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Research Article

Chemical Constituents, Antioxidant, Antimicrobial and Anti-inflammatory Activities of *Erysimum corinthium* Boiss. (Brassicaceae)

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ABSTRACT
Aerial part of *Erysimum corinthium* Boiss (Brassicaceae) was subjected to chromatographic and biological investigations. GLC analysis of fatty acids methyl esters revealed the presence of 13 fatty acids where linolenic acid is the major one (47.9%). Column chromatography of the petroleum ether fraction afforded five compounds: lupeol acetate, lupeol, α-amyrin, β-sitosterol and β-sitosterol-*O*-glucoside. Quercetin-3-*O*-β-D-glucoside and rutin were isolated from ethyl acetate fraction. The structures of these compounds were determined using IR, MS and ^{1}H and ^{13}C-NMR spectral analysis. HPLC analysis of total alcoholic extract and its ethyl acetate fraction indicated the presence of 17 phenolic acids. Ethyl acetate fraction showed strong anti-oxidant activity (SC_{50} 0.95 µg/ml) vs. ascorbic acid (SC_{50} 1.45 µg/ml). Ethyl acetate, petroleum ether and chloroform fractions showed significant antimicrobial activity against the tested organisms with 35-46 % activity compared with standard. The plant showed anti-inflammatory activity where 100 mg/kg of alcoholic extract induced 55% inhibition of rat paw edema equivalent to that of diclofenac sodium (4 mg/kg).

Keywords: *Erysimum corinthium*, sterols, triterpenes, flavonoids, antioxidant, antimicrobial and anti-inflammatory activities.

INTRODUCTION

The plant family Brassicaceae (Cruciferae) is commonly known as the mustard or the cabbage family. It consists of 350 genera and about 3500 species[1]. This family is an economically important family for its many food and oilseed crops, representing an important part of the human diet worldwide as vegetables (cabbage, broccoli and turnip), as well as many members of ornamental plants and noxious weeds[2]. Cruciferous vegetables act as good source of natural antioxidants due to the high levels of carotenoids, tocopherols and ascorbic acid, and strong epidemiological evidence shows that these compounds may protect the human body against damage by reactive oxygen species. Most of the antioxidative effect related to plant food intake is also due to the presence of phenolic compounds, which have been associated with flavour and colour characteristics of fruits and vegetables. So, the popularity and consumption of vegetable *Brassica* species is increasing because of their nutritional value[3,4].

The genus *Erysimum* comprises about 225 species and is one of the most complex genera of the Cruciferae[5]. *Erysimum* plants are characterised by the presence of methyl thioalkyl, methyl sulfinylalkyl and methyl sulfonylalkyl glucosinolates[6]. Medicinally, many species are known in folk medicine for treatment of snake bites, an antimicrobial, biliary colic and wound sores. Also, they enhance the detoxification of carcinogen and some species exhibit hypoglycemic and hypotensive effects[7,8].

In a previous publication, we reported the GC–MS analysis and biological activities of the glucosinolate hydrolysis products of *E. corinthium* where sinigrin, progoitrin, glucoiberin, 3-(methylcarbonyl) propyl glucosinolate, glucocheirolin and glucoerysolin were identified for the first time in this plant[9]. In continuation of our study on this plant, we report here the chemical constituents and the biological activities of its different fractions.

EXPERIMENTAL

Plant material

The aerial parts of *Erysimum corinthium* Boiss. family Brassicaceae was collected in the flowering stage from medicinal plants garden of Faculty of Pharmacy, Zagazig University. The plant was identified by Dr. Sean Edwards, Botanical Herbarium, Manchester University as reported before[9]. Voucher samples are kept in the Pharmacognosy Department Herbarium, Faculty of Pharmacy, Zagazig University.

Apparatus

Melting points were determined on Digital electro-thermal LTD (England). TLC visualization was done using UV lamp GL-58 (λ max 254 and 365 nm). Infrared spectral analysis was recorded in potassium bromide disks on a Pye Unicam SP 3000 and IR spectrophotometer, Jasko, FT/IR-460 plus. Mass spectra were carried out using Shimadzu GCMS-QP5050A mass spectrometer at 70 eV. ^{1}H- and ^{13}C-NMR spectra were obtained by Bruker at 400 MHz and 100 MHz, respectively.

