Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(1); 1-4

ISSN: 0975-4873

Research Article

Phenolic Compounds from *Centaurea horrida* L Growing in Lebanon

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Received: 11th Nov, 16; Revised: 25th Nov,16; Accepted: 5th Dec,16; Available Online: 15th January, 2017

ABSTRACT

Centaurea horrida has been used in folk medicine for many purposes as diuretic, antiinflammatory, hypotensive, antidiarrhetic, mild astringent, bitter tonic, stomachic, digestive, and emmenagogue and in cosmetics. An infusion of this plant is used to treat diabetes and digestive problems Also it is used as a wash for tired eyes and in conjunctivitis. *C.horrida* grown in Lebanon is widely used in the treatment of diarrhea, as antihypertensive and as hypoglycemic.

Phytochemical investigation of the species grown in Lebanon led to the isolation and identification of five phenolic compounds isolated from methanol, ethyl acetate and butanol fractions of Centaurea extracts. Their structures were determined by NMR, MS, UV and IR methods. It is important to mention that the isolated flavonoids fisetin, hispidulin, quercetin, quercetin -3-D- galactoside and caffeic acid were isolated for the first time from *Centaurea horrida.* L growing in the Middle East.

Keywords: *Centaurea horrida*, emmenagogue, flavonoids, spectral identification.

INTRODUCTION

Centaurea is a huge genus belonging to family Asteraceae which contains about 1486 species from the tribe Cynarea[1,2]. Members of the genus are found only in north of the equator, mostly in the Eastern Hemisphere, the Middle East and surrounding regions are particularly species-rich. The species of the genus *Centaurea* are known for their antidiabetic, anti-inflammatory, antipyretic and antibacterial effects[3,4,5].

Centaurea cyanus, commonly known as cornflower or (basket flower) is used as an ingredient in some tea blends and herbal teas[6]. Moreover, many species of this genus have been used traditionally to cure various ailments as malaria and hypertension[7]. In 2014, an experimental study carried out by Karim et al revealed that, a systemic administration of *Centaurea horrida* powdered extract alleviated hyperalgesia in pain conditions in rats due to its phenolic contents[8]. Moreover, Flamini studied the composition of Centaurea horrida growing in Italy and separated flavonoids and phenolic acids proving its antioxidant properties[9].

Plants of genus Centaurea are the subjects of numerous chemicals studies, due to the occurrence of phenolic acids, flavonoids and lactones[10] exerting a great antioxidant activity that explains their folkloric uses in many ailments. *Centaurea horrida* is an indigenous plant growing wild in Lebanon with an antioxidant potential. Therefore, this research aims to isolate the antioxidative phenolic constituents from aerial parts and roots extracts.

MATERIALS AND METHODS

General

The IR spectra were determined on Shimadzu IR spectrophotometer (FT/IR-8300) in KBr discs and the absorption bands were measured in cm^{-1}. The ^{1}HNMR and ^{13}CNMR spectra were recorded on Bruker Avance 500MHZ apparatus. Column chromatography was performed over silica gel (70-230,mesh,Fluka) using petroleum ether, methylene chloride, ethyl acetate and methanol gradients as eluents.UV spectra were determined using Ciba-Corning Double-beam spectrophotometer (2800 spectroscan) and mass spectra were recorded on a AEIMS-50 spectrometer.

Plant material

Fresh *Centaurea horrida* was collected at the flowering stage from the littoral of Beirut in May 2012. The plant was identified by Dr. Georges.Tohme former professor of Taxonomy. A dried specimen (No.PS.13.06) was kept at the Faculty of Pharmacy. The plant was dried under shade at 25°C and the dried aerial parts and roots were grinded separately with a blender.

Extraction and Isolation

Air dried powdered aerial parts and roots (5kg) were macerated at room temperature in 95% ethyl alcohol. The combined alcoholic extracts were concentrated under vacuum to about 480 mls then extracted successively with 3.5 L light petroleum, 4 L methylene chloride, 3.5 L ethyl acetate and 3 L n-butanol. Extracts were freed from solvents under reduced pressure to provide 10 g, 13 g, 8.7g and 25 g respectively. After TLC screening of the four extracts, the chloroform and butanol extracts were selected for this work.

