

SCREENING OF POLYPHENOLIC COMPOUNDS FROM TRADITIONAL MEDICINAL HERBS

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ABSTRACT

The aim of the study was to determine the antioxidant potential of dry herbal and aromatic plants by a qualitative and quantitative screening of polyphenols by HPLC analysis. The results of screening of polyphenolic compounds obtained by RP-HPLC-DAD analysis showed that flower of *Anethum graveolens* has 13-times greater total polyphenols content than its stalk. Flowers of dill were characterized by the highest content of gallic acid (224.0 µg/g), epicatechin (199.5 µg/g), *trans*-caffeic acid (38.6 µg/g), *trans*-p-coumaric acid (76.1 µg/g), kaempferol (10.9 µg/g), and apigenin (49.4 µg/g) in comparison to other analyzed plants from celery family. It was observed that in comparison to root (*Apium graveolens* var. *rapaceum*) and stalk (*Apium graveolens* var. *dulce*), leaves of *Apium graveolens* var. *rapaceum* have higher content of polyphenols (Σ 2,755.0 µg/g). Celery leaves are primarily rich in chlorogenic acid (1,790.2 µg/g), *trans*-ferulic acid (709.5 µg/g), and resveratrol (32.6 µg/g). Fruits of *Tribulus terrestris* were characterized by the highest polyphenols content among all the analyzed plant materials (Σ 4,780.0 µg/g), followed by extract of *Calendula officinalis* (Σ 3,515.0 µg/g). The results of this study confirmed that oregano is a good source of *trans*-caffeic acid, *trans*-ferulic acid, and apigenin. Both types of mint: *Mentha piperita* and *Mentha longifolia* were characterized by the smallest content of polyphenolic compounds among the herbs. In comparison to mint, over 70% greater content of studied biologically active compounds were present in lavender (*Lavandula angustifolia*) and sage (*Salvia officinalis*). In conclusion, the current study provided an experimental evidence that the extract from *Anethum graveolens* flowers and *Apium graveolens* var. *rapaceum* leaves, and also *Calendula officinalis* and *Tribulus terrestris*, might be promising candidates as health-promoting agents.

Keywords: medicinal herbs, polyphenols, HPLC analysis

INTRODUCTION

Polyphenols are chemical compounds found in plants with a very diversified chemical structure. They are important bioactive food ingredients that have strong antioxidant, anti-inflammatory, antiviral, and anticancer effects. Polyphenols are commonly found in plants such as fruits, vegetables, herbs, tea and cereals (Hajimehdipoor *et al.*, 2014). These compounds are not synthesized in animal organisms but must be taken along with plant foods. Content of the polyphenol fraction in the plants also affects their organoleptic characteristics such as taste, colour, appearance, and texture (Katalinic *et al.*, 2006). Depending on the number of aromatic rings and the way they are combined, polyphenols are divided into the following classes: flavonoids, phenolic acids, stilbene and lignans (Grajek, 2007). Examples of the occurrence of polyphenols in plants is shown in Table 1.

The antioxidant properties of polyphenols result from the inhibition of enzymes that are involved in the production of free radicals and through the chelation of transition metal ions that catalyse reactions that result in reactive oxygen species (Fraga *et al.*, 2019). Polyphenols also can inactivate already formed radicals, especially those having a hydroxyl group at ortho or para positions, are very easy to take part in redox reactions in the cells. These compounds easily undergo oxidation due to the undisturbed transfer of protons and electrons (Cutrim *et al.*, 2018). Polyphenols have anti-cancer properties and are a potential source of natural anticancer drugs. Studies show that they inhibit the formation and progression of tumours and also play an important role in inhibiting the

development of existing ones (Fraga *et al.*, 2019). The effect of these substances on cancer consists in inhibiting the formation of blood vessels that forms and supply tumour with nutrients - this reduces the proliferation and metastasis (Grajek, 2004). They also have a property to decrease the activity of enzymes involved in the formation and proliferation of cancer cells, mainly urokinase and ornithine decarboxylase (Hakimuddin *et al.*, 2008). These compounds also protect against the harmful effects of poisonous nitrogenous compounds entering the body along with the food, i.e. in the form of smoked meats, cured meat.

A range of plants was selected for the experiment based on their potential of being a rich antioxidant source and potentially be applied in cancer treatment. Due to their construction and use, plants were divided into two groups: plants from celery family plants and other herbs. Celery plants are: leaves, flowers, and stalk of *Anethum graveolens*, root and leaves of *Apium graveolens* var. *rapaceum* and stalk of *Apium graveolens* var. *dulce*. The herbal plants are *Calendula officinalis*, *Tribulus terrestris*, Finola cultivar of *Cannabis sativa*, *Lavandula angustifolia*, *Mentha longifolia*, *Mentha piperita*, *Origanum vulgare* and *Salvia officinalis* "Purpurascens". It was previously confirmed that *Calendula officinalis* has a positive effect on the central nervous system and has other healing properties. Particularly commonly used are oils extracted from this plant, they are also used as dyes and lubricants. Also, *Lavandula angustifolia* oils and extracts are widely used in natural medicine (Syta *et al.*, 2018). *Tribulus terrestris* extract is used in traditional medicine mainly as a sedative, strengthening, and aphrodisiac. It also has polyphenolic compounds in its composition, which gives it antioxidant properties (Durgawale *et al.*, 2017).

