IJSM International Journal of Secondary Metabolite

Int. J. Sec. Metabolite, Vol. 4: 3 (2017) pp. 452-458

Special Issue 2: *Research Article*

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

Phytochemical Content of *Centaurea polypodiifolia* boiss. var. *polypodiifolia*

Ferda ESER [*1], Ayse SAHIN YAGLIOGLU[2], Ebru AKTAS[3], Adem ONAL[3], Ibrahim DEMIRTAS[2]

[1] Department of Private Security and Protection, Suluova Vocational Schools, Amasya University, Amasya, Turkey
[2] Department of Chemistry, Faculty of Science, Cankiri Karatekin University, 18100 Cankiri, Turkey
[3] Department of Chemistry, Faculty of Science & Art, Gaziosmanpasa University, 60240 Tokat, Turkey

Received: 04 May 2017 – Revised: 18 September 2017 - Accepted: 03 November 2017

Abstract: Various solvents including hexane, $CHCl_3$, EtOAc, and MeOH were used to extract the aerial parts of *C. polypodiifolia* Boiss. var. *polypodiifolia.* Hexane extract was analyzed by GC-MS and nonacosane and octacosane were determined as the main constituents. HPLC-TOF/MS analyses were carried out on $CHCl_3$, EtOAc, and MeOH extracts. The main constituents of $CHCl_3$ extract were detected as 4-hydroxybenzoic acid, vanilic acid, and gentisic acid. Gentisic acid, fumaric acid, chlorogenic acid, and vanilic acid were determined as the main compounds of EtOAc extract. Finally, main constituents of MeOH extract were found as fumaric acid, chlorogenic acid, quercetin-3-*β*-D-glucoside, gentisic acid and diosmin.

Keywords: *Centaurea polypodiifolia*, extract, GC-MS, HPLC-TOF/MS

1. INTRODUCTION

The genus *Centaurea* which belongs to Asteraceae family, comprises nearly 500 species around the World [1]. *Centaurea* species are generally used in folk medicine due to their pharmacological properties such as fever treatment, diabetes, hemoroid, and peptid ulcer [2]. Biological activity studies of *Centaurea* genus revealed that they have exhibited antioxidant, antimicrobial and antipyretic activities [3].

Phytochemical studies of *Centaurea* species showed that the main compound groups of different *Centaurea* species were sesquiterpenes [4-8], lignin compounds [9-11], flavonoids [12-14], and their glycosides. Secondary metabolites especially phenolic compounds are responsible from the biological [15-18] activities. Phenolic and flavonoid compounds, as important phytochemicals, are present in vegetables, fruits and cereal grains. Phytochemicals such as phenolic and flavonoid compounds commonly found in plants have multiple biological effects and play an important role in the defense against cardiovascular disease, aging and cancer [19]. In recent years, the studies related with plant-derived phytochemicals and their biological activities have gained interest that they give natural solutions for health-care [20].

*Corresponding Author E-mail: ferda.eser@amasya.edu.tr

ISSN: 2148-6905 online /© 2017 DOI: 10.21448/ijsm.376888

Therefore, determination of the phytochemical content of the plants is an important step for the treatment and prevention of some diseases.

The objective of the current study is to determine the phytochemical content of different extracts of *Centaurea polypodiifolia* boiss. var. *polypodiifolia*. So, it facilitates the future investigations related with biological activity.

2. MATERIAL and METHODS

2.1 General Experimental Procedures

GC-MS analysis was performed with a Perkin Elmer Clarus 500 Series GC system. Phenolic constituents of the extracts were determined using an Agilent Technologies 6210 Time-of-Flight LC-MS. All solvents used in HPLC analysis were of HPLC grade and purchased from Merck. The extraction solvents were distilled before the extraction processes.