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Extraction and fractionation

About 5.0 kg of the fresh aerial parts of *E. corinthium* were sliced and homogenized with boiling 70% ethanol (10 L.). The homogenate was further boiled for additional 5 min. to insure the inactivation of naturally occurring enzymes and left for complete exhaustion. The extract was evaporated under reduced pressure at 50ºC to give 400 g of greenish-brown semisolid residue. The concentrated ethanolic extract was dissolved in 1 L of MeOH: H_2O (3:7) and exhaustively fractionated into light petroleum, chloroform, and ethyl acetate. Each fraction was dried over anhydrous sodium sulphate and concentrated under vacuum to afford 50, 12 and 5 g, respectively.

Analysis of the fatty acids content

A part of petroleum ether fraction (10 g) was saponified to afford fatty acids[10]. 2 g of fatty acids residue was esterified to fatty acids methyl esters (FAME)[11]. The resulting esters were subjected to GLC analysis against references while the unsaponified matters (USM) were subjected to chromatographic isolation.

GLC analysis of fatty acids methyl esters

It was carried out using GLC-FID analysis by Pye Unicam Series 304 equipped with 10 % polyethylene glycol adipate column (1.5 m × 4 mm). The temperature program was 70ºC to 190ºC at 8ºC/min, 25 min isothermal. Nitrogen was used as carrier gas (30 ml/min). The injector temperature was 250ºC while detector temperature was 300ºC. The identification was based upon comparison of retention time of the sample peaks and the available authentics.

Chromatographic isolation of compounds 1-7

About 3.0 g of USM fraction was subjected to column chromatography on silica gel (2×100 cm, 90 g silica gel) packed with light petroleum and the polarity was increased gradually using chloroform and methanol to afford compounds 1-5. About 3.0 g of the ethyl acetate fraction were submitted to column chromatography of silica gel (2 × 100 cm, 120 g). The column was packed in light petroleum and the polarity was gradually increased with chloroform then methanol to afford compounds 6 and 7.

Acid hydrolysis

Each of compound 5, 6 and 7 (7-10 mg) was subjected to acid hydrolysis[12]. Liberated aglycones and sugars were identified by direct comparison with authentic samples.

HPLC determination of phenolics

The total alcohol extract of the plant and the ethyl acetate fraction were separately subjected to HPLC analysis against reference compounds in order to determine their phenolic constituents[13]. HPLC (Hewlett Packard, series 1050) attached to C18 hypersil BDS column with particle size 5 µm was used. The separation was carried out with methanol and acetonitrile as a mobile phase, flow with 1 ml/min. Diode-array and electro-array detectors were used and the identification was based upon comparison of retention time with available authentics. Peak area was used to calculate phenolic compounds concentration.

Antioxidant activity

Antioxidant activity of the ethyl acetate fraction was carried out according to previously reported method[14]. The deep violet stable radical1,1-diphenyl-2-picrylhydrazyl (DPPH) in the presence of an antioxidant radical scavenger, decolorizes to the pale yellow non-radical form. The change in color and the subsequent fall in absorbance were monitored spectrophotometrically at λ520 nm. A standard calibration curve was plotted using serial dilutions of ascorbic acid in concentrations ranging from 0-25µg/ml in distilled water. This activity is expressed as percentage DPPH radical-scavenging that is calculated according to the following equation:

% DPPH radical-scavenging = (AC-AS)/AC × 100

Where AC is the absorbance of the control solution (containing only DPPH in methanol), AS is the absorbance of sample in DPPH solution. The percentage of DPPH radical-scavenging was plotted against the ethyl acetate fraction concentrations (mg/ml) to determine the concentration of extract required to scavenge DPPH by 50% (SC_{50}). SC_{50} (half maximal scavenging capacity) values for ethyl acetate fraction and ascorbic acid were estimated

Antimicrobial activity

Petroleum ether, chloroform and ethyl acetate fractions were dissolved in DMSO at concentration of 1 mg/ml for antimicrobial screening by filter paper disc assay[15]. The Gram-positive bacteria used were *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis*, while *Escherichia coli*, *Neisseria gonorrhea* and *Pseudomonas aeroginosa* were the used Gram-negative bacteria. *Candida albicans* and *Aspergillus flavus* were the used fungi. The standard antibiotic was tetracycline while the standard antifungal was amphotericin B. After incubation, the diameter of the clear zone of inhibition surrounding the sample was taken as a measure of the inhibitory power of the sample against the particular test organism

$$\text{Percentage of activity} = \frac{\text{Inhibition zone diameter of test sample}}{\text{Inhibition zone diameter of standard}}\ x\ 100$$

Anti-inflammatory activity

The anti-inflammatory activity of the total alcoholic extract and the fatty acids fraction on the rat paw edema induced by carrageenan was studied using the previously reported methods[16]. Diclofenac sodium and dexamethasone were used as reference standards. Since the time course of the effect was followed, it was possible to use the cumulative anti-inflammatory effect during the whole observation period as the area under the curve (AUC). Total inhibition (TI %) was recorded using the following ratio:

TI (%) = [AUC control −AUC treat]×100/AUC control.

