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Flavonoid Constituents of *Cakile maritima* (Scope) Growing in Egypt and Their Biological Activity

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The study of the flavonoidal constituents isolated from the chloroform, ethyl acetate and butanol fractions of aqueous alcoholic extract of *Cakile maritima* (family *Crucifereae*) revealed the isolation and identification of quercetin, quercetin-3-O-rhamnoside, kaempferol-7-O-glucoside, luteolin, luteolin-7-O-glucoside and caffeic acid. Identification of the isolated compounds was carried out by spectroscopic analysis *viz* UV, MS, NMR, TLC, PC and acid hydrolysis of the glycoside compounds. The radical scavenging effects of the tested extracts and isolated compounds on DPPH free radical were observed, in which the flavonoidal compounds showed a strong antioxidant activity. Molluscidal activity of different fractions and isolated compounds of *Cakile maritime* was carried out against *Biomphalaria alexandrina* snails, in which luteolin-7-O-glucoside, kaempferol-7-O-glucoside and quercetin-3-O-rhamnoside showed significant molluscicidal activity (LC_{50} 24, 37 and 40, respectively). It was also found that the quercetin, luteolin and caffeic acid had showed moderate activity.

Key Words: *Cakile maritime*, Family *Crucifereae*, Flavonoidal constituents, Quercetin, Kaempferol-7-O-glucoside, Luteolin, Caffeic acid, Spectroscopic analysis, Radical scavenging, Molluscicidal activity.

INTRODUCTION

Crucifereae is a large family of 3200 species in 375 genera, it is also known as the *Brassicaceae*. It includes vegetable crops, medicinal plants and plants used as food. Several plants of this family are used as antidiabetic, antibacterial[1], antifungal[2], anticancer[3], antirheumatic[4] and show a potent insecticidal effect[5].

The family *Crucifereae* is represented in Egypt by 53 genera and 105 species, the most common ones are *Anastatica*, *Arabis*, *Diplotaxis*, *Zilla*, *Lepidium* and *Cakile*[6,7].

The genus *Cakile* was represented in Egypt by only one species, *Cakile maritima*. The leaves, stems, flower buds and immature seed pods-row are cooked. They are rich in vitamin C but have a bitter taste[8] used mainly as a flavouring. The young leaves can be added to salad, whilst older leaves can be mixed with milder tasting

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leaves and used as potherb[9]. The dried powder of the roots when mixed with cereal flours were used to make bread[10]. A famine food, its only times of scarcity[9] and the seed contain fatty oil[11]. It was found from literature that *Cakile maritima* is known as Sea Rocket and the analysis of the glucosinolates presented in the plant resulted in the release of only very small amounts of mustard oil, the principal product being 1-cyano-4,5-epithiopentane[12]. A knowledge of the biological activities and/or chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but because such information may be of value in disclosing new sources of material. Schistosomiasis is a serious problem that infects millions of people in tropical and subtropical regions[13]. Through last decades many investigations studied the chemical control of molluscan intermediate hosts of human schistosomiasis[14]. Therefore searching for new agents from natural products of different structures and mode of action is one of the important subjects to be considered in the hope to explore more effective control agents. Numerous investigations on naturally occurring molluscicides have been attributed to saponines[14,15], flavonoids[16], diterpenes and sulphur glycosides[17].

Preliminary phytochemical study of *Cakile maritima* growing in Egypt with standard procedures, showed that it contains flavonoids, coumarins, alkaloids, triterpenes, sterols and sulphur glycosides.

Very little information are reported on the phytoconstituents of *Cakile maritima* growing in Egypt and their biological activities. The study of the glucosinolates of the plant revealed the isolation and identification of four glucosinolates, also the lipid constituents were studied and identified[18]. Therefore, the present work deals with the study of flavonoid constituents of the plant. It also deals with a preliminary biological study of the plant extracts against *Biomphalaria alexandrina* snails as well as evaluation of the antioxidant activities of both the total extracts and the isolated compounds using DPPH.

