PHENOLIC PROFILE OF Centaurea aegyptiaca L. GROWING IN EGYPT AND ITS CYTOTOXIC AND ANTIVIRAL ACTIVITIES

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Abstract

Background: Centaurea aegyptiaca L (Asteraceae), is one of the most attractive plants growing wildly in Sinai, and is not well investigated for its phytochemical constituents. This study represents the first in-depth characterization of the phenolic profile of the aerial parts of C. aegyptiaca methanolic extract utilizing liquid chromatography (LC) combined with electrospray ionization (ESI) tandem mass spectrometry (MS/MS).

Material and Methods: Phenolic profile was researched utilizing LC-HRESI-MS-MS. Assessment of cytotoxic activity against four human cancer cell lines (Hep-G2; hepatocellular carcinoma cells, MCF-7; breast adenocarcinoma cells, and HCT-116; colon carcinoma and HELA; cervical carcinoma cells) was performed using 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Antiviral activity was surveyed utilizing cytopathic effect inhibition assay.

Results: A total of sixty-one compounds were tentatively distinguished (twenty-one phenolic acids and their derivatives, thirty-one flavonols and nine flavones) in the negative and positive modes. Centaurea aegyptiaca demonstrated outstanding results against Hep-G2, MCF-7, HCT-116 and HELA cell lines with IC₅₀ of 12.1, 30.9, 11.7 and 19.5 µg/mL respectively compared and doxorubicin as a reference drug. Weak antiviral activity was seen against hepatitis A virus (HAV) and no impact against herpes simplex virus type 1 (HSV 1).

Conclusion: This study provides a better understanding of the chemistry of C. aegyptiaca that announces itself as a promising cytotoxic agent.

Key words: Centaurea aegyptiaca, Cytotoxicity, MTT assay, flavonoid.

Introduction

The Asteraceae (Compositae) is the biggest family of flowering plants with more than 24000 - 30000 species and 1600 - 1700 genera, distributed worldwide aside from Antarctica with an observable financial and medicinal contribution (Funk et. al, 2005).

Centaurea, the fourth biggest genus in the family Asteraceae, consists of 600 species around the world, especially in Mediterranean regions and in Western Asia, (Garcia-Jacas et al, 2000). Around 13 species of Centaurea are growing in Egypt. Centaurea species, have been the object of various phytochemical studies, with a diversity of bioactive phytochemicals and prevalence of flavonoids (Flamini et al., 2001; Shoeb et al., 2007; Seghiri et al, 2009) and sesquiterpene lactones (Koskoulińska et al., 2002, 2005). Centaurea species are claimed for their uses in gastrointestinal and inflammatory disorders, hypotensive and cytotoxic activities, in addition to the anti-bacterial impacts alone or when mixed with different plants (Farrag et al., 1993; Kargoglu et al, 2010; Köse et al., 2007). Centaurea aegyptiaca (Centaurea cancellata Sieber ex Spreng., Calcitrapa aegyptiaca (L.) Sweet, C. iberica) is growing wild in Sinai (St. Katherine), Egyptian eastern desert, the Red Sea coastal strip, and Gebel Elba. Investigations of C. aegyptiaca revealed the presence of guaianolide (Orabi et al, 2013; Sarg et al, 1987) which showed potential cytotoxic activities against liver and larynx carcinoma cell lines. The studies on this species deal basically with sesquiterpene lactones, while flavonoids are not well investigated.

Due to scarcity of information regarding the chemical composition of C. aegyptiaca, it is valuable to carry out an in-depth phytochemical investigation on this plant to characterize its main active constituents utilizing HPLC-MS-MS analysis and Xcalibur software with the screening of its cytotoxic and antiviral activities.

Plant Material

Aerial parts of Centaurea aegyptiaca L. were gathered during the flowering stage in February 2015 from km 67, Cairo-Suez road, Egypt. The plant was identified by Prof. Dr. Abdel-haleem Abdel-mogaly, Department of Plant Taxonomy, Herbarium of Horticultural Research Institute, Agricultural Research Center, Dokki, Cairo, Egypt. A voucher specimen (RS013) was deposited in Herbarium of Faculty of Pharmacy, October University of Modern Sciences and Arts.