The chloroform extract obtained from *Centaurea horrida* was subjected to column chromatography (CC) on silica gel and eluted with CH Cl_3-EtOAc as eluent with

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increasing ethyl acetate content. Material **C1 (**50 mg) was obtained from the fractions 24-28 after evaporation of the solvent. While fractions 29-35 were chromatographed over silica gel. Twenty three fractions (50 ml) each were collected and screened by TlC. Fractions 13-17 were dissolved in methanol and kept aside at room temperature. A dark yellow amorphous solid was afforded and up on purification from methanol produced 30 mg of yellow amorphous powder nominated as material **C2**.The left supernatant was subjected to PTLC on fluorescent silica gel plates system chloroform: ethyl acetate (6:4) R_f 0.76 observed under UV light was scrapped off and subjected to elution with methanol solvent and then the solvent was distilled off to give 40 mg of yellow powder, designated as material **C3**. Moreover the fraction 36- 40 afforded 30mg of yellow powder, m.p.330 °C designated as **C4**.

In addition, butanol extract was chromatographed on silica gel (CC) and eluted with chloroform-ethyl acetate (7:3) to get 30 mg of material **C5** in the form of yellow crystals.

Caffeic acid **(C1)** yellow crystals, m.p.211-213°C. UV (λ max, MEOH) 225,282,374 nm. DCI-MS m/z; [M^++1]; 180. ^{1}HNMR (DMSO, 500 MHz); 7.09 (1H, d, J=2Hz, H-2), 6.8 (1H, d, J=8Hz, H-5), 7 (1H, dd, J=8.2Hz, H-6) and 7.44 (1H, d, J=8Hz, H-8). ^{13}CNMR (DMSO, 500 MHz); 126.1 (C-1), 115 (C-2), 148.5 (C-3), 146 (C-4), 121.5 (C-5), 115.6 (C-6), 145 (C-7), 116.2 (C-8) and 168.4 (C-9).

Quercetin **(C2)** yellow crystals, m.p.316-317°C. UV (λ max, MEOH): 260,375 nm, (MeOH + NaOMe): 260,380 nm, (MeOH + $AlCl_3$): 265,395 nm, (MeOH + $AlCl_3$ + HCl): 265,395 nm ,(MeOH + NaOAc) : 278,400 nm. DCI-MS m/z; [M^++1]; 303. ^{1}HNMR (DMSO, 500 MHz); 6.4 (1H, d, J=2Hz, H-6), 6.2 (1H, d, J=2Hz, H-2') and 6.9 (1H, d, J= 8Hz). ^{13}CNMR (DMSO,500 MHz;148 (C-2), 136.5 (C-3), 176.2 (C-4), 161 (C-5), 98.6 (C-6), 164.3 (C-7), 93.8 (C-8), 156.6 (C-9), 103.4 (C-10), 122.4 (C-1'), 115.5 (C-2'), 147 (C-3'),145,5 (C-4'), 116 (C-5') and 98.6 (C-6').

Hispidulin **(C3)** yellow crystals, m.p.207°C. UV (λ max, MEOH): 215, 270, 335 nm,(MeOH + NaOMe): 275,385nm ,(MeOH+ $AlCl_3$): 275,364 nm ,(MeOH + $AlCl_3$ + HCl): 275,367 nm,(MeOH + NaOAc) : 285,377nm. DCI-MS m/z; [M^++1]; 300. ^{1}HNMR (DMSO,500 MHz); 6.94 (1H, s, H-3), 6.4 (1H, d, J=2 Hz, H-6), 6.2 (1H, s, H-8), 7.92 (1H, dd, J=8.75 Hz, H-2') , 6.93 (1H, dd, J=8.75 Hz, H-3') , 6.93 (1H, dd, J=8.2 Hz, H-5') and 7.92 (1H,dd, J=8.2Hz, H-6'). ^{13}CNMR (DMSO,500 MHz; 146.25 (C-2), 102.3 (C-3), 182.5 (C-4), 153.2 (C-50, 60.4 (C-6), 157.78 (C-7), 94.7 (C-8), 152.86 (C-9), 104,5 (C-10), 121,67 (C-1'), 128.9 (C-2'), 116.4 (C-3'), 161.63 (C-4'), 116.4 (C-5') and 131.8 (C-6').