Data were expressed as mean ± standard error of mean (SEM) of the five animals.

RESULTS AND DISCUSSION

GLC analysis of FAME showed the presence of 13 fatty acids (Table 1). Palmitic acid is the major saturated fatty acid while linolenic acid is the major unsaturated one. The saturated acids represent about 28.19 % of the total acids including; caprylic, lauric, tridecanoic, myristic, pentadecanoic, palmitic, margaric, and stearic. The mon,

Table 1: GLC analysis of fatty acids methyl esters of *E. corinthium.*

Peak No	Retention Time	R.R.T	No. of Carbon: double bond	Common Name	Relative Percentage
1	12.1	0.47	8:0	Caprylic	0.94
2	15.4	0.6	12:0	Lauric	0.70
3	16.1	0.62	13:0	Tridecanoic	0.10
4	19.1	0.74	14:0	Myristic	0.60
5	21.8	0.84	14:1	9-*cis* –Myristoleic	0.30
6	22.5	0.87	15:0	Pentadecanoic	0.15
7	25.9	1	16:0	Palmitic	19.20
8	27.6	1.1	16:1, 9-*cis*	Palmitoleic	0.20
9	33.0	1.3	17:0	Margaric	5.30
10	37.6	1.45	18:0	Stearic	1.20
11	39.5	1.52	18:1	Oleic	4.30
12	44.4	1.7	18:2	Linoleic	17.80
13	52.8	2.03	18:3	Linolenic	47.90
			Total saturated fatty acids		28.19
			Total unsaturated fatty acids		70.50
			Total identified fatty acids		98.69
			U/S		2.5
			UI		184.1

*RRT: Relative retention time to palmitic acid
U/S: Ratio of unsaturated to saturated fatty acids %
UI: Unsaturated index = 1 x monenes + 2 x dienes + 3 x trienes

Table 2: Phenolic contents of total alcoholic extract and ethyl acetate fraction of *E. corinthium*.

Phenolic compound	Concentration (ppm) Total alcoholic extract	Ethyl acetate fraction
Gallic	116.37	24.61
Pyrogallol	--	1749.60
4-Aminobenzoic	96.47	61.72
Protocatechuic	494.57	613.01
Catachein	52.64	61.08
Chlorogenic	258.46	275.56
Catechol	243.16	337.72
Epicatechein	1147.09	503.01
P-OH.Benzoic	505.34	608.06
Caffeic	32.85	73.16
Vanillic	118.25	284.83
Ferulic	28.92	72.35
Ellagic	120.01	130.16
Benzoic	--	270.10
Salicylic	80.02	109.10
Coumaric	18.69	15.13
Cinnamic	0.79	11.91

di and trisaturated acids represent 4.9%, 17.8% and 47.9% respectively. Palmitic, stearic, oleic, linoleic and linolenic acids were reported in nine Brassicaceae plants[17].

Identification of isolated compounds

Chromatographic investigations and spectroscopic analysis afforded seven compounds (1-7) as shown in figure 1.

*Compound 1***:** White needle crystals (40 mg) with m.p. 218-219ºC and R_f 0.95 (petroleum ether-chloroform, 9:1). IR (KBr) cm^{-1}:2938, 2851, 1737, 1640, 1456 1368 and 1023. EI-MS m/z (relative int. %): 468 (M^+, 12.6), 453 (12.4), 409 (2.5), 393 (1.3), 357 (1.0), 276 (4.8), 218 (21.6), 203 (19.9), 189 (43.4), 121 (58.9), 109 (50.7) and 55 (100). Comparison with reported literature[18,19] as well as direct comparison with authentic sample (Co-TLC) confirmed the structure to be lupeol acetate**.**

