



Characterization and Identification of Antioxidant and Bioactive Components of *Beta Vulgaris L.* (Root and Leaves)

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Abstract

This research evaluated the bioactive components of sugar beet roots and leaves. Sugar beet root is mostly consisting of carbohydrates (59.41 ± 0.10 g/100g dry weight), followed by protein and fats (34.05 ± 0.02 g/100g and 0.29 ± 0.05 g/100g dry weight, respectively). The major components of leaves were protein (46.63 ± 0.05 g/100g dry weight), lipids, and carbs (16.16 ± 0.02 g/100g - 6.85 ± 0.10 g/100g dry weight). Total phenolic and flavonoid chemicals (g per 100g). sugar beet leaves (0.597 ± 0.003) (0.374 ± 0.025) were higher than sugar beet root (0.386 ± 0.003) (0.282 ± 0.004) respectively. Scavenging radical effect on 2, 2-diphenyl picrylhydrazyl (DPPH) radical-scavenging activity was studied. The antioxidant as well as total extract yield of sugar beet leaves ($56.96 \pm 6.32\%$) ($31.68 \pm 0.03\%$) were higher than sugar beet root ($56.08 \pm 5.10\%$) ($30.27 \pm 0.16\%$) respectively in methanolic extract. The phenolic and flavonoid contents of the aqueous, ethanolic as well as methanolic extracts were measured by HPLC. The different extracts included 17 phenolic and flavonoid substances. The root and leaves of *Beta vulgaris L.* contained the greatest amount of chlorogenic acid, gallic acid, caffeic acid, syringic acid, catechin, daidzein, quercetin, and ellagic acid.

Key words: - *Beta vulgaris L.* , Sugar beet , antioxidant , phenolic, and flavonoid compounds.

Introduction

One of the most significant crops is the sugar beet (*Beta vulgaris L.*) which also a significant supply of feed and organic elements that improve soil fertility. Europe, Canada, and Russia are examples of northern hemisphere regions with moderate climates where sugar beet is farmed. In Egypt, sugar beet has just lately been included into industrial and agricultural operations. On the other hand, more land is cultivated with sugar beet as well as white sugar. In the Arab Republic of Egypt, sugar beet became the primary source of sugar production. This means that around 80% of sugar cane is produced, with sugar beet accounting for the remaining 20%. With an emphasis on sugar beet processing, Europe is the universe's largest manufacturer of sugar, representing for about 80% of world manufacturing **Rezbová et al., (2013)** and **Abd El-Rahman and El-Geddayw. (2019)**.

Sugar beet is the second-highest sugar crop in the worldwide, sugar beet is a multipurpose industrial production. In addition to producing useful byproducts the same as the sugar and molasses in beet meal, it could be recycled into biofuels, its leaves and roots give animals important nutrients. **Wang, Y. et al., (2024)**.

The primary source of sugar in the world, after sugarcane, is the sugar beet. Other than

sugarcane, tropical sugar beet is a agricultural product that was produced commercially for its high sucrose content. It may be a highly important crop for the manufacturing sugar. The beet's crown, tends to be level with or slightly above the ground, is where the many, broad leaves appear in a tuft. **Tabriz et al., (2015)**.

Moisture (77.30%), carbs (18.33%), proteins (0.51%), total consumption of fiber (2.41%), fats (0.15%), mineral content (0.53%), vitamins (0.09%), and ash (0.60%) make up the average chemical profile of sugar beets. **Görgülü (2025)**. Carbs (46-71%), protein (18-25%), fiber (7-36%), lipids (2-5%), as well as bioactive substances such as phenolic acids, flavonoids, betalains, carotenoids, the chlorophyll, vitamins, and trace minerals are all in presence sugar beetroot leaves. With a high protein content and all the essential amino acids, sugar beetroot leaves have a lot of potential as a plant protein source. **Stoica, et al., (2025)**. Sugar beet (*Beta vulgaris L.*) is a good source of bioactive compounds. However, information on the biological properties of sugar beet root is limited and its beneficial effects have not been completely understood. 10 phenolic compounds have been separated and identified in various parts of sugar beet, including the most abundant

epicatechin (31.16 ± 1.89 mg/100 g), gallic acid (30.57 ± 2.69 mg/100 g), and quercetin-3-O-rutinoside (30.14 ± 3.63 mg/100 g). The biological activity tests indicated that sugar beet roots DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals values of 88.17 ± 05.14 μ g/mL. **Arjeh et al., (2022)**.

Sugar beet leaves polyphenol chemicals that are useful. Significant extraction yields ranging from 18.21% to 37.04% were obtained using all tested extraction methods. The range of the total phenolic content (g GAE/100 g DW) was 0.4504 to 1.7171. **Maravić et al., (2022)**.

