

Original Article

Chemical Investigation of Some *Capparis* Species Growing in Egypt and their Antioxidant Activity

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Capparis cartilaginea and *C. deserti* growing in Egypt were investigated for their glucosinolates and rutin content. From *Capparis cartilaginea* four isothiocyanates were isolated and identified using GC and EI/MS techniques. These compounds were butyl isothiocyanate (1), 6-methylsulphonylhexyl isothiocyanate (2), 7-methylsulphonylheptyl isothiocyanate (3) and 5-benzylsulphonyl-4-pentenyl isothiocyanate (4). In addition to compounds (1) and (2), two other compounds were isolated and identified from *Capparis deserti*. These compounds are 3-methylthiopropyl isothiocyanate (5) and [11-(2-butenylthio)6-undecenyl isothiocyanate] (6). Compounds (1), (2), (5) and (6) are reported in this study for the first time from *Capparis deserti*. The main flavonoid component in the studied species was isolated and identified as rutin by comparing the data with those reported. Also, quantitative evaluation of rutin in the two species was carried out by TLC-densitometric analysis. The antioxidant activity was done using diphenylpicrylhydrazyl (DPPH) radical scavenging method. The butanol fraction from *C. cartilaginea* and *C. deserti* showed the highest antioxidant properties.

Keywords: antioxidant activity – *Capparis cartilaginea* – *Capparis deserti* – glucosinolates – rutin – TLC-densitometry

Introduction

Family Capparaceae comprises 39 genera and 650 species distributed through warm regions (1). The genus *Capparis* comprises 250 species including shrubs, trees and woody climbers. This genus is represented in Egypt by six species (2). Dioscorides recommended the use of the leaves and roots of *Capparis spinosa* L. to reduce swelling and calm the pain of teeth (3).

In the Arabian folk medicine, several *Capparis* species have many uses. The total herb of *Capparis cartilaginea* Decne. is used for bruises, childbirth, earache, headache, paralysis, snakebite and swelling. Also the fruits of *Capparis decidua* (Forssk.) Edgew are used to relieve difficult breathing as a laxative, anthelmintic and for

nervous disorders. Furthermore, the leaves of *Capparis spinosa* are used for treating earache, coughs, expelling stomach worms and for diabetes (4) while the bark is used for the treatment of gout, rheumatism and as a laxative and expectorant for chest diseases. The bark is also used in liver affections. Infusion of stem and root bark is used for diarrhea and febrifuge. Also the flower buds and roots are used as renal disinfectants, diuretic, tonic and for arteriosclerosis and as compresses for the eyes (5).

In 1972, Ahmed *et al.* investigated some Egyptian *Capparis* species for the presence of glucosinolates. The authors isolated 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 (6).

In this study we report the isolation and identification of isothiocyanates from *C. cartilaginea* and *C. deserti*

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growing in Egypt as well as the evaluation of rutin content by TLC-densitometry analysis. Also, the antioxidant activity of different fractions of two species was studied (8).

Experimental

General

GC/MS was done using Finnigan SSQ 7000 under the following conditions: Column: DB5 capillary column, I.D. 0.25 mm; Ionization mode: EI (70-ev); Temperature program: 50–250 (4°C/min); Detector: FID; Sample volume: 2 µl; Mass range: 50–750 mass unit UV absorbances were measured using Shimadzu UV 2401 PC UV-recording spectrophotometer; TLC-densitometry analysis was carried out using CAMAG ATS III and TLC Scanner 3 with CATS 4 software. TLC was carried out on silica gel plates (Merck 60 F254, 20 × 20 cm). One percent solutions of standard rutin and extracts were prepared. The mobile phase was: EtOAc, AcOH, HCOOH, H₂O (10: 1: 1: 1). Wavelength scan at 366 nm.

Plant Material

C. cartilaginea Decne and *C. deserti* Zoh. were collected from Wadi Zaghra, Dahab, South Sinai, Egypt. The plants were identified by Prof. N. El-Hadidi, Prof. of Botany, Faculty of Science, Cairo University, Egypt. The plant samples were air-dried in a good air draft in the absence of direct sunlight and then ground.