Fisetin **(C4)** faint yellow powder, m.p.330°C .UV (λ max, MEOH) 210,280,325 nm ,(MeOH + NaOMe): 285,370 nm, MeOH+ $AlCl_3$): 280,375 nm, ,(MeOH + $AlCl_3$ + HCl): 280,375 nm, 295,330 nm, (MeOH + NaOAc) : DCI-MS m/z; [M^++1] ; 286. ^{1}HNMR (DMSO,500 MHz); 7.93 (1H, d, J=2 Hz, H-5), 7.58 (1H, dd, J=2 .1, 8.4 Hz, H-6), 7.7 (1H, d, J=2 Hz, H-8), 6.92 (1H, d, J=2 Hz, H-2'), 6.915 (1H, d, J=2.4 Hz, H-5') and 7.9 (1H, dd, J=8.5, 2 Hz, H-6'). ^{13}CNMR (DMSO,500 MHz; 145.4 (C-2), 137.6 (C3), 172.4 (C-4), 126.9 (C-5), 115 (C-6), 162.7 (C-7), 102.27 (C-8), 156.9 (C-9), 120 (C-10), 122.9 (C-1'), 128.9 (C-2'), 115.38 (C-3'), 147.68 (C-4'), 116 (C-5') and 115.1 (C-6').

Quercetin 3-D-galactoside **(C5)** yellow powder, m.p.220-230°C .UV (λ max, MEOH) 255.369 nm. DCI-MS m/z; [M^++1]; 464. ^{1}HNMR (DMSO,500 MHz); 6.4 (1H,d, J=2Hz, H-6, H-8), 7.53 (1H,d, J=2Hz, H-2'), 6.82 (1H,d, J=8.45 Hz, H-5'), 7.68 (1H,dd, J=8.4 5, 2Hz, H-6'), 5.38 (1H,d, J=7.4Hz, 1H, sugar), 5.135 (C-2''-OH), 4.85 (C-3''-OH) and 4.44 (C-4''-OH). ^{13}CNMR (DMSO,500 MHz; 145.3 (C-2), 133.9 (C-3), 177.8 (C-4), 156.7 (C-5), 102.29 (C-6), 161.67 (C-7), 99.2 (C-8), 148.97 (C-9), 104.25 (C-10), 122.4 (C-1'), 121.5 (C-2'), 116.3 (C-3'), 115.38 (C-4'), 147.68 (C-5'), 115.6 (C-6'), 94 (C-1''), 71.66 (C-2''), 73.66 (C-3''), 68.3 (C-4'')**,**76.3 (C-5'') and 60.59 (C-6'').

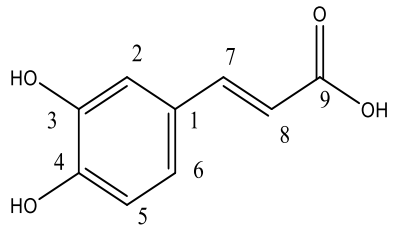
RESULTS AND DISCUSSION

Compounds **C2, C3** and **C5** were found to be 5-hydroxyflavonol derivatives. Compound **C1** was found to be caffeic acid[11]. Compounds **C2, C3** and **C5** were found to be 5-hydroxyflavonol derivatives. UV spectra of compounds **C2** showed the presence of 3-, 5-and 4'-hydroxyl groups. Its MS showed the appearance of the molecular ion peak at m/z=302($C_{15}H_{10}O_7$). Moreover, the ^{13}C-NMR displayed one signal assigned for one carbonyl group at position C-4 at □□176.2 and determined the presence of fifteen carbon atoms. Referring to the literature, the different published data for flavones proved that material C2 is Quercetin[11].

