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Chemical composition and antioxidant activity of the essential oil and various extracts of *Inula graveolens* (L.) Desf.

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ABSTRACT

In this study, chemical composition and *in vitro* antioxidant activity potential of the essential oil and various extracts of *Inula graveolens* (L.) Desf. were evaluated. While identifying the phytochemical composition of the essential oil and extract, GC-MS analyses were used. Chromatographic analysis of the essential oil resulted in identifying twenty compounds representing 99.5% of the total oil. Main constituents of the oil were determined as bornyl acetate (68.5%), borneol (7.7%), camphene (4.6%), *epi*-α-cadinol (4.0%) and eicosane (3.2%), respectively. Antioxidant activity was determined using four complementary test systems named β-carotene/linoleic acid, DPPH free radical scavenging, reducing power, and chelating effect. A strong correlation between the antioxidant activity and phenolic acid contents of the samples was determined. The methanol extract was the most active one in all tested systems. The weakest activity was exhibited by chloroform extract. While methanol extract showed 88.34%, 91.38, and 63.43 activities in β-carotene bleaching, DPPH radical scavenging, and chelating effect tests, respectively, the absorbance value in reducing power assay was measured as 0.273 nm.

1. Introduction

Essential oils obtained from medicinal and aromatic plants have been used by humankind since ancient times in the composition of various medicines. Essential oils are mixtures of terpenoids obtained by water or steam distillation, usually from the aerial parts of plants. With many studies so far, essential oils have been shown to have antimicrobial, antioxidant, anti-inflammatory, analgesic, insecticidal, etc., properties (Abu-Shanab et al., 2005). Essential oils isolated from some plants are used in cancer treatment due to their antiproliferative properties, while others are trendy in the food and perfumery industry (Kelen and Tepe, 2008). The antimicrobial properties of essential oils are of great importance for both producers and consumers, especially in the food industry. Because some essential oils are used as preservatives in canned foods and extend their shelf life (Celiktas et al., 2007). In addition, essential oils are one of the indispensable elements of aromatherapy due to the aromatic phytochemicals they contain (Lee et al., 2012).

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Researchers have conducted numerous studies on the antimicrobial properties of essential oils to combat infections caused by opportunistic and persistent pathogens. Some terpenoids in these oils have found use in the pharmaceutical industry due to their strong bactericidal and fungicidal properties (Tepe et al., 2007). In the coming decades, essential oils are expected to be one of the more frequently used resources in treating infectious diseases (Rios and Recio, 2005).

In addition to their antimicrobial properties, essential oils are also an excellent source of antioxidant compounds. These compounds help to increase the unsaturated fatty acids in animal tissues. In mammals, essential oils also play a hepatoprotective role due to their antioxidant properties. Essential oils can scavenge many reactive radicals such as superoxide, hydrogen peroxide, free hydroxyl radicals, and singlet oxygen due to the antioxidant terpenoids they contain. In this way, essential oils clear the body from reactive radicals (Pérez Gutierrez et al., 2006).

Inula is one of the leading genuses of the Asteraceae family and is generally distributed in Asia, Europe, and Africa. Approximately 90 species represent it. *Inula*, a paraphyletic genus from the

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taxonomical point of view, can grow from a few centimeters to over 3 m in height. Their capitulas are usually straight. There is information that it is used for landscaping purposes in parks and gardens. Smaller species are generally grown in rocky areas, while larger ones are grown in the borders of parks and gardens. Considering the historical records, it is known that the name Inula goes back to ancient Rome (Blanc et al., 2004).

It is known that humans have used some Inula species since ancient times for various reasons from an ethnopharmacological point of view. *Inula* species have been most commonly referred to as an appetizer, expectorant, anti-inflammatory, and diuretic. In addition, there is literature information on the use of Inula species in the treatment of urogenital system diseases such as urinary tract inflammation, cystitis, nephritis, and uremia (Afifi et al., 2015).

This study aimed to reveal the chemical composition and antioxidant activity of the various extracts of *Inula graveolens* L. (Desf.) [Synonyms: *Dittrichia graveolens* (L.) Greuter, *Jacobea graveolens* (L.) Merino, *Cupularia graveolens* (L.) Gren. & Godr.].

