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Two flavone derivatives from *Nepeta cadmea*

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Abstract – Two flavone glycosides were isolated from aerial parts of *Nepeta cadmea*. The structure elucidations were done according to NMR and HR-TOF-MS data. Antioxidant activities of isolated compounds were determined DPPH radical scavenging activity.

Keywords – *Nepeta cadmea, flavone glycosides, antioxidant activity.*

1. Introduction

Genus *Nepeta* (Lamiaceae) contains about 250 species widely spreading in different geographical region such as Asia, North America, North Africa, Europe and Mediterranean region. The genus *Nepeta* is presented by 33 species in the Flora of Turkey of which are endemic [1]. *Nepeta* species are commonly used in Turkish folk medicine as stomachic and stimulants [2]. They have also different biological activity such as anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activity [3]. Many of these properties are related to terpenoids and flavonoids that exist in this genus. *Nepeta cadmea* is endemic to Turkey especially grown in Central Anatolia.

Flavones are naturally occurring secondary metabolites, ubiquitously found in different fruits, vegetables and many plants. The flavones have a great positive impact on human health. The various biological activities exhibited by the flavones are dependent on the nature and position of the substituents on the flavone skeleton. The flavones exhibit a great diversity in their biological activities due to their unique ability to modulate various enzyme systems [4].

In literature, there is lack of information about chemical content of *Nepeta cadmea*. This study reports at the first time isolation of flavonoids from this plant material.

2. Experimental

2.1. Plant Material

The sample of *N. cadmea* was collected from Gaziosmanpasa University Tasliciftlik Campus area in 2011 and authenticated kindly by Asst. Prof. Aşkın AKPULAT (Cumhuriyet University, Faculty of Science and Art, Biology Department, Sivas, Turkey).

2.2. Extraction and Isolation

Air dried and grounded aerial parts of *N. cadmea* (300 g) were extracted with methanol (1 L X 3) for 24 h. Methanolic extracts were combined and filtered off and were concentrated under reduced pressure. The resultant slurry extract was chromatographed over silica gel, eluting with hexane, followed by increasing concentrations of ethyl acetate and methanol (0-100% ethyl acetate and methanol), respectively, to yield 250 fractions. Fractions 44-74 (120 mg) yielded the compound **1** and fractions 88-120 (80 mg) yielded the compound **2**.

2.3. DPPH Radical Scavenging Activity

DPPH radical scavenging activity determination by method described by Blois is the simplest and applicable technique for measuring radical scavenging activity [5]. 0.1 mM solution of DPPH· in ethanol was prepared and 1 mL of this solution was added to 3 mL of isolated compounds and standard solution in methanol at different concentrations (25– 50 µg/mL). Final mixtures were incubated in dark places at room temperature during thirty minutes. The absorbance was measured at 517 nm. A lower absorbance of the reaction mixture indicates higher free radical scavenging activity due to the dark purple color of radicalic DPPH solution bleaches when accept a hydrogen atom from antioxidants.

3. Result and Discussion

Two flavon derivatives were isolated from *Nepeta cadmea.* The compounds were solved in DMSO-d6, structural elucidations were done according ^{1}H, ^{13}C-NMR spectra (Bruker Avence III 400 MHz) and LC-TOF-HRMS.

Compound 1: (4'-O-methylisoscutellarein-7-O-[β-D-allopyranosyl-(1→2)-6"-O-acetyl-β-D-glucopyranoside]), amorphous yellow powder, LC-TOF-HRMS, *m/z*: 665.1786 $[M-H]^-$ (calcd: 665.1716). Signal at δ 8.08 (2 H, d, *J*= 8.84 Hz), 7.12 (2H, d, *J*=8.84 Hz) are characteristic for 4′-substitued B ring. Singlet at δ 6.91 (which is correlated with 103.84, revealed in HETCOR spectra) shows non-substituted C-3 carbon of flavone skeleton and singlet δ 6.68 belonged to C-6 carbon. In addition to, two anomeric proton signals at 5.06

(d, *J* = 7.48 Hz) and 4.91 (d, *J* = 8.01 Hz) in the ^{1}H NMR spectrum, as well as ^{13}C NMR signals at d 100.49 (C-1″) and 102.97 (C-1‴) suggested the presence of two glycoside moiety. Correlation of 4.02-171.3 revealed in HMBC spectrum shows that the acetyl group linked to C-6‴ carbon. As well as the 3.86-162.94 HMBC correlation shows the methoxy group linked to C-4′ carbon. The compound isolated from *Sideritis syriaca* ssp. *syriaca* previously [6].

