Journal of Integrative and Anatolian Medicine
Bütünleyici ve Anadolu Tıbbı Dergisi

Cilt/ Volume: 4 Sayı / Issue: 3
Yıl/Year: 2023

Yayıncı / Publisher
Sağlık Bilimleri Üniversitesi
University of Health Sciences Turkey

Araştırma Makalesi / Research Article, 4(3): 3 - 10, 2023

https://dergipark.org.tr/en/pub/batd/article/1278048
DOI: 10.53445/batd.1278048

Geliş Tarihi:	06 Nisan 2023,	Kabul Tarihi:	11 Mayıs 2023,	Yayın Tarihi:	28 Ağustos 2023
Received:	06 April 2023,	Accepted:	11 May 2023,	Published:	28 August 2023

Quantitative analysis of bioactive compounds by LC-MS/MS from *Inula graveolens*

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ABSTRACT

Medicinal plants have been employed commonly in many countries to remedy of various ailments. In this study, *Inula graveolens* leaves (5.0 g) were extracted with methanol (100 mL) for 24 hours at room temperature. After filtration with Whatman 1 filter pater, the solvent was removed under reduced pressure to yield the crude extract. Quantification of natural products in methanol extract of *Inula graveolens* was determined by LC-MS/MS. Chlorogenic acid was determined as a chief product (3.5 mg/g extract). Furthermore, salicylic acid (0.055 mg/g extract), caffeic acid (0.054 mg/g extract), protocatechuic acid (0.026 mg/g extract), hesperidin (0.016 mg/g extract), rutin (0.0160 mg/g extract) were found in the methanol extract. Since *Inula graveolens* contains important bioactive compounds, it has the possibility to be used in pharmaceuticals and food.

Keywords: *Inula graveolens*, LC-MS/MS, quantitative analysis, bioactive compounds

Inula graveolens'ten LC-MS/MS ile biyoaktif bileşiklerin kantitatif analizi

ÖZET

Şifalı bitkiler birçok ülkede çeşitli rahatsızlıkların tedavisi için yaygın olarak kullanılmaktadır. Bu çalışmada *Inula graveolens* yaprakları (5.0 g) metanol (100 mL) ile 24 saat oda sıcaklığında ekstrakte edildi. Whatman 1 filtre kağıdı ile süzüldükten sonra çözücü, düşük basınç altında uzaklaştırılarak ham ekstrakt elde edildi. *Inula graveolens*'in metanol ekstraktındaki biyoaktif bileşiklerin kantitatif analizi, LC-MS/MS ile belirlendi. Ana ürün olarak klorojenik asit belirlendi (3.5 mg/g ekstrakt). Ayrıca salisilik asit (0,055 mg/g ekstrakt), kafeik asit (0,054 mg/g ekstrakt), protokatekuik asit (0,026 mg/g ekstrakt), hesperidin (0,016 mg/g ekstrakt), rutin (0,0160 mg/g ekstrakt) metanol ekstraktında tespit edildi. *Inula graveolens* önemli biyoaktif bileşikler içerdiğinden ilaç ve gıda da kullanım potansiyeline sahiptir.

Anahtar kelimeler: *Inula graveolens*, LC-MS/MS, kantitatif analiz, biyoaktif bileşikler

