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Assessing ursolic acid contents of some commonly consumed herbs grown in Turkey

Türkiye'de yetişen ve yaygın olarak tüketilen bazı bitkilerin ursolik asit içeriklerinin belirlenmesi

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Abstract
Ursolic acid is a triterpenoid compound in the plant kingdom and has important biological functions. In this study, ursolic acid contents of nine herbs including *Anethum graveolens* (dill), *Camellia sinensis* L. (green tea), *Lepidium sativum* L. (garden cress), *Ocimum basilicum* L. (sweet basil), *Petroselinum crispum* L. (parsley), *Rosmarinus officinalis* L. (rosemary), *Salvia officinalis* L. (sage), *Thymus vulgaris* L. (thyme) and *Urtica dioica* L. (nettle) were determined by High-Performance Liquid Chromatography (RP-HPLC-UV). Based on the results from the analysis of herbs, ursolic acid contents varied between 0.20% and 7.11%. The ursolic acid content was highest in rosemary and sage (7.11±0.20% and 6.63±0.27%, respectively), while the lowest content of ursolic acid was in nettle (0.20±0.10). It is expected from the results of this work to contribute to the knowledge on the nutritional value of commonly consumed herbs in Turkey and encourage their consumption as functional foods.

Keywords: RP-HPLC-UV, Triterpene, Ursolic acid.

Öz
Ursolik asit, önemli biyolojik fonksiyonlara sahip olan ve bitkiler aleminde bulunan bir triterpenoid bileşiktir. Bu çalışmada, Anethum graveolens (dereotu), Camellia sinensis L. (yeşil çay), Lepidium sativum L. (tere), Ocimum basilicum L. (fesleğen), Petroselinum crispum L. (maydanoz), Rosmarinus officinalis L. (biberiye), Salvia officinalis L. (adaçayı), Thymus vulgaris L. (kekik) ve Urtica dioica L. (ısırgan)'i kapsayan 9 bitkinin Yüksek Performanslı Sıvı Kromatografi (RP-HPLC-UV) ile ursolik asit içeriği belirlenmiştir. Bitkilerin ursolik asit içerikleri %0.20 ile %7.11 arasında değişmiştir. En yüksek ursolik asit içeriği biberiye ve adaçayında (sırasıyla %7.11±0.20 ve % 6.63 ±0.27), en düşük ursolik asit içeriği ise ısırganda (%0.20±0.10) bulunmuştur. Bu sonuçlar ile Türkiye'de yaygın olarak tüketilen bitkilerin, besleyici değerleri konusundaki bilgilere katkı sağlanılması ve bu bitkilerin fonksiyonel gıda olarak tüketilmelerinin teşvik edilmesi amaçlanmıştır.

***Anahtarkelimeler**: RP-HPLC-UV, Triterpen, Ursolik asit.*

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1. Introduction

1. Giriş

Pentacyclic triterpenoids are terpenes that have 30 carbons on their skeleton and are found in the food and herbs in form of free acid or aglycones for triterpenoid saponins. A wide range of these compounds have important pharmacological activities. Ursolic acid also known as 3β-hydroxy-urs-12-en-28-oic-acid (UA) belongs to the ursane family. It is a natural pentacyclic triterpenoid carboxylic acid. UA is naturally found in the leaves and berries of natural medicinal plants and in the protective wax-like coatings of fruits. Recently, many studies have investigated the UA contents of some plants and fruits including. Both *in vitro* and *in vivo* studies have demonstrated that UA has biological functions including hepatoprotective, anti-inflammatory, anti-diabetic, anti-HIV, anti-microbial, anti-obesity, and anti-malarial activity (Cimanga et al., 2006; Ataie-Jafari et al., 2008; Jesus et al., 2015). Furthermore, it was found out that UA had marked anti-tumor effects and exhibited cytotoxic activity towards many cancer cell lines (Kim et al., 2011; Gai et al., 2016).