Table 1 Examples of the occurrence of individual polyphenols in plants (Grajek, 2007)

Polyphenol groups	Flavonoid groups	Examples of plants containing polyphenols
Phenolic acids		Raspberries, blackcurrants, wild strawberries, tea
Lignans		Cereal grains, coffee
Stilbenes		Grape seeds, wine
Flavonoids	Catechins	Apricots, cherries, red wine, cocoa, green tea
	Flavones	Celery, parsley, cereal grains
	Isoflavones	Soy beans
	Flavanones	Seeds of peaches, citrus
	Flavanols	Onion, tea
Anthocyanins		Red wine, cereal grains, grapes, berries, cranberries

Antibacterial properties of *Salvia officinalis* "Purpurascens" has been known for long in traditional medicine, mainly used in the form of herbal tea (Neagu et al., 2011). Plant species of the genus *Mentha* are widely known and used due to their organoleptic and healing properties. *Mentha longifolia* and *Mentha piperita* are used as herbal medicines to treat diseases associated with gastrointestinal problems such as nausea, flatulence, vomiting, abdominal pain and cramps (Krzyzanowska et al., 2011). Research related to Finola Cultivar of *Cannabis sativa* suggests that the extracts obtained from this plant are rich in natural nutrients and antioxidants and have an impact on reducing the risk of chronic diseases (Smeriglio et al., 2016). In traditional medicine, *Anethum graveolens* is used as an antimicrobial, anti-diabetic, anti-inflammatory and antioxidant. This plant also has potential anticancer and antiradical properties (Oshaghi et al., 2016). Therapeutic properties, mainly antioxidant, due to their composition also shows *Apium graveolens* (Kooti et al., 2017).

According to the above, aromatic and medicinal plants for centuries have been known for their nutritional and therapeutic properties. As natural sources of active biomolecules, their properties are used in both scientific research and industry. Bioactivity of herbs and plants lie in their content of phytochemicals, including flavonoids, carotenoids, terpenoids, phenolic acids, ascorbic acids, and others (Isbilir et al., 2011). The goal of this study was to evaluate the unexplored source of natural antioxidants in 11 commonly used medicinal plants through screening of polyphenol compounds by HPLC analysis.

MATERIAL AND METHODS

Chemicals

Single-component standards (epicatechin, gallic acid, chlorogenic acid, *trans*-caffeic acid, *trans*-p-coumaric acid, rutin, *trans*-ferulic acid, resveratrol, quercetin hydrate, *trans*-cinnamic acid, apigenin, genistein and kaempferol), acetonitrile (HPLC gradient grade), methanol (HPLC grade), formic acid and phosphoric acid (ACS grade) were purchased from Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Double deionized water (ddH₂O) was treated (18.2 MΩ cm⁻²) in a Simplicity 185 purification system (Millipore SAS, Molsheim, France).

Preparation of calibration solutions and samples

Single-component standard solutions were prepared by dissolving 5 mg of each compound in 10 mL of methanol (HPLC grade). The samples of 11 commonly used plants were divided into two big groups. The first group of plants from celery family was represented by *Anethum graveolens*, *Apium graveolens* var. *rapaceum*, *Apium graveolens* var. *dulce*. The second group of herbal plants was represented by *Calendula officinalis*, *Tribulus terrestris*, Finola cultivar of *Cannabis sativa*, *Lavandula angustifolia*, *Mentha longifolia*, *Mentha piperita*, *Origanum vulgare* and *Salvia officinalis* "Purpurascens". All used plants were obtained from Botanical Garden of Slovak University of Agriculture in Nitra, Slovak Republic. The samples were prepared by extraction of 1 g of dried and grounded plants in 25 mL 80% ethanol (V/V) for 4 hours by horizontal shaker Unimax 2010 (Heidolph Instruments, GmbH, Germany). The extract was filtered through Munktell No 390 paper (Munktell & Filtrak GmbH, Bärenstein, Germany) and stored in closed 50 mL polyethylene (PE) vial tubes. Prior to

HPLC analysis, the extract was filtered through syringe PTFE filters (0.45 µm, 25 mm) (Agilent Technologies, Waldbronn, Germany).

RP-HPLC-DAD analysis of epicatechin

Epicatechin in extracts was determined by HPLC-DAD Agilent 1260 (Agilent Technologies, Waldbronn, Germany). All analyses were performed on a C18 column with reverse-phase Cortecs (4.6 mm x 150 mm x 2.7 µm) (Waters, Massachusetts, USA). The mobile phases consisted of methanol (A) and 0.1% HCOOH in ddH₂O (V/V) (B). The gradient elution was as follows: 0-2 min isocratic elution (20% A and 80% B), 2-15 min linear gradient elution (40% A and 60% B). Post-run was 3 min. The mobile phase flow was 0.6 mL min⁻¹ and the sample injection was 5 µL. Column thermostat was set to 25 °C and the samples were kept at 4 °C in the sampler manager. The detection wavelength was set at 230 nm, 240 nm, and 250 nm, with scanning of the spectrum in the range of 210 – 400 nm. The spectral data were collected and processed using Agilent OpenLab ChemStation software for LC 3D Systems.

RP-HPLC-DAD analysis of the rest of polyphenol compounds

All studied polyphenol compounds were determined by HPLC-DAD Agilent 1260 (Agilent Technologies, Waldbronn, Germany). All analyses were performed on a C18 column with reverse-phase Cortecs (4.6 mm x 150 mm x 2.7 µm) (Waters, Massachusetts, USA). The mobile phases consisted of 0.1% H₃PO₄ in ddH₂O (V/V) (A) and acetonitrile (B). The gradient elution was as follows: 0-1 min isocratic elution (90% A and 10% B), 1-5 min linear gradient elution (85% A and 15% B), 5-10 min (80% A and 20%B) and hold until 12 min, 12-15 min (30% A and 70% B). Post-run was 3 min. The mobile phase flow was 0.6 mL min⁻¹ and the sample injection was 5 µL. Column thermostat was set to 30 °C and the samples were kept at 4 °C in the sampler manager. The detection wavelength was set at 265 nm, 320 nm, and 372 nm, with scanning of the spectrum in the range of 210 – 400 nm. The spectral data were collected and processed using Agilent OpenLab ChemStation software for LC 3D Systems. The compounds were identified by comparing with standards of each identified compound using retention time, the absorbance spectrum profile, and also by running the samples after the addition of pure standards.