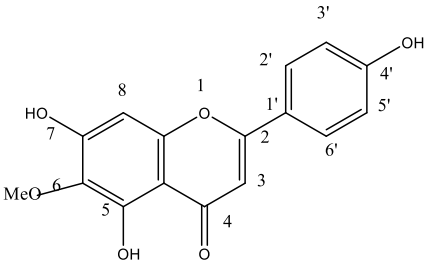
UV spectra of compound **C3** in different shift reagents indicated the presence of free 5-.7-. and 4'- hydroxyl groups**.** MS spectra showed the molecular ion peak at m/z 300 [M^+], calculated for $C_{16}H_{12}O_6$ corresponding to an additional CH_3 group compared to quercetin. ^{1}H-NMR confirmed the presence of ring B protons to be 2', 3', 5' and 6'. The ^{1H}-NMR revealed the presence of one methoxyl group at position C-6 by the signal produced at □ 3.76 for three protons and determined by the appearance of one singlet at □ 6.2 for the proton at position 8. In addition, Dept 135 proves the identity of material C3 to be hispidulin by revealing the presence of six CH groups at positions: C-3,C-8,C-2',C-3',C-5' and C-6' .Comparison of the observed data with those previously reported for hispidulin indicated their similarity[12,13].

EIMS of compound **C4 indicated** the molecular ion peak at m/z= 286 [M^+] calculated for $C_{15}H_{10}O_6$ i.e. fourteen mass units lesser than that of hispidulin. On the other hand, UV spectra of material **C4** indicated the four free hydroxyl groups at positions 3,7,3' and 4'. ^{1}H-NMR assigned the presence of four hydroxyl groups showing their signals at □10.73, 9.03, 9.49 and 9.278 respectively. The ^{13}C-NMR assigned the presence of fifteen carbon atoms with a carbonyl group showing a signal at □172.4 That was also proved by DEPT-135 that revealed the presence of six signals for "CH "at □ 126.9, 115.11, 102.27, 128.9, 116 and 115.1 at positions 5,6,8,2',5'and 6' respectively with the absence of CH_3 group. All the above mentioned and observed data of material **C4** confirmed that it is fisetin[14].

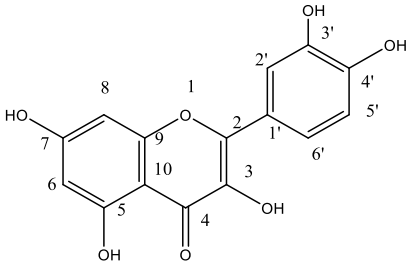
EIMS of compound **C5** indicated the molecular ion peak at m/z= 464 [M^++1] calculated for $C_{21}H_{21}O_{12}$. The



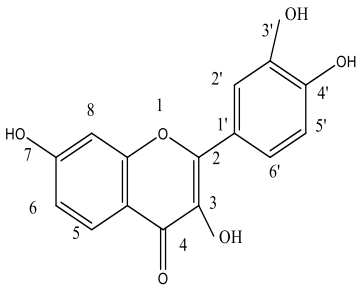
Caffeic acid



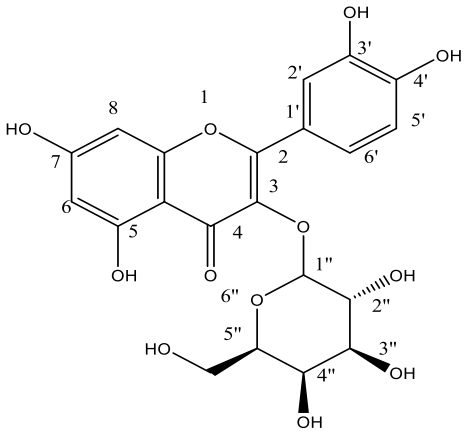
Hispidulin



Quercetin



Fisetin



Quercetin-3-D-Galactoside

Figure 1: The isolated compounds.

glycosidic nature of flavonoid ‘’**C5’’** was first detected by Molisch’s test**.** The ^{1}H-NMR and ^{13}C-NMR for the sugar moiety showed that it is a monoside. The identity of the sugar moiety in “**C5”** was determined as galactoside, as represented by the signals in the ^{1}H-NMR at 5.38 (1H,d,J=7.4) indicating that it is a ß - glycosidic linkage .Otherwise the signals appearing in the^{1}H-NMR at 3.2-3.65 (m,6 H of sugar part) proved that too. Moreover, its ^{1}H-NMR displayed three signals corresponding to free hydroxyl groups in the sugar moiety at 4.4, 4.85 and 5.13 assigned to C-4’’, C-3’’ and C-2’’ respectively. All the above mentioned and observed data of material **C5** confirmed that it is Quercetin 3-D-Galactoside[15]. It is important to mention that quercetin -3-D- galactoside was isolated for the first time from *Centaurea horrida* growing in Lebanon.