EXPERIMENTAL

Cakile maritima (Scope) (Family: *Crucifereae*) was collected from Borg El-Arab near Alexandria during April 2006. The plant was identified by El-Gebaly and El-Kawashty, taxonomists, National Research Centre, Cairo Egypt. The aerial parts of the plant were air dried and ground into fine powder. A voucher specimen was deposited at the herbarium of NRC.

Antioxidant activity: 6-Hydroxy-2,5,7,8-tetra methyl chroman-2-carboxylic acid (Trolox) (Aldrich Chemical Co.), 1,1-diphenyl-2-picryl hydrazyl (DPPH) (Sigma Chemical Co.) and Methanol HPLC grade.

Materials for molluscicidal activity

Snails: The snails were collected from irrigation canals located in Giza governorate, Egypt. They were maintained as stock cultures in a well aeriated glass aquaria containing dechlorinated tap water and fed on fresh lettuce leaves daily at a temperature of 25-27 ºC.

Paper chromatography was carried out on Whatman No. 1 and 3 MM sheets. Column chromatography was performed on Silica gel 60 (Merck), alumina (Al_2O_3, Ved, Lab.) and Sephadex LH-20 (Pharmacia). TLC was performed on Silica gel G (Merck).

Solvent systems: 15 and 20 % acetic acid in water; *n*-butanol:acetic acid:water (4:1:5); ethyl acetate:pyridine:water (12:5:4) and n-butanol:benzene:pyridine:water (5:3:3:1) v/v.

Shimadzu UV. Pc. 2401 spectrophotometer. Mass spectrophotometer GC-MS finnigan mat SSQ 7000 mass spectroscopy 70 ev. Preparative, centrifugally, accelerated, radial, thin layer chromatography by using chromatotron apparatus. ^{1}H NMR spectra were recorded in (DMSO-d_6) using Jeol-Ex-270 MHz spectrometer.

Isolation of flavonoids[19]: About 1.5 kg of defatted dried powder of *Cakile maritima* (Scope) was macerated with 80 % ethanol (3 L x 5 L). The combined alcoholic extracts were evaporated *in vacuo* at 40 ºC. The residue was dissolved in hot distilled water and left over night. The aqueous filtrate was extracted with successive portions of chloroform (3 mL × 200 mL), followed by ethyl acetate (3 mL × 200 mL) and finally with *n*-butanol (3 mL × 200 mL). The chloroform fraction (1.4 g) was subjected to preparative paper chromatography (3 MM, 20 % acetic acid). The flavonoidal bands (R_f = 0.76 and 0.15) were cut and eluted separately with methanol. The eluted fractions were further purified using Sephadex LH-20 column using 90 % methanol. The ethyl acetate fraction (1.1 g) was subjected to preparative PC (3 MM, 20 % acetic acid). The main flavonoidal band (R_f = 0.12, 0.28 and 0.45) were cut and eluted separately with 90 % methanol. The butanol fraction (0.8 g) was subjected to preparative PC (3 MM, 15 % acetic acid in water). The flavonoid component (R_f = 0.55) was isolated and subjected to further purification using Sephadex LH-20 column using 90 % methanol. The eluted fractions were repurified using Sephadex LH-20 column using 80 % methanol as eluent or subjected to centrifugally accelerated rotatory TLC using silica gel 60 PF_{254} discs and eluted with 90 % chloroform in methanol.