Compound 2: (Isoscutellarein-7-O-[β-D-allopyranosyl-(1→2)-6"-O-acetyl-β-D-gluco pyranoside]), amorphous yellow powder, LC-TOF-HRMS, m/z: 651.1628 $[M-H]^-$ (calcd: 651.1559). The ^{1}H and ^{13}C spectrum were similar to **1** with slightly differences in chemical shifts. The presence of hydroxyl group at the C-4′ position instead of methoxy group caused to shift 3′-5′ proton signal to upfield when compared to **1**. The compound isolated from *Veronica thymoides* subsp. *Pseudocinerea* previously [7]. Recorded ^{1}H and ^{13}C chemical shifts in DMSO-d6 are listed in Table 1 (For numbering see Figure 1).

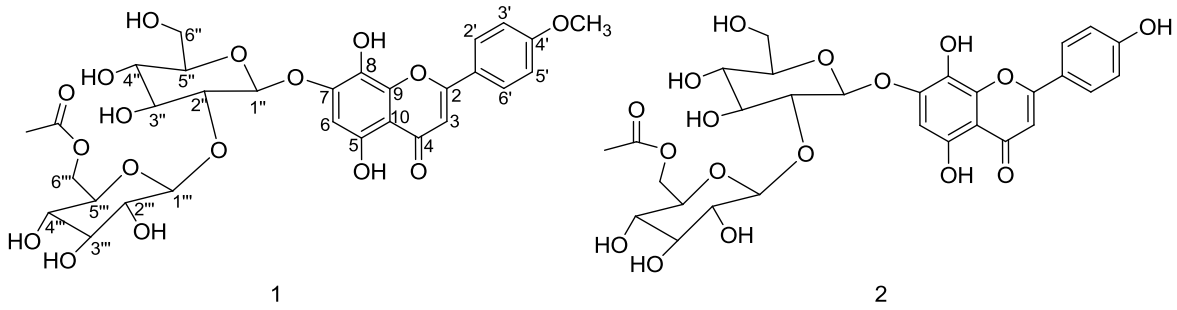

Figure 1. Chemical structures of isolated compounds from *Nepeta cadmea*

Isolated compounds had high free radical scavenging activities. The activities of compounds (**1** and **2**) were higher than BHT and ascorbic acid while lover than BHA (Fig 2). The activity of compound **1** and **2** was smilar. According to our findings, presence of hydroxy or methoxy group at the 4′-position does not considerably contribute to radical scavenging ability. In constast to the 5,8-diOH-7-O-glyc (ring-A) pattern of isolated compounds both **1** and **2** incereased radical scavenging ability. Radical scavenging effects of flavonoids depended on their structure. For example, while quercetin, one of most common flavonoid, have a good radical scavenging ability, apigenin is a weakest one, although it has same flavone skeleton [8]. Quercetin differs from apigenin bearing only one hydroxyl group at C-3′ position in B ring.

Table 1. ^{1}H and ^{13}C chemical shifts of isolated compounds from *Nepeta cadmea*