Thus, the current study objective is to evaluate the chemical components of the root and leaves of *Beta vulgaris L.* Total flavonoid and phenolic substance as well as antioxidant activity were measured.

Materials and methods:

2.1. Materials:

Beta vulgaris L. fresh sugar beet root and leaves were obtained from a local farm in Beba village, Beni Suef Governorate. All samples were taken in 2023. Chemicals applied in these experiments were all-grade and supplied by Sigma and Piochem Company of superior quality as well as purity.

2.2. Analytical methods:

2.2.1. Moisture, total lipids, crude protein, and ash:

These determinations were determined using the procedure of the association of official analytical chemists (A.O.A.C., 2019).

2.2.2. Determination of carbohydrates:

carbohydrate of various samples under research were computed as the difference between 100 and the sum of a percentage quantities of total lipids, protein, moisture, and ash reported by Merrill and Watt (1973).

2.2.3. Preparation of samples:

Sugar beet root and leaves were cleaned with tap water, followed by distilled water. The roots were then hand-peeled and chopped with a sharp knife. These plants were chopped into little pieces, then exposed to air for 10 days and crushed extremely finely. (Beshel et al., 2018).

2.2.4. Preparation of sugar beet root and leaves extract:

The dry powder of beets were extracted using distilled water, ethanol, and methanol 80% liquid solvent in a 1:10 ratio (w/v). A mixture was used as extracting solvents and soaked with distilled water, 80% ethanol and methanol continued under stirring for 4 hours in dark place then kept in dark brown bottles at temperature (20-25 C) in dark place for 2 weeks, combine and shake well every day then, put the extraction solution in 50 mL centrifuge tubed

and centrifuged at 3560 xg for a ten-minute duration. The supernatant was obtained following centrifugation then filtered using syringe filter (0.45 μ m) to clear extract. Several investigations have reported on its effectiveness in gaining phenolic and flavonoids components then a solvent was extracted using a rotary device (IKA-WERKE, Germany). Finally, the extract was stored in refrigerator using the method introduced by Mirmiran et al., (2020), Jahan et al., (2021) and Arjeh et al., (2022).

2.2.5. Determination of total phenolic compounds of sugar beet roots and leaves extracts:

Concentration of total phenols in sample extracts were evaluated by UV spectrophotometer (SM1600UV-vis spectrophotometers, Azzota, USA), according to colorimetric oxidation-reduction process explained by Muntana and Prasong (2010).

2.2.6. Determination of total flavonoids of sugar beet roots and leaves extracts:

The total flavonoid content was measured using the technique discussed by Kumar et al., (2008).

2.2.7. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity:

The electron donating capacity of these extracts were assessed by bleaching a purple-colored DPPH solution using a procedure proposed by Hanato et al., (1988).

2.2.8. HPLC analysis:

HPLC analysis was performed by employing an Agilent 1260 model, USA. the separation was performed using a Zorbax Eclipse plus C18 separation column (4.6 mm x 250 mm i.d., five μ m). The mobile phase was composed of water (A) as well as 0.05% trifluoroacetic acid within acetonitrile (B) at an inflow rate of 0.9 mL/min. The mobile phase gradient was programmed by changing the proportion of solvent A (water with 0.05% TFA) against solvent B (acetonitrile), as follows: 0 min (82% A, 18% B); 0–1 min (75% A, 25% B); 11–18 min (60% A, 40% B); 18–22 min (82% A, 18% B); and 22–24 min (82% A, 18% B). The multi-wavelength device was examined at 280 nm. The injection volume got 5 μ L for every tasted sample. the column heat had been kept at 40 degrees Celsius that standard in house method of National research center-central laboratories network-chromatography lab, Dokki, Giza, Egypt.

2.2.9. Statistical analysis of the data:

The statistical analysis was carried out using One and Two-way ANOVA using SPSS, ver. 27 (IBM Corp. Released 2013). Data were treated as a complete randomization design according to Steel et al., (1997). Multiple comparisons were carried out applying Duncan test the significance level was set at < 0.05 .