INTRODUCTION

The therapeutic use of plants dates back to antique times (Demirtas et al., 2013; Elmastas et al., 2004; Topçu et al., 1999). Plants became the focus of science with the development of spectroscopy (Sahin Yaglioglu et al., 2013). Plants include the secondary metabolites, organic compounds that are produced by plants, fungi, and bacteria (Aksit et al., 2014; Bayir et al., 2014; Erenler et al., 2014; Kaya et al., 2014). Unlike primary metabolites such as carbohydrates, proteins, and fats, which are essential for the progress and growth of the organism, secondary metabolites are not directly included in progress, growth. Instead, secondary metabolites play important roles in the interactions of these organisms with their environment, including defense against predators, competition with other organisms, and attraction of pollinators or other beneficial organisms (Aydin et al., 2016; Erenler et al., 2015; Türkmen et al., 2014). Some examples of secondary metabolites include alkaloids, terpenoids, flavonoids, and phenolic compounds (Elmastas et al., 2016; Erenler, Pabuccu, et al., 2016; Erenler, Sen, Aksit, et al., 2016). Secondary metabolites have many applications in medicine, agriculture, and industry (Erenler, Sen, Yaglioglu, et al., 2016; Erenler, Sen, et al., 2016). For example, many drugs are derived from secondary metabolites, such as the painkiller morphine from opium poppies, the anticancer drug paclitaxel from yew trees, and the antibiotic penicillin from fungi. In addition, secondary metabolites have potential applications in crop protection, as insecticides, fungicides, and herbicides, and in the development of novel materials, such as bioplastics and biofuels. Quantifying secondary metabolites in plants is extremely important for identifying secondary metabolite sources, revealing the potential uses, and isolating bioactive compounds (Erenler, Adak, et al., 2017; Erenler, Demirtas, et al., 2017; T. Karan et al., 2016).

Phenolic acids are a type of organic compound that are widely distributed in plant kingdom. They are a subclass of phenolic compounds and are characterized by the presence of a carboxylic acid group attached to a benzene ring. Phenolic acids are found in a variety of plant-based foods such as fruits, vegetables, grains, and herbs, and are known for their antioxidant and anti-inflammatory properties. Some common examples of phenolic acids include caffeic acid, ferulic acid, gallic acid, and rosmarinic acid. These compounds have been studied for their potential health benefits, including the prevention of chronic diseases such as cancer, cardiovascular disease, and diabetes (Erenler, Meral, et al., 2017; Guzel et al., 2017; Tunay Karan, Altuner, et al., 2017; Tunay Karan & Erenler, 2017).

Inula graveolens (L.) Desf. [Syn. *Dittrichia graveolens*. (Desf.) Greuter] belonging to the Asteraceae family is represented by 90 species and is generally distributed in Asia, Europe and Africa. Previous phytochemical study by LC-MS/MS revealed the chlorogenic acid, quinic acid, hyperoside, protocatechuic acid and quercetin were the major compounds (Silinsin et al., 2018). Inula species were reported to reveal significant biological activity including antioxidant, antibacterial, nematicidal, antiproliferative, cardioprotective, anti-inflammatory, antiseptic properties. In addition, flavonoids, terpenes, xanthanolides, guaianolides were determined in this genus (Tunay Karan et al., 2018).

In this study, bioactive compounds of *Inula graveolens* were determined quantitatively by LC-MS/MS.

MATERIAL AND METHODS

PLANT MATERIAL

Inula graveolens was collected from Tokat in July 2021 and was identified by Dr. Ahmet Ilcim, Mustafa Kemal University in comparison with previously stored sample (No:1823).

EXTRACTION

Aerial part of *Inula graveolens* leaves (5.0 g) were extracted with methanol (100 mL) for 24 hours at ambient temperature. After filtration (Whatman 1 filter paper), the solvent was evaporated by reduced pressure to produce the extract (0.4 g) (Elmastas et al., 2018; Tunay Karan, Erenler, et al., 2017).

LC-ESI-MS/MS ANALYSIS

Quantitative analysis of phenolics was carried out by LC-MS/MS (Agilent Technologies 1260 Infinity II, 6460 Triple Quad Mass spectrometer). Poroshell 120 SB-C18 (3.0 × 100 mm, I.D., 2.7 µm) column was employed (Erenler, Yaman, et al., 2023). *Inula graveolens* leaf extract (50 mg) was weighed in Eppendorf (2.0 mL) and dissolved with methanol (1.0 mL). The hexane was added to this solution and centrifugated for 10 minutes at 9000 rpm. 100 µL was taken from methanol phase and diluted by addition of water (450 µL) and methanol (450 µL). In the last stage, this solution was filtrated (0.22 µm filter) and injected to the instrument. The injection volume was 5.12 µL and flow rate was adjusted as 0.40 mL/min. Formic acid (0.1%) and ammonium formate (5.0 mM) in water A, formic acid (0.1%) and ammonium formate (5.0 mM) in methanol B were used for mobile phase. The gradient program was adjusted as 25% for 1-3 min, 50% for 4-12 min, 90% for 13-21 min, and 3% for 22-25 min for B mobile phase. The column temperature was 40°C. The capillary voltage was 4000 V, nebulizing gas (N_2) flow was 11 L/min, and pressure was 15 psi, gas temperature was 300 °C.

