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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).