The UA contents of nine herbs such as *Ocimum basilicum* L., *Lepidium sativum* L., *Anethum graveolens*, *Camellia sinensis* L., *Urtica dioica* L., *Petroselinum crispum* L., *Rosmarinu sofficinalis* L. and *Salvia officinalis* L. were investigated in the present study. They are commonly consumed as traditional medicines, herbal teas, spices and vegetables. Their utilized parts, common uses, and biological activities are shown in Table 1. *O. basilicum* is cultivated worldwide for essential oil production and consumption for culinary purposes. *L. sativum* is commonly used as a salad ingredient in Anatolia. *A. graveolens* is generally used as a spice and employed in pickles, salads, sauces and soups. *C. sinensis* is one of the most consumed beverages thorough the world and a very important crop for local economy. It prevents catechin oxidation by polyphenoloxidase and provides many benefits to human health (Chan et al., 2011, Di Lorenzo et al., 2013). *U. dioca* is cultivated for the production of commercial chlorophyll used as a green food colorant. *P. crispum* is an aromatic and medicinal commonly used seasoning and flavoring agent for meat and sausages. *R. officinalis* is commonly used as traditional medicine and flavouring food products in Anatolia. *S. officinalis* is commonly used in culinary and as herbal tea in Anatolia. Likewise, *T. vulgaris* is generally used as a medicinal herb, herbal tea and flavouring agent.

The UA extraction technique is a determinant factor that affect the UA extraction yield. Several methods including maceration, heat reflux extraction, soxhlet, accelerated solvent extraction, microwave-assisted extraction, subcritical water and ultrasound-assisted extraction have been tested for the extraction of pentacyclic triterpenoids (Mlyuka et al., 2016; Bernatoniene et al., 2016). These authors stated that ultrasound-assisted extraction is more efficient method, easy to use, and more economical. The ultrasound-assisted extraction enhances the extraction efficiency thanks to the disruption of cell walls and reduction of particle size, enhancing the mass transfer of the cell contents as the result of cavitation bubble collapse. Furthermore, for the qualitative and quantitative analysis, high performance liquid chromatography (RP-HPLC) was revealed to be the most suitable method for the separation and quantification of UA.

To the best knowledge of the authors, no study has reported the UA content of *A. graveolens*, *C. sinensis*, *L. sativum, P. crispum, R. officinalis*, *S. officinalis*, *O. basilicum, U. dioca* and *T. vulgaris* grown in Turkey. Therefore, the present study aimed to evaluate for the first time the UA contents of Turkish *A. graveolens*, *C. sinensis*, *L. sativum, P. crispum, R. officinalis*, *S. officinalis*, *O. basilicum, U. dioca* and *T. vulgaris*. For this purpose, the ultrasound-assisted extraction was used for UA extraction and RP-HPLC-UV was employed for the qualitative and quantitative evaluations.

Table 1. Utilized parts, popular use and biological activities of herbs used in this study
Tablo 1. *Çalışmada kullanılan bitkilerin yararlanılan kısımları, popüler kullanım şekilleri ve biyolojik aktiviteleri.*

Binominal name	Common names	Edible parts	Popular use	Biological activities and role in traditional medicine	References
Ocimum basilicum L.	Sweet basil	Fresh leaves Seeds	Vegetable Herbal tea Spice	Treatment of cough, inflammations, constipation, dyspepsia, diarrhea, aches, warts, worms, kidney malfunctions and pains.	Opalchenova and Obreshkova, 2003
Lepidium sativum L.	Garden cress	Fresh leaves Seeds	Vegetable	Treatment of asthma, bronchitis cough, hypertension, and renal disease. Antibacterial, aphrodisiac, diuretic, expectorant, gastrointestinal stimulant, gastroprotective, laxative, stomachic and diuretic agent.	Rehman et al., 2012
Anethum graveolens	Dill	Fresh leaves Seeds Stems	Vegetable	Treatment of stomachache, indigestion, flatulence, gripe, hiccups and colic. Antihyperlipidaemic, antihypercholesterolaemic, anticancer, antidiabetic, antioxidant and diuretic.	Kaur and Arora, 2010
Camellia sinensis L.	Green tea	Dried leaves	Herbal tea	Reduction of the risk of cancer, cardiovascular diesases, ischemic damage and neurodegenerative diseases. Antioxidant, antidiabetic, antibacterial, anti-inflammatory and anti-HIV activities.	Chopade et al., 2008
Urtica dioica L.	Nettle	Fresh leaves	Vegetable Herbal tea	Good for anemia, diabetes, rheumatism, eczema, diarrhea, prostatic hyperplasia. Antimicrobial, antiulcer, analgesic effects.	Akbay et al., 2003; Gülçin et al., 2004
Petroselinum crispum L.	Parsley	Fresh leaves	Vegetable	Uses as a stomachic, carminative, emmenagogue, abortifacient, and diuretic agent.	Kreydiyyeh and Usta, 2002
Rosmarinus officinalis L.	Rosemary	Dried or fresh leaves	Spice	Antibacterial, antioxidant, and antimutagenic effects. Treatment of diabetes, respiratory disorders, stomach problems and inflammatory diseases.	Oluwatuyi et al., 2004; Bakırel et al., 2008
Salvia officinalis L.	Sage	Dried or fresh leaves	Spice Herbal tea	Anti-inflammatory, antidiarrheal, antiseptic, digestive, diuretic, expectorant, hemostatic, laxative, sedative, spasmolytic and hypoglycemic properties.	Gali-Muhtasib, 2006