Results and Discussion

4.1. Chemical composition of sugar beet (root and leaves)

Chemical examination of sugar beetroot and leaves revealed considerable changes in dry weight across many different plant components. this difference could be attributed to a larger area of surface and exposure to the environment, leaves had a greater moisture level (8.65 g/100g) than roots (4.21 g/100g), as seen in Table 1. The leaves also had a more diverse mineral profile, since their ash concentration, a measure of overall mineral content, was substantially greater (21.71 g/100g) than the roots (2.04 g/100g). the lipid content differed significantly both the roots (0.29 g/100g) as well as the leaves (16.16 g/100g). this shows that sugar

found in beet leaves may be more suitable for manufacturing or lipid-based nutritive applications. The leaves have more protein (46.63g/100g) compared to the root (34.05 g/100g), indicating that they could possibly be used to humans or livestock diets as a form of protein supplement. The root had a total carbohydrate quantity of 59.41 g/100 g, intended it was greater than the leaves 6.85 g/100 g. this is consistent with the main objective of the sugar beet root for the storage of carbs, especially sucrose. In general, the research results indicate that sugar beet leaves include more protein, lipids, ash, as well as moisture than the roots, which are plenty in carbohydrates. The sugar beet leaves are frequently leaving as agriculture trash. It may be used as a useful food ingredient or feed supplemental for its high nutritional content. The findings that were obtained are mostly consistent with those published by **Aramrueang *et al.*, (2017)**.

Table 1. Chemical composition of sugar beet roots and leaves (On a dry weight basis).

Component (%)	Roots	Leaves
Moisture	4.21 ± 0.01^B	8.65 ± 0.03^A
Protein	34.05 ± 0.02^B	46.63 ± 0.05^A
Lipids	0.29 ± 0.05^B	16.16 ± 0.02^A
Ash	2.04 ± 0.03^B	21.71 ± 0.03^A
Total carbohydrate	59.41 ± 0.10^A	6.85 ± 0.10^B

Total carbohydrate = 100 – (Moisture + Protein + Lipids + Ash)

A, B & C: There is no significant difference ($P>0.05$) between any two means, within the same row have the same superscript letter.

4.2. Total phenolic, flavonoid, and antioxidant activity of methanolic, ethanolic and aqueous extracts of sugar beet roots and leaves.

The information contained in Table (2) shows that *Beta vulgaris L.* sugar beet roots and leaves have been extracted by utilizing three vary solvents, namely water, ethanol and methanol. The results confirmed that phenolic, and flavonoids possess highly antioxidant activity. Sugar beet roots and leaves methanol extracts had the phenolic content (0.194 ± 0.011 g/100g extract and 0.212 ± 0.007 g/100g extract, subsequently). ethanolic extracts produced the amount of phenolic substance in both tissues, whereas aqueous was somewhat successful in better extracting phenolics and flavonoids compounds compared with methanolic extracts. In general, and found that flavonoid content followed a similar pattern. The sugar beet leaves methanolic extract had the value (0.146 ± 0.002 g/100 g extract), followed by the root extraction (0.092 ± 0.005 g/100 g extract). Our findings are almost identical to those presented by **Wang *et al.*, (2018)** and **Maravić *et al.*, (2022)**. Additionally, the solvent utilized affected the antioxidant activity of the sugar beet roots and leaves extracts. Methanolic extract of the roots and leaves showed the maximum antioxidant activity ($56.08 \pm 5.10\%$ and $56.96 \pm 6.32\%$, respectively). On the other hand, aqueous extracts showed the lowest antioxidant values ($42.21 \pm 0.45\%$

in roots and $39.82 \pm 4.12\%$ in leaves), ethanolic extracts had a slightly lower activity. This pattern is consistent with the increased quantities of flavonoids and phenolics present in methanolic extracts, indicating the role of these chemicals in scavenging free radicals. these findings are in agreement with previous studies by **Arjeh *et al.*, (2022)**. Sugar beet roots and leaves extract yields were in methanol ($30.22 \pm 0.15\%$ for roots and $31.54 \pm 0.05\%$ in leaves), followed by ethanol and aqueous solvents. Compared to water, methanolic and ethanolic extractions provide higher extract yields, indicating that they are more successful in solubilizing a wide range of bioactive compounds. Interestingly, in all solvent kinds, the extract percentages from the leaves appeared slightly greater than those from the roots.

Table 2. Total phenolic, total flavonoids, antioxidants, and total extract yield of sugar beet root and leaves.

Parameter test	Sample	Solvent		
		Methanolic	Ethanol	Aqueous
Total phenolic (g/100 g extract)	Roots	0.194±0.011 ^{aB}	0.221±0.110 ^{bB}	0.386±0.003^{bA}
	Leaves	0.212±0.007 ^{aB}	0.597±0.003 ^{aA}	0.553±0.004^{aA}
Total flavonoids (g/100 g extract)	Roots	0.092±0.005 ^{bC}	0.213±0.004 ^{bB}	0.282±0.004^{aA}
	Leaves	0.146±0.002 ^{aC}	0.374±0.025 ^{aA}	0.273±0.027^{aB}
Antioxidants activity	Roots	56.08±5.10 ^{aA}	52.20±3.95 ^{aA}	42.21±0.45^{aB}
	Leaves	56.96±6.32 ^{aA}	48.92±4.96 ^{aA}	39.82±4.12^{aB}
	Roots	30.22±0.15 ^{bA}	29.40±0.20 ^{bB}	30.27±0.16^{bA}
Total extract yield (%)	Leaves	31.54±0.05^{aAB}	31.30±0.04^{aB}	31.68±0.03^{aA}

a, b & c: There is no significant difference ($P>0.05$) between any two means for each parameter, within the same column have the same superscript letter.