Statistical analysis

All data were expressed as the mean, where ± is the standard error of the mean (SEM). Statistical analysis was carried out using the Statistica program (version 13.1 for Windows; StatSoft Polska, Krakow, Poland). One-way ANOVA along with Duncan's test was performed as appropriate to determine the statistical significance. The statistical significance was established at P ≤ 0.05.

RESULTS AND DISCUSSION

Screening results of polyphenolic compounds obtained by RP-HPLC-DAD analysis are presented in Table 2-3. Total polyphenolic compounds contents in extracts were quantified as the sum of all RP-HPLC-DAD identified compounds and showed in Figures 1-2. Results are presented in dry weight. All the used polyphenols standards were detected in dill's leaf and flower, where *trans*-caffeic acid and kaempferol were absent in corresponding stalk extract. Furthermore, presented study showed that flower of *Anethum graveolens* has 3-times and 13-times greater polyphenols content (Σ 2,537.0 µg/g) than its leaf (Σ 816.0 µg/g) and stalk (Σ 189.0 µg/g), respectively (Figure 1). Similarly, Shyu et al. (2009) previously confirmed that the flower extract of dill had higher total amounts of polyphenols, flavonoids, anthocyanins, and proanthocyanidins than its corresponding leaf and seed extracts. Flavonoids including epicatechin, myricetin, quercetin, luteolin and kaempferol, and phenolic acids including gallic acid, chlorogenic acid, *p*-coumaric acid, benzoic acid, and *p*-anisic acid could be found in the extract prepared by mentioned authors. In the present work extract obtained from flowers of dill were characterized by the highest content of gallic acid (224.0 µg/g), epicatechin (199.5 µg/g), *trans*-caffeic acid (38.6 µg/g), *trans*-p-coumaric acid (76.1 µg/g), kaempferol (10.9 µg/g) and apigenin (49.4 µg/g) in comparison to other analyzed plants from celery family (Table 2). On the other hand, dill's leaves were characterized by the highest content of genistein (26.3 µg/g). Phenolic compounds isolated from *Anethum graveolens* are considered to be responsible for its antioxidant activity (Jana et al., 2010). Furthermore, Ksouri et al. (2015) showed that aerial parts of dill extracts demonstrated considerable antioxidant and antibacterial activities, providing opportunities to explore dill extracts as biopreservatives. Yazdanparast et al. (2008) also suggested that crude extracts of *Anethum graveolens* L. besides having strong anti-hyperlipidemic effects can improve the biological antioxidant status by reducing lipid peroxidation in liver and modulating the activities of antioxidant enzymes in rats fed with high-fat diet.

It was observed that in comparison to two common forms of celery: root (*Apium graveolens* var. *rapaceum*) and stalk (*Apium graveolens* var. *dulce*), leaves of *Apium graveolens* var. *rapaceum* have the highest content of polyphenols. Celery leaves (*Apium graveolens* var. *rapaceum*) were characterized by over 6-times and

11-times greater average polyphenols content (Σ 2,755.0 $\mu\text{g/g}$) than respectively: a stalk of *Apium graveolens* var. *dulce* and extract of *Apium graveolens* var. *rapaceum* – root (Figure 1). Celery leaves have the highest content of chlorogenic acid (1,790.2 $\mu\text{g/g}$), *trans*-ferulic acid (709.5 $\mu\text{g/g}$), quercetin (29.8 $\mu\text{g/g}$), rutin (69.6 $\mu\text{g/g}$) and resveratrol (32.6 $\mu\text{g/g}$) from analyzed samples from celery family (Table 2).

Considering the analyzed family of entire celery plants, celery leaves have the greatest antioxidant potential based on the greatest average content of polyphenols. Our assumptions have also been confirmed by **Perez-Gutierrez et al. (2018)** who reported the presence of bioactive compounds such as apigenin, lutein, kaempferol, rutin, caffeic acid, chlorogenic acid, ferulic acid and p-coumaric acid in celery. **Yao et al. (2010)** found out that the major phenolic acids in 11 celery cultivars included caffeic acid, p-coumaric acid, and ferulic acid,

while the identified flavonoids included apigenin, luteolin, and kaempferol. **Kaiser et al. (2013)** confirmed that apigenin, caffeic, ferulic and quinic acid derivatives, as well as malonylated and acetylated flavonoid derivatives were identified in celeriac (*Apium graveolens* L. var. *rapaceum* – root). On the other hand, **Liu et al. (2017)** isolated phenolic compounds from the crude extract of celery leaves (*Apium graveolens* L. var. *dulce*) including chlorogenic acid, luteolin, apigenin, and chrysoeriol. Besides confirmation that the crude extract from celery leaves was rich in phenolic ingredients, authors reported that mentioned plants possessed notable bioactivities. Similarly, **Kooti et al. (2017)** aimed that the presence of biological active phytochemical compounds, especially polyphenols are the reason that celery is the most widely used plant in traditional medicine.