2. Materials and methods

2.1 Plant material and extract preparation

Aerial parts of *I. graveolens* were collected from Gaziantep-Karatas highway (steppe land), Gaziantep- Turkey, on 26.09.2020. The plant sample was deposited at the Herbarium of Cumhuriyet University Biology Department (CUFH Voucher No: AA 6753). Details of the extract preparation process were given in the supplementary file.

2.2. Isolation of the essential oil

Details of the essential oil isolation were given in the supplementary file.

2.3. GC-MS analysis conditions

Details of the GC-MS analysis were given in the supplementary file.

2.4. Total antioxidant activity by β-Carotene–linoleic acid method

Details of the β-carotene–linoleic acid method were given in the supplementary file.

2.5. Scavenging effect on 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

Details of the DPPH radical scavenging assay were given in the supplementary file.

2.6. Reducing power

Details of the reducing power assay were given in the supplementary file.

2.7. Chelating effects on ferrous ions

Details of the chelating effect assay on ferrous ions assay were given in the supplementary file.

3. Results and discussion

3.1. Chemical composition of the essential oil

The chemical composition of the essential oil of *I. graveolens* was presented in Table 1. The results revealed that 99.5% of the total oil was identified. Main constituents of the oil were determined as bornyl acetate (68.5%), borneol (7.7%), camphene (4.6%), *epi*-α-cadinol (4.0%) and eicosane (3.2%), respectively.

Table 1. Chemical composition of the essential oil of *I. graveolens*[1]

No	Compounds	Area (%)	Exp. RI	Ident. LRI
1	α-Pinene	0.2	934	939
2	Camphene	4.6	950	954
3	β-Pinene	0.6	974	979
4	Dehydro-1.8-cineole	1.5	986	991
5	Limonene	0.7	1025	1029
6	*Neo*-3-Thujanol	0.8	1150	1154
7	Borneol	7.7	1165	1169
8	Bornyl acetate	68.5	1280	1289
9	β-Caryophyllene	1.8	1410	1419
10	Prenyl benzoate (-3-Methyl-2-butenyl-benzoate)	0.4	1421	MS [1]
11	Drima-7,9(11)-diene	0.5	1470	1473
12	γ-Muurolene	0.4	1479	1480
13	Muurola-4(14),5-diene	0.3	1487	1494
14	β-Atlantol	1.6	1600	1608
15	*Epi*-α-Cadinol	4.0	1640	1640
16	*(E)*-Bisabol-11-ol	0.8	1665	1668
17	Eicosane	3.2	2000	2000
18	Tricosane	0.7	2300	2300
19	Tetracosane	1.0	2400	2400
20	Pentacosane	0.5	2500	2500
21	Total	99.5		
22	α-Pinene	0.2	934	939
23	Camphene	4.6	950	954
24	β-Pinene	0.6	974	979
25	Dehydro-1.8-cineole	1.5	986	991
26	Limonene	0.7	1025	1029
	Neo-3-Thujanol	0.8	1150	1154

[1] MS: 68(100), 105(80), 77(40), 51(15)

The essential oil composition of this plant has previously been reported several times (Blanc et al., 2004; Dinis et al., 1994). According to Dinis et al. (1994), the main constituents of the oils obtained from 20 different plant samples collected from the various locations and at different growing stages were mainly determined as bornyl acetate and borneol (7.6%) typically. Some other samples within the same study showed slight differences in terms of their major compounds. In addition to these major compounds, tau-cadinol was also determined within the oil of these atypical samples. According to another study carried out by Blanc et al. (2004), major compounds of the essential oil of I. graveolens from Lebanese origin were determined as bornyl acetate (70.6-72.3%), *t*-cadinol (1.4-13.4%), borneol (2.7-12.4%) and caryophyllene oxide (1.9-2.3%), respectively. *I. graveolens* is also known as *Dittrichia graveolens* in the literature. The essential oil composition of *D. graveolens* was also studied (Ghosn et al., 2006; Petropoulou et al., 2004). In both studies, bornyl acetate was determined as the major compound. Additionally, *epi*-α-cadinol (30.2%) was defined as the main constituent (Ghosn et al., 2006). In the latter, borneol (60.7%) and β-caryophyllene were identified among the major compounds (Petropoulou et al., 2004).