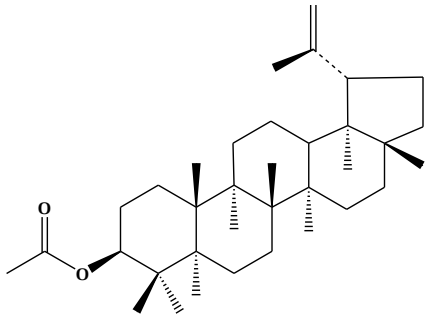
Compound 2: White crystals (20 mg) with m.p. 214-216ºC and R_f 0.75 (petroleum ether-chloroform, 8:2). IR (KBr) cm^{-1}: 3409, 2926, 2860, 1637, 1459, 1378 and 1142. EI-MS: m/z (relative int. %) 426 (M^+, 0.9), 411 (0.2), 393 (0.03), 299 (0.1), 297 (0.2), 257 (1.5), 247 (0.1), 229 (2.6), 231(0.4), 218 (3.8), 207 (1.1), 203 (5.9), 189 (7.3), 177 (0.6), 161(7.1), 121(28.6), 93 (45.6), 81(32.6) and 68 (100).

Direct comparison with authentic sample (Co-TLC) and literature data confirmed the structure to be lupeol[18, 20]. Lupeol and its acetate showed significant hypotensive and hypolipidemic potential which might be associated with their cardioprotective activity[21].

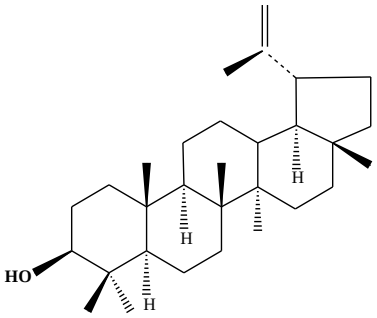
Compound 3: White crystals (45 mg) with m.p. 183-185ºC and R_f 0.38 (petroleum ether-chloroform, 6:4). IR (KBr) cm^{-1}: 3376, 2934, 2860, 1644, 1459, 1379 and 1091**.** EI-MS: m/z (% relative abundance) 426 (M+, 6.6), 411 (2.7), 394 (0.8), 272 (1), 257 (2.8), 243 (1.2), 231 (1.6), 229 (1.8), 218 (100), 207 (9.4), 203 (45.7), 189 (20.5), 175 (9.3), 121 (13.1), 119 (13.2), 109 (15.3), 107 (16), 95 (22.2), 69 (21.5) and 55 (18.5).

Direct comparison (Co-TLC) with authentic amyrins and reported data confirmed that compound 3 is α-amyrin[20,22,23].

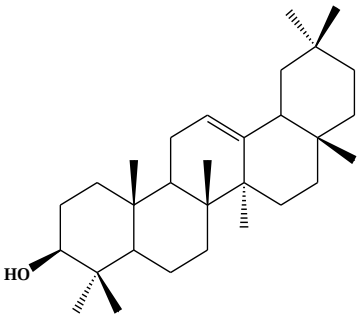
Compound 4: White needle crystals (70 mg), m.p. 136-137ºC and R_f 0.32 (petroleum ether-chloroform, 6:4). IR (KBr) cm^{-1}: 3421, 2957, 2868, 1642, 1463, 1377, 1056 and 1022. EI-MS: m/z (relative int. %): 414 (M^+; 16.8), 396 (28.9), 255 (13.6), 227 (6.7), 213 (16.8), 163 (8.3), 161(21.9), 159 (27.5), 145 (40.7), 133 (24.5), 119 (23.1),