CONCLUSIONS

In this work a phytochemical investigation of aerial and root parts of *Centaurea horrida* and the obtained isolates were assigned for their protons and carbons for the first time. Moreover, caffeic acid, hispidulin, quercetin, fisetin and quercetin 3-D-Galactoside were isolated from *Centaurea horrida* aerial and root parts. It is important to mention that all these compounds were separated for the first time from *Centaurea horrida* growing in Lebanon and fully assigned. All the previously mentioned compounds were responsible for the antioxidant activity of the examined species.

ACKNOWLEDGMENT

The authors are grateful to Miss F.Mostafa (Department of pharmaceutical sciences, Faculty of Pharmacy, AL-Zaytoonah University, Amman, Jordan) for NMR spectra, MS, IR and UV generated for this study. This work was supported by the Faculty of Pharmacy, Beirut Arab University.

REFERENCES

1. Gonzalez C, Macias F A, Massanet G M, Luis F R. Flavonoids from *Centaurea clementei*. J. Nat. Prod.1985; 48 (5), 819-22.
2. Marco J A, Sanz-Cervera J F, Garcia-Lliso V, Susanna A, Garcia-Jacas N. Sesquiterpene lactones, lignans and

aromatic esters from cheirolophus species. Phytochemistry.1994; 37 (4), 1101-7.
3. Garbacki N, Gloaguen V, Damas Bodart P, Tits M, Angenot L. Antiinflammatory and immunological effects of *Centaurea cyanus* flower-heads. J. Ethnopharmacol.1999; 68 (1-3), 235.
4. Chuclá M T, Lamela M, Gato A, Cadavid L. *Centaurea corcubionensis*: A study of its hypoglycemic activity in rats. Plant.Med.1988.; 54.107-9.
5. Massoud R, Kazem N. A revision of *Centaurea* sect. *Centaurea* (Asteraceae) from Iran. Turk J Bot.2014; 38: 969-987.
6. Seghiri R, Boumaza O, Mekkio R, Benayache S, Mosset P, Quintana J, Estévez F, León F, Bermejo Benayach F. A flavonoid with cytotoxic activity and other constituents from *Centaurea africana*, Phytochem. Lett.2009; 2, 114-118.
7. Sarker S D, Dinan L. Moschamide: An unusual alkaloid from the seeds of *Centaurea moschata*, Tetrahedron Lett.1998; 39, 1421-4.
8. Raafat K, Boukhary R, Aboul-Ela M, El-Lakany A. Endogenous Lebanese Plants Treating Diabetes and Related Complications. Nat Prod Chem Res.2013; 1, 112-120.
9. Flamini G, Bulleri C, Morelli J. A new flavonoid glycoside from *Centaurea horrida*. J.N. prod.2000; 63 (5), 662-663.
10. Aktumsek A, Zengin G, Guler GO, Cakmak YS, Duran A. Screening for in vitro antioxidant properties and fatty acid profiles of five Centaurea L. species from Turkey flora. Food chem. Toxicol.2011; 49 (11): 2914-2020.
11. Mirna P, Šaša Likić, Gordana. R. Phenolic Compounds *in Centaurea rupestris* Tissues and Their Antiphytoviral Activity. Croat. Chem. Acta.2014; 87 (1) 79-84.
12. Bicha A, Bentamene O, Benaissa S, Benayache V P, Garcia F, Leon I, Brouard J, Bermejo B, Benayache F. Flavonoids aglycones from *Centaurea maroccana* ,S. Chemistry of natural products .2011; 47(1) 105-106.
13. Sayed A A , Emadeldin M K. Cytotoxic Activities of Flavonoids from *Centaurea scoparia*. Scientific World Journal.2014.
14. Schwarz K, Ternes W. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. II. Isolation of carnosic acid and formation of other phenolic diterpenes. Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung.1992; 195: 99-103.
15. Guido F, Claudia B, Ivano M. Antonio M. A new Flavonoid glycoside from *Centaurea horrida*. J.Nat.Prod.2000; 63(5), 662-663.