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A comparative study of the chemical composition and antioxidant activities of roots, seeds and aerial parts of chicory (*Cichorium intybus* L.)

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Key words: Chicory, *Cichorium intybus* L, chemical compositions, essential oil, GC/MS, water distillation, antioxidant properties.

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Abstract

The chicory plant (*Cichorium intybus* L.) from the sunflower family, is a perennial plant that has a high medicinal value. The aim of this study is to compare the chemical composition and antioxidant activity of roots, seeds and aerial parts of chicory plants. In this study, after collecting plants, the extraction of essential oils was performed using the Water distillation (Clevenger) and used the Gas Chromatography and Gas chromatography–mass spectrometry apparatus for analysis and identification of the essential oils. Of the 74 compositions identified, the major compositions of the essential oil from the chicory root are: Camphor (20.71%), Cymene (15.06%), Gamma-Terpinen (13.24%), Cuminal (10.79%). The major compositions of the essential oil from the seeds are: O-Cymene (11.87%), Cuminal (10.74%), Gamma-Terpinene (10.44%), Thymol (8.24%). The major compositions of the essential oil from the stem and flower are: p-Cymol (17.53%), Gamma-Terpinene (16.31%), Cuminal (12.38%), Carvacrol (9.26%). The major compositions of the essential oil from the leaf are: p-Cymo (17.12%), Gamma-Terpinene (15.18%), Cuminal (10.53%), Thymo (9.38%). In the second part of the study, three different concentrations (0.1, 0.2, 0.3 mg per ml) of the essential oils were prepared. After preparation, the antioxidant activity of each dilution were determined using the DPPH assay. The results showed that the inhibition percent of the essential oils increased with increasing concentration.

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Introduction

Chicory (*Cichorium intybus* L.) belongs to the Asteraceae family and is a biennial plant with many applicationsin the food industry (Bais and Ravishankar, 2001). Many researchers have focused on the investigation of natural products and plant extracts as a source of new bioactive molecules (Renzo *et al.*, 2007; Al-Bakri and Afifi, 2007; Abere *et al.*, 2007).

Cichorium intybus have antibacterial, antifungal, antimalarial, antidiabetic, and cytotoxic activities. The whole plant is also used to treat AIDS, cancer, diabetes, dysmenorrheal, impotence, insomnia, splenitis and tachycardia. The anticancer properties of dietary anthocyanins have been widely evaluated in *in vitro* studies and some animal gastrointestinal cancer models (Espin *et al.*, 2007). Using analytical methods, various studies demonstrated the ability of different chicory varieties to counteract various free radicals, as well as a linear correlation between the phytochemical content and antioxidant capacity of this vegetable (Llorach *et al.*, 2004; Heimler *et al.*, 2007; Lavelli, 2008).

recent study shows an interesting antioxidant activity of the red chicory variety against the oxidative stress response in a eukaryotic cell model system, which suggests, at cellular level, it plays a healthy role (Lante *et al.*, 2011). Studies have shown that chicory preparations possess potent anti-hepatotic activity (Chhaya and Mishra, 1997; Zafar and MujahidAli, 1998; Ahmed *et al.*, 2003), anti-diabetic effects (Pushparaj *et al.*, 2007) and antimalarial activity (Bischoff *et al.*, 2004).

recent study shows an identification of chemical compounds in essential oils from the aerial parts of essential oil of aerial parts of *Cichorium intybus* L. from Iran. Analysis of the chemical composition of essential oil flowering tops of the plant Has been studied in center of Iran (Kashan, Isfahan province), major chemical composition of the essential oil of this plant is as follows:
carvacrol (50.1 %), thymol (13.3 %), cinnamic aldehyde (12.4 %), camphor (4.4 %), carvone (4.1 %), linalool (3.9 %) and α-terpineol (2.1 %), (Haghi *et al.*, 2012).

In this study, we studied of the chemical composition and antioxidant activities of roots, seeds and aerial parts of chicory (*Cichorium intybus* L.).