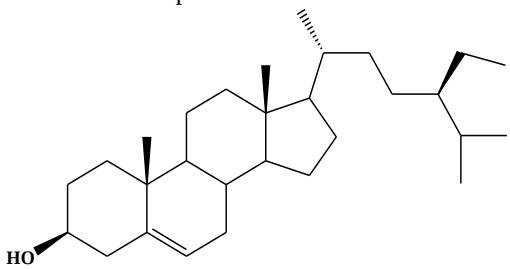
Lupeol acetate



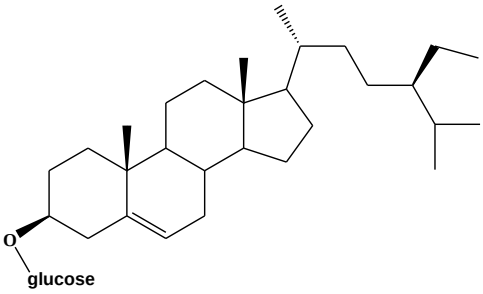
Lupeol



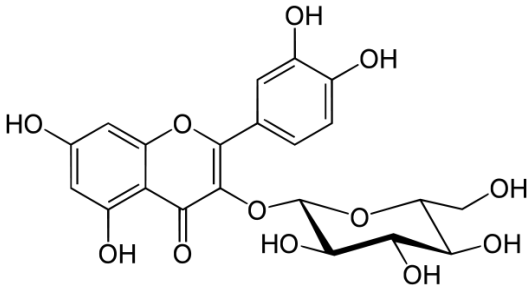
α-Amyrin



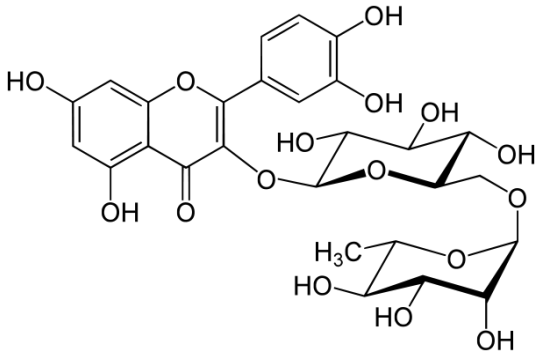
β-Sitosterol



β-sitosterol-*O*-glucoside



Quercetin-3-*O*-β-D-glucoside



Rutin

Figure 1: Compounds isolated from *E. corinthium*

Table 3: Antimicrobial activity of *E. corinthium* fractions

Micro-organism	Inhibition zone diameter (mm/mg sample) (percentage of activity)				
	1	2	3	Standard	
				T	Am
Bacillus subtilis	13 (43)	13 (43)	14 (46)	30	0
Staphylococcus aureus	13 (41)	13 (41)	14 (44)	32	0
Streptococcus faecali	13 (42)	13 (42)	14 (45)	31	0
Escherichia coli	13 (38)	14 (41)	13 (38)	34	0
Neisseria gonorrhea	12 (38)	13 (42)	13 (42)	31	0
Pseudomonas aeroginosa	12 (35)	14 (41)	14 (41)	34	0
Aspergillus flavus	0	0	0	0	0
Candida albicans	13 (35)	14 (37)	14 (37)	-	21

1: Ethyl acetate fraction **2**: Petroleum ether fraction **3**: Chloroform fraction **T**: tetracycline **Am**: amphotericin B

107(27.3), 105 (48.5), 69 (40.0), 67 (44.1), 57 (73.9) and 55 (100)**.**

Direct comparison (Co-TLC) with authentic sterols, compound 4 was proved to be β-sitosterol[20, 24, 25]. α-Amyrin and β-sitosterol were previously isolated from seeds of *E. cheiri*[26].

Compound 5: White amorphous powder (50 mg), m. p. 298-300ºC and R_f 0.13 (petroleum ether-chloroform, 7:3). IR **(**KBr) cm^{-1}: 3408, 2933, 2872, 1634, 1460, 1374, 1163,

