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Original Article

Profile of bioactive compounds of *Capparis spinosa* var. *aegyptiaca* growing in Egypt

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ABSTRACT

The present study was designed to investigate polyphenolic and sulphur contents of the aerial parts of *Capparis spinosa* var. *aegyptia* (Lam.) Boiss., Capparaceae, wildly growing in Egypt. The chemical compositions of the water distilled essential oil were investigated by GC/MS analysis where the major constituent of the oil was methyl isothiocyanate (24.66%). Hydroethanolic extract was evaluated by LC-HRESI-MS–MS in both positive and negative modes. Forty-two compounds were identified including quercetin, kaempferol and isorhamnetin derivatives in addition to myricetin, eriodictyol, cirsimaritin and gallocatechin derivatives. Quercetin tetrahexoside dirhamnoside as well as kaempferol dihexoside dirhamnoside have not been identified before in genus *Capparis*. Phenolic acids, such as quinic acid, *p*-coumaroyl quinic acid and chlorogenic acid were also identified. Evaluation of cytotoxic activity of hydroethanolic extract against three human cancer cell lines (MCF-7; breast adenocarcinoma cells, Hep-G2; hepatocellular carcinoma cells and HCT-116; colon carcinoma) using 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay showed significant effect with IC_{50} values 24.5, 24.4 and 11 μg/ml, compared to Doxorubicin as a standard cytotoxic drug. *C. spinosa* revealed itself as a promising candidate for nutraceutical researches.

Introduction

Capparaceae is a closely related family to the mustard family (Cruciferae) with abundance of glucosinolates and flavonoids (Täckholm, 1974; Inocencio et al., 2000; Kiddle et al., 2001). The genus *Capparis* is represented in Egypt by six species (Täckholm, 1974). *Capparis spinosa* var. *aegyptia* (Lam.) Boiss, (the caper) growing in the Egyptian deserts, is a perennial winter-deciduous plant that bears rounded, fleshy, alternative leaves and thick, shiny, large white to pinkish-white complete flowers. The plant is best known for the edible bud and fruit (caper berry). In Greco-Arab and Islamic medicine, the decoction of root bark is prescribed as deobstruent to liver and spleen, as anthelmintic and anti-inflammatory agents. Decoctions from the root bark have been used in traditional medicines for dropsy, anemia, arthritis, and gout. The stem bark is diuretic (Saad and Said, 2011). The strong flavor of capers is usually due to the very pungent methyl isothiocyanate that is released after an enzymatic reaction with a mustard oil glycoside named glucocapparin (methyl glucosinolate) (Brevard et al., 1992; Romeo et al., 2007; Sozzi et al., 2012).

C. spinosa is considered as a very important source of medicine for antifungal (Ali-Shtayeh and Abu Ghdeib, 1999) anti-inflammatory (Al-Said et al., 1988; Zhou et al., 2010), antidiabetic, antihyperlipidemic (Eddouks et al., 2005), antihypertensive (Baytop, 1984), antihepatotoxic (Gadgoli and Mishra, 1999), potential inhibitor of NF-kappa B (Zhou et al., 2011), and anticarcinogenic (Kulisic-Bilusic et al., 2012).

Quantitation of flavonoid content in Capers revealed it as a very rich source of the flavonols (Inocencio et al., 2000). *C. spinosa* has been an interesting field of study. Estimation of phenolic compounds in the Croatian species revealed the presence of isorhamnetin-3-*O*-rutinoside besides chlorogenic acid derivatives and cinnamoyl-quinic acid derivatives (Siracusa et al., 2011). While in China, flavonoids identified in the fruits were isoginkgetin, and ginkgetin and Sakuranetin (Zhao et al., 2013). Egyptian species have been investigated a long time ago. Six glucosinolates were identified, such as glucoiberin, glucocapparin, sinigrin, glucocleomin, glucobrassicin and glucocapangulin. Also, four flavonoids were isolated from *C. cartilaginea* and *C. deserti* and identified as kaempferol-3-*O*-rutinoside, quercetin-3-*O*-rutinoside, quercetin-7-*O*-rutinoside and quercetin-3-*O*-glucoside-7-*O*-rhamnoside (Ahmed et al., 1972).