2. Material and methods
2. Materyal ve metot

2.1. Materials
2.1. Materyaller

The herbs used in this study were shown in Table 1 The herbs were collected from different locations in Turkey. *L. sativum*, *A. graveolens* and *P. crispum* were harvested from Rize province. *O. basilicum*, *C. sinensis*, *U. dioca*, *R. officinalis S. officinalis* and *T. vulgaris* samples were collected from Mersin province. The fresh samples were dried in a vacuum oven at 60 °C for 8 hours. All samples were ground into powder with a coffee mill, and stored at +4°C until analyses.
All reagents and solvents were analytical grade. UA (98%) was purchased from Sigma-Aldrich Co. (Milwaukee, Wisconsin, USA).

2.2. Methods
2.2. Metotlar

2.2.1. Dry matter content
2.2.1. Kuru madde miktarı

Dry matter content was determined by drying the samples in the oven at 105 °C until to constant weight (AOAC, 2000).

2.2.2. Extraction of ursolic acid
2.2.2. Ursolik asit ekstraksiyonu

The extraction of ursolic acid was carried out adopting the method described in Wójciak-Kosior et al. (2013) with some modifications. Briefly, a portion of the powder (5 g) of the samples was accurately weighed, and placed in a capped glass tube, and then mixed with 100 mL of methanol.

The mixtures were kept for 30 min to allow the solvent to wet the samples and the tubes were subjected to the sonication in an ultrasonic bath (WiseClean WUC-A02H, Daihan Scientific Co., Gangwon-do, Korea) at 48±2°C for 120 min. The extracts were appropriately diluted and then passed through a 0.45 µm membrane filter (Pall Ultipor® N66) for RP-HPLC-UV analysis.

2.2.3. Determination of ursolic acid by RP-HPLC-UV
2.2.3. Ursolik asit miktarının RHPLC-UV ile belirlenmesi

Analysis of UA was done by RP-HPLC (Series 200, PerkinElmer Inc., Massachusetts, USA) equipped with a pump, an autosampler, and a UV-VIS detector. Separation was carried out by using a 150 x 4.6 mm, 5 µm Inertsil® ODS-4 C18 column. RP-HPLC analysis for UA was carried out according to the method described by Taralkar and Chattopadhyay (2012). The mobile phase consisted of a mixture, acetonitrile: methanol (80:20, v/v). The solution was degassed in an ultrasound bath and filtered under vacuum through a membrane (Immobilon®-P PVDF). The flow was 0.5 mL min^{-1}. The column temperature was maintained at 35±0.2°C. The effluent was measured at a wavelength of 210 nm for the detection of UA. To prepare standard solutions, 10.0 mg of UA standard was accurately weighted and dissolved in methanol (10 mL). Volumes of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 10 mL of UA standard solution were placed in 100 mL volumetric flasks and methanol added to a final volume of 100 mL. The solutions were filtered through a 0.45 µm membrane filter and analyzed in RP-HPLC. 10 µL of each solution were injected three times. Identification of UA was made by the comparison of the retention times with those of standard (Figure 1). The retention time for UA is 8.43 min (± 0.2 min). The area under the peak of the UA vs. concentration plot showed a linear fit with a correlation coefficient of 0.999. Quantification was performed on the basis of a linear calibration plot of peak area against concentration. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 1.667 and 5.003 ppm, respectively.

