Frontiers in Life Sciences and Related Technologies 4(1) (2023) 37-42

Contents lists available at Dergipark

Frontiers in Life Sciences and Related Technologies

Journal homepage: http://dergipark.org.tr/en/pub/flsrt

Research article

Phenolic and carotenoid composition of *Rhododendron luteum* Sweet and *Ferula communis* L. subsp. *communis* flowers

Mehmet Emin Seker[1], Aysegul Erdogan[*2]

[1] *Giresun University, Vocational School, Department of Crop and Animal Production, 28600, Giresun, Türkiye*
[2] *Ege University, Application and Research Center for Testing and Analysis, 35100, Izmir, Türkiye*

Abstract

The biologically important potential of polyphenols and carotenoids from plants motivates the exploration of new natural sources and medicinal uses for these chemicals. Plants with colorful flowers are used not only for the benefits of bioactive compounds but also for smart textile materials and as colorants. In this study, quantification of phenolic compounds and carotenoids in *Rhododendron luteum* Sweet and *Ferula communis* L. subsp. *communis* flowers were determined. The flowers of these plants were analysed for the first time in Türkiye. While catechin (297.36±3.42 µg/g), 4-OH benzoic acid (179.28±2.87 µg/g) and salicylic acid (178.98±2.42 µg/g) are found to be relatively higher in *R. luteum* Sweet flowers compared to other phenolic compounds, relatively higher amounts of rutin (335.95±4.32 µg/g) and ferulic acid (367.10±4.11 µg/g) were found in *F. communis* L. subsp. *communis* flowers. On the other hand, lutein and β-carotene were detected in both species, whereas astaxanthin (4.46±0.21 µg/g) was found only in *R. luteum*. This phytochemical information may be important for the proper utilization of these plants as sources of phenolic compounds and carotenoids for a variety of possible commercial applications.

Keywords: *Carotenoids; extraction; Ferula communis* L. subsp. *communis; phenolic compounds; Rhododendron luteum* Sweet

1. Introduction

More than 850 species of *Rhododendron* (Ericaceae) are endemic to Europe, North America, Southeast Asia, China, Japan, and Australia (Heywood, 1993). *Rhododendron luteum* Sweet (Yellow-flowered Rhododendron), which is frequently used as an ornamental plant and in floriculture because of its cold hardiness, is native to Eastern Europe (Caucasus area), north-eastern Lithuania, and Poland (Sawidis et al., 2011). In Türkiye, it is found in the north, particularly along the coast of the Eastern Black Sea, in the North Anatolian Mountains, in a few locations south of the Marmara Sea, and in the southeast Taurus Mountains (Alan et al., 2010; Marin et al., 2014).

The fragrant, funnel-shaped, 1-4 m tall shrub, deciduous plant, with yellow blooms grow in spring alongside or before the leaves and contain poisonous nectar (Marin et al., 2014). The flowers and leaves of *R. luteum* and *R. ponticum* contain a toxic substance called andromedotoxin. The honey produced by bees from these is called mad honey. (Alan et al., 2010). Despite the fact that these two species are toxic to people and animals, they have medical applications (Parfionov, 1987; Alan et al., 2010; Lyko et al., 2022). As a result of research conducted in Türkiye on samples of *R. luteum* leaves, it was determined that the produced extracts exhibited a variety of activities, including suppression of bacterial growth and cytotoxicity. *R. luteum* is well-known for its pharmacological properties and has been discovered to contain a substantial number of polyphenols. (Mahomoodally et al., 2020; Olech et al., 2020). In Turkish traditional medicine, it has been used to cure inflammation and rheumatic discomfort; when used topically, and proven to be effective in treating fungal infections (Popescu and Kopp, 2013). According to another work, Turkish and Chinese traditional

[*] Corresponding author.
E-mail address: aysegul_erdogan@live.com (A. Erdogan).
https://doi.org/10.51753/flsrt.1214172 Author contributions
Received 03 December 2022; Accepted 04 February 2023
Available online 29 April 2023