Besides the previously identified flavonoids, Quercetin-3-*O*-glucose-7-*O*-rhamnoside, quercetin 3-*O*-glucoside and quercetin

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3-*O*-[6‴-α-L-rhamnosyl-6″-β-D-glucosyl]-β-D-glucoside have been identified (Sharaf et al., 1997, 2000).

Studies concerning the sulphur content of *C. spinosa*, revealed the presence of butyl isothiocyanate, methyl isothiocyanate, isopropyl isothiocyanate, and *sec*-butyl isothiocyanate (Afsharypuor and Jazy, 1999; Hamed et al., 2007). Nowadays, *C. spinosa* is also commercially cultivated in several countries for its fruits (Gull et al., 2015).

In the present study, the phenolic composition of the hydroethanolic extract (HEE) was characterized using LC-HRESI-MS–MS (liquid chromatography-high resolution electrospray ionization/mass spectrometry) and X calibur software. While the essential oil was described using GC/MS (Gas chromatography/mass spectrometry). In addition, cytotoxic activity of the HEE was evaluated against different cancer cell lines.

Material and methods

Chemicals

Reagents for HPLC analysis: acetic acid and methanol were of HPLC grade and purchased from Sigma–Aldrich (Steinheim, Germany).

Plant material

Fresh plant material (*Capparis spinosa* var. *aegyptia* (Lam.) Boiss., Capparaceae) was collected from Dahab, South Sinai, Egypt. The plant was identified by Ass. Prof. Dr. Mona Marzouk, Department of Phytochemistry and Plant Systematics, National Research Center, Egypt and a voucher specimen of the aerial parts were kept at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (no. RS 014). The plant samples were air dried in the absence of direct sunlight and ground just before extraction.

Extraction of glucosinolates

Powdered air-dried aerial parts (100 g) were subjected to hydrodistillation for 3 h using Clevenger apparatus. A yellow volatile oil was collected 0.1 ml. The sample oil was collected and freezed till GC/MS analysis (Afsharypuor and Jazy, 1999).

GC–MS analysis of oil

GC–MS analysis of the volatile oil was performed using Hewlett-Packard (HP) 6890 series (Agilent) Gas Chromatography System, interfaced to HP 5973 series (Agilent) mass spectrometer, equipped with an auto-sampler and a single capillary injector. TR-FAME (Thermo 260 M142P) (70% cyanopropyl-polysilphenylene siloxane) capillary GC column (30 m × 0.25 mm, i.d., ×0.25 μm film thickness) was used. Sample size was 1 μl, oven temperature programmed from 50 to 230 °C at 5 °C/min, injector port temperature 200 °C, Carrier gas Helium, Flow rate was 1.5 ml He/min. Identification of the volatile oil constituents was based on comparing their retention times, and mass fragmentation patterns with those of the available references and/or with published data (Adams, 2004) as well as through NIST-MS database library search. The quantitative estimation was carried out by relative peak area measurement.

Preparation of the extract for LC-HRESI-MS–MS

Plant material (100 g) was exhaustively extracted with 80% ethanol. The combined hydroethanolic extracts (HEE) were filtered, concentrated in vacuum at 50 °C, dried and left for HPLC-MS–MS analysis and cytotoxicity evaluation.

LC-HRESI-MS–MS apparatus

The analysis 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 autosampler, binary pump, and PDA detector G 1314 C (SL). Chromatographic separation was performed on a Superspher 100 RP-18 (75 × 4 mm i.d.; 4 μm) column (Merck, Darmstadt, Germany).

Identification of phenolic compounds

The method was performed according to Hassaan et al. (2014). Mobile phase consisted of two solvents, (A) 2% acetic acid (pH 2.6) and (B) 80% methanol. The separation was performed using gradient elution, from 5% to 50% B at 30 °C at a flow rate of 100 μl/min. The ionization technique was an ion spray (pneumatically assisted electrospray). Spectra were recorded in positive and negative ion mode between *m*/*z* 120 and 1500 with capillary voltage, 4000 V 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 measurements, the voltage over the collision cell varied from 20 to 70 eV. Argon was used as collision gas. Data analysis software was used for data interpretation. Sodium formate was used for calibration at the end of LC–MS run. Interpretation for ESI-MS was performed by Xcalibur 2.1 software from Thermo Scientific (Berlin, Germany).