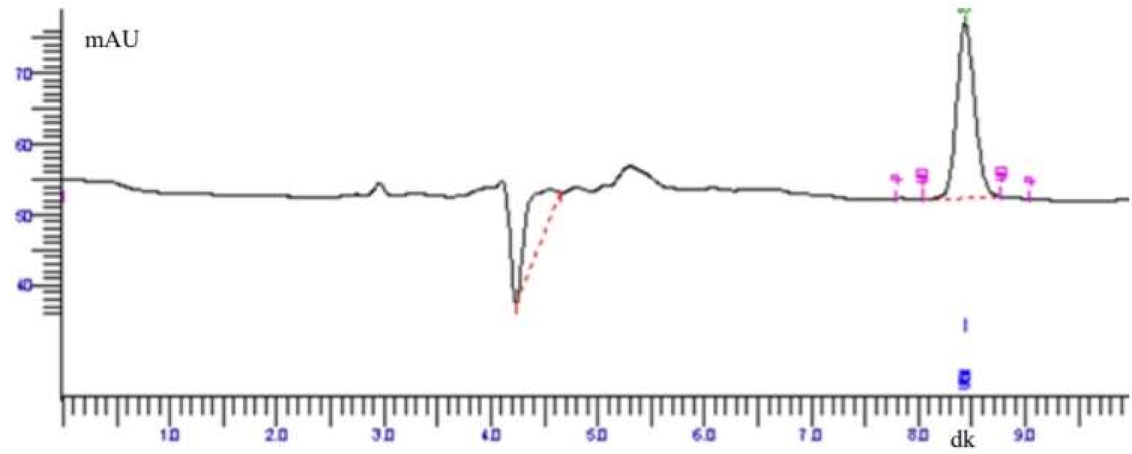

Figure 1. RP-HPLC chromatogram of the standard UA
Şekil 1. *UA standardının RP-HPLC kromatogramı*

2.2.4. Statistical analysis
2.2.4. İstatistiksel analizler

The results are shown as means ± standard deviation. Statistical significance (p<0.05) was measured by one-way ANOVA, followed by Duncan's multiple comparison test.

3. Results and discussion
3. Bulgular ve tartışma

The UA contents of the studied herb samples were given in Table 2. The averages of UA contents in the samples were 7.11%, 6.63%, 2.41%, 1.47%, 0.92%, 0.56%, 0.50%, 0.47% and 0.20% dry weight basis for *R. officinalis*, *L. sativum*, *A. graveolens*, *P. crispum*, *O. basilicum*, *L. sativum*, *C. sinensis*, *T. vulgaris* and *U. dioica*, respectively. As can be seen, the UA contents of the samples were ranged between 0.20% and 7.11%. Wójciak-Kosior et al. (2013) reported UA amounts ranged from 39.1 to 67.3 µg/g (from 0.00391 to 0.00673%) in *L. albi flos*, while Al-Tannak and Novotny (2020) found out that the UA content corresponded to 25.57 µg/g (0.002557%), 28.70 µg/g (0.00287%) and 55.10µg/g (0.00551%) in oregano (Al-Baraka), oregano (Waitro's) and olive leaves, respectively. Likewise, Rubashvili et al.

(2020) mentioned a UA content of 2.412-4.585 mg/g (0.2412-0.4585%) in apple waste. Meng et al. (2019) applied the single factor experimental design to maximize the extraction of UA from *Paulownia flos*. They reported that the UA content of *Paulownia flos* varied from 0.978 to 1.319 mg/g dry weight corresponding to 0.0978-0.1319% dry weight. The extraction of UA from different parts of *G. hederacea* and *G. hirsuta* yielded 2.73-25.15% and 3.47-26.82%, respectively (Grabowska et al., 2021). When comparing our findings to these previous studies, it can be assumed that although the extraction techniques and solvents are determinant factors for the extraction of UA, the Turkish *R. officinalis*, *L. sativum*, *A. graveolens*, *P. crispum*, *O. basilicum*, *L. sativum*, *C. sinensis*, *T. vulgaris* and *U. dioica* can be considered as rich sources of UA. The highest value was in found *R. officinalis* (mean 7.11%) followed by the *L. sativum* (mean 6.63%). The lowest amounts of UA are found nettle (mean 0.20%). *R. officinalis* and *L. sativum* can be regarded as rich sources of UA, since they displayed a higher amount of UA and are generally consumed raw in salads. Razboršek et al. (2007) determined the UA contents of 1.89 mg/g and 4.15 mg/g in dry weight in *R. officinalis* and *S. officinalis* was, respectively, while, Jäger et al. (2009) reported a maximum concentration of 2.95% of UA in dry *R. officinalis* leaves. Janicsák et al. (2006) found that the UA content was 3.825% in *S. officinalis*. Comparing the previous findings to our data, it can be assumed that UA contents in *R. officinalis* and *L. sativum* studied in the represent work are at least two-fold higher than those reported previously.