RESULTS AND DISCUSSION

Quantitative analysis of phenolic compounds in *Inula graveolens* were determined by LC-MS/MS analysis (Table). Quantification of secondary metabolites in the plant plays an important role in the drug development process. Since, quantitative analysis is an important guide for the isolation of target compounds existing in plants for drug potential (Erenler, Demirtas, et al., 2018; Erenler, Telci, et al., 2018).

Polyphenols, which are bioactive compounds, are known as second metabolites in plants. These substances are found in different parts of the plant and are the source of color, smell and taste of the plants. Polyphenols play an important role in resistance against microbial pathogens and protect against radiation and toxins (Haslam, 1974). There is a relationship between the consumption of polyphenol-rich foods and the prevention of diseases such as cancer, coronary heart diseases and osteoporosis. This situation has increased the interest in polyphenols. Phenolic acids and flavonoids are in the class of polyphenols (Del Bo' et al., 2019).

Flavonoids are a class of secondary metabolites revealing interesting and favourable medicinal effects. They display the significant biological properties such as antioxidant, anti-inflammatory, antibacterial, antiviral, anticancer (Erenler, Carlik, et al., 2023). In this study, bioactive compounds in *Inula graveolens* leaf extract were determined quantitatively and chlorogenic acid was found as a major compound with the value of 3.5 mg/g extract. Moreover, salicylic acid (0.055 mg/g extract), caffeic acid (0.054 mg/g extract), protocatechuic acid (0.026 mg/g extract), hesperidin (0.016 mg/g extract), rutin (0.0160 mg/g extract) were determined in *Inula graveolens* leaf extract (Figure).

Table. LC-MS/MS analysis of methanol extract of *Inula graveolens* leaf (mg/g extract)

S. No	Name	RT	Amount
1	Shikimic acid	1.375	nd
2	Gallic acid	3.186	0.0062
3	Protocatechuic acid	5.409	0.0258
4	Epigallocatechin	6.780	nd
5	Catechin	6.839	nd
6	Chlorogenic acid	7.354	3.5167
7	Hydroxybenzaldeyde	7.639	0.0062
8	Vanillic acid	7.750	nd
9	Caffeic Acid	7.814	0.0539
01	Syringic acid	8.353	nd
11	Caffein	8.383	0.0013
12	Vanillin	8.625	0.0055
13	o-coumaric acid	9.425	0.0954
14	Salicylic Acid	9.740	0.0553
15	Taxifolin	9.839	nd
16	Resveratrol	9.670	nd
17	Polydatine	9.774	nd
18	Trans-ferulic acid	10.180	0.0210
19	Sinapic acid	10.429	nd
20	Scutellarin	11.151	nd
21	p-coumaric acid	11.566	nd
22	Coumarin	11.571	nd
23	Protocatehuic ethyl ester	11.638	nd
24	Hesperidin	11.890	0.0160
25	Isoquercitrin	11.730	0.0031
26	Rutin	12.349	0.0635
27	Quarcetin-3-Ksilozid	12.449	nd
28	Kaempferol-3-glucoside	13.283	0.0010
29	Fisetin	13.276	nd
30	Baicalin	13.552	nd
31	Chrysin	14.213	nd
32	Daidzein	14.154	nd
33	Trans-cinnamic acid	14.483	0.0008
34	Quercetin	15.057	nd
35	Naringenin	15.243	nd
36	Silibinin	15.800	nd
37	Hesperetin	15.663	nd
38	Morin	15.937	nd
39	Kaempferol	16.650	nd
40	Baicalein	17.143	nd
41	Luteolin	17.993	nd
42	Biochanin A	17.901	nd
43	Capcaicin	18.211	nd
44	Dihydrocapcaicin	18.615	nd
45	Diosgenin	23.567	nd

Chlorogenic acid is the ester of quinic acid and caffeic acid. Chlorogenic acid is hydrolyzed by the intestinal microflora to various aromatic acid metabolites, including caffeic acid and quinic acid. Chlorogenic acid was reported to display substantial biological activities such as antioxidant, anti-inflammatory, wound healing, antimutagenic, anticarcinogenic, hepatoprotective, anti-diabetic, cardioprotective, antihypertensive, antiobesity, anti-metabolic syndrome, neuroprotective, antimicrobial, potential prebiotic effects (Farah et al., 2019).