Table 2 Screening of polyphenols contents in celery family plants

Polyphenols average amount $\mu\text{g/g}$	Celery family plants					
	<i>Anethum graveolens</i> leaf	<i>Anethum graveolens</i> flower	<i>Anethum graveolens</i> stalk	<i>Apium graveolens</i> leaf	<i>Apium graveolens</i> var. <i>rapaceum</i> root	<i>Apium graveolens</i> var. <i>dulce</i> stalk
Gallic acid	96.6 ^d ±0.0	224.0 ^e ±1.8	28.4 ^b ±1.9	73.4 ^c ±3.8	17.6 ^a ±4.5	13.5 ^a ±0.3
Chlorogenic acid	501.0 ^d ±2.0	1,785.8 ^e ±2.3	81.5 ^a ±0.2	1,790.2 ^f ±1.8	123.8 ^b ±0.4	254.4 ^a ±0.6
<i>trans</i> -Caffeic acid	3.9 ^a ±0.4	38.6 ^d ±0.2	ND	27.7 ^c ±1.3	4.3 ^a ±0.0	16.7 ^b ±0.0
<i>trans</i> -p-Coumaric acid	12.8 ^b ±0.0	76.1 ^c ±0.2	3.7 ^a ±0.3	2.6 ^a ±0.0	6.2 ^a ±6.0	3.4 ^a ±0.2
<i>trans</i> -Ferulic acid	38.8 ^c ±0.1	59.6 ^d ±0.2	7.3 ^a ±0.1	709.5 ^c ±1.0	28.8 ^b ±2.0	39.6 ^c ±0.1
<i>trans</i> -Cinnamic acid	2.6 ^c ±0.0	1.8 ^c ±0.0	0.9 ^a ±0.0	2.1 ^d ±0.0	4.0 ^f ±0.0	1.3 ^b ±0.1
Quercetin hydrate	4.2 ^a ±0.1	24.8 ^d ±0.1	6.1 ^b ±0.3	29.8 ^c ±0.1	ND	19.5 ^c ±0.0
Kaempferol	3.1 ^a ±0.1	10.9 ^d ±0.0	ND	7.6 ^c ±0.0	4.1 ^b ±0.1	ND
Epicatechin	41.9 ^a ±0.2	199.5 ^d ±0.7	36.5 ^b ±0.4	ND	ND	22.4 ^a ±0.1
Apigenin	5.7 ^b ±0.1	49.4 ^d ±0.1	5.8 ^b ±0.2	5.0 ^a ±0.0	7.5 ^c ±0.0	ND
Genistein	26.3 ^d ±1.6	4.8 ^{ab} ±0.0	3.9 ^a ±0.0	4.9 ^{ab} ±0.1	6.8 ^c ±0.0	5.6 ^{bc} ±0.1
Rutin	63.2 ^{de} ±7.5	59.9 ^d ±0.4	14.0 ^a ±0.1	69.6 ^e ±0.5	37.1 ^b ±0.16	48.3 ^c ±0.0
Resveratrol	16.2 ^c ±0.1	2.0 ^c ±0.0	1.1 ^a ±0.0	32.6 ^f ±0.1	1.6 ^b ±0.0	2.2 ^d ±0.1

*ND – Not detected, ± standard error, a-e values with different letters within the same row differ significantly (P<0.05)

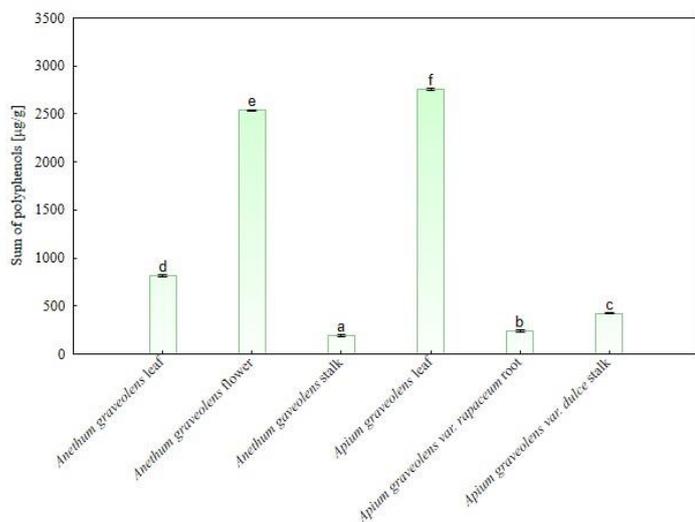


Figure 1 Sum of polyphenols in celery family

By scheduling the analyzed herbal materials according to the highest to the lowest total content of polyphenolic compounds, their order was: *Tribulus terrestris* > *Calendula officinalis* > *Cannabis sativa* L. > *Origanum vulgare* > *Lavandula angustifolia* > *Salvia officinalis* > *Mentha longifolia* > *Mentha piperita* (Table 3).

Fruits of *Tribulus terrestris* were characterized by the highest polyphenols content among all analyzed plant materials (Σ 4,780.0 $\mu\text{g/g}$). Quantitatively, rutin (4,630.0 $\mu\text{g/g}$) accounted for over 96% of all detected polyphenolic substances (Table 3). High levels of rutin were also confirmed by **Ivanova et al. (2010)**, who analyzed *Tribulus terrestris* of Turkey origin. Besides above mentioned flavonoid HPLC analysis confirmed small quantities of gallic acid, *trans*-caffeic acid, *trans*-coumaric acid, *trans*-ferulic acid, resveratrol, quercetin hydrate, *trans*-cinnamic acid, apigenin, and genistein. In addition, **Nagwa et al. (2018)** analyzed flavonoids and phenolic contents in the fruit of *Tribulus terrestris*. Researchers revealed by HPLC analysis the presence of nine identified flavonoids: naringin, rutin, hyperoside, quercetin, naringenin, quercetin, hesperetin, kaempferol, and apigenin. The highest quantities were recorded for (†): hyperoside, naringin, and hesperetin. In the same study, authors identified fourteen phenolic compounds:

pyrogallol (†), gallic acid, protocatechuic acid, catechin, catechol, chlorogenic acid, p-hydroxybenzoic acid, caffeic acid, vanillic acid, ferulic acid, salicylic acid (†), ellagic acid (†), coumaric acid and cinnamic acid.