Cytotoxic activity

The cytotoxicity of HEE was assessed using MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay (Fotakis and Timbrell, 2006) against three human cancer cell lines; breast (MCF-7), liver (HEPG-2) and colon (HCT-116) adenocarcinoma using Doxorubicin® as reference standard. Dose dependent activities were studied from 5 to 50 μg/ml, and the IC_{50} values (concentration which reduced survival to 50%) were estimated from graphic plot. Three separate experiments were performed for each sample.

Results and discussion

GC–MS analysis of the volatile oil

The oil of the dried aerial parts of *C. spinosa* was obtained by water distillation with yields 0.1% w/v. The oil was dark yellow colored showing a strong aromatic odor. GC–MS analysis revealed the identification of twenty-six components (Table 1) amounting for (95.46%) of the oil. The sulfated compounds were present in a relatively high percentage (40.3%), which are responsible for the aroma of *C. spinosa* volatile oil. Methyl isothiocyanate was the major constituent representing 24.66%. Components of the oil, their relative retention times and area percentages were compiled in Table 1. The results of our analyses were in agreement and consistent with those reported previously by Kulisic-Bilusic et al. (2012), where methyl isothocyanate was the major component of the volatile oil of *C. spinosa* collected from central Dalmatia. Phenyl propanoid, terpenoids, isothiocyanate, and n-alkalenes were revealed also as part of the *C. spinosa* oil (Ahmed et al., 1972).

Table 1
GC–MS analysis of the volatile oil constituents of the aerial parts of *C. spinosa*.

Peak no.	Identified compound	RRt[a]	Area%
1	Isopropyl isothiocyanate	0.72	12.44
2	*p*-Cymene	0.84	2.93
3	Methyl isothiocyanate	1	24.66
4	Butyl isothiocyanate	1.17	3.2
5	α-Copaene	1.49	4.57
6	Thujone	1.71	1.81
7	Caryophyllene	1.98	2.91
8	Camphor	2.11	1.66
9	Humulene	2.23	4.24
10	1,2,3,5,6,8a-Hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	2.27	5.56
11	γ-Muurolene	2.31	2.6
12	Unidentified	2.41	1.17
13	3-Cyclohexen-1-one	2.82	1.21
14	3-Methyl-4-isopropylphenol	3.67	3.98
15	Spathulenol	3.69	1.47
16	Muurolol	3.76	2.42
17	6,10-Dimethyl-2-undecanone	3.84	6.5
18	α-Cadinol	3.89	4.26
19	Unidentified	3.91	1.84
20	Unidentified	4.03	0.78
21	Tetracosane	4.22	0.55
22	Eicosane	4.46	1.3
23	Unidentified	4.54	0.75
24	Heptacosane	4.92	2.58
25	1-Naphthalenepropanol	4.99	0.58
26	2-Cyclohexen-1-ol	5.01	0.56
27	1,2-Benzenedicarboxylic acid	5.07	0.91
28	Isobutyl 2-methylpent-3-yl ester	5.35	1.07
29	Phenanthrene	5.45	1.02
30	Dibutyl phthalate	6.24	0.46
Percentage of identified constituents			95.46%
Percentage of unidentified constituents			4.54%
Major constituent			Methyl isothiocyanate

[a] RRt, relative retention time to: methyl isothiocyanate = 6.99 min.

LC-HRESI-MS–MS analysis of phenolic compounds

LC–MS analysis demonstrated the presence of highly glycosylated flavonol in addition to flavone glycosides and phenolic acid derivatives as previously reported in different caper extract (Inocencio et al., 2000). In total, 42 compounds belonging to different classes were identified by LC–MS/MS-HR-ESI in the HEE of the aerial parts of *C. spinosa*. Data concerning the identification of the peaks are shown in Table 2, in which we report the retention time, electrospray ionization mass spectrometry in negative and positive ion mode for all of the compounds detected. The structures of unknown phenolic acids or flavonoids were assessed based on the *m*/*z* of both precursor ion and fragment ion obtained. Moreover, the spectral data were compared with that reported in the literature.