2.2. Plant Material

C. polypodiifolia Boiss. (CPP) was collected from Karacayir/Sivas (39 51 29 N, 36 58 48 E, 1568 m). Identification of the plant material was performed by Prof. Dr. Necati CELİK from Cumhuriyet University and Prof. Dr. Neriman OZHATAY from Istanbul University. A vouchermen deposited the plant material in the herbarium of Cumhuriyet University and Istanbul University, respectively (CUFH 8934; ISTE 85428).

2.3. Extraction Procedure

Flowers of CPP (100 g) were extracted with hexane (1 L x 3) for 24 h. After the process, it was filtered and evaporated. The residue was re-extracted with the same procedure for the following solvents $CHCl_3$, EtOAc, and MeOH, respectively.

2.4. Methylation Procedure

The fatty acid composition of hexane extract was determined by using methylation of the lipid extracts to form fatty acid methyl esters (FAME) for gas chromatography (GC) analysis. For this purpose, 40 mg of extracted oil was dissolved in a tube which contained n-heptane. Then, 2 M KOH (2 mL) was added to the tube, the mixture was shaken vigorously and allowed to reach formation of phases. The upper layer, which contained the FAME was transferred to a vial and diluted with n-hexane for GC analyses [21].

2.5. Gas Chromatography (GC) Analysis

FAME analyses were carried out with a Perkin Elmer Clarus 500 Series GC system equipped with flame ionization detector (FID) and a TR-FAME apolar capillary column (30 m x 0.25 mm and 0.25 m ID). The split ratio was 50:1 port. Helium was used as the carrier gas at a flow rate of 0.5 mL/min. The temperatures of the injector and detector were set to 250 and 260 °C, respectively. An initial column oven temperature of 100 °C was raised to 220 °C at a rate of 2 °C/min. Identification of the FAME peaks was performed by comparing the retention times of each peak with those of authentic standards (Supelco 37 Comp. Fatty acid Mix, 18919) and their mass spectral

2.6. HPLC-TOF/MS Analysis

Phenolic constituents of the plant extracts were determined in an Agilent 1260 Infinity HPLC system coupled with an Agilent 6210 TOF-MS detector and an VYDAC C18 column (25 mm × 300 mm 10 μm). Water (with 2.5 % formic acid) and acetonitrile were used for the mobile phases, A and B, respectively. The column temperature was adjusted to 35°C where the flow rate was 0.8 mL min^{-1}. The injection volume was 200 μL and the elution program was as

follows: 0–1 min, 10% B; 1–12 min, 40% B; 12–14 min, 90% B; 14–17 min, 90% B; 17–18 min, 10% B; 18–25min, 10% B.

3. RESULTS

3.1. Fatty Acid Content

GC-MS analysis results of the hexane extract of CPP was given in Table 1. Main constituent of the hexane extract of CPP was assigned as nonacosane (43.53%).

Table 1. Fatty acid contents of the hexane extract of CPP

No	RT	Compound	Area (%)
Saturated fatty acids			
1	19.771	Lauric acid	0.51
2	22.804	Myristic acid	1.21
3	27.456	Palmitic acid	3.76
4	32.491	Stearic acid	0.85
5	34.763	Arachidic acid	1.05
6	37.2	Behenic acid	0.86
7	38.688	Tricosylic acid	0.12
8	40.468	Lignoceric acid	0.50
9	45.383	Cerotic acid	0.33
10	65.035	Melissic acid	0.19
Mono unsaturated fatty acid			
11	32.188	Oleic acid	2.85
Poly unsaturated fatty acid			
12	32.096	Linoleic acid	2.28
Other compounds			
13	19.903	Myristicin	1.41
14	25.138	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.15
15	25.304	Hexahydrofarnesyl acetone	0.13
16	32.342	Phytol	0.40
17	33.315	Docosane	0.27
18	34.448	Tricosane	3.76
19	34.683	11-Eicosenoic acid, methyl ester	0.43
20	35.552	Tetracosane	0.70
21	35.924	Methyl 14-methyl-eicosanoate	0.11
22	36.8	Pentacosane	3.11
23	37.126	Methyl 11-docosenoate	0.18
24	38.167	Hexacosane	0.47
25	39.964	Octacosane	16.42
26	44.788	Nonacosane	43.53
27	45.583	8-Androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimethyl-	0.22
28	47.488	Triacontane	1.76
29	51.608	Tetratriacontane	9.29
30	56.197	Hexatriacontane	0.30
31	59.934	Stigmasterol	0.49
32	62.406	Hentriacontane	0.67
33	63.504	γ-Sitosterol	0.92
34	65.501	β-Amyrin	0.15
35	68.694	α-Amyrin	0.48
		Total	99.86