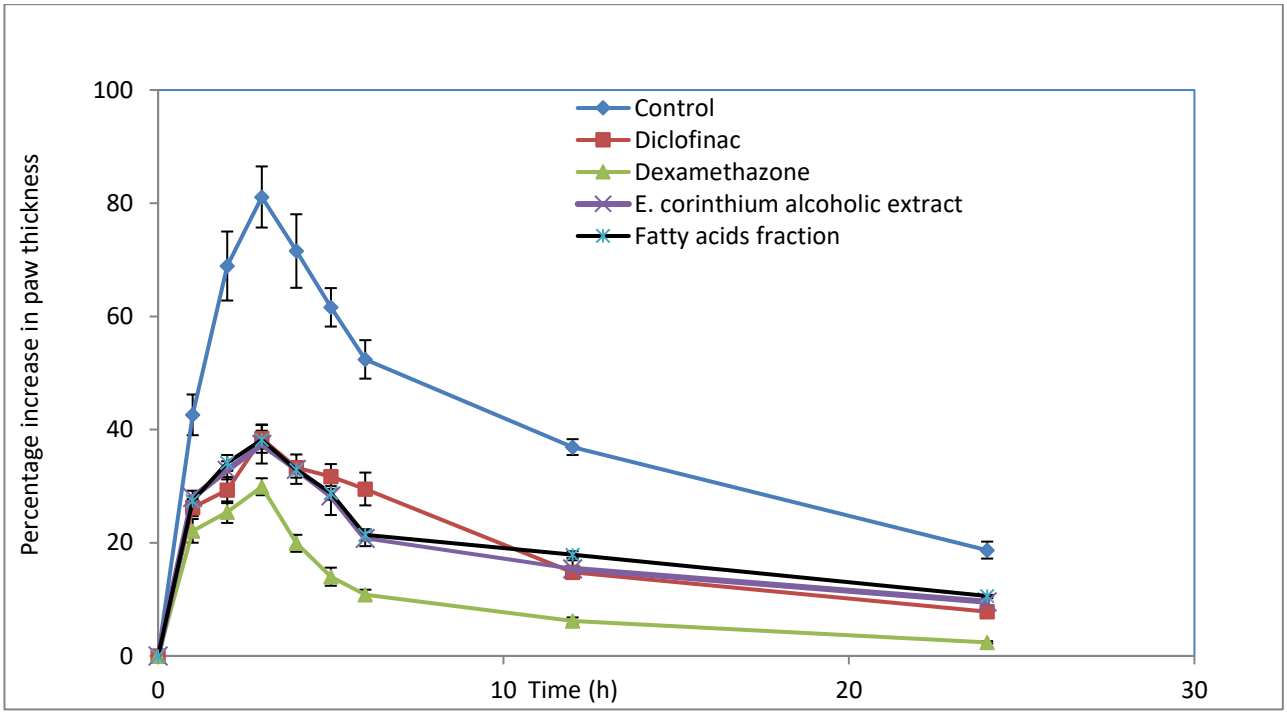


Figure 2: Effect of oral administration of total alcoholic extract and fatty acids fraction (100 mg/kg) of *E. corinthium* on hind paw thickness after induction of edema using carrageenan.

Table 4: Anti-inflammatory potency of *E. corinthium* total extract and its fatty acids fraction (100 mg/kg)

Group	AUC	% inhibition
Control	953 ± 56.31	0%
Diclofenac	441.67 ± 20.43*b	54%
Dexamethasone	229.12 ± 14.48*a	76%
E. corinthium total alcoholic extract	429.35 ± 13.25*b	55%
E. corinthium fatty acids fraction	460.50 ± 17.35*b	52%

*Significantly different from control group.
[a]Significantly different from diclofenac group.
[b]Significantly different from dexamethasone group

1067 and 1024. EI-MS: *m/z* (relative int. %): 574 (M^+; 2.4), 414 (M^+ - sugar, 4), 397 (79), 394 (2), 382 (3), 329 (2), 303 (4), 273 (2), 255 (9), 213 (13), 161 (11), 145 (80), 133 (9), 97 (9), 95 (55), 83 (7), 57 (97) and 55 (100).

Acid hydrolysis afforded one spot identical with β-sitosterol (Co-TLC) and glucose as sugar moiety[27]. The IR data are matched with that reported before[28]. This compound was assigned to be β-sitosterol-*O*-glucoside.

Compound 6: Yellow granules (MeOH, 9 mg) with R_f 0.54 (Ethyl acetate-acetic acid-formic acid-H_2O, 100: 11:11:27). IR (KBr) cm^{-1}: 3407, 2925, 2872, 1656, 1612, 1184, 1097 and 878.

^{1}H-NMR (DMSO-*δ*6): δ 6.19 (1H, *d*, *J* = 2 Hz, H-6), 6.39 (1H, *d*, *J* = 2 Hz, H-8), 6.84 (1H, *d*, *J* = 8.8 Hz, H-5'), 7.57 (1H, *J* = 2 Hz, H-2'), 7.58 (1H, *J* = 2 Hz, H-6'), 5.46 (1H, *d*, *J* = 7.6 Hz, H-1"), 12.64 (1H, *s*, C5-OH) and 3.08-3.24 (sugar protons).

The obtained data are identical with those published for quercetin-3-*O*-β-D-glucoside[12,29,30]. Acid hydrolysis afforded one spot identical with quercetin and glucose as sugar moiety. This was supported by direct comparison (Co-TLC) with authentic quercetin-3-*O*-β-D-glucoside. This compound was reported before in *C. cheiri* and in other Brassicaceae plants[1,31,32].

Compound 7: Yellow crystals (28.0 mg), mp 189-190ºC and R_f 0.33 (ethyl acetate-acetic acid-formic acid-H_2O, 100:11:11:27).