medicine employ *R. luteum* to treat acute and chronic bronchitis, rheumatism, arthritis, asthma, pain, inflammation, hypertension, and muscular and metabolic problems (Erdemoglu et al., 2008; Jaiswal et al., 2012; Lin et al., 2014). Chronic inflammation and oxidative stress are linked to several disorders, including cancer, cognitive dysfunctions, rheumatoid arthritis, and heart disease. While anti-inflammatory drugs can cause problems such as damage to the gastric mucosa, a decrease in gastric acid production, diarrhea or nausea, or painful side effects (Bindu et al., 2020), on the contrary, secondary metabolites derived from plant-based medicines have been demonstrated to possess either directly or indirectly anti-inflammatory and antioxidant properties without causing significant adverse effects (Pietta, 2000; González et al., 2015). Numerous studies demonstrate that polyphenols, such as flavonoids, have powerful antioxidant and anti-inflammatory properties (Olech et al., 2020). Antioxidants such as ascorbic acid, carotenoids, and phenolic compounds are chemicals generated by medicinal plants that help inhibit lipid peroxidation (Ceylan et al., 2019). Therefore, analysing the contents of herbal medications appears to be a potential option for determining the levels of secondary metabolites present in their composition and for discovering new natural and safe agents.

Due to the presence of polyphenolic compounds, carotenoids, and antioxidant properties, the flowers of *R. luteum* exhibit anti-inflammatory, antiviral, antibacterial, anticancer, anti-diabetic, immunomodulatory, cardioprotective, and hepatoprotective effects. (Demir et al., 2016). In tests with extracts of its leaves and flowers, it was shown that the extract showed specific cytotoxicity against colon and liver cancer cells relative to normal fibroblast cells (Demir et al., 2016; 2018). According to the study findings, *R. luteum* can be an excellent source of antioxidant and anticancer natural agents owing to their potential to inhibit the multiplication of cancer cells (Demir et al., 2018).

It has been utilized historically as an antidysenteric agent in the treatment of anti-hysteria and dysentery (Gunther, 1959; Heywood, 1972). *Ferula communis* L. (Giant fennel) is one of the most well-known ancient medicinal herbs. (Miski, 1986; Akaberi, 2015). *Ferula*, which is a member of the *Apiaceae* family, contains around 170 species (Rahali et al., 2019) that are scattered from Central Asia westward and through the Mediterranean area to northern Africa. *F. communis* is a 1-2.5 m tall, thickly rooted, hairless, latex-containing perennial aromatic plant with fragrant leaves (Rahali, 2021). The pharmacological properties of the genus *Ferula* are well-documented in both human and veterinary medicine. Its use as traditional medicine is preferred to treat a number of diseases (Singh, 1998). As medicinal applications of the genus *Ferula*, several studies have documented anti-carcinogenic (Saleem et al., 2001), anti-diabetic (Iranshahy and Iranshahi, 2011), anti-bacterial, anti-ulcerative, and anti-inflammatory benefits (Li et al., 2015; Rahali, 2021). However, reports indicate that *F. communis* is a highly dangerous and hazardous plant for people and animals (Rahali, 2021; Marchi, 2003). This is because *F. communis* exists in nature with two distinct chemical structures (chemotypes). Chemically hazardous *F. communis* and non-toxic *F. communis* are often found in distinct geographic locations (Akaberi et al., 2015). According to published research, the secondary metabolites of *F. communis* vary substantially based on regional conditions (Maggi et al., 2016).

According to classical medical texts such as Dioscorides, this plant has a wide variety of medicinal uses. For instance, crushed fresh seeds have been used to evacuate bloody contents from the mouth, relieve diarrheal stomach discomfort, and cure snake bites (Akaberi et al., 2015). For instance, it is utilized locally as a traditional cure to treat skin problems in Saudi Arabia, whilst roasted flower buds are employed to treat fever and diarrhea (Collenette, 1985). In general, polyphenols have significant anti-carcinogenic, anti-inflammatory, anti-diabetic, anti-osteoporotic, and anti-degenerative activities against the development of malignancies, cardiovascular diseases, diabetes, and osteoporosis (Graf et al., 2005). It is important to investigate the contents of plants used for therapeutic purposes in different regions. In this study, the contents of some phenolics and carotenoids in the flowers of *R. luteum* Sweet and *F. communis* L. subsp. *communis* plants were investigated. The secondary metabolite contents of these flowers were detected for the first time in Türkiye.

2. Materials and methods

2.1. Chemicals and reagents

Catechin, taxifolin, epicatechin, gallic acid, caffeic acid, ferulic acid, ellagic acid, protocatechuic acid, protocatechuic aldehyde, vanillin, p-coumaric acid, resveratrol, salicylic acid, rutin, 4-OH-benzoic acid, rosmarinic acid, oleuropein standards, and HPLC grade methanol used during extraction was purchased from Merck (Darmstadt, Germany) from Sigma Aldrich (Steinheim, Germany). The carotenoid standards (lutein, β-carotene and astaxanthin) in this study were obtained from CaroteNature (Switzerland).