3.2. Phenolic Compounds of the CPP Extracts

The study was conducted to investigate the phenolic compounds of $CHCl_3$, EtOAc, and MeOH extracts of CPP. The phenolic compound profile of CPP extracts were given in Table 2. The results of the HPLC-TOF/MS analysis revealed that the main constituents of $CHCl_3$ extract were 4-hydroxybenzoic acid (0.168 g kg^{-1}), followed by vanilic acid (0.133 g kg^{-1}), and gentisic acid (0.112 g kg^{-1}). Main phenolic compounds of EtOAc extract were gentisic acid (1.220 g kg^{-1}), fumaric acid (0.990 g kg^{-1}), chlorogenic acid (0.866 g kg^{-1}), and vanilic acid (0.571 g kg^{-1}). HPLC-TOF/MS results of MeOH extract showed that the main constituents of the extracts were fumaric acid (137.010 g kg^{-1}), chlorogenic acid g kg^{-1}), quercetin-3-β-D glucoside (4. 407 g kg^{-1}), gentisic acid (2.567 g kg^{-1}), and diosmin (1.954 g kg^{-1}), respectively. It is observed that, while the main phenolic constituents of MeOH extract are determined as fumaric acid, chlorogenic acid, quercetin-3-β-D glucoside, gentisic acid, and diosmin; EtOAc extract doesn't contain these phenolics except gentisic acid (Table 2).

Table 2. Phenolic compounds of CPP extracts determined by HPLC-TOF/MS

	CPP		
Compound	$CHCl_3$ (g/kg)	EtOAc (g/kg)	MeOH(g/kg)
Chlorogenic acid		0.866	16.305
4-hydroxybenzaldehyde		0.020	
Vanilic acid	0.133	0.571	0.797
Gentisic acid	0.112	1.220	2.567
4-hydroxybenzoic acid	0.168	0.404	1.533
Kafeic acid		0.333	0.756
Rutin			0.021
Protocatechuic acid		0.184	0.951
Naringenin	0.011	0.046	0.411
Salisilic acid		0.005	
Wogonin	0.007		
Fumaric acid		0.990	137.010
Quercetin-3-β-D glucoside		0.287	4.407
Sinapic acid		0.081	0.143
Diosmin		0.364	1.954
Morin		0.129	0.634

The standard compounds used for HPLC-TOF/MS analysis include the following phenolics besides the phenolics in Table 2: hesperidin, apigenin-7-glucoside, rosmarinic acid, protocatechuic acid ethyl ester, caftaric acid, quercetin, *p*-coumaric acid, kaempferol, ferulic acid, chicoric acid, ellagic acid, resveratrol, biochanin, eupatorin, cinnamic acid, syringic acid, apigenin, scutellarin, and neohesperidin. The HPLC-TOF/MS chromatograms of the CPP extracts were shown in Figure 1.