Compared to the literature data given above, the essential oil of I. graveolens commonly consists of borneol, bornyl acetate, and cadinol derivatives. As can be seen from the results presented in Table 1, the main compounds identified in the present study are highly inconsistent with those published before.

3.3. Antioxidant activity

It is possible to determine the antioxidant activities of plant extracts or essential oils, or individual phytochemicals with more than one test system. For antioxidant activity results to be consistent, researchers are expected to use at least two different test systems together. In each antioxidant test system, test samples may exhibit different activity profiles. These differences can be mainly affected by the solvent used for the extraction, the experimental medium temperature, the chemicals used, etc.

Lipid peroxidation in organisms is one of the most important causes of cellular damage. The main factor causing lipid peroxidation is free radicals. Free radicals can cross-link membrane lipids. Therefore, lipid oxidation causes severe damage to cell membranes, the main components of which are largely lipids (Lanzetta et al., 1991). Oxidation of lipids can cause many diseases, especially ischemia, as it increases the sensitivity of phospholipids to phospholipase (Esterbauer et al., 1991; Sevanian et al., 1981) and increases membrane calcium permeability (Weglicki et al., 1984).

A strong correlation between the antioxidant activity and phenolic acid contents of the samples was determined. In β-carotene/linoleic acid test system, the most potent activity was exhibited by methanol extract, of which inhibition value is 88.34% (Table 2). This value is greater than that of synthetic antioxidant BHA (86.48%). This activity is followed by acetone extract. On the other hand, essential oil and ethanol extract showed almost the same activity profile. Chloroform extract showed the weakest activity in this test system.

Table 2. Antioxidant of the essential oil and different solvent extracts of Inula graveolens in β-carotene/linoleic acid and DPPH test systems[1]

Antioxidant activity in β-carotene/linoleic acid test system			
Samples	**Inhibition (%)**		
Essential oil	55.40 ± 0.12		
Chloroform extract	42.14 ± 0.70		
Acetone extract	71.26 ± 0.19		
Acetonitrile extract	45.20 ± 0.42		
Ethanol extract	56.19 ± 0.38		
Methanol extract	88.34 ± 0.57		
BHA	86.48 ± 1.93		
BHT	92.14 ± 0.15		
DPPH free radical scavenging capacity (%)			
Samples	**0.2 mg/ml**	**0.4 mg/ml**	**0.8 mg/ml**
Essential oil	14.85 ± 0.02	26.50 ± 0.24	56.19 ± 0.46
Chloroform extract	6.85 ± 1.25	12.52 ± 0.50	26.92 ± 0.41
Acetone extract	18.27 ± 0.34	33.40 ± 0.21	71.80 ± 0.24
Acetonitrile extract	8.56 ± 0.25	15.65 ± 0.56	33.65 ± 0.53
Ethanol extract	18.11 ± 0.17	32.32 ± 0.50	68.53 ± 0.63
Methanol extract	24.15 ± 0.40	43.10 ± 1.22	91.38 ± 0.24
BHA	92.83 ± 0.84	nt [2]	nt
BHT	81.41 ± 0.00	nt	nt

[1] Values expressed are means ± S.D. of three parallel measurements
[2] nt: Not Tested

Plant extracts, essential oils, or phytochemicals can terminate oxidant chain reactions by reacting with peroxyl radicals. Many researchers agree that natural antioxidants derived from plant sources effectively terminate free radical reactions (Bagchi et al., 1997). In addition, it is known that L-tryptophan (one of the precursors of secondary metabolites) reacts with phenolic aldehydes in foods to form phenolic tetrahydro-β-carboline alkaloids. This compound can effectively scavenge 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (Shimada et al., 1992).

As presented in Table 2, all samples showed a concentration-dependent activity in DPPH free radical scavenging assay. In this test system, methanol extract showed the most substantial radical scavenging effect. The scavenging value exhibited by this extract was measured as 91.38% at 0.8 mg/mL concentration. This is followed by acetone and ethanol extracts. The radical scavenging capacity of the essential oil was determined as 56.19% at 0.8 mg/mL. In this test system, the weakest activity was exhibited by chloroform extract at all concentration values.