Material and Methods

Preparation of the plant extract

Three hundred grams of plant material were exhaustively extracted with methanol. The combined methanolic extracts (CME) were filtered, concentrated under vacuum at 50°C, dried and left for LC-HRESI-MS-MS analysis, and to evaluate the cytotoxic and antiviral activities.
The absorbance of untreated cells was considered as 100%. The percentage cell viability was calculated with the Microsoft Excel®. Percentage cell viability was calculated as follows: % Cell viability = \((\text{Mean Abs control} - \text{Mean Abs test metabolite}) / \text{Mean Abs control}) \times 100\). STATA statistical analysis package was used for the dose response curve drawing in order to figure IC_{50}. The viability of control and treated cells were explored at the Regional Center for Mycology and Biotechnology (RCMB). The study was performed on a Bruker micro-TOF-Q Daltonics (API) Time-of-Flight mass spectrometer (Bremen, Germany), coupled to 1200 series HPLC system (Agilent Technologies, Waldbronn, Germany), equipped with a high-performance auto-sampler, binary pump, and PDA detector G 1314 C (SL). Chromatographic separation was performed on a Superspher 100 RP-18 (75 x 4 mm i.d.; 4 µm) column (Merck, Darmstadt, Germany).

### Table 1: Phenolic acids and their derivatives tentatively identified in Centaurea aegyptiaca

| Rt (min.) | [M-H]- m/z | Fragment ions | Tentatively identified     | Ref.           |
|-----------|-------------|---------------|----------------------------|----------------|
| 1         | 1.68        | 341.11        | 179.08 (-162) 161.17 143.09 119.09 113.04 | Caffeoyl hexoside Dartora et al, 2011 |
| 2         | 3.26        | 191.06        | 111.01 173.16 127.10 85.05 | Quinic acid Dartora et al, 2011 |
| 3         | 3.59        | 493.16        | 191.21 330.43 402.4 473.24 | Quinic acid deriv Dartora et al, 2011 |
| 4         | 8.34        | 315.07        | 133.07 (-162) 165.14 135.19 109.09 225.2 (-90) 195.05 (-120) | Protocatechuc acid hexoside deriv Chen et al, 2012 Valverdu-Queralt et al., 2015 |
| 5         | 9.27        | 515.14        | 179.06 353.23 341.17 191.08 | Dicaffeoyl quinic acid Chen et al, 2012 |
| 6         | 12.45       | 353.09        | 191.11 179.13 135.15 | Chlorogenic acid Dicaffeoyl quinic acid Dartora et al, 2011 |
| 7         | 14.90       | 285.06        | 153.10 109.18 | Protocatechuc acid pentoside Dartora et al, 2011 |
| 8         | 14.91       | 447.12        | 315.21 411.22 271.25 152.18 163.10 179.15 | Protocatechuc acid pentoside hexoside Dartora et al, 2011 |
| 9         | 23.52       | 499.15        | 191.15 337.26 | 3 caffeoyl 5 coumaroyl quinic acid Jaiswal et al, 2010 |
| 10        | 15.31       | 337.09        | 163.14 | 3-p-Coumaroyl quinic acid Jaiswal et al, 2010 |
| 11        | 24.30       | 337.09        | 173.08 | 4p-Coumaroyl quinic acid Jaiswal et al, 2010 |
| 12        | 19.64       | 529.16        | 173.13 191.11 367.21 355.21 | 4-Feruloyl quinic acid isomer Jaiswal et al, 2010 |
| 13        | 21.54       | 447.11        | 152.12 401.19 285.26 241.24 163.16 177.2 401.26 285.18 152.15 163.16 | Protocatechuc acid hexoside pentoside Jaiswal et al, 2010 |
| 14        | 21.96       | 193.05        | 134.11 149.07 | Feralic acid Jaiswal et al, 2010 |
| 15        | 22.25       | 417.10        | 241.18 152.07 285.22 163.15 179.15 | Dihydroxybenzoic acid-O-dipentoside Beelders et al, 2014 |
| 16        | 23.62       | 529.16        | 173.13 193.08 | 4-Feruloyl-5-Caffeoyl quinic acid Dartora et al, 2011; Jaiswal et al, 2010 |
| 17        | 35.14       | 367.10        | 173.16 191.14 | 4-Feruloyl quinic acid Jaiswal et al, 2010 |
| 18        | 42.74       | 367.10        | 191.12 173.13 193.14 | 5-Feruloyl quinic acid Jaiswal et al, 2010 |
| 19        | 32.15       | 352.09        | 163.05 119.09 101.08 113.18 | Coumaroyl hexoside Jaiswal et al, 2010 |
| 20        | 59.68       | 543.21        | 349.21 367.20 193.22 173.13 | Diferuloylquinic acid isomer Jaiswal et al, 2010 |
| 21        | 60.69       | 473.11        | 311.27 341.20 | Dicaffeoyltartaric acids (chloric acids) Jaiswal et al, 2010 |
Table 2: Flavonoids tentatively identified in *Centaurea aegyptiaca*