One of the greatest amounts of polyphenols (Σ 3,515.0 $\mu\text{g/g}$) was also detected for *Calendula officinalis*. All analyzed polyphenolic compounds were detected by HPLC analysis. Chlorogenic acid (2,098.0±19.4 $\mu\text{g/g}$) was identified to be the dominant component followed by *trans*-cinnamic acid (396.6±0.4 $\mu\text{g/g}$), *trans*-p-coumaric acid (385.4±0.3 $\mu\text{g/g}$) and rutin (266.9±0.8 $\mu\text{g/g}$). Also, **Sytar et al. (2018)** confirmed that the leaf extracts of *Calendula officinalis* L. among investigated extracts of the representative family *Asteraceae* have been shown to have the highest total phenolic, total flavonoid contents and antioxidant activity. According to the aforementioned study, the most abundant polyphenolic acid was syringic acid (3.7 mg/g). **Frum (2017)** reported that the greatest quantity of phenolic compound in *Calendula officinalis* flowers was 3.10 mg/100 g vegetal product (v.p.) for rutin, followed by 2.79 mg/100 g v.p. for syringic acid. The researcher also confirmed small quantities of cinnamic acid, gallic acid, resveratrol, and ferulic acid. Differently than in this work **Frum (2017)** not reported presence of quercetin, chlorogenic acid, caffeic acid and (+)- catechin, which can be caused by other sources of used raw material and different extraction condition. The results obtained by **Rigane et al. (2013)** revealed phenolic fingerprint composed of gallic acid, coumarin (scopoletin-7-O-glucoside) and three flavonoids (quercetin-3-O-glucoside, isorhamnetin-3-O-glucoside, and rutin) which is mostly with accordance to our work. **Hernández-Rosas et al. (2018)** revealed by HPLC-UV-DAD a rich profile of phenolic compounds from petals of *Calendula officinalis* 70% hydroalcoholic extract where the major flavonoids were rutin > quercetin > catechin and major acid-phenols were identified as ferulic acid > coumaric acid > vanillin > gallic acid > caffeic acid. Authors observed that rutin was the most abundant polyphenol present in the extract (58.7%). All of these compounds were also detected in the present study but mutual proportions and quantities of each phenols were different.

Successively, *Cannabis sativa* L. showed greater content of polyphenols (Σ 1,233.0 $\mu\text{g/g}$) than herbal spices from *Lamiaceae* family. Our results demonstrated that *Cannabis sativa* was characterized by the highest content of gallic acid (169.7±1.6 $\mu\text{g/g}$). **Flores-Sanchez et al. (2008)** identified 20 flavonoids mainly belonging to the flavone and flavonol subclasses. Also, **Ross et al. (2000)** reported the presence of the apigenin, luteolin, kaempferol, and quercetin, as well as unique flavones: cannflavin A and cannflavin B. The flavones and flavonols found in *Cannabis* exert a wide range of biological effects. They present anti-inflammatory, anticancer and neuroprotective properties (**Andre et al., 2010**).

Lamiaceae family is considered a rich source of terpenoids, iridoid glycosides, flavonoids, phenolic acids and other phenolic compounds (Naghbi et al., 2005). The aforementioned observation was successfully confirmed in our experiment on the example of *Origanum vulgare* which possessed the greatest total polyphenol content (Σ 1,027.0 $\mu\text{g/g}$) from the entire Lamiaceae family studied. Additionally, Spiridon et al., (2011) confirmed that major phenolic acids identified in the *Origanum vulgare* L. were ferulic and p-coumaric acid and caffeic acid which is in accordance with our results. As shown in Table 3, our study also confirmed that oregano is a good source of: *trans*-caffeic acid, *trans*-ferulic acid, and apigenin. Health-promoting characteristic of apigenin which includes anxiolytic (Murti et al., 2012) and oestrogenic properties (Wangand et al., 1998) has been previously reported. Both types of mint: *Mentha piperita* and

Mentha longifolia were characterized by the smallest content of polyphenolic compounds from whole herbal plants group (Table 3). However, it has to be pointed out that *Mentha longifolia* showed the highest content of quercetin (25.4 $\mu\text{g/g}$) and genistein (37.5 $\mu\text{g/g}$) of all screened plant materials. Our results demonstrated that there were no significant differences in polyphenols content between analysed types of mint. In comparison to mint, over 70% greater content of studied biologically active compounds were presented in lavender (*Lavendula angustifolia*) and sage (*Salvia officinalis*). *Lavendula angustifolia* was very rich in epicatechin (469.4 $\mu\text{g/g}$) where in *Salvia officinalis* increased content of resveratrol (92.5 $\mu\text{g/g}$) were detected.