A, B & C: There is no significant difference ($P>0.05$) between any two means, within the same row have the same superscript letter.

4.3. Identification of bioactive components in aqueous, ethanolic and methanolic extracts of sugar beet roots and leaves by HPLC

Results mentioned in Table (3) as well as figs (1 to 6) showed the chemical composition of the aqueous, ethanolic as well as methanolic extracts of sugar beet roots and leaves were demonstrated.

The information in Table (3) confirmed the existence totally 17 phenolics and flavonoids compounds in *Beta vulgaris L.* aqueous, ethanolic and methanolic extracts. In this section, the comparison was made between sugar beet roots and leaves, as well as among their aqueous, ethanolic, and methanolic extracts. The aim was to identify which plant part and which extraction solvent yielded the highest concentrations of individual phenolics and flavonoids compounds.

Sugar beet roots has the largest amounts of chlorogenic acid (19.53 $\mu\text{g/mL}$) and gallic acid (2.07 $\mu\text{g/mL}$), while the lowest amounts are methyl gallate (0.08 $\mu\text{g/mL}$). Sugar beet leaves have the largest amounts of chlorogenic acid (78.43 $\mu\text{g/mL}$), syringic acid (45.98 $\mu\text{g/mL}$), catechin (15.52 $\mu\text{g/mL}$), and the lowest amounts of kaempferol (0.02 $\mu\text{g/mL}$) in aqueous extract.

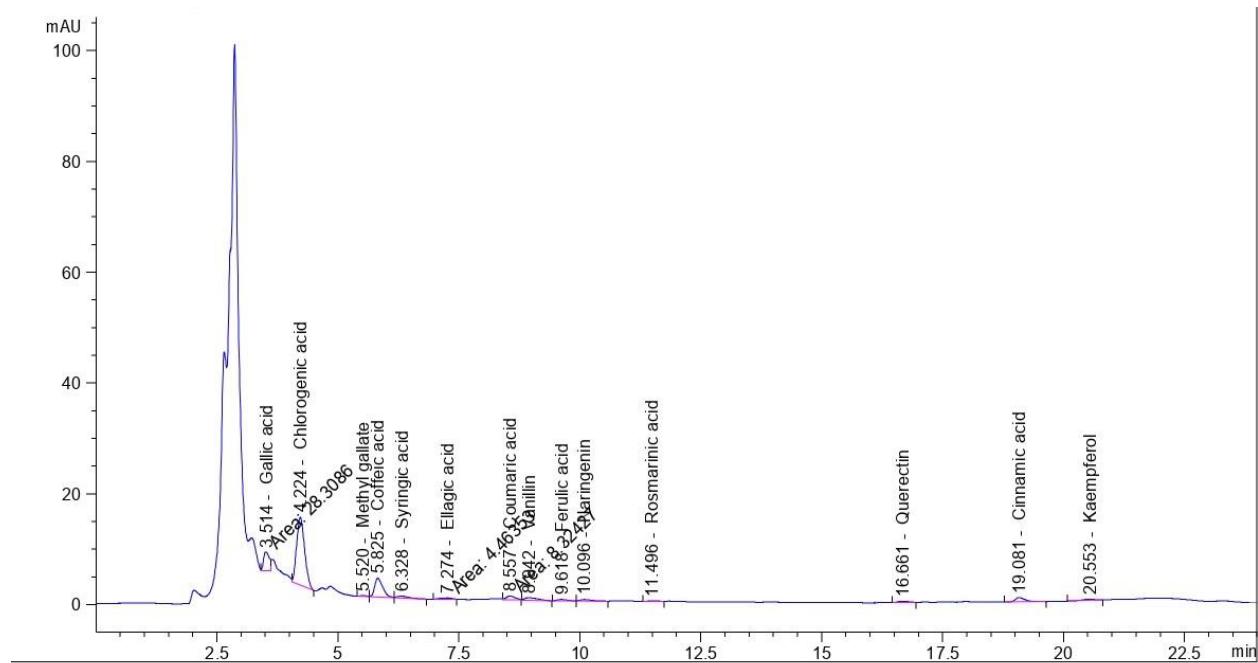
Sugar beet root has the most significant amounts of chlorogenic acid (4.04 $\mu\text{g/mL}$), quercetin (0.83 $\mu\text{g/mL}$), as well as caffeic acid (0.64 $\mu\text{g/mL}$), while the lowest amounts are cinnamic acid (0.08 $\mu\text{g/mL}$).