| No | RT  | [M-H]$^-$ m/z | Fragment ions | [M-H]$^-$ m/z | Fragment ions | Tentatively identified compound | Ref.          |
|----|-----|---------------|---------------|---------------|---------------|--------------------------------|--------------|
| 1  | 38.23 | 563.14 | 437.26 [M-H-90] 443.31 [M-H-120] 353.22 [M-H-120-90] 383.25 | ------ | Apigenin 8C-hexoside 6C-pentoside | Ibrahim et al., 2015 |
| 2  | 38.83 | 785.21 | 315.19 [isorh, M-H-2hexose-rh], 623.26 [M-H-162] 477.23 [M-H-162-146] 300.16 | 787.24 | 625.14 [M+H-162] 479.17 [-162-146] 317.13 | Isorhamnetin dihexoside rhamnoside |
| 3  | 40.46 | 479.08 | 317.15 [M-H-162] 357.15 | 481.10 | 319.08 [M+H-162] | Quercetagenin –O- hexoside |
| 4  | 40.57 | 317.16 | 271.15 287.13 166.03 139.14 299.18 243.14 227.17 179.06 | 319.12 | 273.09 301.03 245.07 181.02 153.08 136.37 | Quercetagenin |
| 5  | 41.36 | 625.14 | 317.16 [M-H-308] 461.25 341.18 | 627.16 | 481.18 [M+H-146] 319.14 [M-H-146-162] 463.23 [M+H+162] | Quercetagenin-rhamnoside-hexoside (rutinoside) |
| 6  | 42.22 | 641.27 | 317.15 [M-H-2hexose] 243.22 225.30 479.35 [M-H-162] 447.23 | ------ | Quercetagenin dihexoside |
| 7  | 44.10 | 639.16 | 477.19 [M-H-162] 315.18 [M-H-162-162] 447.22 300.26 271.21 | 641.17 | 479.17 [M+H-162] 317.16 [M+H-162-162] | Isorhamnetin –O-dihexoside |
| 8  | 44.36 | 623.13 | 285.15 [M-H-162-176] 447.22 [M-H-176] 337.23 357.13 257.18 243.26 | 625.14 | 287.12 449.17 [M+H-162] | Kaempferol hexoside glucuronide |
| 9  | 44.55 | 463.09 | 301.14 | 465.10 | 303.14 | Quercetin –O-hexoside |
| 10 | 45.46 | 301.18 | 283.13 271.16 255.17 227.10 179.07 8165.10 151.18 | 303.12 | 283.14 273.13 179.14 169.06 137.02 | Quercetin |
| 11 | 48.01 | 269.14 | 251.16 209.23 225.26 165.27 189.26 | 271.21 | 242.13 253.15 153.10 225.20 211.17 r48.70 | Apigenin |
| 12 | 48.31 | 431.10 | 311.22 [M-H-120] 341.25 [M-H-90] | 433.11 | 415.16 367.16 337.14 313.17 | Apigenin C-glucoside (Isovitexin) |
| 13 | 49.05 | 639.16 | 331.20 [M-H-162-146] 316.20 | - | Patuletin hexoside rhamnoside | Ibrahim et al., 2015 |
| 14 | 49.08 | 493.10 | 331.16 [M-H-162] | - | Patuletin hexoside |
| 15 | 49.10 | 331.05 331.08 | 316.17 317.18 [M-H-14] | 333.06 | 318.09 301.12 155.05 169.10 | Patuletin |
| 16 | 49.37 | 623.15 | 315.14 300.22 271.18 | 625.18 | 479.17 [M+H-146] 317.07 [M-H-146-162] 463.20 [M+H+162] | Isorhamnetin 3-O-hexoside 7-O-rhamnoside |
| 17 | 49.51 | 609.18 | 301.18 [M-H-308] 269.14 199.22 | 611.19 | 449.10 [M+H-162] 271.11 303.13 [M+H-146] 465.10 [quercetin+162] | Quercetin hexoside rhamnoside |
| Bakr et al., Afr J Tradit Complement Altern Med. (2016) 13(6):135-143 |
|---|---|---|---|---|
| 18 | 49.73 | 285.19 | 267.11 253.27 239.12 185.13 137.06 | 287.13 R159 269.22 259.14 241.14 213.12 164.89 153.02 | Kaempferol | Schmeda- Hirschmann et al., 2015 |
