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Original Article

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Chemical Analysis of the Flavonoid, Antioxidant and Antimicrobial Activities of Chaerophyllum macropodum (Boiss.) Extract

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Abstract

Flavonoids are secondary metabolites produced mainly in almost all terrestrial plants and fruits, where they provide UV protection and color. They have a fused ring system consisting of an aromatic ring and a benzopyran ring with a phenyl substituent. Their biological activities have an impact on human health so that they serve as target molecules to develop new drugs. From methanolic extract of aerial parts of Chaerophyllum macropodum (Boiss.) (Umbelliferae family), a diglycosylated flavonoid derivative namely 4′,5-O-dirhamnosid-3'-butanoat-7-hydroxyflavone and a phthalic acid ester namely dioctylphthalate have been isolated by column chromatography (CC) and preparative TLC. Those structures were elucidated by UV, 1H- and 13C- NMR, HMBC, EI-MS and IR spectra. The antimicrobial activity of the methanol and aqueous extracts of aerial part were determined against seven Gram-positive and Gram-negative bacteria (Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, S. epidermidis, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae), as well as three fungi (Candida albicans, Saccharomyces cerevisiae and Aspergillus niger). The bioassay showed that both extracts exhibited moderate antimicrobial activity. The antioxidant activity of methanol and aqueous extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. The results indicate that methanol extract from aerial parts of C. macropodum possess considerable antioxidant activity. The highest radical scavenging activity was detected (IC50 = 65 μg/mL). This study reveals that the methanolic extract of this plant is attractive sources of flavonoid, especially the essential ones, as well as of effective natural antioxidants.

Keywords: Chaerophyllum macropodum, Umbelliferae, Methanolic extract. 4′, 5-O-dirhamnosid-3'-butanoat-7-hydroxyflavone, dioctylphthalate, antimicrobial activity, antioxidant activity.

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INTRODUCTION

Throughout time, many plants have been used for the treatment of mental problems. A good number of those are alkaloid-containing plants, which are known to interact strongly with receptors in the central nervous system, but in recent years it has become clear that flavonoids may also play a role in enzyme and receptor systems of the brain, exerting various effects on the central nervous system, including prevention of the neurodegeneration associated with Alzheimer's and Parkinson's diseases [1]. Flavonoids possess various biological activities, besides their effects on the CNS. They have attracted attention as free radical scavengers with antioxidant activity. They are yellow, blue or red, and function as both UV-protections for the plant and pollination aids by providing specific colors or patterns to flowers. Over 6,000 flavonoids have been identified and plants where flavonoids are thought to, or are proven to be
active constituents, include species with a long history of use as traditional folk medicines in Europe. Chamomile flowers (Matricaria recutica L. Asteraceae family) have for centuries been used for their calming effect, which is due to apigenin [2].
Our previous report on the methanolic extract of Tanacetum parthenium from North-west Iran showed that its flavonoids were Flavonol, Kaempferol, Fisetin and Naringenin [3]. In addition, identification of the flavonoids from Zosimia absinthifolia (Umbelliferae family) and Galium verum (Rubiaceae family) are found in the literature [4, 5].
The genus Chaerophyllum is represented in Iranian flora by eight species, among which two are endemic: C. nivale Hedge et Lamond and C. khorassanicum Czern.ex Schischk. [6, 7]. Chaerophyllum species are widely distributed in Europe, Iran, Caucasus and Central Asia, while C. macropodum is only known wild species in Iran and Turkey. During flowering, it is known to be a good source of honey. The plant is also used in traditional healing practices in most of those countries. Fresh stems and leaves are some times added to salads, while tea made of dried leaves and roots is used as herbal remedy to soothe cough, allergies and sore throat. Few investigations have been made on the composition of Chaerophyllum species. The essential oil compositions of two Chaerophyllum species growing in Azerbaijan, C. bulbosum L. and C. macrospermum (Willd. ex Spreng.) Fisch, et Mey., were reported [8,9].
However, only a small part of their components was identified. In the oil of C. bulbosum, 18 components were identified, amounting to only 32% of the total oil, linalool (18.3%) being the major component. The oil of C. macropodum aerial parts from Iran was reported to be predominated by α-pinene (23.0%), β-pinene (17.3%) and fenchyl acetate (13.8%) as the major constituents. The oil was richer in monoterpene than sesquiterpene hydrocarbons [10].
The fruits of C. hirsutum from Germany contained β-pinene (25%), [11]. The oil of C. macrospermum (Spreng.) Fisch, et C.A. Mey, growing in Iran, was obtained from aerial parts, 16 components were identified with high percentage (97.4%) of monoterpenes with (E)-β-ocimene (40.0%) as the main constituent [12]. In the oil of C. bulbosum ssp. bulbosum growing in Greece, sesquiterpenes (20.9%) and alkanes (14.2%) occurred in amounts similar to that encountered in the oil of C. macropodum. Apiole (37.1%) was reported as the main constituent of the oil of C. bulbosum ssp. bulbosum [13]. To the best of our knowledge, this is the first report on the flavonoids from aerial parts and those antioxidant and antibacterial activities of Chaerophyllum macropodum Boiss. from Iran.