the results are in good agreement with those reported by El-Beltagi *et al.*, (2018). In sugar beet leaves the highest quantities were chlorogenic acid (61.08 $\mu\text{g/mL}$), syringic acid (59.00 $\mu\text{g/mL}$), gallic acid (28.55 $\mu\text{g/mL}$), rosmarinic acid (13.04 $\mu\text{g/mL}$) and the lowest quantities were hesperetin (0.05 $\mu\text{g/mL}$) as well as kaempferol (0.03 $\mu\text{g/mL}$) in ethanolic extract. the results are in good agreement with those reported by Maravić, *et al.*, (2022).

In sugar beet root the highest quantities were, chlorogenic acid (12.74 $\mu\text{g/mL}$), gallic acid (3.49 $\mu\text{g/mL}$), caffeic acid (1.71 $\mu\text{g/mL}$), quercetin (1.71 $\mu\text{g/mL}$) while the lowest quantities were kaempferol (0.07 $\mu\text{g/mL}$) and methyl gallate (0.06 $\mu\text{g/mL}$). the findings are excellent and similar with the findings recorded by Arjeh *et al.*, (2022). In sugar beet leaves the highest quantities were, chlorogenic acid (112.92 $\mu\text{g/mL}$), syringic acid (73.34 $\mu\text{g/mL}$), gallic acid (65.61 $\mu\text{g/mL}$), ellagic acid (21.20 $\mu\text{g/mL}$), catechin (17.49 $\mu\text{g/mL}$) and the lowest quantities was hesperetin (0.06 $\mu\text{g/mL}$) in methanolic extract.

Table 3. Phenolics and flavonoids compounds of aqueous, ethanolic and methanolic extract of sugar beet and their leaves analyzed by HPLC.

Phenolics and Flavonoids compounds	Sugar beet root	Sugar beet leaves	Sugar beet root	Sugar beet leaves	Sugar beet root	Sugar beet leaves
	Conc.(μ g/mL)					
	aqueous		ethanolic		methanolic	
1 Gallic acid	2.07	9.78	0.24	28.55	3.49	65.61
2 Chlorogenic acid	19.53	78.43	4.04	61.08	12.74	112.92
3 Catechin	0.00	15.52	0.00	0.00	0.00	17.49
4 Methyl gallate	0.08	4.11	0.00	0.97	0.06	1.11
5 Caffeic acid	2.05	2.29	0.64	4.92	1.71	5.94
6 Syringic acid	0.35	45.98	0.12	59.00	0.44	73.34
7 Rutin	0.00	0.00	0.00	0.00	0.00	0.00
8 Ellagic acid	0.45	12.42	0.00	12.40	0.62	21.20
9 Coumaric acid	0.30	0.30	0.15	8.53	0.37	16.56
10 Vanillin	0.34	2.64	0.11	4.41	0.31	5.25
11 Ferulic acid	0.20	0.81	0.18	5.01	0.39	5.66
12 Naringenin	0.40	2.24	0.19	5.35	0.48	6.59
13 Rosmarinic acid	0.16	4.65	0.00	13.04	0.17	16.30
14 Daidzein	0.00	4.05	0.46	1.09	0.96	1.30
15 Quercetin	0.39	5.59	0.83	11.52	1.71	2.62
16 Cinnamic acid	0.22	0.82	0.08	1.40	0.18	1.60
17 Kaempferol	0.09	0.02	0.00	0.03	0.07	0.08
18 Hesperetin	0.00	0.00	0.00	0.05	0.00	0.06
Total known	26.63	189.65	7.04	217.35	23.7	353.63

