Phytochemical investigation of *Corchorus olitorius* and *Corchorus capsularis* (Family Tiliaceae) that grow in Egypt

Amal Z. Hassan[a], Maged K.G. Mekhael[a], Atef G. Hanna[a], András Simon[b], Gábor Tóth[b], Helmut Duddeck[c]

[a]Department of Chemistry of Natural Compounds, National Research Centre, Cairo, Egypt, [b]Institute of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Budapest, Hungary, [c]Institute of Organic Chemistry, Leibniz University Hannover, Hannover, Germany

Correspondence to Dr. Maged K.G. Mekhael, Department of Chemistry of Natural Compounds, National Research Centre, 33 El Buhouth Street, Dokki, Cairo 12622, Egypt.
Tel: +20 233 370 933; fax: +20 333 70931; e-mail: magedkgm@hotmail.com

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Background and objective
Corchorus (Family Tiliaceae) is a genus of annual herbs. Nearly 40 species are known to occur in nature and distributed in the tropics of both hemispheres. Because of the wide medicinal applications of compounds isolated thereof, the present investigation deals with the isolation and structure elucidation of some phytochemicals from *Corchorus olitorius* (molokheya) and *Corchorus capsularis* that grow in Egypt.
Materials and methods
Phytochemical investigation of the seeds and different plant organs of both *C. olitorius* and *C. capsularis* was achieved applying different separation techniques. Petroleum ether extraction followed by saponification of the extract led to the isolation of phytosterols, hydrocarbons and fatty acids. Essential oils were obtained from the leaves by extraction with methylene chloride. Methanolic extraction led to the isolation of cardiac glycosides. Identification of isolated compounds was realized through R_f values, shift reagents and spectroscopic tools such as ultraviolet and nuclear magnetic resonance. The fatty acids were identified using gas liquid chromatography.
Results and conclusion
A study of the lipid contents (fatty acids, phytosterols and hydrocarbon components) of seeds, roots, leaves and stems of *C. olitorius* as well as the seeds and vegetative part of *C. capsularis*, which grow locally in Egypt, was carried out. The identification of the lipid content was achieved by comparing the retention time of their peaks in gas liquid chromatography with those of authentic samples. Gas chromatography/mass spectrometry study of the chemical constituents of the essential oils of the leaves of *C. olitorius* and *C. capsularis* led to the identification of 11 and 21 compounds with a total concentration of 24.7 and 62.9%, respectively. Cedrane-5-one (17.7%) and γ-terpinene (12.1%) represent the major compounds in each plant, respectively. Phytochemical investigation of *C. olitorius* led to the isolation of raffinose **I**, coroloside **II**, glucoevatromonoside **III**, erysimoside **IV** and olitoriside **V** and gluco-olitoriside **VI**. Meanwhile, the study of the vegetative parts of *C. capsularis* led to the isolation of 3-*O*-glucopyranosyl-β-sitosterol **VII**. The isolated compounds were identified by spectral tools (hydrogen-1, carbon-13-nuclear magnetic resonance, electron ionization mass spectrometer).

Keywords:
cardiac glycosides, *Corchorus capsularis*, *Corchorus olitorius*, essential oils, fatty acids, hydrocarbons, phytosterols

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Introduction

Plants containing cardiac glycosides have been of great demand since a long time. Efforts are constantly directed to discover and introduce cardiotonic drugs in therapy [1]. The importance of the genus *Corchorus* (family Tiliaceae) arises from its economic importance, as well as being a good source of a wide range of cardiac glycosides [2]. Several workers reported the isolation of products such as corchorin, corchorgenin, capsularin, corchoritin, olitoriside and corchortoxin from the seeds of *Corchorus olitorius* [3]. The occurrence of corchorin was reported decades ago [4] and was revealed to be identical with strophanthidin [5]. Several cardiac glycosides have been isolated from *C. olitorius* by several research groups [6–9].