IR (KBr) cm^{-1}: 3427, 2928, 2872, 1649, 1612, 1184, 1093 and 872.

^{1}H-NMR (400 MHz, DMSO- δ6): δ ppm 6.19 (1H, d, *J* = 1.6 Hz, H- 6), 6.38 (1H, d, *J* = 1.6 Hz, H-8), 6.83 (1H, d, *J* = 8 Hz, H-5'), 7.53 (1H, s, H-2'), 7.55 (1H, d, *J* = 2 Hz, H-6'), 5.34 (1H, d, *J* = 7.50 Hz, H-1"), 5.09 (1H, broad s, H-1'"), 12.59 (1H, *s*, C5-OH), 3.7 (1H d, *J* = 10.4 Hz, H-6"), 3.21 -3.29 (1H m, H-6"), 3.21 -3.29 (6H, m, sugar protons), 3.05-3.09 (2H, tr, sugar protons) and 0.99 (3H, d, *J* = 6 Hz, H-6'").

^{13}C-NMR (100 MHz, DMSO-δ6): δ ppm 156.43 (C-2), 133.30 (C-3), 177.37 (C-4), 161.22 (C-5), 98.7 (C-6), 164.13 (C-7), 93.6 (C-8), 156.61 (C-9),103.95 (C-10), 121.6 (C-1'), 116.26 (C-2'), 144.76 (C-3'), 148.43 (C-4'), 115.23 (C-5'), 121.18 (C-6'), 101.19 (C-1"), 74.08 (C-2"), 75.91 (C-3"), 68.25 (C-4"), 76.45 (C-5"), 67 (C-6"), 100.75

(C-1'''), 70.56 (C-2'''), 70.01 (C-3'''), 70.38 (C-4'''), 71.85 (C-5''') and 17.74 (C-6''').

^{1}H-NMR and ^{13}C-NMR data are matching with those reported for rutin[12,33,34].

Acid hydrolysis afforded one spot identical with quercetin and two sugars identical with rhamnose and glucose (Co-TLC). This was confirmed by direct comparison (Co-TLC, mp) with authentic rutin. In a previous report, rutin was isolated from the seeds of *E. cheiranthoides* L[35].

Several species within the family *Brassicaceae* were surveyed for their flavonoid profiles. In these studies, flavonol glycosides were the only flavonoids present in leaves and flowers of the genera *Brassica* and *Sinapis*. In a chemosystematic survey on wild *Brassica* relatives, 21 different flavonoid glycosides, all based on the flavonol skeleton were identified[36]. Moreover, quercetin derivatives were reported for other species e.g. *Diplotaxis erucoides, Eruca sativa* and *Diplotaxis tenuifolia*[37,38]. According to the available literature, this is the first report on isolation of compounds 1-7 from *E. corinthium*.

HPLC determination of phenolics

Phenolic compounds are a large group of phytochemicals widespread in the plant kingdom. Depending on their structure they are classified into simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids. Phenolic compounds have received considerable attention for being potentially protective agents against cancer and heart diseases, in part because of their potent antioxidant properties and their ubiquity in a wide range of commonly consumed foods of plant origin.

The total alcohol extract of the plant and the ethyl acetate fraction were separately subjected to HPLC analysis against reference compounds. The compounds were identified by comparing the retention time of their peaks and authentics of phenolics injected under the same conditions of the experiment. The results were recorded in Table (2). The results indicated that pyrogallol is the major compound for ethyl acetate fraction while epicatechein is the major one for total extract.

A review for the occurrence of phenolic acids in Brassicaceae stated that ferulic, coumaric and other hydroxyl cinnamic acid derivatives are common in vegetable *Brassica* crops[1]. Vanillic, protocatechuic, syringic, *p*-coumaric, ferulic, caffeic and chlorogenic acids were reported in rapseed[39].

It is worthy to note that some peaks cannot be identified due to the limited number of available authentics. These results are consistent with another studies carried out on some plants belonging to Brassicaceae. Chlorogenic acid was found to be the major phenolic compound in leafy Brassica species like kale, cabbage and brussels sprouts[40]. It is also found in Brassica vegetables e.g. cauliflower in addition to *Lepidium sativum* L., *Cronopus didyma* L., *Capsella bursa-patoris* Moench, *Raphanus sativus* L., *Cardamine africana* L. and *Alyssum montanum* L[41]. Gallic, ferulic, chlorogenic, p-coumaric and caffeic acids were also reported in cauliflower and broccoli[42]. Moreover, many other phenolic acids like gallic, protocatechuic, p-hydroxybenzoic, vanillic, syringic, salicylic, p-coumaric, caffeic, ferulic and sinapic acids were detected as major compounds in kale[43].