Flavonoids were the major identified components with predominance of flavonol class especially quercetin derivatives (fifteen compounds). New compounds were identified such as quercetin tetrahexoside and quercetin tetrahexoside dirhamnoside. Kaempferol derivatives have been also identified (seven compounds), isorhamnetin derivatives (six compounds) in addition to myricetin, eriodictyol, cirsimaritin and gallocatechin derivatives. Sugar moieties consist of hexosides, deoxyhexosides and pentosides as deduced from the loss of 162, 146 and 132 u respectively (Ibrahim et al., 2015).

Quercetin (compound 26) was tentatively identified with its base peak at *m*/*z* 271.15 and characteristic peaks at *m*/*z* 255, 179 and 151 (Martucci et al., 2014), Quercetin tetrahexoside (compound 7), tentatively identified for the first time in this genus, showed a precursor ion at *m*/*z* 949.24 [M−H] and the MS/MS led to a product ion at *m*/*z* 301.16 denoting a quercetin derivative. In the positive ionization, precursor ion peak appeared at *m*/*z* 951.26 with product ions at 789.24 (M+H-hexose), 627.34 (M+H-dihexose), 465.23 (M+H-trihexose) and 303.19 (M+H-tetrahexose) and denoting the quercetin.

Compound 11, was tentatively identified as quercetin trihexoside rhamnoside with a precursor ion peak at 933.25 [M−H] and product ion peak at *m*/*z* 609.28 [M−H-dihexose]. Positive ionization showed a precursor ion at [M+H] at *m*/*z* 935.26 with product ions at 789.1 [M+H-rhamnose], 627.09 [M+H-rhamnose-hexose], 465.15 [M+H-rhamnose-dihexose] and 303.13 [M+H-rhamnose-trihexose]. Compound 20, tentatively identified as quercetin dirhamnoside hexoside showed a precursor ion peak at *m*/*z* 755.2 [M−H] with product ions at *m*/*z* 593.3 [M−H-hexose], 447.1 [M−H-hexose-rhamnose], 301.2 [M−H-hexose-dirhamnose] and a characteristic peak for quercetin at 271.21. Positive ionization showed similar fragmentation with [M+H] at *m*/*z* 757.22.

Compound 34, with a precursor ion peak [M−H] at *m*/*z* 1095.25 and [M+H] at *m*/*z* 1097.32 showed product ion peaks at *m*/*z* 936.39 [M+H-hexose], 773.31 [M+H-dihexose], 627.19 [M+H-dihexose-rhamnose], 465.11 [M+H-trihexose-rhamnose], 303.07 [M+H-tetrahexose-rhamnose] was tentatively identified as quercetin tetrahexoside rhamnoside. Compound 40 tentatively identified as quercetin dihexoside dirhamnoside, showed a precursor ion peak [M−H] at *m*/*z* 917.23 and positive ionization [M+H] at *m*/*z* 919.27 with product ion peak at 611.14 [M+H-hexose-rhamnose], 465.15 [M+H-hexose-dirhamnoside] and 303.19 [M+H-dihexose-dirhamnoside].

Kaempferol is the second major identified flavonoid (compound 15) with [M−H] at *m*/*z* 285.04 and differentiated from luteolin by the presence of characteristic peaks at *m*/*z* 213.13 (Cuyckens and Claeys, 2004). Several kaempferol derivatives were identified, however, compound 36, tentatively identified as kaempferol rutinoside hexoside was not identified before in *C. spinosa* and showed [M-H] at *m*/*z* 755.2 and positive ionization [M+H] at *m*/*z* 757.22

Table 2
Peak assignment of metabolites in hydroethanolic extract of *C. spinosa* using LC-HRESI-MS–MS in the negative and positive modes.