Materials and methods

Plant material

The aerial parts of *Cichorium intybus* L were collected during the flowering period of June 2014, from Khorasan, Iran. Each sample of the fresh aerial parts (about 30 kg, the root, stem and leaves were separated manually with sharp knife and then washed with distilled water) of Cichorium intybus L were hydrodistilled using the pilot distillation apparatus in order to obtain distillate. Distillate was extracted with hexane. The organic layer was separated, which revealed that the color of the oils were yellow. They were dissolved in hexane (Merck), dried over anhydrous sodium sulphate and stored at 4–6°C.

GC/MS analysis

An Agilent 7890 gas chromatograph with mass spectrometry detector 5975C inert MSD (Agilent Corporation, USA) was used for sample analysis. Chromatography conditions were set according to AOAC method No. 963/22 (27), and a capillary column of DB-35 MS, at length of 30 meters and diameter 0.25 mm, and a Polar Silica of thickness 0.25 micrometers. The injector was set at 250°C and ion source at 200°C, as chromatography temperature parameters. The oven was programmed to operate at an initial temperature of 80°C for 20s, then 80°C to 240°C at 4°C min^{-1}, and 240°C for 10 min. Helium gas was used as the carrier gas, with a column flow of 1.4 $ml.min^{-1}$ and split rate of 1:30. 1.0 mm^3 of the sample was injected. The energy for the EI source of the Agilent mass spectrometer was 70 eV. The mass unit was monitored to range from 30 to 450 m/z. Identification of components in the oil was based on retention indices, relative to n-alkanes and computer matching with the WILLEY 275.L library, as well as by making a comparison between the fragmentation

patterns of mass spectra and those reported in the literature (Adams, 2001).

Result

With a detailed study of retention times of the compounds, Kovats retention indices, and mass spectra, it was found that the amounts of certain substances are similar in different parts of the chicory plant (Figure1-4).

Table 1. The chemical composition of the essential oil of different parts of *Cichorium intybus* L.

Compound	%Area root	%Area seed	%Area Stem and Flower	%Area Leaf
Alpha-Thujene	0.53	0.40	0.53	0.60
Alpha-Pinene	3.39	2.55	3.72	3.54
Camphene	1.48	0.77	1.87	1.80
Verbenene	0.22	-	-	0.21
Beta-Pinene	5.54	4.77	6.50	6.19
Beta-Myrcene	0.94	0.80	1.11	1.12
Alpha- Phellandrene	5.03	3.67	6.28	5.95
Alpha.Terpinene	0.30	0.32	0.23	0.23
Cymene	15.06	-	-	-
Alpha-Propyl	1.57	2.44	-	-
Beta.Phellandrene	0.90	-	-	-
1,8-Cineole	1.12	0.92	-	-
Gamma.Terpinene	13.24	10.44	16.31	15.18
Terpinolene	0.30	0.22	0.33	0.33
Alpha-Thujone	0.46	-	-	-
Beta-Thujone	2.17	-	-	-
Camphor	20.71	6.60	2.32	2.54
Borneol	0.57	-	-	-
Terpinene-4-ol	0.33	0.29	0.20	-
Alpha- Terpineol	0.43	-	-	-
Myrtenal	0.84	0.19	0.41	0.62
Chrysanthenone	0.14	-	1.44	3.52
Cuminal	10.79	10.74	12.38	10.53
Carvacrol	0.26	-	9.26	5.95
Azulene	1.48	-	0.43	-
Alpha- Copaene	0.14	-	-	0.19
Beta-Bourbonene	0.14	0.21	-	-
Beta-Cubebene	0.33	0.32	-	0.88
Beta-Elemene	0.19	0.15	0.19	1.21
Cis-Jasmone	0.24	-	-	-
Dlepi-Alpha-Cedren	2.98	-	-	-
Trans-Caryophyllene	1.17	0.63	1.05	3.12
Aromadendrene	0.17	-	-	0.16
Beta-Farnesene	0.40	0.61	0.34	0.37
Alpha.Humulene	0.20	-	0.20	0.22
9-epi-E- Caryophyllenol	0.31	-	-	0.18
Beta-Bisabolene	0.14	3.20	0.45	0.37
Delta-Cadinene	0.31	0.40	0.26	0.74
Calarene	0.20	-	0.21	0.24
E- Nerolidol	0.78	0.40	0.41	0.45
Spathulenol	0.75	0.44	0.53	0.61
Caryophyllene oxide	1.05	0.41	0.77	0.85
Beta-Eudesmol	0.68	0.22	-	-
Alpha-Sinensal	0.26	0.23	-	-
Phytol	0.21	0.21	0.21	0.84
Tetradecanoic acid	0.19	0.15	0.16	0.19
n-Hexadecanoic acid	0.89	-	-	-