Olitoriside was isolated from the seeds of *C. olitorius* [10], whereas corchoroside A was isolated from the seeds of *Corchorus capsularis* [11]. Moreover, olitoriside, gluco-(1→6) olitoriside, olitoriusin and erysimoside were isolated from the seeds of *C. olitorius*, and their

structure was elucidated by fast atom bombardment mass spectrometry, carbon-13-nuclear magnetic resonance (^{13}C-NMR) and some chemical transformations [5]. The isolation of the glycosides coroloside, glucoevatromonoside, deglucoroloside and corchorusosides A, B, C, D and E from the seeds of *C. olitorius* was also reported [12,13].

The roots of both *C. capsularis* and *C. olitorius* were reported to contain three ursane triterpenes namely corosin, corosolic acid and ursolic acid [14].

The volatile components of the natural fresh leaves and cupric chloride-treated leaves of *C. olitorius* were prepared and subjected to gas chromatography/mass spectrometry (GC-MS) analysis [15]. A total of 45 and 49 components were identified in the control and treated leaves, respectively. *cis*-3-Hexen-1-ol, *cis*-4-hexen-1-ol, terpinolene, sabinene and phytol were found to be the major compounds in the control leaves, whereas those of the treated leaves were found to be *cis*-4-hexen-1-ol, *cis*-3-hexen-1-ol, tetradecanal and phytol [15,16].

C. olitorius and *C. capsularis* are widely cultivated for their fibre [17]. Fatty acid methyl esters of the leaves of *C. olitorius* are rich in ω3-octadecatriene fatty acid or α-linolenic acid (49%) [18]. Oleic acid, palmitic acid, stearic acid, behenic acid, linoleic acid and linolenic acid were found in seeds of *C. olitorius,* wherein linolenic acid was in the range of 1.82–1.90 mg/g. The physicochemical properties and fatty acid components of *C. olitorius* seeds oil were also studied [19–22]. Four higher fatty acids, corchorifatty acids A, B, C, D, E and F, an undecanoic acid and trihydroxy fatty acid were isolated from the less polar fraction of molokheya [23].

Certain phenolic compounds isolated from *C. olitorius* jute showed great antioxidation, inflammatory inhibition and cytotoxic activities [24,25]. Moreover, new flavonol glycosides were isolated from the leaves of *C. olitorius;* corchorusides A and B exhibited α-glucosidase inhibitor activity [26]. Dry oils were isolated from the leaves and stem of *C. olitorius* [27].

C. olitorius exhibited diverse biological activities including: antioxidant [28], antitumor [29], hypoglycaemia [30], antimicrobial [31], anti-inflammatory, analgesic [32], antiobesity [33], gastroprotective [34] and wound healing effects [35].

Accordingly, the objectives of this study were as follows:

(1) To compare between fatty acids, sterols and hydrocarbons of different organs of both *C. olitorius* and *C. capsularis* using gas liquid chromatography (GLC).
(2) To examine the essential oil of the leaves of both species using GC-MS.
(3) To identify the secondary metabolites of the seeds of *C. olitorius* and the vegetative part of *C. capsularis* that grow in Egypt.
(4) To elucidate the structure of the isolated compounds using spectroscopic methods (^{1}H-NMR and ^{13}C-NMR).

To our knowledge, no previous papers were published on the comparison between fatty acids, sterols and hydrocarbons and essential oils of both *C. olitorius* and *C. capsularis* that grow locally. Therefore, this study is of deemed interest.

Materials and methods

General instrumental procedures

Melting point was determined using electrothermal melting point apparatus, uncorrected. Ultraviolet (UV) was measured using Shimadzu UV-visible spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance DRX-500 Spectrophotometer (Bruker, Germany) and Varian-500 Spectrophotometer (Agilent, California, USA). Electron ionization mass spectrometer spectra were taken on HP; MS-5988 (Hewlett Packard, California, USA). GLC was performed on Hewlett Packard-HP 6890 series (Hewlett Packard, California, USA), and GC-MS were recorded on Trace GC 2000 (Thermo Quest Corporation, Milan, Italy) and Finnigan SSQ 7000 MS by Thermo Scientific (Finnigan Corporation), Waltham, USA.