**Fig (1).** Phenolics and flavonoids compounds of aqueous extract in sugar beet roots were analyzed by HPLC.

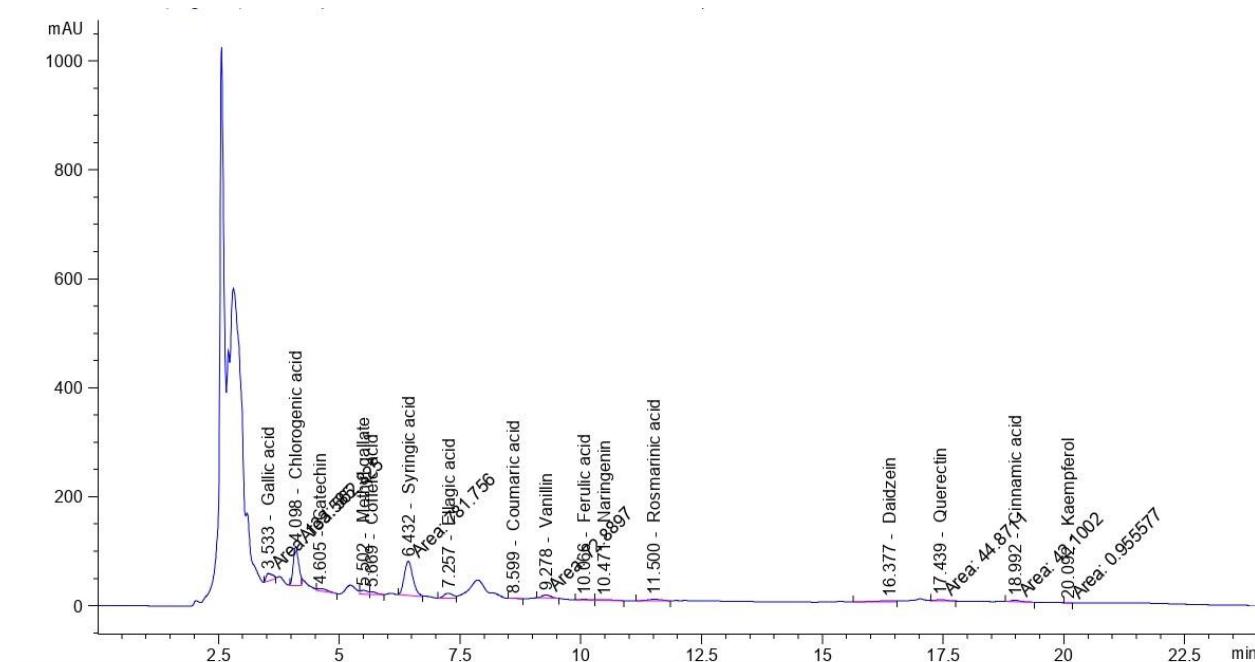


Fig (2). Phenolics and flavonoids compounds of aqueous extract in sugar beet leaves were analyzed by HPLC.

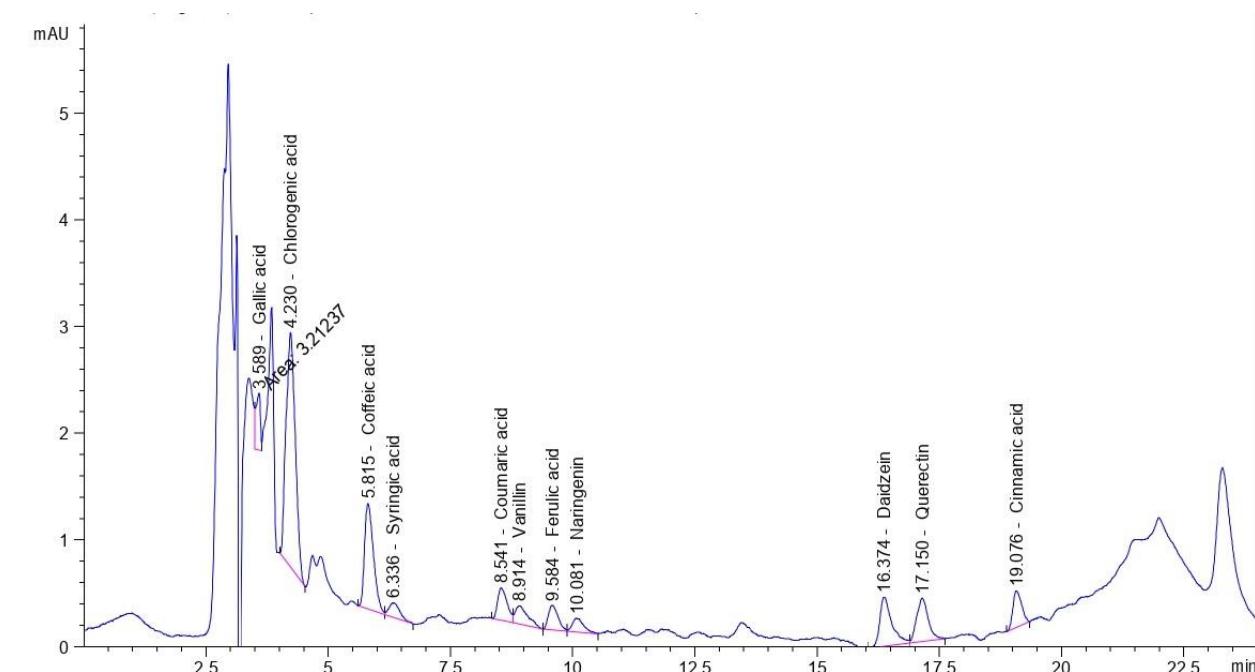


Fig (3). Phenolics and flavonoids compounds of ethanolic extract in sugar beet roots were analyzed by HPLC.