EXPERIMENTAL

General experimental:

The IR spectra were determined on a Bruker Tensor 27 spectrometer. The 1H-NMR and 13C - NMR spectra were recorded on a Bruker AM 300 spectrometer. Column chromatography was performed over silica- gel (70-230 mesh, Merck,) using petroleum ether, AcOEt, methanol gradients as elution solvents. UV spectra were recorded on a Perkin- Elmer Lambda 12 spectrophotometer and Mass spectra were recorded on an AEI MS-50 spectrometer.

Plant materials:

The aerial parts of Chaerophyllum macropodum was collected in June 2009 from Givi-Khalkhal road (Ardabil province) in northwest of Iran at an altitude of 1400m. A voucher specimen (No: 027) has been deposited at the Herbarium of the Agriculture Research Centre (A.R.C.) Ardabil, Iran.

Extraction and isolation:

Dried and finely powdered C. macropodum aerial parts (600g) were extracted with methanol in a Soxhlet apparatus during 2 days. The concentrated total extract (72g) was extracted with petroleum ether, CHCl3, EtOAc and n-BuOH, respectively. A part of the EtOAc portion (3g) was subjected to silica gel column chromatography (70-230 mesh, Merck), eluted with an equivalent petroleum ether, AcOEt, methanol stepwise gradients to obtain 32 fractions (15mL each). Fractions 7-13 after solvent evaporate were in turn chromatographed over silica gel with CHCl3: MeOH mixture to provide 16 subfractions. Subfraction 7 (145 mg) was rechromatographed on silica gel into 20 fractions (20×15 mL.) using as eluents an 8.5:1.5, CHCl3: MeOH mixture. The combined fractions 6 to 10(24 mg) were further purified on a preparative TLC to give compound 1 (14 mg). A portion of the AcOEt (0.37 g of fractions 19- 26 after removal of solvent) was chromatographed over a small column (15cm × 1.5 cm) with AcOEt: MeOH (8.25: 1.75) as eluents. A total of 14 fractions were collected. The combined fractions 8 to 14(48 mg) according to TLC analysis were further purified on a preparative TLC to give compound 2(19 mg). The flavonoid were readily identified as 4′, 5-O-dirhamnosid-3'-butanoat-7-hydroxyflavone, and dioctylphthalate by comparing their physical and spectroscopic data with those reported in the literature [14-16].

Antioxidant activity tests:

The DPPH assay was carried out according to the modified method [17]. Briefly, 0.5 mL of DPPH in ethanol (0.1 mM) was added to 1 mL of extracts in different concentrations (0.1-1.6 mg/mL) and kept in the dark for 10 min. The absorbance of the resulting solution was recorded on a spectrometer at 520 nm against a blank of alcohol. Vitamin C was used as reference antioxidant. DPPH scavenging activity was expressed as IC50 values (μg extract/mL) for comparison. IC50 value of each sample defined as the concentration of sample required for the 50 % decrease in absorbance of the blank, was calculated.

Antimicrobial activity:

The in vitro antibacterial and antifungal activities of the extract was evaluated by the disc diffusion method (DDM) using Mueller-Hinton agar for bacteria and Sabouraud Dextrose agar for fungi [18]. Discs containing 30 μL of the methanol extract were used and growth inhibition zones were measured after 24 hr and 48 hr of incubation at 37°C and 24°C for bacteria and fungi, respectively. Gentamicin and tetracycline for bacteria and nystatin for fungi were used as positive controls. The microorganisms used were: Bacillus subtilis ATCC 9372, Staphylococcus epidermidis ATCC 12228, Enterococcus faecalis ATCC 15753, Staphylococcus aureus ATCC 25923,

Klebsiella pneumoniae ATCC 3583, Pseudomonas aeruginosa ATCC 27852, Escherichia coli ATCC 25922, Aspergillus niger ATCC 16404, Candida albicans ATCC 5027 and Saccharomyces cerevisiae ATCC 9763.

RESULTS AND DISCUSSION

The 1H-NMR spectrum of compound 1 displayed the characteristic signals of the luteolin nucleus.

