutritive benefits of chicory (*Cichorium intybus*) as an ideal complementary and/or alternative livestock feed supplement. *The Scientific World Journal*, 2017(1), 7343928.
- Ordonez, A. A. L., Gomez, J. D., & Vattuone, M. A. (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food chemistry*, 97(3), 452-458.
- Petropoulos, S. A., Levizou, E., Ntatsi, G., Fernandes, Â., Petrotos, K., Akoumianakis, K., ... & Ferreira, I. C. (2017). Salinity effect on nutritional value, chemical composition and bioactive compounds content of *Cichorium spinosum* L. *Food Chemistry*, 214, 129-136.
- Saybel, O. L., Rendyuk, T. D., Dargaeva, T. D., Nikolaev, S. M., & Khobrakova, V. B. (2020). Phenolic compounds and immunomodulating activity of chicory (*Cichorium intybus* L.) extract. *Pharmacognosy Journal*, 12(5).
- Singh, R., & Chahal, K. K. (2018). *Cichorium intybus* L: A review on phytochemistry and pharmacology. *International Journal of Chemical Studies*, 6(3), 1272-1280.
- Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteu reagent. *Methods in Enzymology*, 299: 152-178.
- Steel, R.G.D., Torrie, J.H. and Dickey, D. (1997). Principles and procedures of statistics: a biometrical approach, 3rd ed. McGraw Hill Book Co., Inc., New York.

- Street, R. A., Sidana, J., & Prinsloo, G. (2013). Cichorium intybus: Traditional uses, phytochemistry, pharmacology, and toxicology. *Evidence-Based Complementary and Alternative Medicine*, 2013.
- Varotto, S., Lucchin, M., & Parrini, P. (2000). Immature embryos culture in Italian red chicory. *Plant cell, tissue and organ culture*, 62(1), 75-77.
- Zengin G, Sarikurkcu C, Aktumsek A. (2020). Phenolic constituents, antioxidant and enzyme inhibitory activities of *Cruciata taurica* Endemic to Turkey. *Chemistry & Biodiversity*. 17(2):e1900506.