Table 3 Screening of polyphenols content in herbal plants

Polyphenols average amount $\mu\text{g/g}$	Herbal plants							
	<i>Origanum vulgare</i>	<i>Salvia officinalis</i>	<i>Lavendula angustifolia</i>	<i>Mentha piperita</i>	<i>Mentha longifolia</i>	<i>Cannabis sativa</i> L.	<i>Calendula officinalis</i>	<i>Tribulus terrestris</i>
Gallic acid	164.7 ^d ±0.8	51.7 ^b ±0.2	40.6 ^{ab} ±27.9	38.9 ^{ab} ±8.2	16.0 ^a ±0.0	169.7 ^d ±1.6	76.9 ^c ±9.4	95.9 ^e ±2.4
Chlorogenic acid	26.0 ^b ±0.3	22.5 ^b ±3.0	ND	ND	ND	13.8 ^{ab} ±0.0	2,098.0 ^e ±19.4	ND
<i>trans</i> -Caffeic acid	206.2 ^f ±0.4	196.4 ^e ±1.9	46.0 ^b ±0.0	70.5 ^d ±0.2	59.0 ^e ±0.2	10.5 ^a ±0.1	66.4 ^d ±4.7	8.2 ^a ±0.3
<i>trans</i> -p-Coumaric acid	14.1 ^a ±0.1	26.6 ^d ±0.6	7.8 ^a ±0.1	8.2 ^a ±1.7	9.6 ^{ab} ±0.1	528.4 ^f ±0.3	385.4 ^e ±0.3	11.4 ^b ±2.6
Rutin	25.8 ^a ±1.6	33.6 ^a ±0.1	134.4 ^c ±0.5	82.9 ^b ±17.4	81.6 ^b ±0.5	94.7 ^b ±0.6	266.9 ^d ±0.8	4,630.0 ^e ±1.6
<i>trans</i> -Ferulic acid	300.0 ^e ±0.9	27.8 ^b ±0.0	13.5 ^a ±0.4	26.7 ^b ±0.1	35.3 ^c ±5.9	263.1 ^d ±0.6	27.4 ^b ±0.1	12.3 ^a ±0.0
Resveratrol	15.5 ^e ±0.1	92.5 ^b ±0.3	24.0 ^f ±0.1	11.5 ^d ±0.0	10.0 ^e ±0.0	60.7 ^a ±0.1	1.3 ^a ±0.1	2.8 ^b ±0.3
Quercetin hydrate	21.2 ^d ±0.3	10.2 ^a ±0.1	4.4 ^a ±0.1	7.2 ^b ±0.0	25.4 ^e ±1.2	4.7 ^b ±0.1	12.3 ^c ±0.0	5.6 ^{ab} ±2.5
<i>trans</i> -Cinnamic acid	36.8 ^d ±0.0	27.1 ^c ±0.1	16.1 ^b ±0.1	4.1 ^a ±0.1	3.6 ^a ±0.9	42.8 ^c ±0.0	396.6 ^f ±0.4	3.8 ^a ±0.2
Apigenin	80.9 ^b ±0.1	73.6 ^b ±0.2	6.6 ^d ±0.0	3.5 ^b ±0.2	20.4 ^d ±0.0	12.1 ^e ±0.1	2.4 ^a ±0.0	5.6 ^c ±0.0
Genistein	11.1 ^d ±0.1	13.9 ^e ±0.4	17.8 ^e ±0.0	16.7 ^f ±0.0	37.5 ^b ±0.1	4.9 ^b ±0.0	5.8 ^b ±0.5	4.3 ^a ±0.0
Kaempferol	80.2 ^a ±0.1	129.3 ^a ±1.1	5.7 ^b ±0.2	30.6 ^c ±0.1	82.3 ^c ±0.1	3.4 ^a ±0.0	5.1 ^{ab} ±2.2	ND
Epicatechin	44.5 ^a ±0.1	16.6 ^a ±0.3	469.4 ^b ±196.4	73.7 ^a ±0.0	97.8 ^a ±0.3	23.9 ^a ±0.4	170.5 ^a ±3.7	ND

*ND – Not detected, ± standard error, a–g values with different letters within the same row differ significantly (P< 0.05)

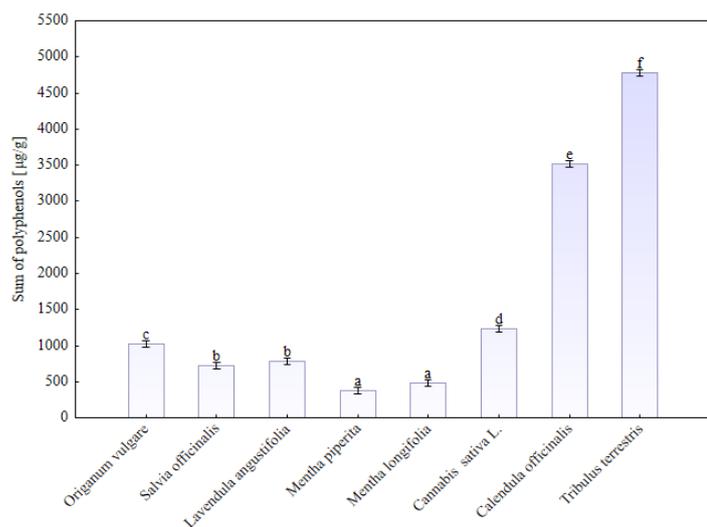


Figure 2 Sum of polyphenols in herbal plants family

CONCLUSION

Screening of polyphenolic compounds in alcoholic extracts of 11 commonly used plants in traditional medicine, were performed by RP-HPLC-DAD method using epicatechin, gallic acid, chlorogenic acid, *trans*-caffeic acid, *trans*-p-coumaric acid, rutin, *trans*-ferulic acid, resveratrol, quercetin hydrate, *trans*-cinnamic acid, apigenin, genistein, and kaempferol. The comparative study showed qualitative and quantitative differences between single polyphenolic components. Total polyphenolic compounds content varied depending on the part of the tested plant (flower/stalk/leaf) and its botanical classification. In conclusion, the flowers of *Anethum graveolens* and leaves of *Apium graveolens* var. *rapaceum* and also *Calendula officinalis* flowers and *Tribulus terrestris* fruits, may be used as a source of health-promoting phytochemicals for developing new natural, safe and cost-effective food additives or pharmaceuticals.