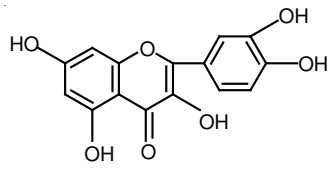
Determination of scavenging effect on DPPH radicals[20]: The decrease of the absorbance at 516 nm of the DPPH solution after addition of the sample (extract of plant materials) was measured in a glass cuvette. An aliquot of 0.1 mL M. methanol solution of DPPH was mixed with the methanolic solution of the sample, so that the relative concentration of plant materials *versus* the stable radical in the cuvette was 0.13. Then the solution with tested sample was shaken vigorously. The absorbance was measured at the start and after 20 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

$$\text{RSA}\,(\%) = 100\,\% \times \frac{\text{Abs of blank 516 nm} - \text{Abs of sample 516 nm}}{\text{Abs of blank 516 nm}}$$

Molluscicidal activity: Measurment of LC_{50} of the extracts and the isolated fractions. (**a**) Stock solutions (100 ppm) of extract or isolated fractions were prepared separately by dissolving 100 mg in 1 mL ethanol and then diluted with dechlorinated water (1 L). Series of dilution that would permit the computation of LC_{50}-LC_{90} values were used. (**b**) 10 mature snails (8-10 mm diameter) were immersed in suitable beaker containing 100 mL of the tested concentration. Three replicates were employed in each concentration beside a control groups containing ethanol and water only. The exposure period was 24 h. The molluscicidal activity of the studied extracts were done according to standard procedure[21] and the effectiveness of the extracts was expressed in terms of LC_{50} and LC_{90} *via* statistical analysis according to the procedure of Litchfield and wilcoxon[22].

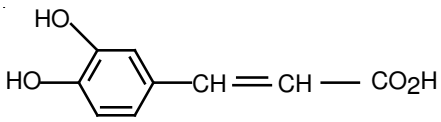
RESULTS AND DISCUSSION

Quercetin (compound 1): The flavonoidal band (R_f = 0.15) isolated from the chloroform fraction by preparative PC (3 MM, 20 % acetic acid) and eluted by methanol, gave after purification on Sephadex LH-20 column using 90 % methanol a single flavonoidal compound corresponding to that of quercetin (19 mg) which identified by PC, TLC, UV[23] (Table-1) and MS [M^+ 302 ($C_{15}H_{10}O_7$) and fragments at m/e 274, 152, 153, 134] which are characteristic for that of quercetin[24].



Quercetin

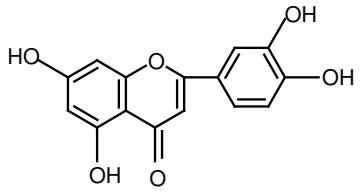
Caffeic acid (compound 2): The flavonoidal band (R_f = 0.76) isolated from the chloroform fraction by using preparative PC (3 MM, 20 % acetic acid) and eluted by methanol, gave after purification on Sephadex LH-20 column using 90 % methanol, a single phenolic compound corresponding to that of caffeic acid (17 mg) which identified by PC, TLC, UV[25] (Table-1) and MS [M^+] 180 and fragments at m/e 163 (M^+-OH), 145 (M^+-H_2O, OH), 136. These fragment pattern is in agreement with that reported for caffeic acid[23,25].



Caffeic acid

Luteolin (compound 3): The flavonoidal band (R_f = 0.12) isolated from the ethyl acetate fraction by preparative PC (3 MM, 20 % acetic acid) and eluted by methanol, gave after purification on Sephadex LH-20 column using 90 % methanol,

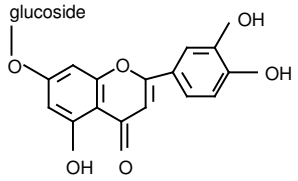
a single flavonoidal compound corresponding to that of luteolin (19 mg) which identified by TLC, PC, UV[23] (Table-1) and MS [M^+] 286 which corresponds to the molecular formula $C_{15}H_{10}O_6$, in addition to fragment ions at m/e 258 (M^+-CO), 153, 152, 137, 134 which are in agreement with those reported for luteolin[24].