Plant material

Seeds, leaves, stems and roots of *C. olitoris* were bought from the local market. The seeds, leaves and stems of *C. capsularis* were collected from the gardens of National Research Centre. The plants were identified by Dr M. El Gibaly at the Plant Taxonomy Department, National Research Centre, Cairo, Egypt. Voucher specimens are deposited at the Herbarium of National Research Centre, Dokki, Cairo, Egypt. Authentic Reference Material were available at the Department of Chemistry of Natural Compounds, National Research Centre, Cairo, Egypt.

Phytosterols and hydrocarbons

The dry powder (100 g) of each of the seeds, roots, leaves and stems of *C. olitorius* and the seeds and

vegetative part of *C. capsularis* was exhaustively extracted with petroleum ether (40–60°C) at room temperature to afford oily extracts. Each of the oily extracts was treated with 50 ml of 10% alcoholic KOH for 2 h; 50 ml water was added, and the solution was extracted with chloroform. The combined organic phase was washed with water until alkali free and dried over anhydrous Na_2SO_4. The solvent was distilled off to give the unsaponified fraction. The identification of the sterols content was achieved by comparing the retention time of their peaks with those of authentic material (Table 1).

Fatty acids fraction

The aqueous alkaline solution remaining after the separation of the unsaponifiable matter was acidified with dilute HCl. The liberated fatty acids were exhaustively extracted with chloroform several times. The combined organic phase was washed with water until it was neutral to litmus paper. The chloroform layer was dried over anhydrous Na_2SO_4, and the solvent was distilled off to give the fatty acid fraction.

The fatty acids were identified through their methyl esters using GLC. The isolated fatty acids were methylated by refluxing in absolute methanol (15 ml) containing 5% sulphuric acid (0.5 ml) for about 1 h on a water bath. The solution, after cooling, was diluted with water and exhaustively extracted with chloroform. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was distilled off. The fatty acids were determined by a comparative study of their methyl esters with authentic samples (Table 2).

Essential oils of the leaves of *Corchorus olitorius* and *Corchorus capsularis*

The fresh leaves (100 g) of *C. olitorius* and *C. capsularis* were exhaustively extracted with methylene chloride (500 ml) at room temperature. The solvent was distilled off under vacuum at a temperature not exceeding 30°C. The residue was extracted with diethyl ether, followed by drying over anhydrous sodium sulphate, and the solvent was left to evaporate at room temperature. The essential oil components were identified using GC-MS analysis. The results are presented in Tables 3 and 4.

Isolation of phytochemicals from *Corchorus olitorius* seeds

The ground seeds of *C. olitorius* (5 kg) were exhaustively extracted with 80% methanol (25 l) at room temperature and then filtered off. The residual mass was washed with 80% methanol (2.5 l) until free from cardiac glycosides (negative Kedde reagent) [36]. The combined methanolic extract was concentrated to 2 l under reduced pressure at 50°C and left overnight at room temperature. The white precipitate obtained was filtered off and dried to give 150 g of white precipitate. It was crystallized from methanol and, finally, from methanol/ethyl acetate to give 15 g of compound **I** (melting point 90°C).

The mother liquor was exhaustively extracted with chloroform (1 l). The solvent was distilled off under reduced pressure to give 250 g of cardiac glycoside mixture. Two hundred grams of the glycosidic mixture was resolved on column chromatography packed with Silica gel 60 (Merck, Germany) (2000 g, 80 cm×15 cm). It was eluted, at first, with benzene followed by addition of increasing proportions of methanol during the course of fractionation. The collected fractions (250 ml each) were followed-up by thin-layer chromatography using ethyl acetate/methanol/water (16 : 1 : 1, 16 : 2 : 1 and 16 : 3 : 1) as developing systems. The cardiac glycoside compounds were detected using Kedde spraying reagent [36].