Glucosinolates

The defatted plant material was air-dried and extracted with 80% ethanol. The combined alcoholic extracts were concentrated *in vacuo* at 50°C, dissolved in hot distilled water, filtered and left overnight in the refrigerator to remove any resinous material. Two-thirds of the clear aqueous filtered extract was successively partitioned with chloroform, ethylacetate and finally with butanol. Each extract was dried over anhydrous sodium sulfate (Merck, Germany) and evaporated *in vacuo* at 50°C.

The remaining third of the clear aqueous filtrate was diluted with distilled water and allowed to pass slowly through a column of acidic aluminum oxide (Anionotropic, 800 g, Sigma chemical Co., activity grade-I, type WA-I acidic). The column was washed with distilled water till the elute was colorless and then eluted with 2% aqueous potassium sulfate solution. The collected fractions were evaporated under reduced pressure at about 45°C till dryness. The aqueous residue was extracted with hot ethanol and filtered. The combined ethanol extract was concentrated *in vacuo* at 45°C and the residue was

dissolved in distilled water and lyophilized to give a brownish residue (total glucosinolate fraction).

Enzymatic Hydrolysis

About 1 g of the total glucosinolates of *C. cartilaginea* was dissolved in 10 ml double-distilled water. The solution was buffered with sodium citrate at pH 6. Five milligram of myrosinase (Sigma-Aldrich, Germany) were added and incubated at 28°C for 24 h. The enzymatic hydrolysis was blocked by saturating the solution with sodium chloride. The hydrolysate solution was exhaustively partitioned with diethyl ether. The combined ether extract was washed with distilled water, dehydrated over anhydrous sodium sulfate and evaporated *in vacuo* at 30°C. Semisolid yellow (isothiocyanates) residue was obtained and was subjected to GC/MS analysis. (9)

Flavonoids

Paper chromatographic investigation [Whatman No. 1, 15% acetic acid, *n*-butanol: acetic acid: water (4: 1: 5 and 3: 1: 1)] of ethyl acetate and butanol fractions of both *C. cartilaginea* and *C. deserti* revealed the presence of a main component which was visualized by spraying with 1% alcoholic AlCl₃ and NA reagent. Preparative PC (3 MM, 15% aqueous acetic acid) followed by Sephadex LH₂₀ column (90% methanol) was used for isolation and purification of the main flavonoidal compound.

Thin Layer Densitometric Assay

Sample Solutions

One percent of standard and samples stock solutions were prepared.

Method

A CAMAG automatic TLC Scanner with CATS evaluation software was used with the following settings: wavelength 520 nm, scanning speed 20 mm s⁻¹, multi level calibration via peak area by polynomial regression, silica gel 60 F254 (20 × 20 cm, Merck) plates were used. Samples were applied band wise using CAMAG automatic TLC sampler III under the following conditions: distance from lower edge = 10 mm, band length = 8 mm, track distance = 12, No. of applications = 14.

Determination of Scavenging Effect on DPPH Radicals (ref)

The decrease of the absorbance at 516 nm of the DPPH solution after addition of the sample (plant materials) was

measured in a glass cuvette. An aliquot of 0.1 ml M. methanol solution of DPPH was mixed with the methanolic solution of the sample, so that the relative concentration of plant materials versus the stable radical in the cuvette was 0.14, then the solution with tested sample was shaken vigorously. The absorbance was mentioned at the start and after 30 min. after being kept in the dark against a blank of methanol without DPPH. All tests were run in duplicate and averaged. The antioxidative of these samples were compared with Trolox where

$$\% \text{ RSA} = 100\%$$

$$\times \frac{\text{Abs of blank 516 nm} - \text{Abs of sample 516 nm}}{\text{Abs of blank}_{516 \text{ nm}}}$$

Results and Discussion

Isothiocyanates of *Capparis cartilaginea*

The GC spectrum of the obtained glucosinolate aglucones of *C. cartilaginea* revealed the presence of four different compounds; (1), (2), (3) and (4) with the following different percentages (65.03%), (29.86%), (0.066%) and (0.914%), respectively.