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REFERENCES

ANDRE, C.M., LARONDELLE, Y., EVERS, D. 2010. Dietary Antioxidants and Oxidative Stress from a Human and Plant Perspective: A Review. *Current Nutrition & Food Science*. 6, 2–12. <http://dx.doi.org/10.2174/157340110790909563>

CUTRIM, C., CORTEZ, M. 2018. A review on polyphenols: Classification, beneficial effects and their application in dairy products. *International Journal of Dairy Technology*. 71(3) 564-578. <https://doi.org/10.1111/1471-0307.12515>

DURGAWALE P.P., DATKHILE K.D. 2017. Study of Polyphenol Content and Anti- Oxidative Potential of *Tribulus terrestris* Dry Fruit Extract. *International Journal of Pharmacognosy and Phytochemical Research*. 9(5), 716 721. <http://dx.doi.org/10.25258/phyto.v9i2.8154>

FLORES-SANCHEZ, I.J., VERPOORTE, R. 2008.Secondary metabolism in *Cannabis*. *Phytochemistry Reviews*. 7, 615–639. <http://dx.doi.org/10.1007/s11101-008-9094-4>

FRAGA, C., CROFT, K., KENNEDY, D., TOMÁS-BARBERÁN, F. 2019. The effects of polyphenols and other bioactives on human health. *Food&Function*. 10, 514. <https://doi.org/10.1039/c8fo01997e>

FRUM, A. 2017. HPLC Determination of Polyphenols from *Calendula Officinalis* L. Flowers. *Acta Universitatis Cibiniensis. Series E: Food Technology*. 21(2). <http://dx.doi.org/10.1515/auaft-2017-0020>

GRAJEK, W. Przeciwnutleniaacze w żywności. Aspekty zdrowotne, technologiczne, molekularne i analityczne. *Wydawnictwo Naukowo-Techniczne*, Warszawa 2007.

GRAJEK, W., 2004. Rola przeciwutleniaaczy w zmniejszaniu ryzyka wystąpienia nowotworów i chorób układu krążenia. *Żywność. Nauka. Technologia. Jakość*, 1(38), 3-11.

HAJIMEHDIPOOR, H., GOHARI, A.R., AJANI, Y., SAEIDNIA, S. 2014. Comparative study of the total phenol content and antioxidant activity of some medicinal herbal extracts. *Research Journal of Pharmacognosy* 1(3), 21–25.

HAKIMUDDIN, F., TIWARI, K., PALIYATH, G., MECKELING, K. 2008. Grape and wine polyphenols down-regulate the expression of signal transduction genes and inhibit the growth of estrogen receptor–negative MDA-MB231 tumors in nu/nu mouse xenografts. *Nutrition Research*. 28, 702-713. <https://doi.org/10.1016/j.nutres.2008.06.009>

HERNÁNDEZ-ROSAS, N.A., GARCÍA-ZEBADÚA, J.C., HERNÁNDEZ-DELGADO, N., TORRES-CASTILLO, S., FIGUEROA-ARREDONDO, P., MORA-ESCOBEDO R. 2018. Polyphenols profile, antioxidant capacity, and *in vitro* cytotoxic effect on human cancer cell lines of a hydroalcoholic extract from *Calendula officinalis* l. petals. *Revista Especializada en Ciencias Químico-Biológicas*. 21(1), 54-64. <http://dx.doi.org/10.22201/fesz.23958723e.2018.0.150>

ISBILIR, S.S., SAGIROGLU, A. 2011. Antioxidant Potential of Different Dill (*Anethum Graveolens* L.) Leaf Extracts. *International Journal of Food Properties*. 14(4), 894-902. <https://doi.org/10.1080/10942910903474401>

IVANOVA, A., LAZAROVA, I., MECHKAROVA, P., TCHORBANOV, B. 2010. HPLC Method for Screening of Steroidal Saponins and Rutin as Biologically Active Compounds in *Tribulus Terrestris* L., *Biotechnology &*