The fractions obtained by benzene/methanol (80 : 20) were combined, and the residue (1.5 g) was

Table 1 Gas liquid chromatography of sterols and hydrocarbons of *Corchorus olitorius* and *Corchorus capsularis*

Compounds	Room temperature	*Corchorus olitorius*				*Corchorus capsularis*	
		Seeds (%)	Roots (%)	Stems (%)	Leaves (%)	Seeds (%)	Vegetative part (%)
Tetradecane	18.87	33.58	22.79	15.80	13.57	–	–
Hexadecane	21.65	30.42	7.27	7.76	10.05	16.49	8.70
Heptadecane	23.33	33.07	–	7.06	–	–	10.33
Octadecane	24.96	–	55.54	13.81	5.12	45.13	20.66
Nonadecane	27.51	1.91	–	–	9.76	9.87	7.45
Eicosane	28.58	–	–	–	15.24	–	–
Cholesterol	32.84	0.88	9.92	14.62	19.28	28.52	12.46
Campasterol	33.85	–	–	–	–	–	24.98
β-Sitosterol	36.09	–	–	8.39	7.62	–	10.39
Stigmasterol	37.32	–	–	9.37	–	–	4.38
β-Amyrin	41.80	0.86	4.49	23.18	1.93	–	–

Table 2 Gas liquid chromatography of fatty acids of *Corchorus olitorius* and *Corchorus capsularis*

Compounds	Room temperature	*Corchorus olitorius*				*Corchorus capsularis*	
		Seeds (%)	Roots (%)	Stems (%)	Leaves (%)	Seeds (%)	Vegetative part (%)
Palmitic acid (16 : 0)	33.39	40.60	14.90	59.94	32.94	81.68	54.10
Heptadecanoic acid (17 : 0)	36.12	–	–	5.52	4.85	–	–
Stearic acid (18 : 0)	37.94	49.48	68.8	27.84	58.67	–	10.66
Oleic acid (18 : 1)	38.47	9.93	6.22	–	–	11.51	10.96
Linoleic acid (18 : 2)	39.64	–	6.14	6.70	3.54	0.79	9.42
Arachidic acid (20 : 0)	42.55	–	3.94	–	–	6.02	14.86

Table 3 Gas liquid chromatography/mass spectrometry of essential oils from the leaves of *Corchorus olitorius*[a]

Compounds	Room temperature	Concentration (%)
trans–*cis*-Farnesol	20.52	0.44
Cedran-5-one	25.16	17.77
Carvacrol methyl ether	28.48	0.34
Piperonal	29.05	0.63
l-Menthone	30.06	0.23
Isobutyl salicylate	31.49	0.16
Ethyl salicylate	32.12	0.27
trans-Phytol	33.69	0.99
cis-β-dihydroterpineol	38.28	0.40
trans-β-dihydroterpineol	38.61	0.80
Methyl tiglate	46.47	2.73

[a]Twenty-nine compounds corresponding to 75.2%, not identified.

Table 4 Gas liquid chromatography/mass spectrometry of essential oils from the leaves of *Corchorus capsularis*[a]

Compounds	Room temperature	Concentration (%)
α-Pinene	6.87	0.80
Sabinene	7.99	7.51
p-Cymene	10.03	0.93
γ-Terpinene	10.81	12.19
trans-Carveol	15.27	0.79
trans-Carvyl acetate	15.49	0.46
Estragole	16.65	5.33
Umbellulone	17.82	8.86
Cedran-5-one	22.37	0.65
δ-Elemene	28.39	0.29
Neocedran-5-ol	29.22	0.24
cis-Methyl isoeugenol	30.49	0.41
Tetrahydrolinalool	33.32	0.80
Terpene-1-ol	34.23	3.15
Myrtenal	34.39	6.78
cis-Verbenol	39.25	0.24
Isomenthyl acetate	39.64	0.30
Artemisyl acetate	40.45	0.36
Carvacrol methyl ether	41.37	10.01
E-Ocimenone	42.58	0.43
Piperonal	44.76	2.44

[a]Nineteen compounds corresponding to 37.03% not identified.

rechromatographed on a column of Silica gel 60 (300 g, 50 cm×3 cm). It was eluted, first, with chloroform, followed by addition of increasing proportions of methanol during the course of elution. The chloroform/methanol (9 : 1) fractions were collected, the solvent was distilled off and the residue was precipitated from methanol/diethyl ether to give 10 mg of compounds **II** and **III**.