Identification of Compound (1)

The EI-mass spectrum of compound (1) showed a molecular ion peak at m/z 115 [M^+ , 11%] where other fragments at m/z 101 [$M^+ - CH_2$, 27%], 73 [$M^+ - (CH_2)_3$, 68%] and 58 [$M^+ - CH_3 - (CH_2)_3$, 32.7%] were found to be the same as that of molecular formula C_5H_9NS . The fragmentation pattern of compound (1) was found to be identical to that of butyl isothiocyanate [$CH_3(CH_2)_3N=C=S$] (7).

Identification of Compound (2)

The EI-mass spectrum of compound (2) showed a molecular ion peak at m/z 221 [M^+ , 4.5%] which corresponds to the molecular formula $C_8H_{15}NO_2S_2$. Other fragments at m/z 220 [$M^+ - 1, CH_2$, 32.7%], 206 [$M^+ - CH_3$, 14.5%], 205 [$M^+ - CH_4$, 100%], 142 [$M^+ - CH_3SO$, 3%] and 57 [$NSC - 1$, 15.5%] were detected. Thus, compound (2) can be identified as 6-methylsulfonylhexyl isothiocyanate [$CH_3SO_2(CH_2)_6 - N=C=S$] (Khaled, 2002).

Identification of Compound (3)

The EI-mass spectrum of compound (3) showed a molecular ion peak at m/z 235 [M^+ , 3.5%] which corresponds to the molecular formula $C_9H_{17}NO_2S_2$ in

addition to other fragments at m/z 220 [$M^+ - CH_2$, 5.5%], 205 [$M^+ - CH_3CH_2$, 20%], 92 [$M^+ - (CH_2)_6NCS$, 22.7%] and 58 [$M^+ - CH_3SO_2(CH_2)_7$, 36.4%]. From this fragmentation pattern compound (3) could be identified as 7-methylsulfonylheptyl isothiocyanate [$CH_3SO_2(CH_2)_7 - N=C=S$] (7).

Identification of Compound (4)

The EI-mass spectrum of compound (4) showed a molecular ion peak at m/z 281 [M^+ , 10.9%] which corresponds to the molecular formula $C_{13}H_{15}NO_2S_2$ in addition to other fragments at m/z 223 [$M^+ - NCS$, 3.6%], 207 [$M^+ - CH_3NCS$, 15.4%], 167 [$M^+ - CH=CH-CH_2+CH_2NCS$, 27.3%], 149 [$M^+ - SO_2CH=CH-(CH_2)_3$, 100%] and 91 [$M^+ - SO_2CH=CH-(CH_2)_3 - NCS$, 70%]. From these data, compound (4) could be identified as 5-benzylsulfonyl-4-butenyl isothiocyanate (Tait *et al.*, 1962).

Isothiocyanates of *Capparis deserti*

The GC spectrum of the obtained glucosinolate aglucones of *C. deserti* revealed the presence of four different compounds, two of them were identified as compounds (1) and (2) with different percentages (68.67%) and (20.18%); respectively. The other two compounds (5) (0.105%) and (6) (0.123%) were identified as follows.

Identification of Compound (5)

The EI-mass spectrum of compound (5) showed a molecular ion peak at m/z 147 [M^+ , 4.5%] which corresponds to the molecular formula $C_5H_9NS_2$ in addition to other fragments at m/z 101 [$M^+ - CH_3S$, 21%], 91 [$M^+ - NCS$, 36.4%] and 73 [$M^+ - CH_3S(CH_2)_2 - 1$, 100%]. So, compound (5) could be identified as 7-methylthiopropyl-isothiocyanate [$CH_3S(CH_2)_3 - NCS$] (7).