Linolic acid	0.25	-	-	-
Sabinene	-	1.05	-	-
O-Cymene	-	11.87	-	-
3,8-p-Menthadiene	-	0.47	-	-
Linalool	-	0.52	0.86	0.77
L-Menthone	-	6.16	-	-
Menthol	-	4.46	-	-
Pulegone	-	4.96	-	-
Piperitone	-	4.42	0.18	0.34
Thymol	-	8.24	6.74	9.38
2,6-dimethoxy Phenol	-	0.14	-	-
Eugenol	-	0.20	-	-
Alpha-Cedrene	-	3.20	3.35	-
Bicyclogermacrene	-	0.24	-	-
Alpha-Farnesene	-	0.66	0.34	-
Carotol	-	0.23	-	-
Dillapiole	-	0.29	-	-
Palmitinic acid	-	0.82	0.85	0.94
Phthalic acid	-	0.80	-	-
p-Cymol	-	-	17.53	17.12
Alpha-Cubebene	-	-	0.54	-
Beta-Selinene	-	-	0.16	-
Dill apiole	-	-	0.25	0.37
(Z,Z)-9,12-Octadecadienoic acid	-	-	0.17	-
4-Terpineol	-	-	-	0.21
Beta-Terpineol	-	-	-	0.29
Carotol	-	-		0.47

Table 2. Common components in essential oils of different parts of *Cichorium intybus* L.

Compound	%Area root	%Area seed	%Area Stem and Flower	%Area Leaf
Alpha-Thujene	0.53	0.40	0.53	0.60
Alpha-Pinene	3.39	2.55	3.72	3.54
Camphene	1.48	0.77	1.87	1.80
Beta-Pinene	5.54	4.77	6.50	6.19
Beta-Myrcene	0.94	0.80	1.11	1.12
Alpha- Phellandrene	5.03	3.67	6.28	5.95
Alpha.-Terpinene	0.30	0.32	0.23	0.23
Gamma.-Terpinene	13.24	10.44	16.31	15.18
Terpinolene	0.30	0.22	0.33	0.33
Camphor	20.71	6.60	2.32	2.54
Myrtenal	0.84	0.19	0.41	0.62
Cuminal	10.79	10.74	12.38	10.53
Beta-Elemene	0.19	0.15	0.19	1.21
Trans-Caryophyllene	1.17	0.63	1.05	3.12
Beta-Farnesene	0.40	0.61	0.34	0.37
Beta-Bisabolene	0.14	3.20	0.45	0.37
Delta-Cadinene	0.31	0.40	0.26	0.74
E- Nerolidol	0.78	0.40	0.41	0.45
Spathulenol	0.75	0.44	0.53	0.61
Caryophyllene oxide	1.05	0.41	0.77	0.85
Phytol	0.21	0.21	0.21	0.84
Tetradecanoic acid	0.19	0.15	0.16	0.19

The major compositions of the essential oil from the chicory root are: Camphor (20.71%), Cymene (15.06%), Gamma-Terpinen (13.24%), 10.79%). The major compositions of the essential oil from the seeds are: O-Cymene (11.87%), Cuminal (10.74%), Gamma-Terpinene (10.44%), Thymol (8.24%). The major compositions of the essential oil from the stem and flower are: p-Cymol (17.53%), Gamma-Terpinene (16.31%), Cuminal (12.38%), Carvacrol (9.26%). The major compositions of the essential oil from the leaf are: p-Cymo (17.12%), Gamma-Terpinene (15.18%), Cuminal (10.53%), Thymo (9.38%). The results are shown in Table 1.

