Eurasian J. Bio. Chem. Sci. 1(1): 29-32, 2018

EJBCS

Eurasian Journal of Biological and Chemical Sciences

Journal homepage: www.dergipark.gov.tr/ejbcs

Determination of Phytochemical Contents of Some Medicinal Aromatic Plants (*Echinacea pallida, Melissa officinalis, Hypericum perforatum and Sideritis syriaca*) Belonging to Antalya Region

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Abstract: *Echinacea pallida* (Nutt.) Nutt., *Melissa officinalis* L. (lemon balm), *Hypericum perforatum* L. (St. John's wort) *and Sideritis syriaca* L. are valuable for its pharmaceutical, medicinal and agricultural properties. These plants were extracted with methanol/chloroform (1:1) solvent, filtered, and solvents were removed by rotary evaporator to get four separate extracts. Quantifications of chemical constituents of extracts were determined by TOF-LC/MS and GC-MS. The main compounds of extracts for *E. pallida* were 4-hydroxybenzoic acid caffeic acid, for *M. officinalis* were eupatorin and diosmin, for *S. syriaca* chlorogenic acid and fumaric acid, for *H. perforatum* quercetin-3-β-D-glucoside and morin. The fatty acid contents of *E. pallida* is palmitic acid composition (30.07%), of *M. officinalis* is octadecatrienoic acid (43.39%), *H. perforatum* is octadecatrienoic acid (31.37%) and of *S. syriaca* is linoleic acid (34.89%).

Keywords: *Echinacea pallida, Melissa officinalis, Hypericum perforatum and Sideritis syriaca*, HPLC-TOF/MS, GC-MS

Antalya Bölgesine Ait Olan Bazı Tıbbi Aromatik Bitkilerin (Echinacea pallida, Melissa officinalis, Hypericum perforatum ve Sideritis syriaca) Fitokimyasal İçeriğinin Belirlenmesi

Özet: *Echinacea pallida* (Nutt.) Nutt., *Melissa officinalis* L. (lemon balm), *Hypericum perforatum* L. (St. John's wort) *ve Sideritis syriaca* L. farmasötik, tıbbi ve tarımsal özelliklerinden dolayı değerlidir. Bu bitkiler metanol / kloroform (1: 1) çözücüsü ile ekstrakte edildi, süzüldü ve dört ayrı ekstraktı elde etmek için çözücüler döner buharlaştırıcıyla uzaklaştırıldı. Ekstraktlar içerisindeki kimyasal bileşiklerin miktarları HPLC-TOF/MS ve GC-MS cihazları ile belirlendi. *E. pallida* ektresi için 4-hydroxybenzoic acid ve caffeic acid, *M. officinalis* ekstresi için eupatorin ve diosmin, *S. syriaca* ekstresi için chlorogenic acid ve fumaric acid, *H. perforatum* ekstresi için quercetin-3-β-D-glucoside ve morin bileşikleri ana bileşen olarak belirlenmiştir. *E. pallida* ekstresi içerisinde yağ asidi bileşeni (30.07%) oranında Palmitic acid, *M. officinalis* ektresinde (43.39%) oranında octadecatrienoic acid, *H. perforatum* ekstresinde (31.37%) oranında octadecatrienoic acid ve *S. syriaca* ekstresinde (34.89%) oranında linoleic acid bileşikleri ana bileşen olarak bulunmuştur.

Anahtar Kelimeler: *Echinacea pallida, Melissa officinalis, Hypericum perforatum and Sideritis syriaca*, HPLC-TOF/MS, GC-MS

1. Introduction

In our modern World, chemical preservatives/additives from unnatural sources and environmental pollution have a big contribution to the emerge of today's main illnesses. We can see the effects of these chemicals on the body and the different tissues and organs in the diseased individuals. It is seen that the incidence of illnesses of individuals exposed to environmental pollution is very high compared to those who are naturally fed and are away from environmental pollution. Natural nutrition begins with natural consumption first. The dirty atmosphere created by drugs, consumed food and crowded city life causes very intense chemical exposures on living individuals and causes negative effects on the body over time.