2.2. Sampling area

The flowers of *Rhododendron luteum* Sweet and *Ferula communis* L. subsp. *communis* plants were collected from Şebinkarahisar district of Giresun. The identification of these species was made by Dr. Rena Hüseyinoğlu. The flowers obtained from these plants were dried in an oven at 40°C for 48 hours (Fig. 1).

Fig. 1. Photographs of dried plant flowers (A) *Rhododendron luteum* Sweet and (B) *Ferula communis* L. subsp. *communis*.

2.3. Extraction of phenolic compounds and carotenoids

The extraction of phenolic compounds was performed via Soxhlet extraction. For this purpose, 5 g of each sample was ground in a blender and placed in a Soxhlet cartridge. Methanol (150 ml) was added to the apparatus and extraction was carried out. After this process, the resulting mixture was filtered, and the solvent was evaporated with a rotary evaporator at 40°C and 175 mbar. After, the samples were dissolved in 25% methanol-water and stored at -20°C until LC-MS/MS analysis.

For the extraction of carotenoids, a modified protocol was applied to the plant samples reported in a previous study (Erdogan et al., 2020). Each sample was weighed (1 g) and $CaCO_3$ (1 g) was added to each. The mixture was extracted using 20 ml of ethanol containing 0.01 percent pyrogallol in an ultrasonic bath (Elmasonic S80H) for 15 minutes at 30 degrees Celsius. The solution was subsequently centrifuged at 5000 rpm for two minutes. After separating the filtrate, the residual biomass was re-extracted with new ethanol until its discoloration.

After vacuum filtering using 47 mm 0.20 m nylon filter paper, the mixed filtrates were finally collected (Sartorius). In the dark, the resulting extracts were saponified for two hours with 10% methanolic KOH. To halt the saponification process, 10.0 ml of 10% (w/v) Na_2SO_4 was added. The carotenoids were then extracted using 10.0 ml of diethyl ether.

This procedure was repeated until no yellow/red hue was noticed in the supernatant. The mixture was then treated with $CaCl_2$ to eliminate any leftover water, filtered using nylon filter paper, and evaporated to dryness at 40°C and 400 mbar using a rotary evaporator (Stuart RE 400). The residue was subsequently diluted in 5 ml of methanol and kept at -20°C for LC-MS/MS analysis.

2.4. Analysis of phenolic compounds and carotenoids by LC-MS/MS

LC-MS/MS analysis of phenolic compounds using C_{18} column (ODS Hypersil, 4.6 x 250mm 5μm) at 30°C and flow rate of 0.7 ml min^{-1} (Seker et al., 2021). The mobile phase information and operating conditions of the LC-MS/MS are given in Table 1.

Table 1
LC-MS/MS conditions for the analysis of phenolic compounds in plants.

	Solvent program		
	Time (Min.)	A % (Water) containing % 0.1 Formic acid	B % (Methanol)
LC-MS/MS parameters	0	100	0
	1	100	0
	22	5	95
	25	5	95
	30	0	100
Flow rate	0.7 ml/min		
Column oven temperature	30°C		
Column specifications	ODS HYPERSİL C_{18} 4,6*250 mm 5um		
Injection volume	20 µl		
Analysis time	34 min		
Ionization source	ESI		

For the quantification of carotenoids, C_{30} carotenoid column was used (YMC, 4.6 x 250mm 5μm) at 25°C and flow rate of 1.0 ml/min (Erdogan et al., 2022). The mobile phase information and operating conditions of the LC-MS/MS are given in Table 2.

