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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 IC_{50} 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 (Koukoulitsa 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; Kargıoglu 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.

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Cytotoxic activity

The viability of control and treated cells were explored at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University. The CME was tested against the following human tumor cell lines: hepatocellular carcinoma (HepG-2), breast adenocarcinoma (MCF-7), colorectal carcinoma (HCT-116), cervix adenocarcinoma (HELA) utilizing MTT (3-(4, 5-dimethylthiazolyl-2)- 2, 5-diphenyltetrazolium bromide) test in triplicate (Fotakis and Timbrell, 2006). Untreated cells represented the negative control while doxorubicin was the positive control. The absorbance was measured at 570 nm using a microplate ELISA reader (Sun Rise TECAN, Inc, USA). The absorbance of untreated cells was considered as 100%. The results were determined by three independent experiments (Wilson, 2000).

The percentage cell viability was calculated with the Microsoft Excel®. Percentage cell viability was calculated as follows: % Cell viability = (Mean Abs control – Mean Abs test metabolite X 100)/ Mean Abs control
Where: Abs: absorbance at 570 nm

The graphic plots were used for estimation of the 50% inhibitory concentration (IC_{50}). STATA statistical analysis package was used for the dose response curve drawing in order to figure IC_{50}.

Antiviral activity

Cytopathic effect inhibition assay was performed following Hu and Hsiung, (1989). The cultures were treated with two fold serial dilutions of *C. aegyptiaca* extract. Six wells were utilized for every concentration of *C. aegyptiaca* extract. Untreated vero cells in absence of CME represented the control. Inverted microscope was used for watching the virus in the control wells every 24hr until complete viral-induced cytopathic effects (CPE). Antiviral activity was evaluated by the inhibition of cytopathic effect in comparison with control where scoring of the protection level offered by *Centaurea* extract was estimated (Vijayan et al., 2004). The experiment was performed thrice. Acyclovir, the treatment clinically used for herpetic viral disease, was utilized as a positive control (Dargan, 1998).

LC-HRESI-MS-MS apparatus

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 × 4mm 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 compound	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 derv	
4	8.54	315.07	153.07 (-162) 165.14 135.19 109.09 225.2 (-90) 195.05 (-120)	Protocatechuic acid hexoside	Chen et al, 2012 Vallverdu-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	Protocatechiuc acid pentoside	
8	14.91	447.12	315.21 411.22 271.25 152.18 163.10 179.15	Protocatechiuc acid pentoside hexoside	
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 5caffeoylquinic acid isomer	Jaiswal et al 2010
13	21.54 21.69	447.11	152.12 401.19 285.26 241.24 163.16 177.2 401.26 285.18 152.15 163.16	Protocatechuic acid hexoside pentoside	
14	21.96	193.05	134.11 149.07	Ferulic acid	Vallverdu-Queralt et al., 2015
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	325.09	163.05 119.09 101.08 113.18	Coumaroyl hexoside	Vallverdu-Queralt et al., 2015
20	59.68	543.21	349.21 367.20 193.22 173.13	Diferuloylquinic acid isomer	
21	60.69	473.11	311.27 341.20	Dicaffeoyltartaric acids (chicoric acids)	

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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	473.26 [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	Pajero et al., 2004
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	Lin and Harnly, 2010
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-176]	Kaempferol hexoside glucuronide	
9	44.55	463.09	301.14	465.10	303.14	Quercetin -*O*-hexoside	Vallverdu-Queralt et al., 2015.
10	45.46	301.18	283.13 271.16 255.17 227.10 179.07 8165.10 151.18	303.12	285.14 273.13 179.14 169.06 137.02	Quercetin	Vallverdu-Queralt et al., 2015.
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 rt48.70	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)	Irahim et al., 2015.
13	49.05	639.16	331.20 [M-H-162-146] 316.20		-	Patuletin hexoside rhamnoside	Parejo et al., 2004
14	49.08	493.10	331.16 [M-H-162]			Patuletin hexoside	Parejo et al., 2004
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	Parejo et al., 2004
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	Stintzing et al., 2004 Ibrahim et al., 2015
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	

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					431.15 413.26		
18	49.73	285.19	267.11 253.27 239.12 185.13 137.06	287.13 RT59	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			Quercetin glucuronide	
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] 383.21 353.16	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 gucuronide	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	

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			301.21 [M-H-176-162] 219.17				
37	64.24	521.33	359.45 [M-H-162] 503.39 [M-H-18] 489.26 [M-H-32] 475.22 341.44 315.21	523.35	343.13 [M+H-180] 361.13 [M+H-162] 505.21 [M+H-18] 318.14 325.13 307.23	Centaurein (jaceidin hexoside)	Flamini et al., 2001
38	68.03	299.06	284.06 [M-H-15]	301.07	286.09 [M+H-15] 147.11 129.12	Hispidulin	Flamini et al., 2001
39	69.35	507.35	345.41 [M-H-162] 331.10 299.35 161.19 417.41	509.13	347.17 [M+H-162] 303.12 449.17 [M+H-60]	Axillarin hexoside	Parejo et al., 2004
40	74.61	609.41	315.27 [M-H-162-132] 293.34 246.23 225.09 447.39 [M-H-162]		--	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.

Table 3: Cytotoxic activity of *Centaurea aegyptiaca* extract

Cell line	HepG-2		MCF-7		HCT-116		HELA	
	CME	Dox	*CME*	Dox	*CME*	Dox	*CME*	Dox
IC_{50} (µg/mL)	12.1 ± 3.89	0.467 ± 0.2	30.9 ± 2.62	0.426 ± 0.35	11.7 ± 1.44	0.23 ± 0.17	19.5± 2.73	0.456± 0.35

(CME) and Doxorubicin standard (Dox) against hepatocellular carcinoma cells (Hep-G2), Human breast adenocarcinoma cells (MCF-7), colon carcinoma cells (HCT-116) and cervical cell carcinoma (HELA)

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 revealed the presence of compounds with a hexose (galactose or glucose), pentose (arabinose or xylose), deoxyhexose (rhamnose) and glucuronic acid unit, respectively (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 dicaffeoyl 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 furfuracea* (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 8*C*-glycoside and 6*C*-glycoside, while compounds **24** and **37** were identified as hispidulin hexoside, and hispidulin, respectively.

Methoxylated flavonol represented the majority of identified flavonols 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 dihexoside rhamnoside. Quercetagenin (compound **4**), with its derivatives quercetagenin *O*-hexoside, rutinoside, and dihexoside 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 hexoside 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 hexoside glucuronide with the characteristic loss of 176 in the positive and negative mode. Kaempferol hexoside 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 hexoside 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 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-acetyl-β-galactopyranoside and quercetin 3-*O*-[(2,3,4-triacetyl-α-rhamnopyranosyl)1"→6"]-4-acetyl-β-galactopyranoside from *Gentiana spicata*, a descendant from *Centaurea* family (Handoussa et al., 2009).

Cytotoxic activity

When the IC_{50} 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, Shoeb 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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