The benzene/methanol (70 : 30) fractions were combined, and the residue (2.5 g) was rechromatographed on Silica gel 60 column (400 g, 80 cm×3 cm). It was eluted, first, with chloroform, followed by adding increasing proportions of methanol during the course of elution. The chloroform/methanol (8 : 2) fractions were combined, and the solvent was distilled off. The residue (1 g) was purified on Sephadex LH-20 (Merck, Germany) (400 g, 80 cm×3 cm) using methanol (800 ml) as an eluent to give 50 mg of compounds **IV** and **V**.

The benzene/methanol (60 : 40) fractions were combined and the residue (3 g) was rechromatographed on Silica gel 60 (400 g, 80 cm×3 cm) using chloroform as an eluent, followed by increasing methanol proportions during the course of elution. The residue obtained from chloroform/methanol fractions (8.5 : 1.5) was crystallized several times from methanol/diethyl ether to give 15 mg of compound **VI**.

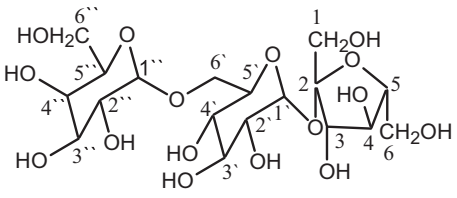
Compound I

It was identified as raffinose (galactose–glucose–fructose) (**I**, Fig. 1) from its ^{1}H and ^{13}C-chemical shift values (Table 5).

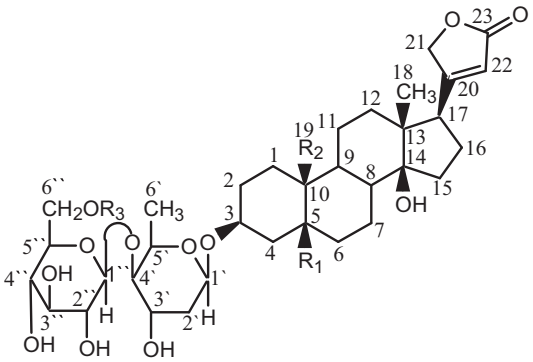
Compounds II and III

^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra showed that the mixture was composed of two compounds, ca 2 : 1. It was having the same glycone and differed only in the stereochemistry at C-4 of the first sugar. The mixture could not be detected by using the available laboratory chromatographic tools. It was found that it was composed of coroloside (**II**) as the major compound and glucose-evatromonoside (**III**) as the minor compound. The structure of both compounds was established using the NMR experiments [one-dimensional ^{1}H-NMR and ^{13}C-NMR, DEPT, z-