Position	1		2	
	^{1}H*	^{13}C	^{1}H	^{13}C
2	-	164.09	-	164.40
3	6.91 (1H, s)	103.81	6.82 (1H, s)	103.08
4	-	182.87	-	182.60
5	-	151.06	-	150.99
6	6.70 (1H, s)	99.87	6.68 (1H, s)	99.85
7	-	152.62	-	152.66
8	-	128.93	-	127.48
9	-	144.24	-	144.22
10	-	106.04	-	105.20
1’	-	123.29	-	125.65
2’-6’	7.13 (2H, d, J=8.60 Hz)	115.01	6.94 (2H, d, J=8.68 Hz)	116.34
3’-5’	8.09 (2H, d, J=8.60 Hz)	128.93	7.98 (2H, d, J=8.68 Hz)	129.09
4’		162.91	-	162.10
1’’	5.08 (1H, d, J=7.40 Hz)	100.48	5.07 (1H, d, J=7.36 Hz)	100.05
2’’	3.58 (1H, m)	82.93	3.58 (1H, m)	81.67
3’’	3.49 (overlap with H_2O)	77.56	3.48 (overlap with H_2O)	77.46
4’’	3.27 (1H, m, overlap with 2’’’)	69.63	3.25 (1H, m, overlap with 2’’’)	69.70
5’’	3.50 (overlap with H_2O)	75.98	3.52 (overlap with H_2O)	76.14
6’’	3.74/3.50 (2H, diastrophic)	60.96	3.74-3.50 (2H, diastrophic	60.98
1’’’	4.93 (1H, d, J=7.92 Hz)	102.96	4.92 (1H, d, J=8.00 Hz)	102.20
2’’’	3.27 (1H, m, overlap with 4’’)	71.95	3.27 (1H, m, overlap with 4’’)	72.02
3’’’	3.90 (1H, m)	71.19	3.88 (1H, m)	71.50
4’’’	3.42 (1H, overlap with H_2O)	67.26	3.43 (1H, overlap with H_2O)	67.62
5’’’	3.50 (1 H, overlap with H_2O)	75.98	3.51 (1 H, overlap with H_2O)	75.07
6’’’	4.02 (1H, brs)	63.95	4.03 (1H, brs)	61.35
$OCO\underline{C}H_3$	1.87 (3H, s)	20.91	1.88 (3H, s)	21.42
$O\underline{C}OCH_3$	-	170.82	-	170.85
$O\underline{C}H_3$	3.86 (3H, s)	56.01	-	-

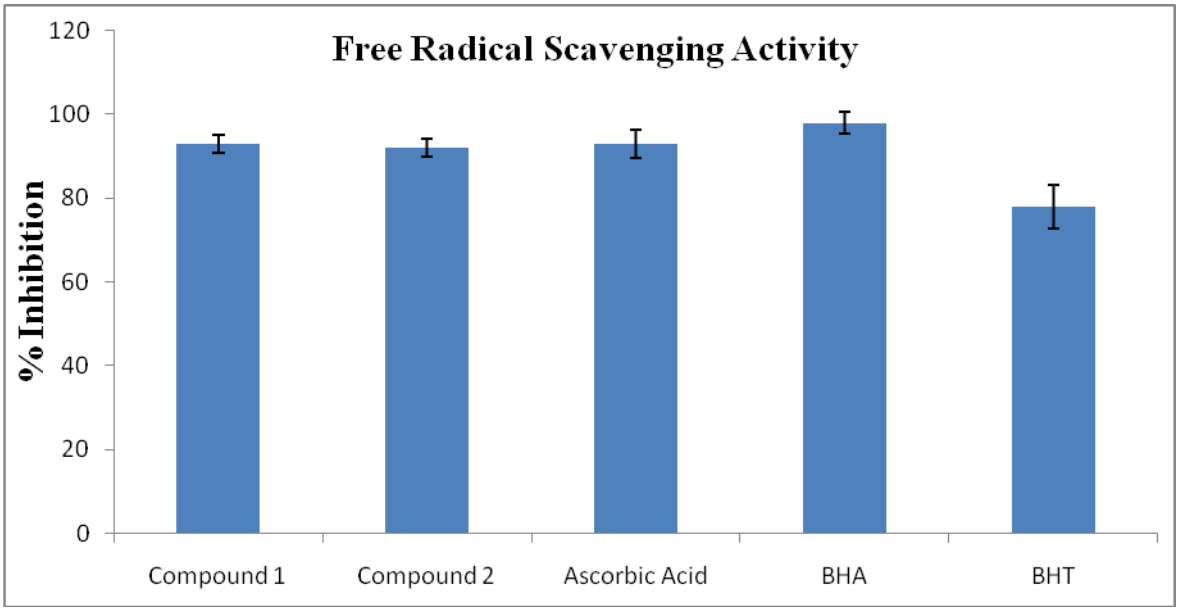

Figure 2. Free radical scavenging activity of isolated compounds and standarts.

4. Conclusions

Natural antioxidants which take place synthetic ones have gained increasing interest among scientific community because of benefit to human health without risk of many diseases such a cancer. Intake of antioxidant flavonoids from foods (fruits, vegetables, wines and tea) is an important health-protecting factor. Therefore, the determination of presence of antioxidant flavonoids in wild or cultivated plants is crucial event in order to finding new sources of flavonoids.

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