Luteolin

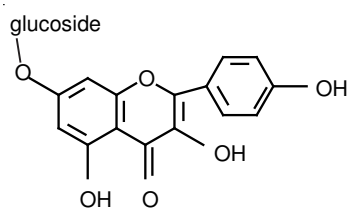
Luteolin-7-glucoside (compound 4): The flavonoidal band (R_f = 0.28) isolated from the ethyl acetate fraction by preparative PC (3 MM, 20 % acetic acid) and eluted by 80 % methanol, gave after purification by using centrifugally accelerated rotatory TLC using silica gel 60 PF_{254} discs and elution with chloroform/methanol (9:1), a single flavonoidal compound corresponding to that of luteolin-7-glucoside (31 mg) which identified by TLC, PC, UV[23] (Table-1). Further confirmation was performed by carrying out ^{1}H NMR, which found to be identical for that of luteolin-7-glucoside[23]. Acid hydrolysis (2N HCl) gave luteolin which was identified by TLC, PC, UV[15] (Table-1) and MS [M^+] 286, in addition to fragment ions at m/e 258, 153, 152, 137, 134 which are characteristic to luteolin[24]. The sugar moiety was identified as glucose by PC on Whatman No. 1, ethyl acetate:pyridine:water (12:5:4) and *n*-butanol: benzene:pyridine:water (5:1:3:3) as solvent systems.

Kaempferol-7-O-glucoside (compound 5): The flavonoid band (R_f = 0.45) isolated from the ethyl acetate fraction, gave after purification by preparative PC followed by centrifugally accelerated rotatory TLC using silica gel 60 PF_{254} discs and elution was carried out with 90 % chloroform in methanol a single flavonoidal



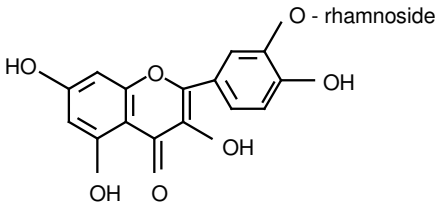
Luteolin-7-glucoside

compound (kaempferol-7-O-glucoside) which was identified by using PC, TLC and UV[23] (Table-1). Acid hydrolysis gave kaempferol which was identified by TLC, PC, UV and MS [M^+] 286 which corresponds to the molecular formula $C_{15}H_{10}O_6$, in addition to fragment ions at 259, 153, 152, 135, 121 which are characteristic for kaempferol[24]. Further confirmation was performed by carrying out ^{1}H NMR (DMSO-d_6) which found to be identical to that reported for kaempferol[23]. The sugar moiety was confirmed as glucose [PC, Whatman No. 1, ethyl acetate: pyridine:water (12:5:4), sprayed with aniline phthalate].



Kaempferol-7-O-glucoside

Quercetin-3-O-rhamnoside (compound 6): The flavonoidal compound (R_f = 0.55) isolated from the butanol fraction by preparative PC, gave after purification on Sephadex LH-20 column using 90 % methanol, a single flavonoid compound (quercetin-3-O-rhamnoside, 27 mg) which was identified by PC, TLC and UV[23] (Table-1). Acid hydrolysis (2N HCl) gave quercetin which was identified by m.p. 313 ºC, TLC, PC, UV and MS [M^+] 302 ($C_{15}H_{10}O_7$) and fragment ions at m/z 274, 153, 152, 134, which are characteristic for quercetin[24]. The sugar moiety was confirmed as rhamnose [PC, Whatman No. 1, ethyl acetate:pyridine:water (12:5:4), sprayed with aniline phthalate.