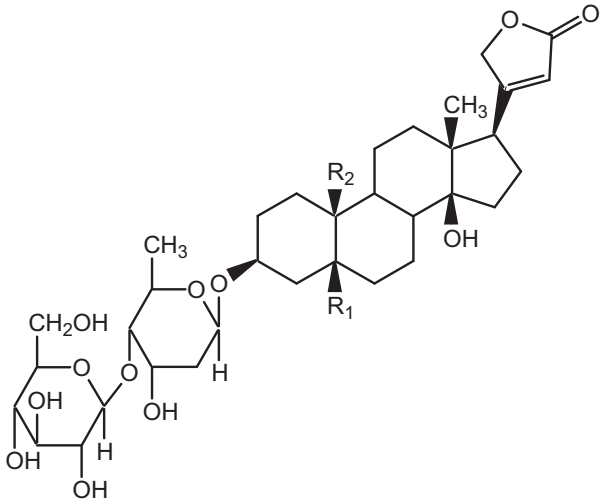
Figure 1



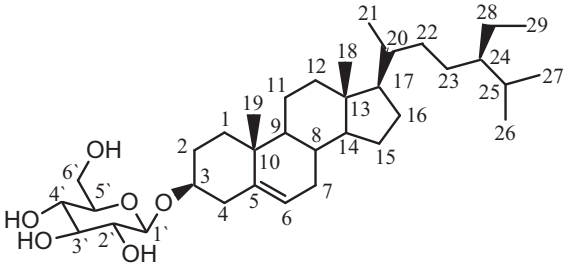
I Raffinose



II Coroloside $R_1 = H, R_2 = CH_3, R_3 = H$
V Olitoriside $R_1 = OH, R_2 = CHO, R_3 = H$
VI Gluco-olitoriside $R_1 = OH, R_2 = CHO, R_3 = \beta$-D-glucopyranosyl



III Gluco-evatromonoside $R_1 = H, R_2 = CH_3$
IV Erysimoside $R_1 = OH, R_2 = CHO$



VII 3-*O*-β-D-Glucopyranosyl-β-sitosterol

Structure of some isolated compounds from Corchorus olitorius and Corchorus capsularis and C-atoms' numbering used in their nuclear magnetic resonance structure elucidation.

Table 5 ^{1}H-nuclear magnetic resonance and ^{13}C-nuclear magnetic resonance chemical shifts of raffinose I

Fructose	δ ^{13}C	δ ^{1}H	Glucose	δ ^{13}C	δ ^{1}H	Galactose	δ ^{13}C	δ ^{1}H
1-CH_2OH	64.32	3.62	1′ β	93.45	5.41 *d*	1′′ β	100.59	4.895 *d*
2	105.31	–	2′ α	73.10	3.45 *d-d*	2′′ α	83.49	3.77–3.75[a]
3	75.38	4.10	3′ α	74.48	3.72	3′′ α	79.25	4.1
4	70.53	3.79–3.76[a]	4′ α	72.03	3.32	4′′ β	71.07	3.90
5	71.47	3.8–3.75[a]	5′ α	73.32	4.07 *m*	5′′ α	72.46	3.86
6-CH_2OH	62.78	3.737–3.69[a]	6′ a	68.32	3.90 *d-d*	6′′ a	63.15	3.8–3.75[a]
			b		3.75 *d-d*	b		
			$J_{1',2'}$=3.8 Hz, $J_{2',3'}$=9.8 Hz, $J_{3',4'}$=$J_{4',5'}$=9.5 Hz					

[a]Overlapped signals.

total correlation spectroscopy (z-TOCSY) and two-dimensional ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and ^{1}H–^{13}C-heteronuclear multiple bond correlation (HMBC)] (Tables 6–8).

Compounds IV and V

^{13}C-NMR and DEPT spectra showed that the mixture is composed of two compounds (ca 2 : 1) having the same glycone and differing only in the stereochemistry at C-4 of the first sugar. The

Table 6 ^{1}H-nuclear magnetic resonance and ^{13}C-nuclear magnetic resonance chemical shifts of the aglycone moieties