| 19 | 50.52 | 315.05 | 300.20 | 317.07 302.11 | Isorhamnetin | Pajero et al., 2004 |
| 20 | 53.79 | 477.10 | 315.18 [M-H-162] 301.19 357.19 [M-H-120] 415.35 | 479.12 317.13 [M+H-162] 419.94 360.94 | Isorhamnetin 3-O-hexoside | Pajero et al., 2004. |
| 21 | 50.54 | 593.15 | 285.15 [M-H-146-162] 429.28 531.28 269.17 | 595.17 287.13 [M+H-162-146] 449.11 [M+H-146] 213.27 | Kaempferol O-hexoside-rhamnoside | Ibrahim et al., 2015. |
| 22 | 53.13 | 477.07 | 301.13 [M-H-176] 415.29 | | | |
| 23 | 54.10 | 431.10 | 269.18 [M-H-162] 311.16 [M-H-120] 433.11 | 271.10 [M+H-162] 415.21 | Apigenin hexoside | |
| 24 | 54.5 | 461.11 | 299.19 [M+H-162] 339.17 | --- | Hispidulin hexoside | Akkal et al., 2003 |
| 25 | 55.91 | 593.28 | 269.29 [M-H-162-162] 431.36 [M-H-162] 417.36 285.24 245.27 225.20 | 595.30 577.22 [M+H-18] 415.16 [M+H-180] 253.21 235.20 325.14 271.27 | Apigenin -O- dihexoside |
| 26 | 56.13 | 521.09 | 317.16 [M-H-204] 399.29 429.21 | 523.11 319.09 [M+H-204] 503.25 455.14 | Quercetagenin acetyl hexoside | Parejo et al., 2004 |
| 27 | 56.95 | 505.10 | 301.16 [M-H-204] 343.21 [M+H-162] 461.30 [M-H-44] 179.09 151.13 | 507.11 303.12 [M+H-204] 385.22 447.23 [M+H-60] | Quercetin acetyl hexoside | Oszmiański et al., 2015 |
| 28 | 57.03 | 491.20 | 476.28 [M-H-15] 315.23 [M-H-176] 329.22 [M+H-162] 431.32 [M-H-60] 175.16 161.12 | | Isorhamnetin glucuronide |
| 29 | 58.59 | 607.26 | 269.27 [M-H-176-162] 431.37 [M-H-176] 383.21 355.16 | 609.28 253.08 235.14 429.03 [M+H-180] 431.28 | Apigenin glucuronide hexoside |
| 30 | 58.70 | 461.07 | 285.18 219.23 175.11 339.24 | 461.09 287.13 [M+H-176] 445.24 [M+H-18] 175.07 | Kaempferol glucuronide | Schmeda-Hirschmann et al., 2015 |
| 31 | 59.03 | 625.12 | 463.19 [M-H-162] 315.15 301.19 [M-H-162-162] | | Quercetin 3, 7 di-O-hexoside | Ibrahim et al., 2015. |
| 32 | 61.34 | 447.09 | 327.14 [M-H-120] 357.06 [M-H-90] 387.31 [M-H-60] 285.29 429.28 241.25 175.16 | 449.11 287.13 431.19 383.12 329.17 276.17 246.19 | Luteolin C-hexoside | Brito et al., 2014 |
| 33 | 61.87 | 491.21 | 285.16 473.36 431.41 [M-H-60] 315.24 [M-H-176] 329.33 [M+H-162] 175.13 179.18 167.19 | 493.23 33.13 475.29 [M+H-15] 269.155.05 | Kaempferol methyl ether glucuronic acid | Schmeda-Hirschmann et al., 2015 |
| 34 | 62.41 | 535.11 | 331.19 [M-H-204] 373.09 [M-H-162] 471.31 [M-H-62] | 537.23 478.98 333.18 [M+H-204] 419.88 375.31 [M+H-162] | Patuletin acetyl hexoside |
| 35 | 63.18 | 519.11 | 315.23 [M-H-204] 357.36 [M-H-162] | 521.13 317.12 [M+H-204] 501.27 359.34 [M+H-162] | Isorhamnetin acetyl hexoside |
| 36 | 63.76 | 639.17 | 519.27 [M-H-120] 549.24 [M-H-90] 607.34 [M-H-32] 463.22 [M-H-176] | --- | Quercetin C-hexoside glucuronide |
### Bakr et al., Afr J Tradit Complement Altern Med. (2016) 13(6):135-143