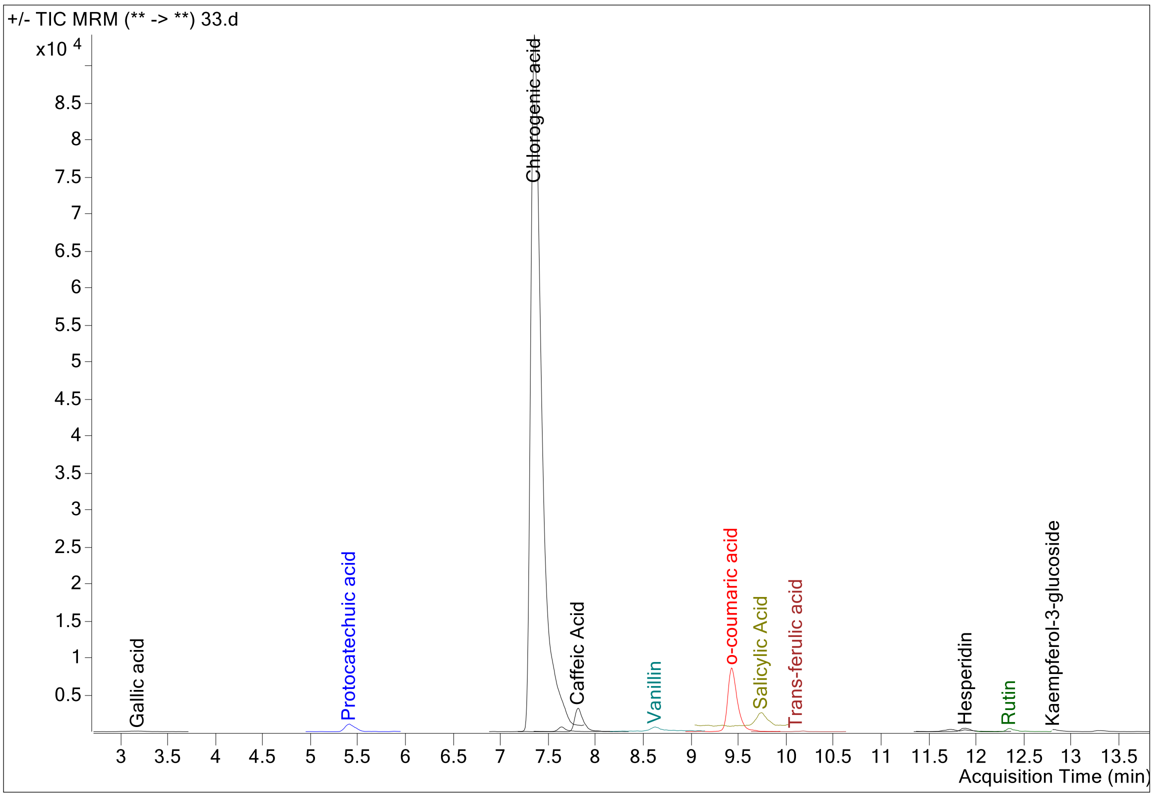

Figure. Chromatogram of methanol extract of *Inula graveolens* leaves

CONCLUSION

Phytochemistry of *Inula graveolens* leaves was investigated. The phytochemistry of *Inula graveolens* leaves was investigated. Due to the bioactive compound content, *Inula graveolens* is a promising agent for the drug development process. The major product chlorogenic acid enhances the significance of this plant. In addition, *Inula graveolens* could be an effective source for the isolation of chlorogenic acid in high quantities. This study will guide future studies in the field of *Inula graveolens*. It is extremely important to reveal its chemical content, especially for its use in food and pharmacology.

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