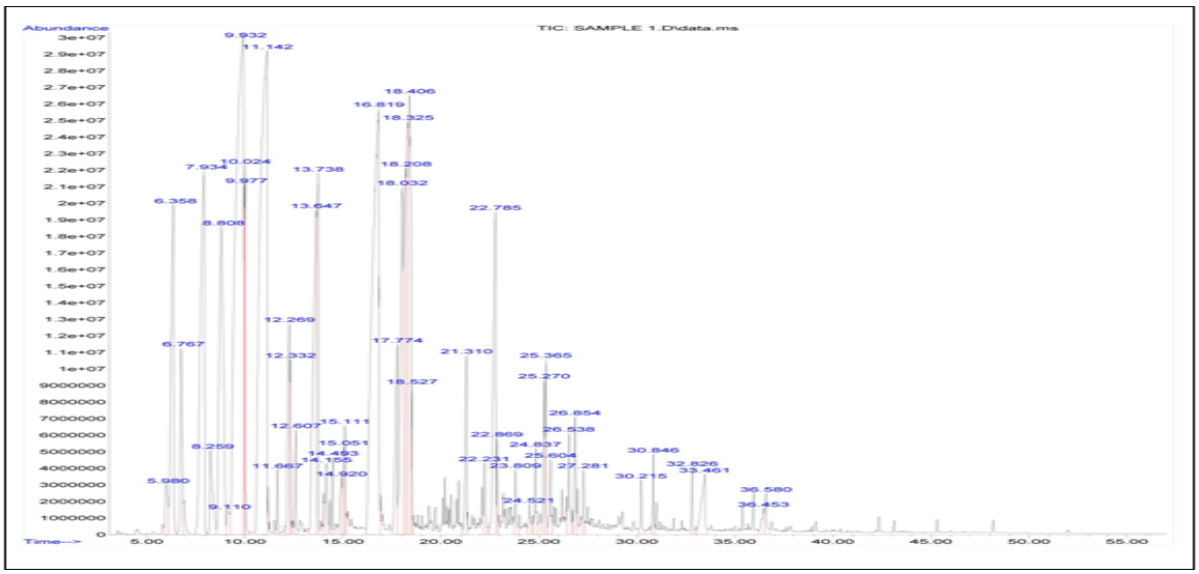

Fig. 1. Chromatogram of the essential oil of root of *Cichorium intybus* L.