| PPM | Mass (Da) | Monoisotopic Mass (Da) | Precursor Ion Mass (Da) | Comment |
|-----|-----------|------------------------|-------------------------|---------|
| 37  | 64.24     | 521.33                 | 301.21 [M-H-176-162] 219.17 | 523.35 |
|     |           |                        | 359.45 [M-H-162] 503.39 [M-H-18] 489.26 [M-H-32] 475.22 341.44 | 315.21 |
|     |           |                        | 343.13 [M+H-180] 361.13 [M+H-162] 505.21 [M+H-18] 318.14 325.13 307.23 |         |
|     | 68.03     | 299.06                 | 284.06 [M-H-15]         | 301.07 |
|     | 69.35     | 507.35                 | 345.41 [M-H-162] 331.10 299.35 161.19 417.41 | 509.13 |
|     | 74.61     | 609.41                 | 315.27 [M-H-162-132] 293.34 246.23 225.09 447.39 [M-H-162] | --     |
| 38  | 147.11    | 286.09 [M+H-15] 147.11 129.12 | 284.06 [M-H-15]         |         |
| 39  | 303.12    | 449.17                 | 347.17 [M+H-162] 303.12 449.17 [M+H-60] |         |
| 40  | 449.17    | 303.12                 | 347.17 [M+H-162] 303.12 449.17 [M+H-60] |         |