No	Rt (min)	Compounds	$[M-H]^-$ *m/z*	Negative ionization MS/MS	$[M+H]^+$ *m/z*	Positive ionization	References
1	1.71	Gluconic acid	195.05	195.05 129.07 177.1 159.07 99.07	197.08	197.08 179.09 135.10	Felipe et al. (2014)
2	1.89	Quinic acid	191.02	191.02 111.08 173.04	193.05	193.05 175.01 161.11 147.04 133.03	
3	22.23	Chlorogenic acid	353.09	353.09 191.11 269.21 293.2 179.19	355.12	355.10 337.19 193.06 201.04 267.01	Martucci et al. (2014)
4	30.34	Kaempferol-*O*-glucoside	447.09	447.09 285.14 (M−H-hex) 325.21 327.21	449.11	449.11 287.11	Rodrigo et al. (1992)
5	45.23	Quercetin-*O*-glucoside	463.09	463.09 301.19 (M−H-hex) 343.16 (M−H-120)	465.10	465.10 303.10 287.13	Martucci et al. (2014)
6	33.31	*p*-Coumaroyl quinic acid	337.06	337.06 191.16 179.15 135.22 293.2	339.10	339.10 320.22 293.14 279.11 206.18 176.16 130.1	Martucci et al. (2014)
7	33.81	Quercetin tetrahexoside	949.24	949.24 625.30 (M−H-dihexose) 301.16 (M−H-tetrahexose) 787.40 (M−H-hexose) 463.25 (M−H-trihexose)	951.26	951.26 931.43 789.24 (−162) 627.34 (−162) 465.23 (−162) 303.19 (−162)	
8	34.97	Quercetin trihexoside	787.3	787.3 621.29 625.39 (M−H-hexose) 463.31 (M−H-dihexose) 301.24 (M−H-trihexose)	789.23	789.23 303.14 627.23 (M+H-hex) 465.17 (M+H-dihex)	Qu et al. (2013)
9	35.43	Epigallocatechin hexoside	467.12	467.12 305.24	469.17	469.17 307.02 435.91 169.07 144.05 289.24	
10	35.81	Quercetin rhamnoside hexoside	609.15	609.15 301.21 (M−H-Rh-hex) 447.28 (M−H-hex) 549.28 271.21 255.09 179.1	611.16	611.16 303.12 (M+H-rh-hex) 465.06 (M+H-rh)	Falé et al. (2013)
11	36.20	Quercetin trihexoside rhamnoside	933.25	933.25 609.28 (M−H-dihex) 301.17 (609-rh-hex)	935.26	935.26 789.1 (M+H-rh) 627.09 (M+H-rh-hex) 465.16 (M+H-rh-dihex) 303.13 (M+H-rh-trihex)	
12	36.73	Acacetin hexoside	445.21	445.21 385.27 283.19	447.23	447.23 225.04 386.94 429.25 207.15 189.23	Lin and Harnly (2010)
13	54.70	Kaempferol	285.04	285.04 255.11 241.22 229.18 213.13 163.2 151.13 96.97	287.08	287.08 241.11 269.19 213.07 164.99 152.99 133.05 121.05	Martucci et al. (2014)
14	39.17	Eriodictyol hexoside	449.11	449.11 287.15 (M−H-hex) 387.28 281.23 151.09 137.15	451.12	451.12 289.15 (M+H-hex) 433.22 268.08	Zhao et al. (2013)
15	39.38	Isorhamnerin hexoside rutinoside	785.11	785.11 623.28 (−M−H-hex) 315.12 (M−H-hex-rh-hex)	787.23	787.23 317.21 (M+H-rh-dihex) 641.13 (M+H-rh) 479.15 (M+H-rh-hex)	Kim and Park (2009)
16	40.23	Eriodictyol-7-*O*-rutinoside	595.17	595.17 287.11 (M−H-rutinose)	597.18	597.18 435.08 (M+H-hex) 289.14 (M+H-hex-rh) 577.26 451.07 399.2	Zhao et al. (2013)
17	40.27	Eriodictyol	287.06	287.06 151.08 125.18 135.07	289.07	289.07 163 271.13 152.98 179.03 143.96	Zhao et al. (2013)
18	41.5	Eriodictyol glucuronide	463.09	463.09 287.18 301.17 403.23 311.26 175.09 151.09			Li et al. (2012)
19	43.21	Quercetin glucoside-*O*-rutinoside	771.2	771.2 609.15 (-hex) 463.21 (-rh) 301.26 (-rh)	773.21	773.21 465.18 611.10 303.14 627.15	Sharaf et al. (2000)
20	43.91	Quercetin dirhamnoside hexoside	755.2	755.2 593.3 (-hex) 447.1 (-rh) 301.2 (-rh) 271.21	757.22	757.22 624.16 595.37 611.16 449.24 303.14 287.21	
21	44.62	Myricetin rutinoside	625.14	625.14 316.1 317.16 (M−H-rh-hex) 285.12 271.20 479.20 (M−H-rh) 607.26 179.08	62.16	627.16 319.12 (M+H-rh-hex) 464.22 481.11 (M+H-rh)	Sójka et al. (2009)
22	44.82	Cirsimaritin hexoside rhamnoside	621.15	621.15 313.15 (M−H-rh-hex) 285.2 257.3	623.16	623.16 477.09 (M+H-rh) 315.11 (M+H-162)	