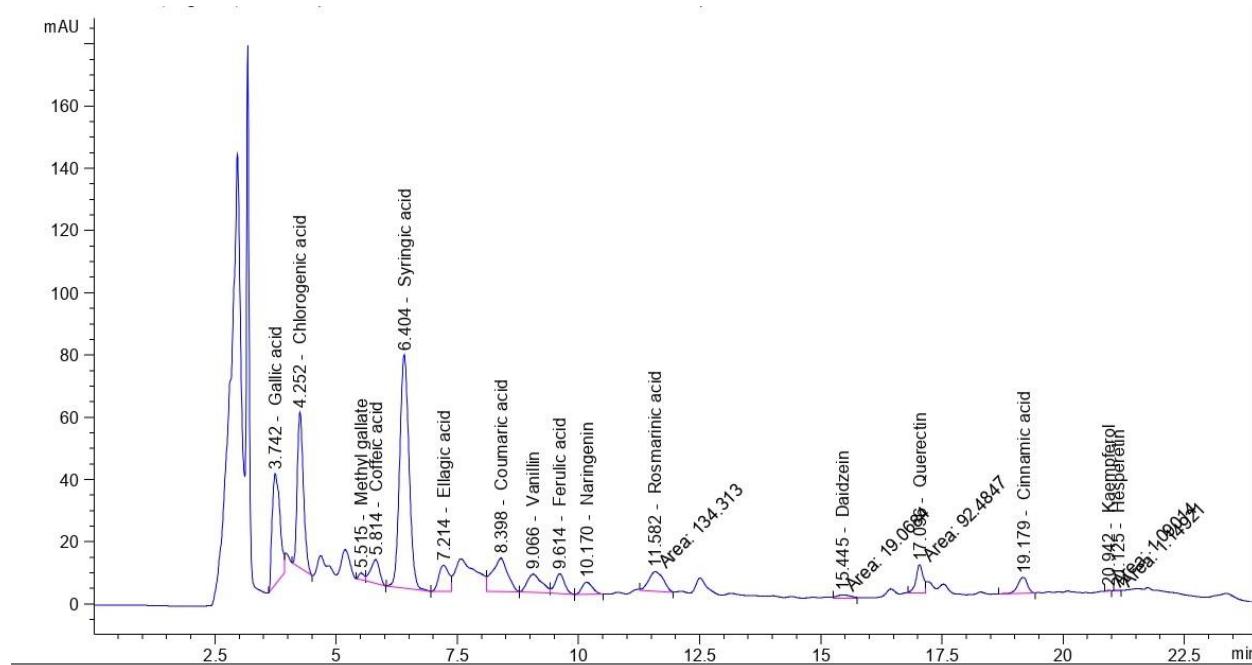


Fig (4). Phenolics and flavonoids compounds of ethanolic extract in sugar beet leaves were analyzed by HPLC.