| Centaurein (jaceidin hexoside) | Flamini et al., 2001 |
|--------------------------------|----------------------|
| Hispidulin                     | Flamini et al., 2001 |
| Axillarin hexoside             | Parejo et al., 2004  |
| Isorhamnetin hexoside pentoside|                      |
**Identification of Phenolic Compounds**

*Centaurea aegyptiaca* CME was investigated following Hassaan et al., (2014). The mobile phase consists of two solvents, (A) 2% acetic acid (pH 2.6) and (B) 80% methanol. Gradient elution was used at a flow rate of 100 μL/min, from 5% to 50% B at 30°C. Ion spray (pneumatically assisted electrospray) ionization system was utilized. Spectra were recorded in positive and negative ion mode between m/z 120 and 1,500 with capillary voltage, 4000V and heated dry nitrogen gas temperature, 200°C and flow rate 10 L/min. The gas flow to the nebulizer was set at pressure 1.6 bar. For collision-induced dissociation (CID) MS-MS estimations, the voltage over the collision cell ranged between 20 to 70 eV. Argon was utilized as collision gas. Sodium formate was utilized for calibration toward the end of LC-MS run. Interpretation for ESI-MS was performed by Xcalibur 2.1 software from Thermo Scientific (Berlin, Germany).

**Results**

**Phenolics identified**

The phytochemical fingerprint of *C. aegyptiaca* was determined using an LC-HRESI-MS-MS. Analyses were carried out by using a full scan and MS2 data-dependent operative mode.

The combination of ESI positive and negative modes permitted the tentative identification of a total of sixty-one compounds by the interpretation of their fragmentation patterns combined with the available literature information. Twenty-one phenolic acid and their derivatives have been identified in the negative mode only (Table 1), while thirty-one flavonol and nine flavones were tentatively identified based on their MS/MS fragmentation in the negative and positive modes and listed in table 2.

**Cytotoxic activity**

*Centaurea aegyptiaca* CME was investigated against four human cell carcinoma (HepG-2, MCF-7, HCT-116, and HELA) using MTT assay. The higher potency was observed with HCT-116 followed by HepG-2 then HELA cells (Table 3) with IC_{50} less than 20 μg/mL compared with doxorubicin as a standard cytotoxic agent with a range of 0.46-0.23 μg/mL.

**Antiviral activity**

*Centaurea aegyptiaca* CME exhibited weak antiviral activity against hepatitis A virus (HAV), and no effect was observed with herpes simplex virus type 1 (HSV 1).

**Discussion**

Liquid chromatography/tandem mass spectrometry (LC/MS/MS), represents the most popular technique used to unravel the complexity of phenolic compounds. Those non-volatile, polar and thermally labile compounds are found as complex mixtures in plants [Parejo et al., 2004]. *Centaurea aegyptiaca* has not been studied for its phenolic content before.

**Phenolic acids and their derivatives**

Chlorogenic acids (CGAs) are a group of esters formed between a quinic acid and certain trans-cinnamic acids, most commonly caffeic, p-coumaric, and ferulic acid (Clifford et al, 2006).

The neutral loss of 162, 132, 146, 176 mass units commonly corresponds to the fragments for the corresponding fragments for the hexose, pentose, and hexose (Cuyckens and Claeys 2004). Compound 1 was identified as caffeoyl hexoside with base peak 179, while compound 2 was tentatively identified as quinic acid (MS2 at m/z 111, 173). Many derivatives were assigned as dicafeoyl quinic acid (compound 5; MS2 at m/z 191, 353), chlorogenic acid (compound 6; MS2 at m/z 191, 179), caffeoyl coumaroyl quinic acid (compound 9; MS2 173, 191), 4 and 5 p-coumaroyl quinic acid (compounds 10 and 11; 163, 191 respectively) in addition to several isomers of Feruloyl caffeoyl quinic acid with various fragmentation pattern (compound 12 and 16). Phenolic acid glycosides, such as protocatechuic acid hexoside (compound 4), protocatechuic acid pentoside (compound 7) and protocatechuic acid pentoside hexoside (compound 8), giving the corresponding fragments for the loss of a hexose [M-H-162], a pentose [M-H-132] and the consecutive loss of both. In addition, coumaric acid hexosides (compound 19) was identified based on the fragments at m/z 163 (loss of a hexose moiety) and the typical fragment of coumaric acid at m/z 119 (Fischer et al., 2011). Compound 15 was assigned as dihydroxybenzoic pattern previously reported with the same fragmentation pattern and attempted to be protocatechuic acid dipentoside. Many isomers of feruloyl quinic acid have been identified with different fragmentation pattern (Compound 17 and 18; MS2 173 and 191 respectively) (Table 1).
Flavonoid glycosides