Table 2. Ursolic acid contents of herbs used in this study.
Tablo 2. *Çalışmada kullanılan bitkilerin ursolik asit içerikleri.*

Common name	Binominal name	Ursolic acid (%)	Mean±std
Dill	*Anethum graveolens*	0.96-3.43	2.41±0.98b
Garden cress	*Lepidium sativum* L.	0.35-0.93	0.56±0.23de
Green tea	*Camellia sinensis* L.	0.47-0.55	0.50±0.04de
Nettle	*Urtica dioica* L.	0.13-0.37	0.20±0.10e
Parsley	*Petroselinum crispum* L.	1.26-1.64	1.47±0.18c
Rosemary	*Rosmarinus officinalis* L.	6.86-7.28	7.11±0.20a
Sage	*Salvia officinalis* L.	6.26-6.97	6.63±0.27a
Sweetbasil	*Ocimum basilicum* L.	0.67-1.37	0.92±0.32d
Thyme	*Thymus vulgaris* L.	0.43-0.54	0.47±0.04de

The different letters in the same column denote the significant differences ($p < 0.05$).

UA contents of *A. graveolens* and *P. crispum* were reported for the first and their values were found lower than those of *R. officinalis* and *L. sativum*. *C. sinensis* and *L. sativum* displayed low UA content of 0.50% and 0.56% dry weight, respectively, and could be considered as poor UA sources. Regarding *O. basilicum*, our result is in accordance with Silva et al. (2008) who have determined 0.27% dry weight of UA in *O. basilicum* and 2.02% dry weight in *O. tenuiflorum*. In contrast, Jäger et al. (2009) have reported a higher value of UA in *T. vulgaris* when compared to our result. The lowest amount of UA found in *U. dioica* is consistent with Shailajan et al. (2014) who have mentioned low UA content ranged from 0.125 and 0.14 mg/g. Similarly, Bourgeois et al. (2016) have applied an optimization design to optimize the extraction conditions of UA from *U. dioica* and determined low amount of UA ranged from 0.99 to 32.41 µg/g dry weight in *U. dioica* having anti-aging and antioxidant activity. These differences may be due to the extraction methods, analysis methods, geographical origins, growing season, and agricultural practices.

4. Conclusion

4. Sonuç

In this study, UA contents of herbs that are generally used as raw (parsley, dill and cress), herbal tea or spices (rosemary, nettle, sage, thyme, sweet basil and green tea) in Turkey have been determined. The UA content was highest in rosemary and sage (7.11±0.20% and 6.63±0.27%, respectively), while the lowest content of UA was in nettle (0.20±0.10). In general, the UA content of herbs grown in Turkey was higher than that reported by many researchers. It has been shown that ultrasound-assisted extraction is an effective method for the recovery of UA from herbs. UA and its esters are virtually insoluble in water, thus, their bioavailability in the body is limited. Therefore, the use of these herbs as fresh or spice instead of consuming as herbal tea may help to increase the bioavailability of UA. Further studies should be done on the bioavailability of UA in these herbs.

Author contribution

Yazar katkısı

Oscar Zannou: Writing- Original draft, **Burak İpekci**: Visualization, Investigation, **İlkay Koca**: Conceptualization, Supervision, **Halil İbrahim Odabaş**: Writing- Original draft, Writing-Reviewing and Editing.

Declaration of ethical code

Etik beyanı

The author(s) of this article declares that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Conflicts of interest

Çıkar çatışması beyanı

The authors declare that they have no conflict of interest

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