Table 2 (*Continued*)

No	Rt (min)	Compounds	$[M-H]^-$ m/z	Negative ionization MS/MS	$[M+H]^+$ m/z	Positive ionization	References
23	59.85	Isorhamnetin hexoside	477.27	477.27 315.34 (M−H-hex)	479.12	479.12 317.09 (M+H-hex) 301.15 360.86 419.93 459.27	
24	45.53	Isorhamnetin dihexoside dirhamnoside	931.27	931.27 767.38 (M−H-hex) 621.35 (M−H-hex-rh) 315.19 300.08 357.35 785.40	933.29	933.29 317.21 (M+H-dirh-dihex) 914.45 771.29 (M+H-hex) 769.29 625.22 (M+H-hex-rh) 463.2 (M+H-rh-dihex)	
25	46.75	Gallocatechin	305.07	305.07 290.23 97.01 225.13 275.22 259.21	307.11	307.11 130.06 289.18 262.15 242.16 204 307.13 265.16 289.20 247.17 178.12 144.11	Hossain et al. (2010)
26	47.64	Quercetin	301.04	301.04 271.15 255.15 257.22 179.15 151.1 229.93	303.04	303.04 285.05 302.85	Martucci et al. (2014)
27	47.9	Quercetin-3-*O*-hexose-*O*-pentoside	595.13	595.13 301.16 (M−H-hex-pen) 285.14 271.22 433.43 (M−H-hex) 463.27 191.16 179.13	597.15	597.15 303.12 (M+H-pen-hex) 287.12 465.10 (M+H-pen) 449.11	Martucci et al. (2014)
28	48.19	Kaempferol hexoside dirhamnoside	739.21	739.21 575.3 649.43 429.29 284.18 255.1	741.22	741.22 595.05 (M+H-rh) 449.07 (M+H-dirh) 287.13 (M+H-dirh-hex)	Rodrigo et al. (1992)
29	48.50	Apigenin 8C-glucoside	431.1	431.1 311.22 (M−H-120) 341.18 (M−H-90) 413.21 312.18	433.11	433.11 415.23 313.16 (M+H-120) 397.23 367.18 271.16 (M+H-hex)	Abad-García et al. (2008)
30	49.05	Kaempferol hexoside rhamnoside	593.15	593.15 285.21 (M−H-308) 473.24 (M−H-120) 503.24 (M−H-90)	595.16	595.16 287.05 (M+H-rh-hex) 449.11 (M+H-rh) 576.38 431.24	Ferreres et al. (2011)
31	49.36	Isorhamnetin 3-O rutinoside	623.16	623.16 315.22 (-rutinose) 314.15 459.2 460.38 503.14 (−120)	625.18	625.18 317.12 (M+H-rh-hex) 479.02 (M+H-rh)	Roriz et al. (2014)
32	50.00	Quercetin dihexoside	625.14	625.14 301.2 (M−H-dihex) 463.14 (M−H-hex) 285.26 271.2	627.16	627.16 465.11 (M+H-hex) 303.10 (M+H-dihex)	Tedesco et al. (2015)
33	51.22	Kaempferol glucuronide	461.07	461.07 285.18 381.27	463.09	463.09 343.14 427.21 299.11 268.23	Martucci et al. (2014)
34	51.90	Quercetin tetrahexoside rhamnoside	1095.29	1095.29 609.3 (rutin) 787.28 (M−H-hex-rh) 483.2 933.44 (M−H-hex) 302.33	1097.32	1097.32 627.19 (M+H-rh-dihex) 936.39 (M+H-hex) 773.31 (M+H-dihex) 465.11 (M+H-trihex-rh) 303.07 (M+H-tetrahex-rh) 449.19 (Q-rh) 611.09 (rutin) 787.39	
35	54.13	Isorhamnetin	315.1	315.1 271.2 285.18 287.11 300.11	317.07	317.07 302.09 285.1 271.13 257.12 165.07 139.02	Sánchez-Rabaneda et al. (2004)
36	54.24	Kaempferol rutinoside hexoside	755.2	755.2 285.15 (M−H-hex-rh) 593.32 (M−H-hex)	757.22	757.22 449.12 (M+H-hex-rh) 595.15 (M+H-hex) 611.10 (M+H-rh) 287.09 (M+H-trihex-rh)	
37	54.49	Myricetin hexoside rutinoside	787.17	787.17 625.24 (M−H-hex) 479.23 (M−H-hex-rh) 317.21 (M−H-dihex-rh)	789.19	789.19 481.14 (M+H-hex-rh) 319.2 (M+H-dihex-rh)	
38	55.18	Isorhamnetin glucuronide	491.08	491.08 315.20	493.1	493.1 317.16	Dueñas et al. (2008)
39	55.47	Quercetin acetyl hexoside	505.13	505.13 353.29 459.29 485.31 323.24 301.20 151.03 179.12 191.09			Navarro-González et al. (2015)
40	58.05	Quercetin dihexoside dirhamnoside	917.23	917.23 609.31 (M−H-308) 301.16 (M-H-308)	919.27	919.27 611.14 (M+H-308) 465.15 (M+H-rh) 449.16 303.19 (M+H-hex)	