	Coroloside II and glucoevatromonoside III		Erysimoside IV and olitoriside V	Gluco-olitoriside VI	
	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{13}C	δ ^{1}H
1	33.50	α 2.19, β 1.73	24.2	25.30	α 2.14, β 1.32
2	28.30	2.18	25.3	26.00	α 1.60, β 1.96
3	74.43	4.05	74.96	76.50	α 4.18
4	31.11	α 1.83, β 1.47	35.70	37.00	α 1.58, β 2.18
5	38.09	1.95	75.34	75.40	–
6	27.80	1.6–1.7 *m*	39.00	37.30	α 1.63, β 2.10
7	22.30	α 1.29, β 1.45	19.00	19.00	α 2.13, β 1.69
8	42.82	1.65	42.70	42.70	β 1.96
9	37.00	1.73	40.30	40.50	α 1.71
10	36.44	–	56.20	56.30	–
11	22.40	α 1.32, β 1.79	23.40	23.40	α 1.53, β 1.21
12	41.90	1.50	40.15	40.60	α 1.45, β 1.49
13	51.18	–	50.80	50.30	–
14	86.56	–	86.04	86.10	–
15	31.48	1.48	32.50	32.50	α 2.14, β 1.69
16	28.00	α 1.28, β 1.90	28.00	28.00	α 2.17, β 1.89
17	52.25	2.82	51.85	51.90	α 2.89
18	16.40	0.89	16.25	16.30	0.85
19	24.00	0.96	210.05	210.00	10.06
20	178.57	–	178.30	178.30	–
21	75.46	a 5.04, b 4.90	75.42	75.40	a 5.02, b 4.91
22	117.88	5.90	118.02	118.00	5.90
23	177.37	–	178.26	177.30	–

Table 7 ^{13}C-nuclear magnetic resonance chemical shifts of the glycone moieties

No.	Coroloside II Boivinose	Glucoevatromonoside III Digitose	Erysimoside IV Digitose	Olitoriside V Boivinose	Gluco-olitoriside VI Boivinose
1′	97.69	96.96	98.33	99.22	99.20
2′	35.36	39.17	38.10	37.80	35.20
3′	66.69	68.65	68.48	66.71	66.70
4′	76.03	84.34	84.08	76.04	76.00
5′	70.16	69.75	69.88	70.30	70.30
6′	17.50	18.80	18.65	17.31	17.50
	Glucose	Glucose	Glucose	Glucose	Glucose
1″	102.30	105.88	105.88	102.57	102.50
2″	75.03	74.59	75.17	74.96	74.90
3″	78.20	77.83	78.21	78.01	78.10
4″	71.98	71.26	71.24	71.94	71.70
5″	77.98	75.18	78.02	77.50	77.50
6″	63.09	62.39	62.29	63.02	70.50
					Glucose
1‴	–	–	–	–	105.20
2‴	–	–	–	–	75.30
3‴	–	–	–	–	78.10
4‴	–	–	–	–	72.00
5‴	–	–	–	–	78.00
6‴	–	–	–	–	62.90

mixture is composed of erysimoside (**IV**) as the major compound and olitoriside (**V**) as the minor one. The structure of each compound was established using NMR experiments (one-dimensional ^{1}H-NMR, ^{13}C-NMR and DEPT, two-dimensional HMQC and HMBC) (Tables 6–8).

Compound VI

It was identified as gluco-(1→6)-olitoriside (**VI**) from its NMR spectroscopic measurements (one-dimensional ^{1}H-NMR, ^{13}C-NMR and DEPT, selective TOCSY and two-dimensional ^{1}H–^{1}H-COSY, rotating-frame overhauser spectroscopy, HSQC–TOCSY, HSQC and ^{1}H–^{13}C-HMBC) (Tables 6–8).

Table 8 ^{1}H-nuclear magnetic resonance chemical shifts of the glycone moieties

No.	Coroloside II Boivinose	Glucoevatromonoside III Digitose	Gluco-olitoriside VI Boivinose
1′	4.88	4.91	4.90
2′	α 1.93, β 1.68	[a]	α 1.70, β 1.95
3′	4.16	4.30	4.15
4′	3.48	3.30	3.49
5′	4.04	3.88	4.06
6′	1.24	1.30	1.26
	Glucose	Glucose	Glucose
1″	4.31	4.37	4.30
2″	3.23	3.38	3.22
3″	[a]	[a]	3.35
4″	3.29	3.37	3.30
5″	[a]	[a]	3.46
6″	a 3.88, b 3.67	a 3.83, b 3.10	a 4.14, b 3.77
			Glucose
1‴	–	–	4.41
2‴	–	–	3.20
3‴	–	–	3.34
4‴	–	–	3.29
5‴	–	–	3.24
6‴	–	–	a 3.87, b 3.67

Boivinose: $J_{1,2\alpha}$=9.7; $J_{1,2\beta}$=2.2; $^{2}J_{2\alpha,2\beta}$=12.5; $J_{2\alpha,3}=J_{2\beta,3}=J_{3,4}$=3; $J_{4,5}$=1.6; $J_{5,6}$=6.5 Hz. Glucose: $J_{1,2}$=7.8; $J_{2,3}=J_{3,4}=J_{4,5}$=9.5; $J_{5,6}$=2.0; $J_{5,6b}$=6.6; $^{2}J_{6a,6b}$=12.0 Hz. [a]Overlapped signals.