Compound 1 was obtained in the form of yellow amorphous solid, mp 184-187°C. The molecular formula, C31H35O15 was obtained on the basis of the 13C-NMR and Mass spectra analysis. The identification of the compounds was supported by comparison with published data of related compounds [19- 22]. The combination of 1H- , 13C -NMR, and HMBC correlation spectral data of 1 indicated the presence of a propanoat group [δH 2.2(2H, t, H-2′′), 1.6(2H, m, H-3′′), 0.9(3H, t, H-4′′) with the corresponding δC 39.7, 22.9 and 15.1, respectively]. Two methyl groups were observed on the spectrum which assignment to two groups of sugar at δH 3.2 to 3. 9(Table 1) and those two methyl groups [δH 0.98(3H, d, J=11.3Hz, H-G′6) and (δH 0.95(3H, d, J=11.2Hz, H-G6)].

1H-NMR spectrum of compound 1 showed three ABX type phenyl protons at δH 6.8 (1H, d, J = 8.2 Hz, H-5'), 7.2 (1H, dd, J = 8.2, 1.9 Hz, H-6'), 7.4 (1H, d, J = 1.9 Hz, H-2')], two doublet signals at δH 6.87 (1H, d, J= 1.9Hz, H-6) and 7.84 (1H, d, J= 1.9Hz, H-8). The 13C-NMR spectrum showed 31 signals corresponding to six -CH- and nine quaternary carbons in aromatic moiety [23]. 13C–NMR spectrum indicated the presence of two carbonyl carbons, which showed signal at δ183.2(C-4) and 183.9 ppm(C-1''). The other signals of 13C- NMR spectrum showed at δ167.2 (C- 2), 103.4 (C- 3), 158.9 (C- 5), 118.0(C-6), 128.7(C-1′), 129.2(C- 2′), 121.3 (C-3′), 164.7(C- 4′), 126.1(C-5′) and 129.1(C- 6′) (Table1).

Table 1: 1H, 13C-NMR and HMBC data of compounds 1 (CD3OD).

NO	δH	δC	HMBC
2	-	167.2	-
3	6.64	103.4	C-10, C-1'
4	-	183.9	-
5	-	158.9	-
6	6.87	118.0	C-8, C-10
7	-	101.6	-
8	7.84	129.6	C-6, C-10
9	-	146.2	-
10	-	101.1	-
'1	-	128.7	-
'2	7.41	129.2	C-2, C-4', C-6'
'3	-	121.3	-
'4	-	164.7	-
'5	6.80	129.8	C-1', C-3'
'6	7.21	129.1	C-2', C-4'
"1	-	183.2	-
"2	2.20	39.7	C-4"
"3	1.61	22.9	C-1"
"4	0.90	15.1	C-2"
G1	4.81	92.1	C-4', C-G3,C-G5
G'1	5.20	93.0	C-5, C-G'3,C- G'5
G2-G5	3.2-3.5	56-80	-
G'2-G'5	3.6-3.9	60-80	-
G6	0.95	20.3	-
G'6	0.98	20.9	-

The portion of sugar signals were observed in δH 3.2- 3.9 that corresponding to six -CH- carbons in δC 60.0- 78.3 ppm. Significant HMBC correlations were observed
between H-G1 and C-4', and between H-G'1 and C-5, confirming the location of the sugar groups G and G', respectively (fig. 2). The UV spectrum exhibited absorption maxima at 283 nm and 369 nm that are characteristic absorption bands of a flavanone skeleton [14].

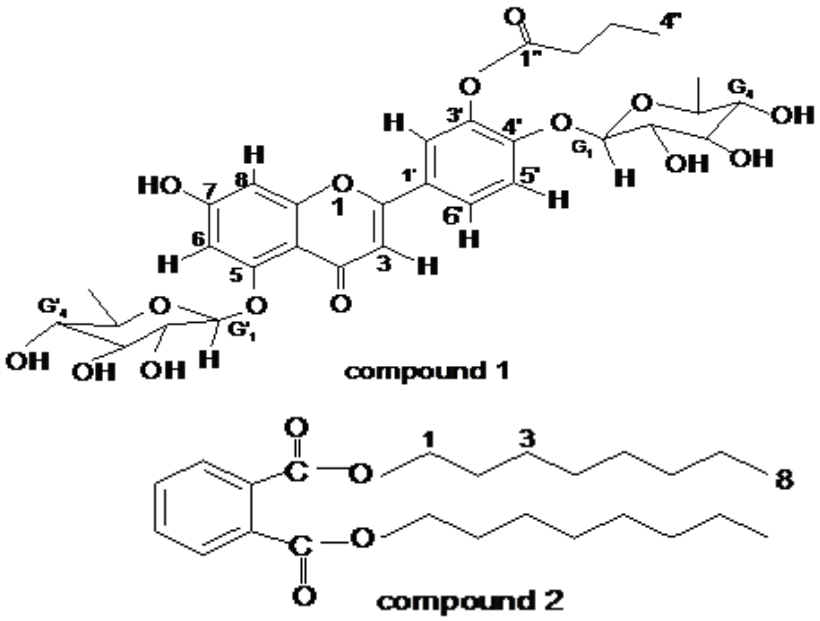

Figure 1: Structures of compounds 1 and 2.