Based on previous information concerning *Centaurea* genus, methoxylated derivatives of flavone and flavonol glycosides were expected. Through this study, *C. aegyptiaca* was claimed as a rich source of flavonoids. In the MS identification of C-glycosides, the key fragmentations utilized were [M-H-60], [M-H-90] and [M-H-120]. Apigenin and hispidulin that were previously identified in *Centaurea furfurosea* (Akkal et al., 2003) represented the principle identified flavones. Compounds 1, 11, 12, 23, 25 and 29 were tentatively identified as apigenin derivatives with [A-H] at m/z 269.14 and characteristic peaks at m/z 225 and 209. Compounds 12 and 23 were tentatively identified as Apigenin 8C-glycoside and 6C-glycoside, while compounds 24 and 37 were identified as hispidulin hexoside, and hispidulin, respectively. Methoxylated flavonol represented the majority of identified flavonoids including isorhamnetin and patuletin derivatives in addition to jaceidin, axillarin. Compound 2, with a [M-H] at m/z 785 and MS2 ions at m/z 315 (M-324, - dihexose moiety, - rhamnose), 623 [M-H-162] and 477 [M-H-162-146] was tentatively identified as Isorhamnetin dihexose rhamnuronide. Quercetagenin (compound 4), with its derivatives quercetagenin O-hexoside, rutinoside, and dihexose with loss of 162, 308 and 324 daltons respectively were also tentatively identified (Compounds 3, 5 and 6), in addition to the acetylated derivative (compound 26), with [M-H] at m/z 521 and MS2 at 317 and the characteristic loss of 204 denoting acetylated hexose and tentatively identified as quercetagenin acetyl hexoside. Kaempferol, (compound 18), was also identified with its characteristic peak in the positive mode at m/z 213. Compound 8 was tentatively identified as kaempferol hexose glucuronide with the characteristic loss of 176 in the positive and negative mode. Kaempferol hexose rhamnoside was also identified with [M+H] at m/z 595 and characteristic fragments at m/z 287, 449 [M+H-146] and 213 (compound 21). Quercetin (Compound 10), was observed with six derivatives tentatively identified as quercetin-O-hexoside (compound 9), quercetin rutinoside (compound 17) with the characteristic loss of 308 daltons, quercetin glucuronide (compound 22), quercetin dihexoside (compound 31), quercetin hexose glucuronide (compound 36) and the acetylated derivative of quercetin hexoside (compound 27) with MS2 at m/z 301 [M-H-204], 343 [M-H-162] and 461 [M-H-44]. Patuletin (compound 15) and its derivatives, patuletin hexoside (compound 14) and hexoside rhamnoside (compound 13) have been also identified with [A-H] 331. Acetylated hexoside derivatives (compound 34), has been also identified with [M-H] at m/z 535 and base peak 331 [M-H-204] and characteristic fragment at m/z 373 [M-H-162]. Centaurein (compound 37), was tentatively identified with [M-H] at m/z 521 and characteristic fragments at m/z 359 [M-H-162] indicating its aglycone jaceidin. Patuletin and Centaurein were previously identified in *Centaurea* species (Akkal et al., 2003; Flamini et al., 2001).

Acetylated derivatives have been reported before as, quercetin 3-O-[(2,3,4-triacetyl-α-rhamnopyranosyl)]1-→6] 3-acetylated-β-galactopyranoside and quercetin 3-O-[(2,3,4-triacetyl-α-rhamnopyranosyl)]1-→6] 4-acetylated-β-galactopyranoside from *Gentiana spicata*, a descendant from *Centaurea* family (Handoussa et al., 2009).

Cytotoxic activity

When the IC50 value in carcinoma cells, after incubation between 48 and 72 hrs, is under 20 μg/mL, the cytotoxic activity was commonly considered (Boik, 2001). *C. aegyptiaca* CME demonstrated significant activity against colorectal and hepatocellular carcinoma. This promising cytotoxic action was related to the increased number of OH groups and presence of 3'- OMe as reported by Jeong et al. (2007). Cytotoxic activity was previously reported in various *Centaurea* species because of phenolic, flavonoid and sesquiterpene contents (Erol-Dayi et al., 2011, Medjroubi et al., 2005, Shoenb et al., 2007, Saroglou et al., 2005 and Ahmed et al, 2014).

Conclusion

*Centaurea aegyptiaca* is a rich source of phenolic and flavonoid glycosides contributing to a powerful cytotoxic activity that may represent a good candidate for further studies and reflects its traditional uses.

Conflict of interest

The authors announce that there are no conflicts of interest.

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