Table 2 (*Continued*)

No	Rt (min)	Compounds	$[M-H]^-$ *m/z*	Negative ionization MS/MS	$[M+H]^+$ *m/z*	Positive ionization	References
41	60.68	Myricetin hexoside	479.16	479.16 317.18 (M−H-hex) 273.18 258.29 447.31 461.34 389.12 (M−H-90) 359.28 (M−H-120)	481.19	481.19 319.17 (M+H-hex) 464.25 302.14 285.21 191.14	Raal et al. (2015)
42	62.14	Kaempferol dihexoside dirhamnoside	901.24	901.24 593.23 (M−H-rh-hex) 285.2 (M−H-dirh-dihex) 739.44 (M−H-hex)	903.27	903.27 884.47 (M+H-H_2O) 287.16 (M+H-dirh-hex) 757.16 (M+H-hex) 595.23 (M+H-rh-hex) 449.08 (M+H-dirh-hex)	

rh, rhamnose; hex, hexose; Q, quercetin; pen, pentose.
Fragment ions are listed in order of relative abundances. Ions in boldface indicate the most intense product ion (100% relative intensity).

Table 3
Cytotoxic activity of the HEE of the aerial parts of *Capparis spinosa* and Doxorubicin standard against human breast adenocarcinoma cells (MCF-7), hepatocellular carcinoma cells (Hep-G2) and colon carcinoma cells (HCT-116).

Cell line	MCF-7		Hep-G2		HCT-116	
	C. spinosa HEE	Doxorubicin	*C. spinosa* HEE	Doxorubicin	*C. spinosa* HEE	Doxorubicin
IC_{50} (μg/ml)	24.5 ± 2.23	0.426 ± 0.35	24.4 ± 1.87	0.467 ± 0.2	11 ± 1.54	0.23 ± 0.17

Data are presented as mean ± SD of IC_{50} (μg/ml) from 3 independent experiments, triplicate for each.

with product ion peaks at 611.10 [M+H-rhamnose], 449.12 [M+H-rhamnose-hexose] and 287.09 [M+H-rhamnose-dihexose].