Medicinal aromatic plants have very useful medical features due to the wide variety of components that they contain. It is known that the use of plants known as alternative medicine for this medical purpose is as old as human

history. Currently, preparations from many drug plants that use modern environment are used. The share allocated to the Ar-Ge work in the world is quite high. However, due to the relatively high cost of synthetic drugs and the adverse side effects, the popularity of alternative medicine is increasing day by day. Turkey has a very rich medicinal plant flora which is endemic due to its climate characteristics. Although we have this potential, our country still lacks enough scientific research on plants.
H. perforatum plant species, known as yellow kantaron, koyun kıran, kan otu, yaraotu and mayasıl otu, belongs to the family Clusiaceae and covers about 400 species worldwide (Curtis and Lersten, 1990). In Turkey, 70 species were found (Baytop et al. 1999). In our country, this plant species is generally distributed in the Black Sea, Aegean, Marmara, Middle and Eastern Anatolia regions (Davis et al. 1988; Davis et al. 1965; Güner et al. 2000). These plant wounds are useful for the treatment of cancer, diabetes mellitus, chronic rheumatism, stomach ulcers, gastrointestinal diseases, diuretic sedative, liver-bile disorders, antidepressant (De Smet and Nolen, 1996; Linde et al. 1985), cold sores, worm-lowering, antiseptic wound healing (Baytop et al. 1999; Özyurt et al. 1992), throat infections, jaundice, bronchitis, diarrhea and dysentery.
E. pallida is spreading from North America to the whole world. These plant species are used as disinfectant for wound, burn healing, mumps, insect bite, mouth and pharynx, as a pain reliever in the abdomen and headache, as an antidote in snake bites and poisonings, as a blood cleanser, with coughs, colds, measles and gonorrhea (Prica et al. 1998). Melissa officinalis L. (Lamiaceae, Labiatae) is a perennial herbaceous plant. Melissa plant has effects such as scorpion insertions, irritation of the teeth, menstruation by women, sputum diseases, mouth odor, skin itchiness, relieving shortness of breath, relieving shortness of breath, digestive ciliator (Sina et al. 2000; Kültür et al. 2007; Şaşkara et al. 2004; Baykan et al. 2011; Uzun et al, 2004).
S.syriaca The plant is used to make an aromatic herbal tea. This plant is used for its anti-inflammatory and analgesic properties for treatment of gastric ulcers (Menghini et al. 2005). The aerial parts are used for their antioxidative properties (Armata et al. 2008). Common uses include treatment of stomach ache, stomach pain, painkiller, throat inflammation, neural appeaser, and colds (Fakir et al. 2009).
We have identified content analyzes of these medically specific plants that have been grown in their natural environment to help alleviate these negative and better understand the value of these plants. For this purpose, the chemical contents of *E. pallida*, *M. officinalis*, *H. perforatum* and *S. syriaca* plants were determined by GC-MS and HPLC-TOF / MS instruments collected from the highly consumed Antalya province.

2. Materials and Method

2.1. Plant materials

These plants were collected from Antalya, Karaman-Sarıveliler, Uctas, 1905 m, in 2015 June, Turkey A voucher specimen has been deposited at the Herbarium of Faculty of Science, Cankiri Karatekin University.

2.2. Preparation of Extracts Using Different Solvents

Plants were cut into small pieces with liquid nitrogen. These parts were extracted with methanol-chloroform (1:1 v/v) for five times at room temperature. Then extracts were filtered through Whatman No. 2 filter paper and concentrated to dryness under vacuum. The crude extracts were stored under adequate conditions until the time of analysis.

2.3. GC-MS Analysis

Fatty acid and volatile components analysis of plants extracts were carried by GC-MS performed on an Agilent Technologies model 7890 gas chromatograph equipped with a 5975 Triple Axis Detector Mass spectrometer. Analyzes were carried out using HP-5 ms capillary column (30 m x 250 m x 0.25 µm film thickness, 5%-phenylmethylpolysiloxane). Ultra-pure helium was used as a carrier gas at a flow rate of 1 ml/min, splitless 2 µL injections were used. Electron impact (EI) ion source were analyzed at 70 eV. Injector, ion source and interface temperatures were 250, 250 and 270 °C. Oven temperature programme was arranged as follow: starting temperature was 100 °C. The temperature was kept at 100 °C for 10 min, then increased to 200 °C at a 10 °C/min rate, and held for 10 min, then 25 °C/min to 270 °C for 36 min, held for 20 min. total run time 84 min. Compounds in samples were identified comparing with those in the NIST and WILEYsearch database. Mass spectra were recorded in the m/z 50–550 mass range.
For GC-MS analysis, approximately 40 mg of extracts was weighed. The extracts were dissolved by the addition of 3 mL of KOH solution prepared in 2 M methanol. 3 ml of hexane was added to the solution and vortexed for 2 minutes. After a few minutes of waiting, two phases were observed. The esterified and hexane-phase supernatant was carefully separated from the lower phase. Hexane parts placed in vials.