Molecules with chelating ability reduce the redox potential of biochemical reactions. As a result, they contribute to the stabilization of the oxidized forms of metal ions. Therefore, secondary metabolites with chelating properties can act as good antioxidants. Chain oxidation reactions initiated by metal ions are important causes of oxidation-related spoilage in foods (Herraiz et al., 2003). Due to these properties, metal ions are also associated with cancer and arthritis cases (Gordon, 1990). Ferrous ions are one of the most effective pro-oxidants and are widely available in foods (Halliwell et al., 1995).

In the present study, the chelating ability of the extracts and essential oil toward ferrous ions was also investigated. Table 3 also shows the chelating effects of the samples obtained from *I. graveolens*. In this test system, EDTA was also used as a standard on ferrous ions. According to the data presented in the table, the most potent chelating agent was determined as methanol extract (63.43%). On the other hand, chloroform extract's metal chelating ability was found weak compared with the other test materials. The essential oil also showed a weak activity profile. The chelating effect of EDTA was determined as 99.74%.

Table 3. Chelating effect and reducing power of the essential oil and different solvent extracts of Inula graveolens[1]

Chelating effect			
Samples	**(%)**		
Essential oil	27.35 ± 0.52		
Chloroform extract	18.79 ± 0.85		
Acetone extract	36.68 ± 0.32		
Acetonitrile extract	22.30 ± 0.09		
Ethanol extract	47.57 ± 0.81		
Methanol extract	63.43 ± 0.75		
EDTA	99.74 ± 0.15		
Reducing power (absorbance at 700 nm)			
Samples	**0.2 mg/ml**	**0.4 mg/ml**	**1.0 mg/ml**
Essential oil	0.045 ± 0.006	0.100 ± 0.008	0.171 ± 0.003
Chloroform extract	0.024 ± 0.005	0.053 ± 0.003	0.091 ± 0.002
Acetone extract	0.037 ± 0.012	0.082 ± 0.005	0.140 ± 0.002
Acetonitrile extract	0.025 ± 0.009	0.057 ± 0.004	0.098 ± 0.006
Ethanol extract	0.054 ± 0.006	0.120 ± 0.002	0.204 ± 0.001
Methanol extract	0.072 ± 0.004	0.160 ± 0.006	0.273 ± 0.008
BHA	2.303 ± 0.064	nt [2]	nt
BHT	1.258 ± 0.121	nt	nt

[1] Values expressed are means ± S.D. of three parallel measurements
[2] nt: Not Tested

Reducing molecules generally donate a hydrogen atom to free radicals, reducing their tendency to react. Therefore, the reaction chain that causes oxidative damage can be broken by reducing molecules (Bagchi et al., 1997; Yamaguchi et al., 1998). The basis of the reducing power test applied in the present study is based on the principle that the Fe^{3+}/ferricyanide complex turns into Fe^{2+} in the presence of antioxidant compounds (Hatano et al., 1988).

Table 3 also shows the reducing power of the samples. The activity increased with concentration. The reducing power of methanol extract was determined as 0.273 nm at 1.0 mg/mL. The reducing

power of BHA and BHT were measured as 2.303 and 1.258 nm, respectively, at 0.2 mg/mL.

4. Conclusions

In this study, antioxidant activities of extracts and essential oil obtained from the aerial parts of *I. graveolens* with solvents of different polarities were investigated. In the tests, the essential oil showed moderate antioxidant activity, while the activity of the methanol extract was comparable to the positive control. Therefore, it was concluded that the methanol extract of *I. graveolens* could be considered as one of the new and alternative sources of antioxidant compounds. However, further chromatographic analyzes are needed to determine the bioactive compounds responsible for the activity in the methanol extract.

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None.

Conflict of Interest

The author confirms that there are no known conflicts of interest.

CRediT authorship contribution statement

H. Askin AKPULAT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing, Review & Editing.
Saliha Seyma SAHINLER: Conceptualization, Data curation, Formal analysis, Resources, Software, Visualization.

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Supplementary file

The supplementary file accompanying this article is available at https://dergipark.org.tr/en/download/journal-file/23546.

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