Identification of Compound (6)

The EI-mass spectrum of compound (6) showed a molecular ion peak at m/z = 297 [M^+ , 4.5%] and $M^+ - 1$ at m/z = 296 (9%) that corresponds to the molecular formula $C_{16}H_{27}NS_2$ in addition to other fragments at m/z = 264 [$M^+ - SH$, 22.7%] and 101 (22.7%) due to the presence of [$CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - N=C=S$] which cyclized to the stable form (Fig. 1). Also, a fragment at m/z 55 (100%) was due to the presence of [$CH_3 - CH=CH - CH_2$]. Compound (6) was identified as 11-(2-butenyl thio) 6-undecenyl isothiocyanate (7). This is the first report of isolation and identification of butyl isothiocyanate; 6-methylsulfonylhexyl isothiocyanate; 3-methylthiopropyl-isothiocyanate

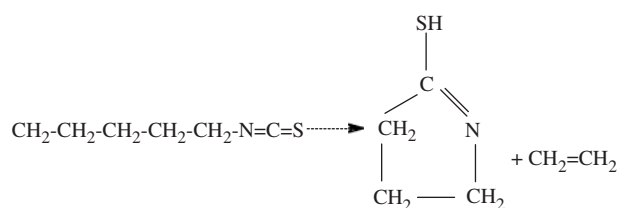


Figure 1. EI mass spectrum of compound (6).

Table 1. UV Spectral data (nm) of the isolated rutin

Additios to MeOH	None	NaOMe	AlCl ₃	AlCl ₃ + HCl	NaOAc	NaOAc/ H ₃ BO ₃
Rutin	250	275	273	276	269	265
	268 sh	332	306 sh	298	328 sh	301
	296 sh	406	430	357 sh	387	384
	354			404		

and 11-(2-butenyl thio) 6-undecenyl isothiocyanate from *C. deserti*.

Identification of the Main Flavonoidal Compound (rutin)

Preparative PC followed by Sephadex LH₂₀ column gave a single flavonoidal compound corresponding to that of rutin (UV data: Table 1). Acid hydrolysis gave quercetin which was identified by TLC, PC, UV and MS (M^+ 302, (C₁₅ H₁₀ O₇)) and fragments at m/e 274, 152, 153, 134. Glucose and rhamnose were confirmed by PC [Whatman No. 1, ethyl acetate: pyridine: water (12: 5: 4)]

Quantitative Determination of Rutin by TLC-Densitometer

The standard rutin has been subjected to TLC-Densitometric evaluation under suitable conditions. This technique proved that, it is suitable for the detection of rutin under the selected conditions. Standard curve of authentic rutin ($R_f = 0.56$) were made with serial concentration starting from 200 nl to 1200 nl on the TLC stationary layer. The prepared samples under investigation were analyzed under the same conditions as the standard rutin. The rutin concentrations, in the different prepared samples, were calculated automatically using CATS 4 Software.

The results revealed that the highest percent of rutin was found in butanol fraction of *C. deserti* (12.5%) followed by ethylacetate fraction of the same species (9.84%). On the other hand, the rutin in the butanol fraction of *C. cartilaginea* was found only in 5.6%, while in the ethylacetate fraction, rutin could not be detected in the tested concentrations up to 12000 nl (Table 2). The radical scavenging effect of the tested extracts on DPPH free radical was performed (Table 3). The butanol fractions of both *C. deserti* and *C. cartilaginea* had the

Table 2. Rutin determination in *Capparis Species*

Capparis species	Fraction	Rutin%
<i>C. cartilaginea</i>	Butanol	5.6
	ethylacetate	not detected
<i>C. deserti</i>	Butanol	12.5
	ethylacetate	9.84

Table 3. Antioxidant activity of different plant extracts

Capparis species	<i>Capparis Cartilaginea</i> % antioxidant activity	<i>Capparis deserti</i> % antioxidant activity
Extract		
Tolorox	94.96	94.96
MCM	0	0.6
80% aq. EtOH	10.87	6.95
Water	0	0
CHCl ₃	66.75	65.73
EtOAc	0.1	23.38
BuOH	77.24	72.94
Glucosinolates	0	0
Pet. Ether	0	0

highest antioxidant activity followed by the CHCl₃ extracts. Also, EtOAc of *C. deserti* showed mild antioxidant properties. MCM extract, total glucosinolates, EtOAc and pet. Ether extracts of both species showed no antioxidant activity. The results are expressed as radical scavenging activity (%RSA) as shown in the table.

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