2.4. HPLC-TOF/MS Analysis

Phenolic components analysis of extracts were determined by Agilent Technology of 1260 Infinity HPLC System was coupled with 6210 Time of Flight (TOF) LC/MS detector and ZORBAX SB-C18 (4.6 x100mm, 3.5µm) column. The mobile phases consisted of the A (ultra pure water with 0.1% formic acid) and B (acetonitrile-HPLC grade). Flow rate was 0.6 mL min-1 and column temperature was 35°C. Injection volume was 10 µL. The solvent program was as follow: 0.min 10% B; 0-1.min 10% B; 1-20.min 50% B; 20-23.min 80% B; 23-25.min 10% B; 25-30. min 10% B. Ionization mode of HPLC-TOF/MS instrument was negative and operated with a nitrogen gas temperature of 325 °C, nitrogen gas flow of 10.0 L min-1, nebulizer of 40 psi, capillary voltage of 4000 V and finally, fragmentor voltage of 175 V. For sample analysis, dried crude extracts (250 ppm) were dissolved in methanol. Samples were filtered passing through a PTFE (0.22 µm) filter by an injector to remove particulates.

For HPLC-TOF/MS analysis, about 2 mg was weighed from each extracts. On the extracts were dissolved by adding 2 ml of methanol and 1000 ppm stock solutions were prepared. From the stock solutions, 200 ppm new solutions were prepared and transferred to the vials and analyzed by the device.

3. Results

In this study, phytochemical contents of plants with some medicinal properties collected from Antalya province were carried out. For this the plants were extracted in the methanol/chloroform solvent system. The fatty acid components in the plants were determined by GC-MS instrument and are given in Table 1. According to the results obtained; more component analysis of *H. perforatum* plant extract was determined among these plants and Octadecatrienoic acid is the main component. It was also determined that essential oil components were present in the extract. Linoleic acid compound in *S. syrica* plant extract, Palmitic acid compound in *E. pallida* extract and octadecatrienoic acid compound as main component in M. officinalis extract were determined as main components.
Analysis of the phenolic components of extracts was quantitatively performed on HPLC-TOF/MS and results of analysis are given in Table 2. HPLC-TOF/MS chromatogram of these extracts is shown in Figure 2.

Table 1. GC-MS Analysis results of hexane extract of plants

RT (min)	Compounds name	*S. syriaca*	*E. pallida*	*H. perforatum*	*M. officinalis*
16.53	Capric acid		6.39		
18.54	Caryophyllene			0.30	
19.37	γ-Muurolene			0.26	
19.84	Lauric acid			0.52	
19.96	γ-Cadinene			0.15	
20.04	beta-Cadinene			0.30	
21.06	Caryophyllene oxide			0.36	
22.90	Myristic acid	0.32	2.73	1.70	1.14
24.92	Pentadecylic acid			0.69	
27.48	Palmitoleic acid	0.23		0.27	
27.61	Palmitic acid	21.70	30.07	25.39	31.85
32.18	Linoleic acid	34.89	15.16	29.71	13.99
32.27	Octadecatrienoic acid		20.65	31.37	43.39
32.43	Trans Linoleic acid			1.58	
32.44	Oleic acid		1.52		2.23
32.56	Stearic acid		20.65	4.13	6.32
34.50	Heptacosane			0.23	
34.57	Eicosenoic acid		4.85	0.16	
34.84	Arachidic acid		6.13	1.09	1.08
37.30	Behenic acid		2.03	0.45	

Table 2. Quantitative phenolic component analysis of plants extracts by HPLC-TOF/MS