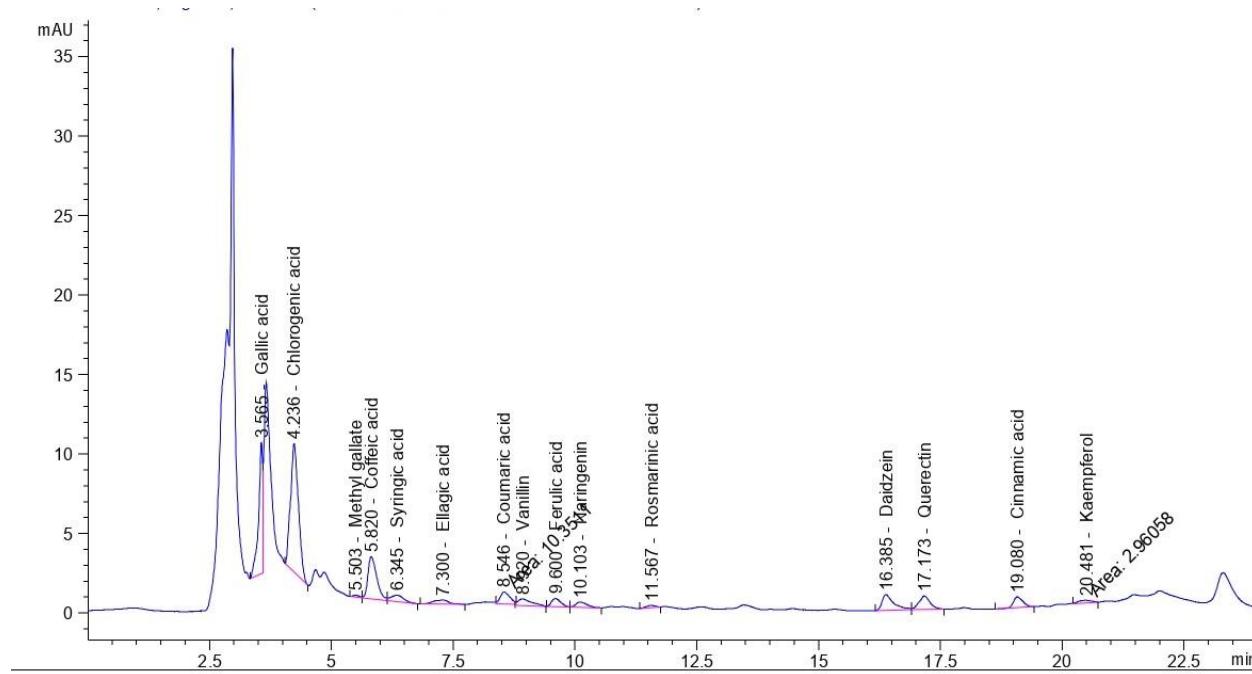


Fig (5). Phenolics and flavonoids compounds of methanolic extract in sugar beet roots were analyzed by HPLC.

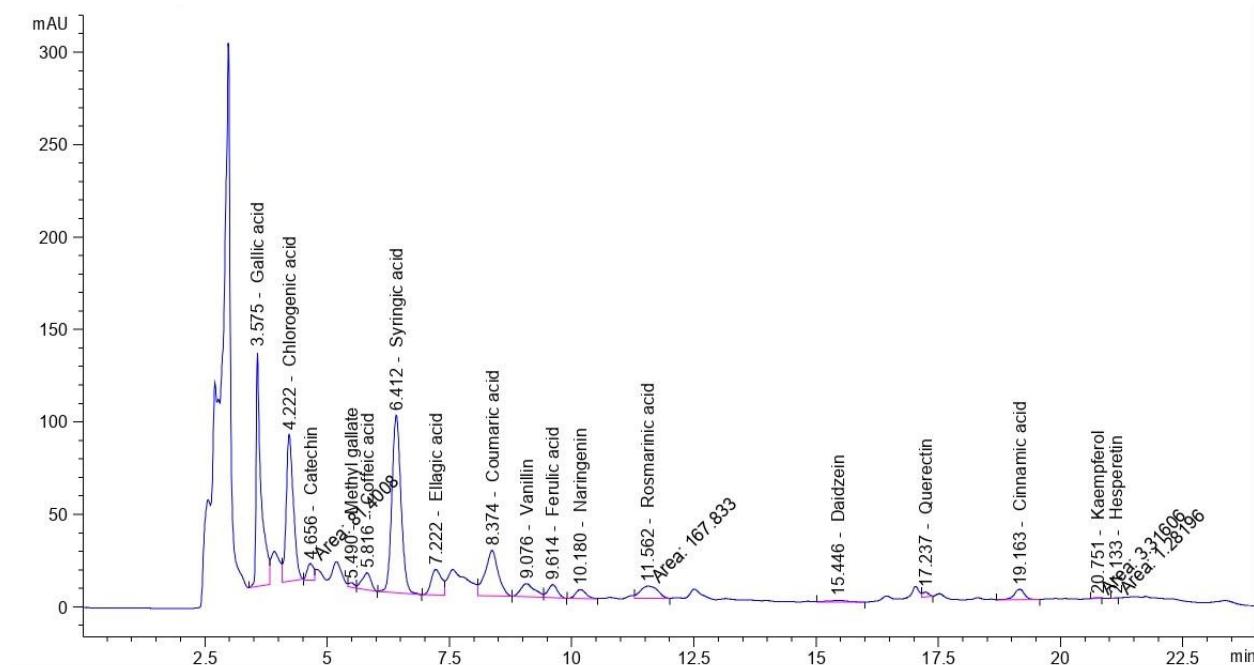


Fig (6). Phenolics and flavonoids compounds of methanolic extract in sugar beet leaves were analyzed by HPLC.

Conclusion

In summary, this present research demonstrates that sugar beet roots as well as leaves include a wide range of essential phytochemical components. The methanolic extract contains an important bioactive compounds that have antioxidant activity which, acts like an effective natural antioxidant source.

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