The compound was readily identified by comparing their physical and spectroscopic data with those reported in the literature [24-26]. From these results, compound 1 was identified as 4′, 5-O-dirhamnosid-3'-butanoat-7-hydroxyflavone.
Compound 2 (ester derivative) showed the molecular formula C24H38O4 by MS (m/z 390.6), and 13C- NMR data. It was obtained in the form of oily. The NMR spectrum of 2 has signals for the ester compound with the octyl group (Table1). The ester was readily identified by comparing their physical and spectroscopic data with those reported in the literature and books. From the above described spectral evidence, compound 2 was identified conclusively as dioctylphthalate (fig. 1).

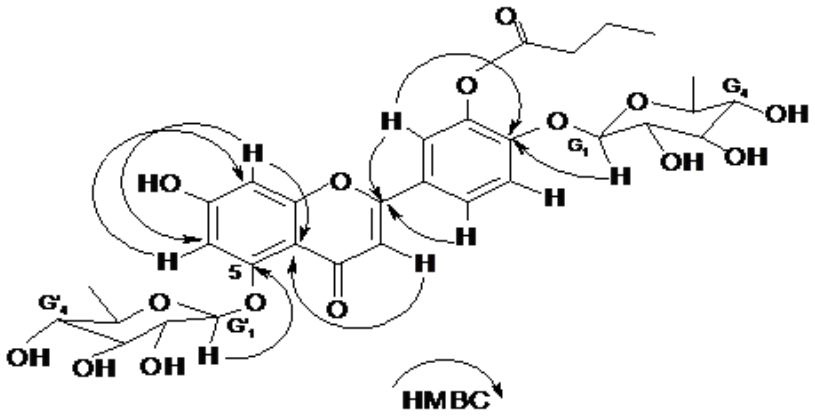

Figure 2: The selected HMBC correlation of compound 1.

The antioxidant activity of methanol and water extracts obtained from C. macropodum was also reported for the first time. Results obtained in the antioxidant study of the samples are shown in Table 2. Antioxidant activity was tested according to the DPPH (2, 2-diphenyl-1-pycrylhydrazile) radical scavenging method. The both extracts scavenged the DPPH radical in a dose-dependent manner, and the DPPH radical scavenging activity (IC50) was decreased in the following order: methanol > aqua (Table 2). According to this data, methanol extract was the most efficient free radical scavenger by the lowest IC50 value of 65 μg/mL among both the extracts. The activity of the reference antioxidant (vitamin C) was much higher than that of aqua extract.

Table 2: DPPH free radical scavenging activity of methanol and aqua extracts of C. macropodum and standard antioxidant, vitamin C.

No	Sample(extract)	IC50 (μg/mL)
1	methanol	65
2	water	115
3	Vitamin C(Ref.)	27

The methanol and water extracts from C. macropodum was tested against four Gram-positive and three Gram-negative bacteria, as well as three fungi. The results, presented in Table 3, show that the methanol extract exhibited a good biological activity against all tested fungi and bacteria except for a resistant Gram-negative bacteria, E. coli, as well as a fungi, Aspergillus niger. The most sensitive microorganisms were Bacillus subtilis and Staphylococcus epidermidis, with inhibition zones of (17.1-21.3) and (14.7-19.1) mm, respectively. Other microorganisms were found to be less sensitive to the extracts with inhibition zones ranged from 8 to 14 mm. It is conceivable that the antimicrobial property of the methanol and water extracts from C. macropodum might be ascribed to its effective compounds such as flavonoids.

Table 3: Antimicrobial activity of the methanol and water extracts of Chaerophyllum macropodum.

	Zone of inhibition (mm) *				
Microorg	Extracts		Antibiotics		
	MeOH	H2O	Genta	Nyst	Tetra
B. subtilis	21.3	17.1	NTb	NT	22.2
S. epidermidis	19.1	14.7	NT	NT	34.1
E. faecalis	11.3	10.7	NT	NT	9.4
S. aureus	16.6	14.9	NT	NT	21.6
K. pneumoniae	9.3	8.7	20.3	NT	NT
P. aeruginosa	9.7	8.1	11.5	NT	NT
E. coli	NAa	NA	24.7	NT	NT
A. niger	8.6	NA	NT	16.3	NT
C.albicans	12.5	14.2	NT	18.9	NT
S. cerevisiae	15.9	12.3	NT	18.1	NT

a NA: Not Active; b NT: Not Tested.
**Inhibition zone diameter (mm), including diameter of sterile disk 6 mm*

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