RT (min)	Compounds name	*M. officinalis*	*S. syriaca*	*E. pallida*	*H. perforatum*
3.19	Fumaric acid		140.79	2.29	
4.50	Gentisic acid	37.29	3.88	44.61	26.99
5.46	Chlorogenic acid		202.55	38.18	21.22
5.79	Catechin				19.03
6.96	4-hydroxybenzoic acid	93.08	13.10	166.47	4.43
7.08	Protocatechuic acid		5.60	77.88	
7.65	Caffeic acid			347.86	
7.87	Vanillic aicid		2.45	30.46	
8.08	Syringic acid	89.03	6.58		39.30
9.23	Rutin	1.58	1.06	33.78	
9.73	Scutellarin	6.30			
9.76	Quercetin-3-β-D-glucoside	5.67		45.93	330.44
10.50	Naringin	7.93	2.19		
10.61	Diosmin	263.95	22.79		
10.76	Hesperidin		24.21		
10.87	Apigetrin		33.31		
11.08	Neohesperidin		14.58		
13.01	Morin	61.99	3.96	86.72	119.80
13.12	Salicylic acid		6.52		
14.02	Quercetin				56.51
15.16	Sinnamic acid		18.40		18.02
15.64	Apigenin		1.49		
18.91	Eupatorin	1403.91			
20.26	Galangin				1.08
20.53	Biochanin A			0.45	

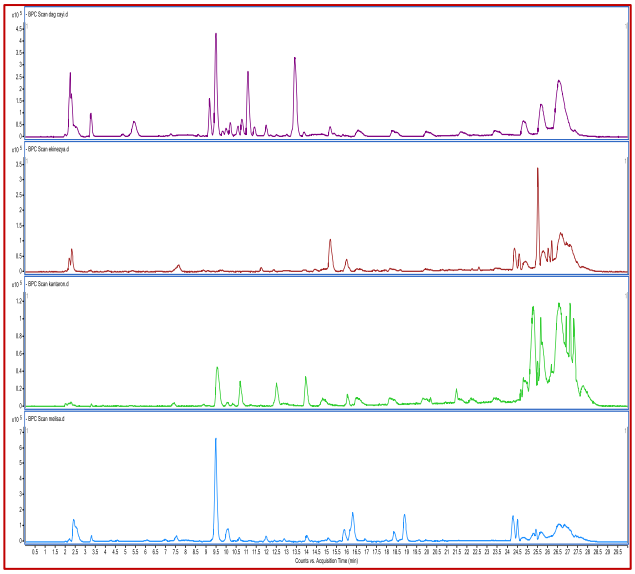

Figure 1. HPLC-TOF/MS TIC chromatogram of plants extracts

Table 2 shows the results of the phenolic compounds contained in plants extracts. The quantitative analysis of a total of 25 compounds in the extracts was defined with HPLC-TOF/MS.

It has been determined that very high amounts of Eupatorin and Diosmin flavon compounds are found in the presence and high amount of phenolic acids in *M. officinalis* plant. It has been determined that very high amounts of chlorogenic acid and fumaric acid are present in the *S. syriaca* plant. It was determined that the composition of caffeic acid and 4-hydroxibenzoic acid in the *E. pallidia* plant. It was determined that quercetin-3-β-D-glucoside and morin compounds are present in very high amounts in *H. perforatum* plant.

4. Discussion

These plant species used in this study are among the plant species known and sold in medicinal where in transmitting the Turkey. These plant species are in the first place among exported plants. It is due to the phenolic and volatile components in these plants that they have medicinal properties. In particular, the results of HPLC-TOF analysis demonstrate that the amounts of the components are high and thus illuminate that the therapeutic effects are derived from phenolic compounds which are present in too much amounts. *M. officinalis* plant has activity against many cancer cells (Androutsopoulos, 2008). Eupatorin found in *M. officinalis* has been shown to have anti-inflammatory effects in a mouse ear oedema, human gastric adenocarcinoma (MK-1), human uterus carcinoma (HeLa), murine melanoma (B16F10) cell lines (Nagao et al. 2002), highly metastatic murine colon carcinoma cells (26-L5) (Tezuka et al. 2000).

5. Conclusions

E. pallida, M. officinalis, H. perforatum and S. syriaca have excellent biological activities and they contain medicinally valuable compounds. Therefore, these species could be used in food and pharmaceutics industries.

Acknowledgements

The authors thank to State Planning Organization, Turkey (DPT 2003K120510) for the financial support, University of Çankırı Karatekin for grant (BAP, 2014L10).

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