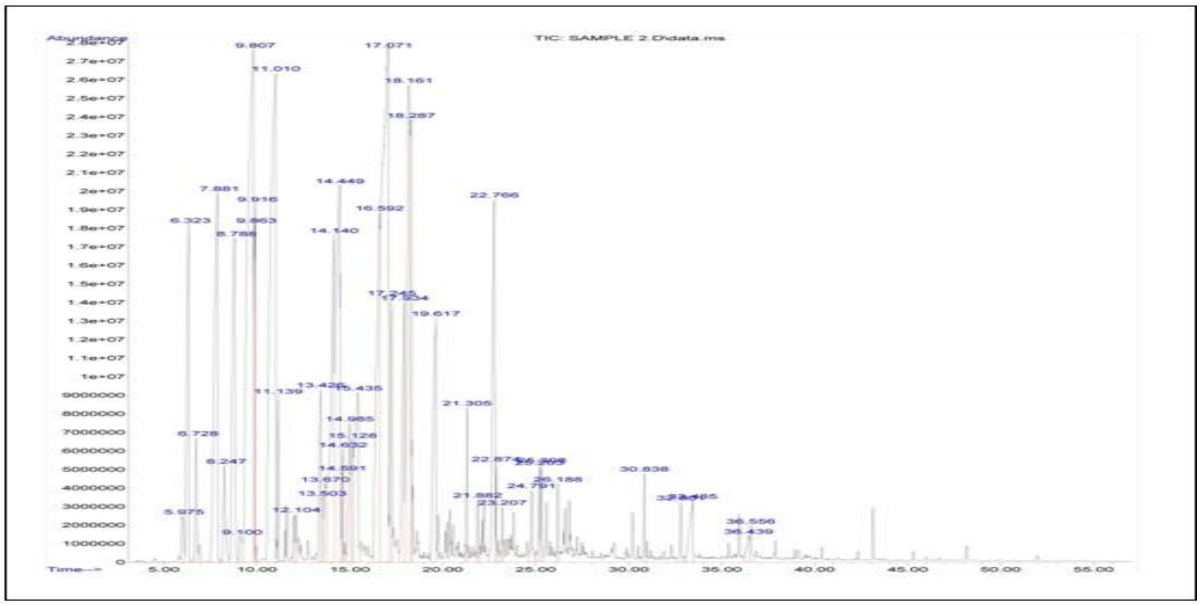

Fig. 2. Chromatogram of the essential oil of seed of *Cichorium intybus* L.

In a comparison of the composition table, Table 1, we found that 22 components of the essential oil compositions were common (Table 2). Camphor in the essential oil from the root was the most abundant compound**.**

Analysis of antioxidant activity

In the DPPH system, antioxidants react with DPPH stable radicals which makes them pale or colorless. Color reduction of the sample is directly related to the antioxidant's power. Antioxidants such as cysteine,

glutathione, ascorbic acid, tocopherol, and polyhydroxy aromatic compounds (e.g. hydroquinone, pyrogallol, etc.) regenerate DPPH radicals by giving a hydrogen or an electron, which subsequently makes essential oils pale or even colorless. Increasing the concentration of phenolic compounds or the degree of hydroxylation of phenolic compounds will increase radical scavenging activity of essential oils or extracts. Butyl hydroxy toluene (BHT) was used in this method to compare the antioxidant activity of the essential oils with industrial samples. Table 3 shows the comparison of the inhibitory activity of the chicory extracts. Diagrams comparing the inhibitory activity of chicory essences are shown in Figures 5-7 for three concentrations, namely 1.0, 2.0 and 3.0 mg/ml.

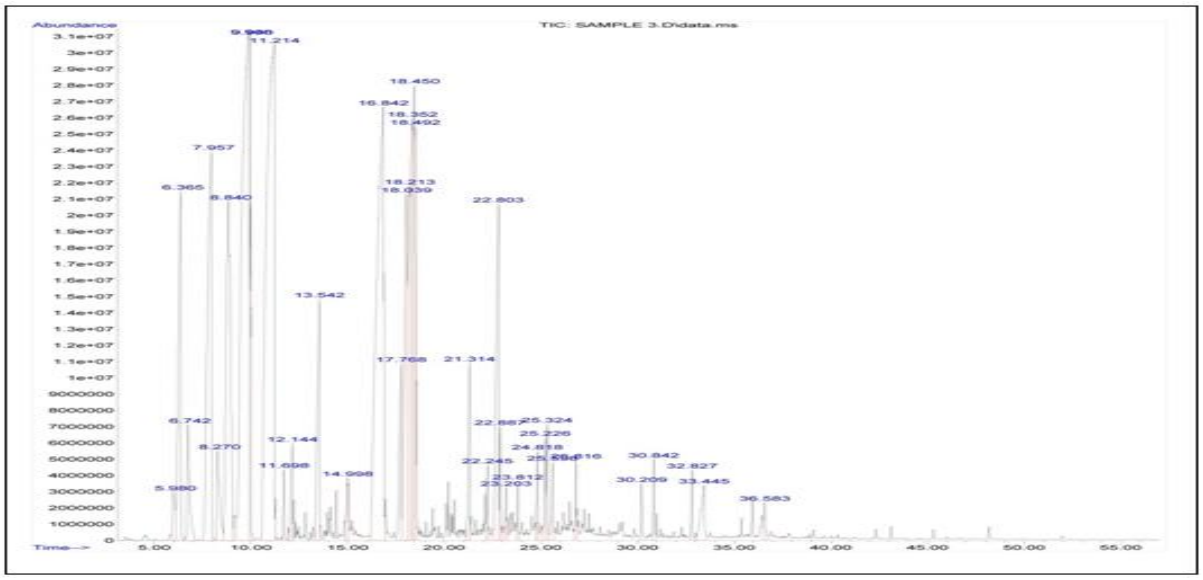

Fig. 3. Chromatogram of the essential oil of stem and flower of *Cichorium intybus* L.

