

Phenolic Compounds from *Centaurea horrida* L Growing in Lebanon

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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 teas⁶. Moreover, many species of this genus have been used traditionally to cure various ailments as malaria and hypertension⁷. 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 contents⁸. Moreover, Flamini studied the composition of *Centaurea horrida* growing in Italy and separated flavonoids and phenolic acids proving its antioxidant properties⁹.

Plants of genus *Centaurea* are the subjects of numerous chemicals studies, due to the occurrence of phenolic acids, flavonoids and lactones¹⁰ 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 ¹HNMR and ¹³CNMR 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 CH₂Cl₂:EtOAc as eluent with

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 TIC. 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. ¹H-NMR (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). ¹³C-NMR (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 + AlCl₃): 265,395 nm, (MeOH + AlCl₃ + HCl): 265,395 nm, (MeOH + NaOAc) : 278,400 nm. DCI-MS m/z; $[M^+ + 1]$; 303. ¹H-NMR (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). ¹³C-NMR (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+ AlCl₃): 275,364 nm ,(MeOH + AlCl₃ + HCl): 275,367 nm,(MeOH + NaOAc) : 285,377nm. DCI-MS m/z; $[M^+ + 1]$; 300. ¹H-NMR (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'). ¹³C-NMR (DMSO,500 MHz); 146.25 (C-2), 102.3 (C-3), 182.5 (C-4), 153.2 (C-5), 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+ AlCl₃): 280,375 nm, ,(MeOH + AlCl₃ + HCl): 280,375 nm, 295,330 nm, (MeOH + NaOAc) : DCI-MS m/z; $[M^+ + 1]$; 286. ¹H-NMR (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'). ¹³C-NMR (DMSO,500 MHz); 145.4 (C-2), 137.6 (C-3), 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. ¹H-NMR (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). ¹³C-NMR (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 acid¹¹. 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(C₁₅H₁₀O₇). Moreover, the ¹³C-NMR displayed one signal assigned for one carbonyl group at position C-4 at $\square\square$ 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 Quercetin¹¹.

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 C₁₆H₁₂O₆ corresponding to an additional CH₃ group compared to quercetin. ¹H-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 \square 3.76 for three protons and determined by the appearance of one singlet at \square 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 C₁₅H₁₀O₆ 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'. ¹H-NMR assigned the presence of four hydroxyl groups showing their signals at \square 10.73, 9.03, 9.49 and 9.278 respectively. The ¹³C-NMR assigned the presence of fifteen carbon atoms with a carbonyl group showing a signal at \square 172.4 That was also proved by DEPT-135 that revealed the presence of six signals for "CH "at \square 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 CH₃ group. All the above mentioned and observed data of material **C4** confirmed that it is fisetin¹⁴. EIMS of compound **C5** indicated the molecular ion peak at m/z= 464 $[M^+ + 1]$ calculated for C₂₁H₂₁O₁₂. The

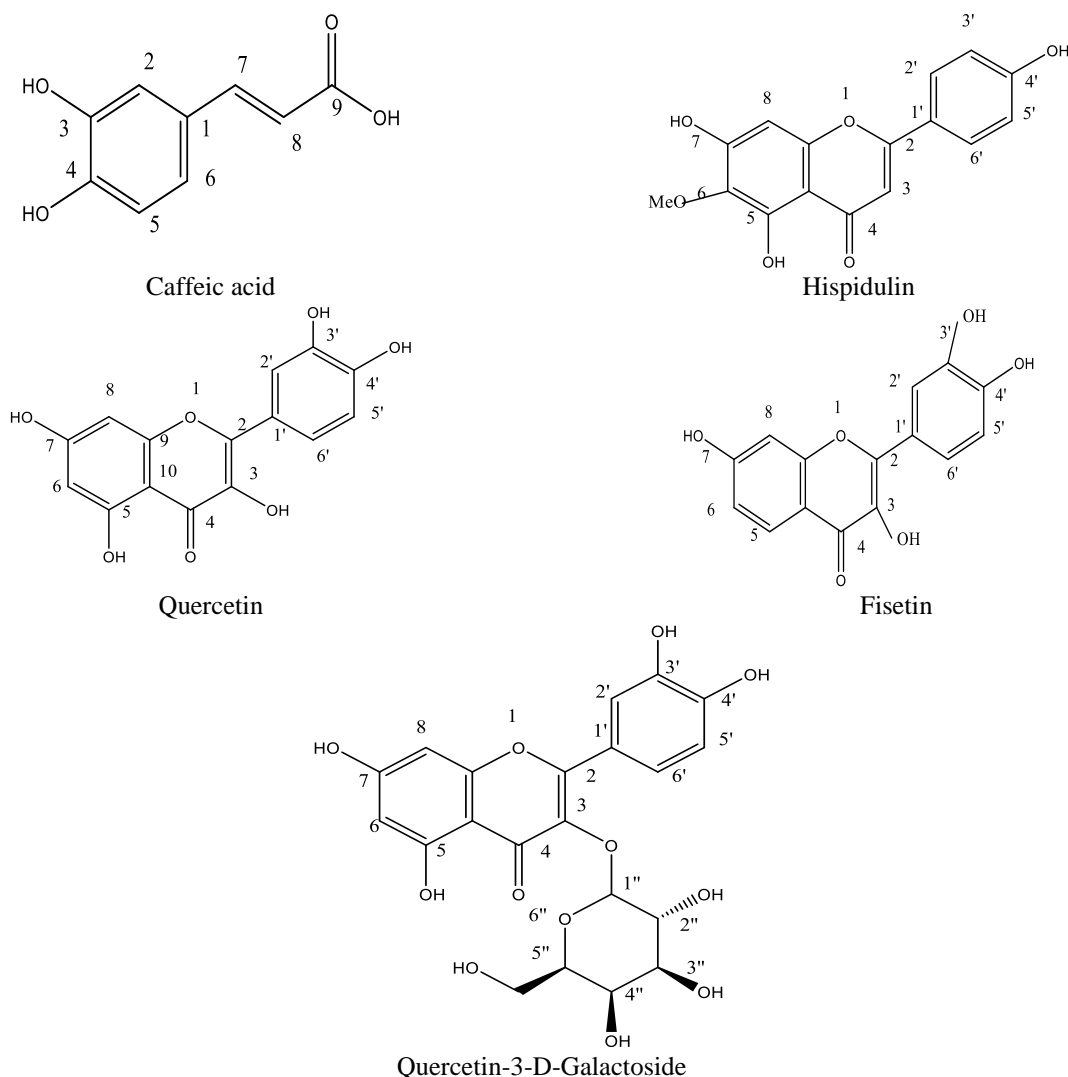


Figure 1: The isolated compounds.

glycosidic nature of flavonoid ‘‘C5’’ was first detected by Molisch’s test. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ 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 $^1\text{H-NMR}$ at 5.38 (1H,d,J=7.4) indicating that it is a β - glycosidic linkage. Otherwise the signals appearing in the $^1\text{H-NMR}$ at 3.2-3.65 (m,6 H of sugar part) proved that too. Moreover, its $^1\text{H-NMR}$ 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-Galactoside¹⁵. 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.

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