- Biotechnological Equipment. 24(1), 129-133. <http://doi.org/10.1080/13102818.2010.10817826>
- JANA, S., SHEKHAWAT, G.S. 2010. Phytochemical Analysis and Antibacterial Screening of *in vivo* and *in vitro* Extracts of Indian Medicinal Herb: *Anethum graveolens*. *Research Journal of Medicinal Plants*. 4(4), 206-212. <https://doi.org/10.3923/rjmp.2010.206.212>
- KAISER, A., KATJA, I.H., KAMMERER, D.R., CARLE, R. 2013. Evaluation of the effects of thermal treatments on color, polyphenol stability, enzyme activities and antioxidant capacities of innovative pasty celeriac (*Apium graveolens* L. var. rapaceum (Mill.) DC.) products. *European Food Research Technology*. 237(3), 353-365. <https://doi.org/10.1007/s00217-013-1998-6>
- KATALINIC, V., MILOS, M., KULISIC, T., JUKIC, M. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*. 94 (4), 550-557. <https://doi.org/10.1016/j.foodchem.2004.12.004>
- KOOTI W., DARAEI N. 2017. A Review of the Antioxidant Activity of Celery (*Apium graveolens* L). *Journal of Evidence-Based Complementary & Alternative Medicine*. 22(4), 1029-1034. <https://doi.org/10.1177/2156587217717415>
- KRZYŻANOWSKA J., JANDA B., PECIO L., STOCHMAL A., OLESZEK W., CZUBACKA A. PRZYBYS M., DROSZEWSKA T. 2011. Determination of Polyphenols in *Mentha longifolia* and *M. piperita* Field-Grown and In Vitro Plant Samples Using UPLC-TQ-MS. *JOURNAL OF AOAC INTERNATIONAL*. 94(1).
- KSOURI, A., DOB, T., BELKEBIR A., LAMARI L., KRIMAT S., METIDJI H. 2015. Total Phenolic, Antioxidant, Antimicrobial Activities and Cytotoxicity Study of Wild *Anethum graveolens* L. *International Journal of Pharmacognosy and Phytochemical Research*. 7(6), 1025-103.
- LIU, G., ZHUANG, L., SONG, D., LU, C., XU, X. 2017. Isolation, purification, and identification of the main phenolic compounds from leaves of celery (*Apium graveolens* L. var. dulce Mill/Pers.). *Journal of Separation Science*. 40, 472-479. <https://doi.org/10.1002/jssc.201600995>
- MURTI, K., PANCHAL, M.A., GAJERA, V., SOLANKI, J. 2012. Pharmacological properties of *Matricaria recutita*: a review. *Pharmacologia*. 3(8), 348-351. <http://dx.doi.org/10.5567/pharmacologia.2012.348.351>
- NAGHIBI, F., MOSADDEGH, M., MOHAMMADI, S., GHORBANI, A. 2005. Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. *Iranian Journal of Pharmaceutical Research*. 2, 63-79. <http://dx.doi.org/10.22037/ijpr.2010.619>
- NAGWA, M.A., SEHAM, S.D.H., DOHA, A.M., MANAL S.A., DINA, M.G., GAMAL, A. 2018. Phytochemical and Biological Studies of *Tribulus terrestris* L. Growing in Egypt. *International Journal of Pharmacology*. 14, 248-259. <https://doi.org/10.3923/ijp.2018.248.259>
- NEAGU, E., ROMAN G.P., RADU G.L. 2011. Antioxidant capacity of some *salvia officinalis*. *Revue Roumaine de Chimie*. 56(7), 777-782.
- OSHAGHI E.A., KHODADADI I., TAVILANI H., GOODARZI M.T. 2016. Aqueous Extract of *Anethum Graveolens* L. has Potential Antioxidant and Antiglycation Effects. *International Journal of Molecular Sciences*. 41(4), 328-333.
- PEREZ-GUTIERREZ, R.M., MUÑIZ-RAMIREZ, A., GARCIA-CAMPOY, A.H., MOTA-FLORES, J.M. 2018. Polyphenols of leaves of *Apium graveolens* inhibit *in vitro* protein glycation and protect RINm5F cells against methylglyoxal-induced cytotoxicity. *Functional Foods in Health and Disease*. 8(3), 193-211. <https://doi.org/10.31989/ffhd.v8i3.399>
- RIGANE, G., BEN-YOUNES, S., GHAZGHAZI, H., BEN SALEM, R. 2013. Investigation into the biological activities and chemical composition of *Calendula officinalis* L. growing in Tunisia. *International Food Research Journal*. 20(6): 3001-3007.
- ROSS, S.A., MEHMEDEC, Z., MURPHY, T.P., EL-SOHLI, M.A. 2000. GC-MS analysis of the total delta9-THC content of both drug- and fiber-type *Cannabis* seeds. *Journal of Analytical Toxicology*. 4, 715-717. <http://doi.org/10.1093/jat/24.8.715>
- SHYU, Y.S., LIN, J.Y., CHANG, Y.T., CHIANG C.J., YANG, D.J. 2009. Evaluation of antioxidant ability of ethanolic extract from dill (*Anethum graveolens* L.) flower. *Food Chemistry*. 115, 515-521. <https://doi.org/10.1016/j.foodchem.2008.12.039>
- SMERIGLIO A., GALATI E.M., MONFIRTE M.T., LANUZZA F., D'ANGELO V., CICOSTA C. 2016. Polyphenolic Compounds and Antioxidant Activity of Cold-Pressed Seed Oil from Finola Cultivar of *Cannabis sativa* L. *Phytotherapy Research*. 30, 1298-1307. <https://doi.org/10.1002/ptr.5623>
- SPIRIDON, I., COLCERU, S., ANGHEL, N., TEACA, C.A., BODIRLAU, R., ARMATU, A. 2011. Antioxidant capacity and total phenolic contents of oregano (*Origanum vulgare*), lavender (*Lavandula angustifolia*) and lemon balm (*Melissa officinalis*) from Romania. *Natural Product Research*. 25 (17), 1657-1661. <http://dx.doi.org/10.1080/14786419.2010.521502>
- SYTAR, O., HEMMERICH, I., ZIVCAK M., RAUH C., BRESTIC M. 2018. Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. *Saudi Journal of Biological Sciences*. 25, 631-641. <http://dx.doi.org/10.1016/j.sjbs.2016.01.036>
- WANG, C., KURZER, M.S. 1998. Effects of phytoestrogens on DNA synthesis in MCF-7 cells in the presence of estradiol or growth factors. *Nutrition and Cancer*. 31, 90-100. <http://dx.doi.org/doi:10.1080/01635589809514686>
- YAO, Y., SANG, W., ZHOU, M., REN, G. 2010. Phenolic Composition and Antioxidant Activities of 11 Celery Cultivars. *Journal of Food Science*. 75(1). <https://doi.org/10.1111/j.1750-3841.2009.01392.x>
- YAZDANPARAST R., BAHRAMKIA S. 2008. Evaluation of the effect of *Anethum graveolens* L. crude extracts on serum lipids and lipoproteins profiles in hypercholesterolaemic rats. *Daru-Journal of Faculty of Pharmacy*. 16(2), 88-94.