3. Results and discussion

Ferula communis L. subsp. c*ommunis* is widely used in traditional medicine for a wide variety of ailments. Fresh plant materials, raw extracts, and isolated components of *F. communis* are reported to exhibit many antidiabetics, antimicrobial, antiproliferative and cytotoxic properties (Akaberi et al., 2015). There is a similar study on phenolic component analysis in *F. communis* flowers realized by Rahali et al. (2019). In the study, a total of 11 phenolic compounds were analyzed. These; tannic, gallic, syringic acid, ferulic, chlorogenic acids and catechin, catechin hydrate, resorcinol, coumarin, quercetin and flavone. Among them, catechin, gallic acid and ferulic acid were analyzed. While catechin could not be detected in this study, the amounts of gallic acid and ferulic acid were found to be 6.99 µg/g and 63.70 µg/g, respectively. The highest amount found was resorcinol with 148.02 µg/g. Gallic acid and catechin could not be detected in our study. The amount of ferulic acid was determined as 367.10 µg/g. This result is approximately 5.76 times more than the flowers collected from Tunisia. *Ferula* flowers are characterized by the presence of ferulic acid. Apart from these, unlike the studies in the literature, in *Ferula* flowers; protocatechuic acid, protocatechuic aldehyde, caffeic acid, vanillin, taxifolin, oleuropein and 4-OH-benzoic acid, salicylic acid and rutin were detected. The major phenolic compounds determined in *F. communis* flowers were ferulic acid and rutin with content of 367.10 µg/g and 335.95 µg/g, respectively. Apart from these, the amounts of salicylic acid and 4-OH-benzoic acid in the flowers were found to be higher than the other phenolic compounds.

Table 2
LC-MS/MS conditions for the analysis of carotenoids in plants.

	Solvent program			
	Time (Min.)	A % (Methanol)	B % (Water)	C % (tert- butyl-methyl ether)
LC-MS/MS parameters	0	70	5	25
	5	60	5	35
	10	45	0	55
	154	25	0	75
Flow rate	1.0 ml/min			
Column oven temperature	25°C			
Column specifications	YMC C_{30} carotenoid column 4.6*250 mm 5um column			
Injection volume	20 µl			
Analysis time	15 min			
Ionization source	APCI			

The abovementioned is the first study to detect phenolic compounds in *F. communis* flowers. In this context, our study is the first for *F. communis* in Türkiye and the second for *F. communis* flowers in the world. Also, many different phenolic compounds analyzed in this study. These polyphenols in flowers are known to effectively inhibit the oxidation process due to their hydroxyl groups and cyclic structures. It is also reported that various parts of this plant show antibacterial and cytotoxic activities (Akaberi et al., 2015). Another plant collected from Şebinkarahisar region is *Geranium ibericum* subsp. *jubatum.* When compared to *Geranium* flowers, it is seen that compounds other than catechin, gallic acid, and protocatechuic acid are quite high in *Ferula* flowers (Seker et al., 2021).

Due to its antioxidant effects and low toxicity, ferulic acid is currently widely employed in the food and cosmetics industry (Goleniowski et al., 2013). It has been observed that rutin, which has a combination of antioxidant activity from radical scavenging, xanthine oxidase inhibition, and chain-breaking effects (Itagaki et al., 2010), protects several types of cancer via a variety of pathways and performs a number of essential biological functions. Studies have demonstrated that rutin reduces the initiation and progression of several forms of cancer by inhibiting the development of numerous diseases (Nouri et al., 2020).

When literature studies regarding *Rhododendron luteum* Sweet are examined, the studies on the leaves of this plant are present, but there is no study on the phenolic component content of the flowers. For this reason, there is no study in which the obtained results can be compared. The results obtained can only be compared with the results obtained from the leaves of *R. luteum* or the phenolic component contents found in the flowers of *R. ponticum* or other plants.

Accordingly, the results of the investigation of phenolic compounds in *R. luteum* flowers will be the first in the literature. Among these results, the highest amount belongs to catechin with 297.36 µg/g. Apart from that, 4-OH-benzoic acid 179.28 µg/g and salicylic acid 178.98 µg/g were determined in *R. luteum* flowers. Protocatechuic acid, p-coumaric acid and gallic acid were also detected in the extracts. When we look at the studies on *R. luteum* leaves in the literature, it is seen that these values are 4-5 times lower than the amounts found in the leaves (Łyko et al., 2022). These values seem low when compared to another *Rhododendron* species, *R. ponticium* (Malkoc et al., 2016).

When compared to *Ferula communis*, the highest number of phenolic components determined in *Ferula* flowers was rutin and ferulic acid, while no ferulic acid was detected in *R. luteum* and the amount of rutin was found to be at a very low level of 4.35 µg/g. On the contrary, while catechin, epicatechin, *p*-coumaric acid and gallic acid were not found in *Ferula*, these amounts were found relatively high in *R. luteum* flowers. It is known that catechin has protective properties for human health due to its antioxidant properties (Zanwar et al., 2014). Although the amounts of catechin, gallic acid and ellagic acid were lower in *R. luteum* compared to *Geranium* flowers with high antioxidant properties, the amounts of all other phenolic compounds determined were higher than *Geranium* flowers (Seker et al., 2021).