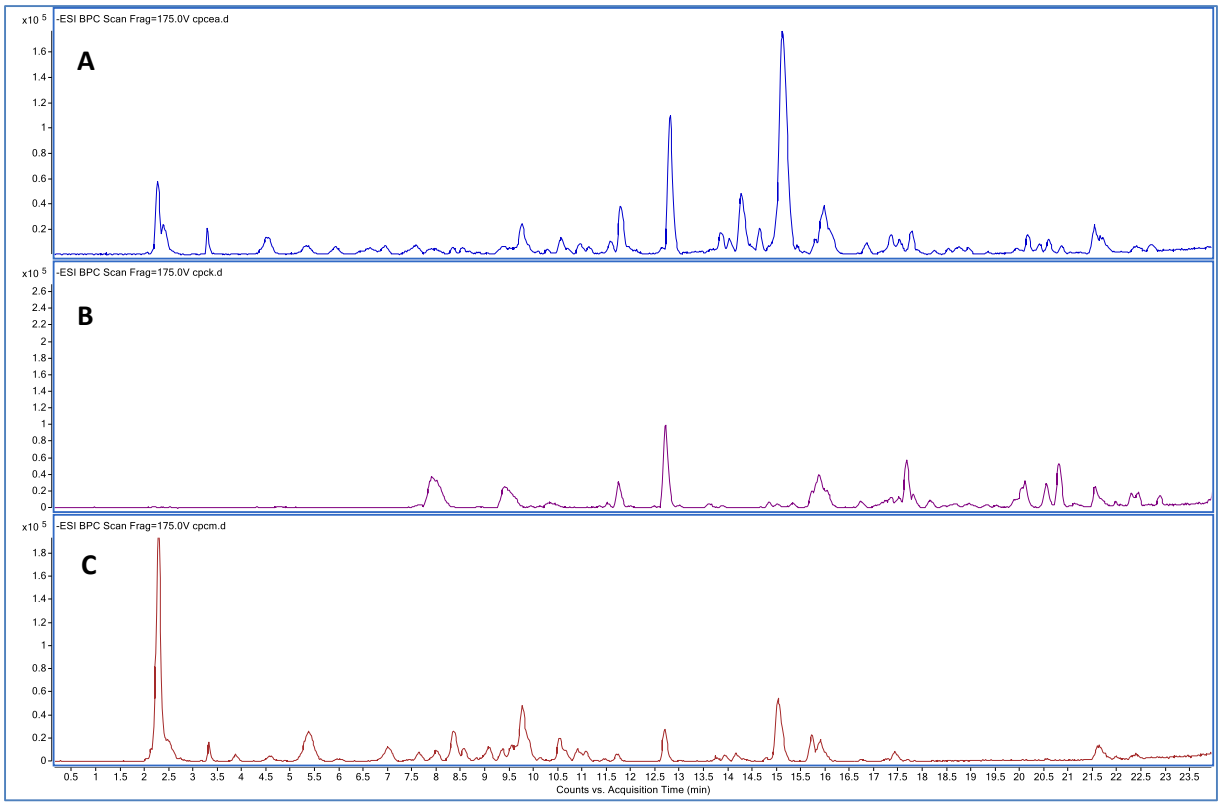

Figure 1. HPLC-TOF/MS chromatograms of $CHCl_3$ (A), EtOAc (B), and MeOH (C) extracts of CPP

4. CONCLUSION

The presence of the phytochemicals plays an important role in the treatment or prevention of many diseases due to their potential biological activities. Therefore, it is important to know the phytochemical content of plants. In this study, different extracts of the flowers of *Centaurea polypodiifolia* Boiss. var. *polypodiifolia* were screened for their secondary metabolite contents using GC-MS and HPLC-TOF/MS, respectively. The results demonstrated that the main constituent of the hexane extract was nonacosane. Main phenolic compounds of $CHCl_3$, EtOAc, and MeOH extracts were determined as 4-hydroxybenzoic acid, gentisic acid, and fumaric acid, respectively.

Acknowledgements

This study was funded by Scientific Research Projects, Gaziosmanpasa University, Turkey (2015/92). We thank to Prof. Dr. Necati Celik from Cumhuriyet University and Prof. Dr. Neriman Ozhatay from Istanbul University for identification of the plant material. We also thank to Huseyin Aksit for his help in analyses.

Conflict of Interests

Authors declare that there is no conflict of interests.

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