Compound 42, identified as kaempferol dihexoside dirhamnoside, showed a precursor ion peak [M-H] at *m/z* 901.24 and positive ionization [M+H] at *m/z* 903.27 with product ions at *m/z* 757.16 [M+H-rhamnose], 595.23 [M+H-rhamnose-hexose], 449.08 [M+H-dirhamnose-hexose] and 287.16 [M+H-dirhamnose-dihexose].

Isorhamnetin has been previously identified in *Capparis*, however, its derivatives have not. Compound 15, with a precursor ion [M-H] at *m/z* 785.11 and [M+H] at *m/z* 787.23 and daughter ion peak at 641.13 [M+H-rhamnose], 479.15 [M+H-rhamnose-hexose] and base peak at *m/z* 317.21 [M+H-rhamnose-dihexose] was identified as Isorhamnetin hexoside rutinoside. Compound 23 was tentatively identified as isorhamnetin hexoside with [M−H] at *m/z* 477.27 while compound 24, tentatively identified as isorhamnetin dihexoside dirhamnoside with a precursor ion peak [M−H] at *m/z* 931.27 and [M+H] at *m/z* 933.29, product ions in the positive ionization at 771.29 [M+H-hexose], 625.22 [M+H-hexose-rhamnose], 463.2 [M+H-dihexose-rhamnose] and 317.21 [M+H-dihexose-dirhamnose].

These results were in agreement with Gull et al. (2015) and Behnaz et al. (2013) who reported *C. spinosa* leaves to be a good source of kaempferol, quercetin, rutin and isorhamnetin. While Siracusa et al. (2011) reported rutin, kaempferol, 3-*O*-rutinoside, and isorhamnetin 3-*O*-rutinoside as major flavonoids in *C. spinosa*.

Myricetin and its derivatives have not been previously identified in *Capparis*, however, it was tentatively identified with its characteristic peak at *m/z* 317 in the negative ionization and *m/z* 319 in the positive ionization.

Flavones were also present. Compound 29, was identified as apigenin 8-*C*-glucoside with precursor ion at *m/z* 433 [M+H] in the positive ionization mode. The product ions obtained with cleavage of sugar ring have been proposed as diagnostic ions, where *m/z* 313 is observed and *m/z* 283 diagnostic of 6-*C*-glucoside is absent (Abad-García et al., 2008). *C*-glycosyl flavones produces MS fragmentation pattern including dehydration and cross ring cleavage of the glucose moiety that produces 0,2 cross ring cleavage [M−H-120] and 0,3 cross ring cleavage [M−H-90] (Martucci et al., 2014).

Phenolic acids were also identified as quinic acid, *p*-coumaroyl quinic acid and chlorogenic acid.

Cytotoxic activity of the HEE

HEE of *C. spinosa* showed promising cytotoxic activity. In the US National Cancer Institute Plant Screening Program, a crude extract is generally considered to have *in vitro* cytotoxic activity if the IC_{50} value in carcinoma cells, following incubation between 48 and 72 h, is less than 20 μg/ml (Boik, 2001). HCT-116 was found to be the most sensitive to HEE with cell viability less than 50% at the concentration of 11 ± 1.54 μg/ml. Investigation showed difference in sensitivity of different cancerous cells to the *Capparis* extract as has been observed with many other species (Fouche et al., 2008). Potency against different cancer cell lines was presented in Table 3. Flavonoids have been reported to exhibit prooxidant cytotoxicity against cancer cells through the ROS-triggered mitochondrial apoptotic pathway, therefore, responsible for the promising cytotoxicity of *C. spinosa* against various cancer cell lines (Zhang et al., 2015).

Conclusion

C. spinosa is a rich source, not only of sulphur compounds, but also with phenolic and flavonoid glycosides contributing to its powerful cytotoxic activity. *C. spinosa* is suggested to be a good candidate for use in natural medicine with historical background.

Author's contributions

RO suggested the point, wrote the manuscript, carried out extraction procedures and interpreted LCMS analysis. MH interpreted the GC/MS data of essential oil and cytotoxic activity of HEE and revised the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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