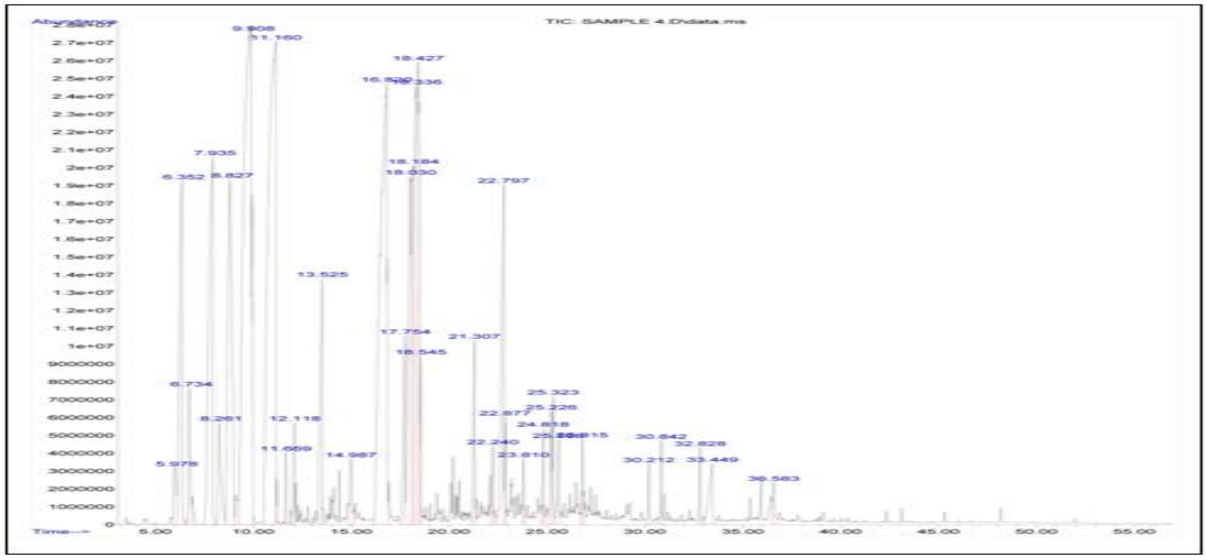

Fig. 4. Chromatogram of the essential oil of leaf of *Cichorium intybus* L.

Conclusion

In this study, we obtained 74 different compounds from the essential oils of various parts of chicory plants: 48 compounds were obtained from the essential oil from the root, which composes of 99.78% of its total components; 46 compounds were obtained from the essential oil from seeds, which composes of 98.23% of its total components; 39 compounds were obtained from the essential oil from the stem and flower, which composes of 99.07% of its total

components; and 40 compounds were obtained from the essential oil from the leaf, which composes of 98.82% of its total components.

The detailed study of retention times of the compounds, Kovats retention indices, and mass spectra, it was found that the amounts of certain substances were similar in different parts of the chicory plants. A total 22 components of the essential oils compositions were common across all of the extracted essential oil. Camphor (20.71%) from the essential oil from the roots was the most abundant compound, and γ-Terpinen (16.31%) from the essential oil from the stem and flower was the second most abundant. The least abundant compound was β-Bisabolene (0.14%) from the essential oil from roots.
In regards to antioxidant activity, root essential oil has the lowest inhibition percentage, whereas the stem and flower essential oil has the highest inhibition percentage at 0.1 mg/ml concentration. For both 0.2 and 0.3 mg/ml concentrations, the lowest and highest inhibition percentage was observed in the seed and leaf essential oils, respectively. We can conclude that the inhibition percentage increases with an increased concentration, and the leaf samples have more antioxidant activities compared to other samples.

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