Antioxidant activity

The ethyl acetate extract of the plant possessed a strong antioxidant activity against DPPH radicals as concluded from its low SC_{50} value (0.95 µg/ml) compared with the standard antioxidant activity of ascorbic acid (SC_{50} 1.45 µg/ml). Antioxidant activity of ethyl acetate fraction is highly correlated to its contents of flavonoids and phenolic acids. In a previous study, the high antioxidant activity of cabbage samples collected from different regions of Europe was attributed mainly to its phenolic contents[44]. It was reported that quercetin-3-*O*-β-D-glucoside and rutin exhibited strong antioxidant activity when tested by DPPH assay with SC_{50} values 0.011 and 0.016 mM respectively[12]. In another study, rutin showed DPPH radical scavenging activity of 33%[34].

Antimicrobial activity

The interest in natural plant products as antimicrobials is supported by the necessity to reduce the use of conventional antibiotics in food preservation and to overcome the emergence of antibiotic resistance in bacterial pathogens[45].

The antimicrobial activities of petroleum ether, chloroform and ethyl acetate fractions are given in Table (3). All tested fractions showed inhibition of growth for the tested organisms, except *Aspergillus flavus*, which means that *E. corinthium* has the ability to inhibit the growth of Gram-positive and Gram-negative bacteria, as well as fungi.

It was reported that glucosinolates hydrolysis products of leaves, seeds and roots of *E. corinthium* demonstrated antimicrobial activity against the same microorganisms used in the current study which was attributed mainly to isothiocyanates, nitriles and other volatiles[9].

Phenolic fractions extracted from kale leaves (*B. oleracea*), rich in quercetin and kaempferol derivatives, significantly inhibited the growth of Gram-positive bacteria *S. aureus* and *B. subtilis*, which are known as major respiratory pathogen in humans[43].

The antimicrobial activity of ethyl acetate fraction is related to its flavonoids and phenolic acids contents. Rutin, which has both anti-arthritic and antifungal effects, could safely be administered into the blood circulation for treatment of septic arthritis caused by *C. albicans*[46]. It was reported that caffeic acid, p-coumaric acid and rutin inhibited the development of the isolated food contaminant *S. aureus*[47].

α-Amyrin (a major compound of petroleum ether fraction), which was identified in several plants had shown anti-microbial and anti-inflammatory activities[48].

Anti-inflammatory activity

As shown in figure 2 and table (4), the total plant extract and the fatty acids fraction at a dose of 100 mg/kg significantly decreased the rats hind paw edema compared to the control group. In addition, the results obtained from AUC calculation showed that the potency of the anti-inflammatory activity of the extract (100 mg/kg) is biologically equivalent to that of Diclofenac sodium at a dose of 4.0 mg/kg (Table 4).

Linolenic acid has the capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism and could be one of the compounds responsible for the antiinflammatory activity of petroleum ether fraction[49,50]. Pharmacological studies of triterpenoids indicated their anti-inflammatory activity in addition to, anticancer, anti-ulcerogenic, antimicrobial, antiviral (including anti-HIV), analgesic, antioxidative, hepatoprotective and other activities[51]. So, the presence of lupeol acetate, lupeol and α-amyrin in petroleum ether fraction potentiate the anti-inflammatory activity.

β-sitosterol isolated from the petroleum ether fraction was reported as a potent antiinflammatory, anti-pyretic, antioxidant and anti-ulcer compound. Moreover, it showed a positive effect on male hair loss, prostatic carcinoma and breast cancer. In Europe, β-sitosterol is usually prescribed for benign prostatic hypertrophy[21]

CONCLUSION

Chromatographic investigation and spectroscopic analysis of *E. corinthium* aerial part afforded isolation and characterization of lupeol acetate, lupeol, α-amyrine, β-sitosterol, β-sitosterol-*O*-glucoside, quercetin-3-*O*-β-D-glucoside and rutin for the first time from this plant in addition to identification of 17 phenolic acids. The plant showed significant antioxidant, antimicrobial and anti-inflammatory activities.

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