In addition to phenolic compounds, the flowers/leaves of many plants may contain various carotenoids. In fact, various flowers of the same species might have varied quantities of total carotenoids. These may be the result of soil qualities, production conditions, and other variables, such as floral parts or analytical parameters. The majority of carotenoids in published research on various flowers are xanthophylls such as lutein and zeaxanthin. Various amounts of lutein may be found in the following plants: Chrysanthemum (11.78-307.22 g/g dry weight), snapdragon (14.1 g/g dry weight), garden nasturtium (350-450 g/g fresh weight), Mexican marigold (1062 g/g fresh weight), and pansies (51.1 g/g dry weight). It has also been observed that edible flowers, leafy greens, and root vegetables have significant levels of carotene. For instance, spinach (4 mg/100 g) in summer sun (358.1 mg/100 g fw), Mexican marigold (8.55 mg/100 g fw), and squash blossoms (1.01-13.35 mg/100 g fw) have greater carotene concentrations than carrots (0.3 mg/100 g fw) (Singh et al., 2001). However, related plants have not been shown to contain astaxanthin. In a research, *Cucurbita maxima* was shown to have trace quantities of astaxanthin (Seroczyńska et al., 2006). According to the findings of our investigation, the acquired carotenoid levels are quite high. Specifically, the lutein levels reported from both species are astonishingly high. On the other hand, a rather significant level of astaxanthin, which was exclusively detected in *R. luteum*, was discovered. Table 4 presents a summary of the outcomes.

Table 3

Quantitative results of phenolic compounds in plant flowers (µg g^{-1}).

Phenolic compounds	*F. communis*	*R. luteum*
Gallic acid	ND	59.98±0.60
Protocatechuic acid	16.50±0.24	88.26±0.73
Protocatechuic aldehyde	7.59±0.12	5.34±0.06
Catechin	ND	297.36±3.42
Epicatechin	ND	16.62±0.37
Caffeic acid	5.55±0.06	3.60±0.05
Vanilin	31.05±0.45	22.44±0.30
Taxifolin	10.35±0.18	4.14±0.06
p-Coumaric acid	ND	78.97±1.14
Ferulic acid	367.10±4.11	ND
Rosmarinic acid	ND	ND
Oleuropein	0.20±0.01	ND
4-OH-Benzoic acid	58.14±0.42	179.28±2.87
Salicyclic acid	59.40±0.68	178.98±2.42
Rutin	335.95±4.32	4.35±0.07
Resveratrol	ND	ND
Ellagic acid	ND	0.44±0.01

ND: Not detected
Data are represented as the mean ±SD (standard deviation) of three measurements

Table 4

Quantitative results of carotenoid in plant flowers (µg g^{-1}).

Carotenoids	*F. communis*	*R. luteum*
Lutein	633.72±12.20	207.63±3.58
Astaxanthin	ND	4.46±0.21
β-carotene	23.16±0.69	36.57±1.34

ND: Not detected
Data are represented as the mean ±SD (standard deviation) of three measurements

4. Conclusion

According to the findings of this study, some of the reported therapeutic benefits of *Rhododendron luteum* and *Ferula communis* flowers may be attributable to the phenolic compounds and carotenoids that were identified. The first detailed description of the flowers of these plants will make a significant contribution to the literature. Even though both blooms are yellow, the phenolic and carotenoid contents of the two plants differ greatly.

The phenolic and carotenoid contents of these plants may contribute to their anti-inflammatory, antiviral, antibacterial, anticancer, anti-diabetic, immunomodulatory, cardioprotective, and hepatoprotective medicinal properties. To better comprehend the origins of these effects, it is necessary to identify the many secondary metabolites present in the structure of these plants. Additionally, at subsequent phases, the amount of essential oil in the flowers of these plants and the amount of phenolic components in the leaves can be investigated and published.

Acknowledgments: The authors would like to acknowledge Ömer Kayır from Hitit University Scientific Technical Application and Research Center (HÜBTUAM) for the LC-MS/MS analyses. They are also thankful to Assoc. Prof. Dr. Rena Hüseyinoğlu for the identification of plants used in this study.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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Cite as: Seker, M. E., & Erdogan, A. (2023). Phenolic and carotenoid composition of *Rhododendron luteum* Sweet and *Ferula communis* L. subsp. *communis* flowers. *Front Life Sci RT*, *4*(1), 37-42.