Quercetin-3-O-rhamnoside

TABLE-1
UV, SPECTRAL DATA (nm) OF THE ISOLATED FLAVONOIDS AND THEIR CORRESPONDING AGLYCONES

Flavonoides	UV abs. in MeOH	NaOMe	$AlCl_3$	$AlCl_3$+HCl	NaOAc	NaOAc+ H_3BO_3
Quercetin 1	371, 297sh, 265, 253	325, 248sh	451, 335, 272	461, 334, 303sh, 270	389, 329, 275, 251	390, 299sh, 257
Caffeic acid 2	322, 290sh, 253	348, 305, 253	392, 358, 294, 269	380, 352, 294, 277, 262sh	370, 321, 274	345, 266, 254sh
Luteolin 3	354, 294sh, 265sh, 255	410, 326, 267	432, 328sh, 300sh, 273	411, 362sh, 295, 265	375, 319, 279	383, 295sh, 264
Luteolin-7-O-glucoside 4	346, 288sh, 263, 251	401, 313sh, 267sh	431, 329, 301sh, 271	386, 353, 278, 266sh	386, 321sh., 266	431sh, 371, 258
Kaempferol-7-O-glucoside 5	355, 264, 251	396, 303sh, 266	431, 353, 263	422, 347, 302sh, 264	418sh, 383sh, 268,	373, 324sh 265sh
Quercetin-3-O-rhamnoside 6	366, 301sh, 262	413, 325, 272	435, 329sh, 271	408, 363sh, 304sh, 264	, 381, 323, 274	383, 295sh, 265

In the series of searching of natural antioxidants which are preferred over synthetic antioxidants, we tried to isolate new antioxidant compounds from medicinal plants, in particular from the plants which were not investigated before. Therefore, we studied the flavonoidal compounds of *Cakile maritima* (scope) and the different extracts and isolated compounds were evaluated for their antioxidant activity against DPPH.

The analysis of the flavonoid constituents of *Cakile maritima* (scope) revealed the presence of quercetin, quercetin-3-O-rhamnoside, kaempferol-7-O-glucoside, luteolin, luteolin-7-O-glucoside and caffeic acid. Identification of the isolated compounds was carried out by spectroscopic analysis *viz* UV, MS, NMR, TLC, PC and acid hydrolysis of the glycoside compounds. On reviewing the literature, it was found that this is the first record of the flavo-noids in Cakile maritime. It was also found that preparative, centrifugally, accelerated, radial, thin layer chromatography is a good technique to purify the fractionated flavonoidal compounds.

The radical scavenging effects of the tested extracts and isolated compounds on DPPH free radical were observed, in which the flavonoidal compounds showed a strong antioxidant activity (Table-2).

TABLE-2
RADICAL SCAVENGING EFFECT OF SAMPLES ON DPPH FREE RADICAL

Tested compounds	Absorbance 516/ reaction period (min)		RSA (%)
	10 min	20 min	
Trolox	0.022	0.023	95.63
Chloroform ext.	0.075	0.078	85.174
Ethyl acetate ext.	0.061	0.063	87.98
Butanol ext.	0.075	0.081	84.78
Quercetin (**1**)	0.065	0.066	79.16
Caffeic acid (**2**)	0.052	0.057	89.43
Luteolin (**3**)	0.043	0.064	91.28
Luteolin-7-O-glucoside (**4**)	0.048	0.052	90.31
Kaempferol-7-O-glucoside (**5**)	0.061	0.064	87.88
Quercetin-3-O-rhamnoside (**6**)	0.068	0.071	86.53

The absorbance readings at each reaction period are the means of two measurement.

TABLE-3
MOLLUSCICIDAL ACTIVITY OF DIFFERENT FRACTIONS AND ISOLATED COMPOUNDS

Plant fractions and isolated compounds	LC_{50} (ppm)	LC_{90} (ppm)
Chloroform extract	61	72
Ethyl acetate extract	42	95
Butanol extract	46	67
Quercetin	66	88
Caffeic acid	59	81
Luteolin	73	99
Luteolin-7-O-glucoside	24	38
Kaempferol-7-O-glucoside	37	55
Quercetin-3-O-rhamnoside	40	66

Molluscicidal activity of different fractions and isolated compounds of *Cakile maritima* were carried out against *Biomphalaria alexandrina* snails in which luteolin-7-O-glucoside, kaempferol-7-O-glucoside and quercetin-3-O-rhamnoside showed significant molluscicidal activity (LC_{50} 24, 37 and 40, respectively). It was also found that quercetin, luteolin and caffeic acid showed moderate activity (Table-3).

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