Isolation of a glucositosterol from *Corchorus capsularis*

The air-dried vegetative part of *C. capsularis* (500 g) was extracted with 80% methanol (2 l) at room temperature. The methanolic extract was concentrated under vacuum at a temperature not exceeding 40°C. The remaining aqueous extract (500 ml) was extracted with chloroform (500 ml), and the solvent was distilled off to give an oily residue (2.5 g). It was resolved on silica gel column (200 g, 70 cm×1.5 cm). The column was eluted at first with benzene, followed by a gradual increasing in ethyl acetate proportions during the course of fractionation. The fractions (50 ml each) were monitored by thin-layer chromatography, and similar fractions were combined. Benzene/ethyl acetate fractions (1 : 9) gave 0.2 g of crude compound **VII**. It was crystallized from methanol to yield 25 mg with a melting point of 265°C. It was identified as 3-*O*-β-d-glucopyranosyl-β-sitosterol (**VII**) from its mass spectrometry (MS), infrared (IR) and NMR spectroscopic measurements [one-dimensional ^{1}H, attached proton test (APT) and two-dimensional ^{1}H–^{1}H-COSY, HMQS and HMBC] (Table 9).

Results and discussion

Phytosterols and hydrocarbons

The finely powdered seeds, roots, leaves and stems of *C. olitorius*, as well as the seeds and vegetative part of *C. capsularis* were separately extracted with petroleum ether at room temperature to give oily substances. Each oil fraction was separately saponified [37]. The total sterol contents were found to be 1.8, 0.6, 1.6 and 1.4% (dry weight basis) in the seeds, roots, leaves and stems of *C. olitorius* and 0.05 and 0.12 (dry weight bases) in the seeds and vegetative part of *C. capsularis*, respectively.

The identification of the sterols' content was achieved by comparing the retention time of their peaks in GLC with those of authentic samples (Table 1).

GLC of the unsaponifiable fractions of the investigated parts showed that the seeds and roots contain mainly cholesterol, in addition to the triterpenoid compound β-amyrin. In the leaves, cholesterol, β-sitosterol and β-amyrin were detected. In the stems, cholesterol, β-sitosterol, stigmasterol and β-amyrin were present (Table 1). It was noticed that cholesterol represents the major compound in the leaves (19.28%), stems (14.62%), roots (9.92%) and seeds (0.88%), whereas the triterpenoid compound, β-amyrin, represents the major compound in stems (23.18%). β-Sitosterol is present only in stems (8.39%) and leaves (7.62%). In contrast, stigmasterol is present only in the stems (9.37%) (Table 1).

Estimation of hydrocarbon contents showed that octadecane is the major compound in the roots (55.54%), followed by the stems (13.81%) and leaves (5.12%). A large quantity of tetradecane was present in the seeds (33.58%), followed by the roots (22.79%), the

Table 9 ^{1}H and ^{13}C chemical shifts of 3-*O*-β-d-glucopyranosyl-β-sitosterol VII

Carbon	δ ^{13}C	δ ^{1}H
1	36.82	α 1.79, β 0.98
2	29.26	α 1.82, β 1.47
3	76.91	3.44
4	38.31	α 2.34, β 2.11
5	140.45	–
6	121.19	5.30
7	31.36	α 1.93, β 1.39
8	32.20	1.48
9	49.60	0.88
10	36.21	–
11	20.59	α 1.45, β 1.38
12	39.68	α 1.93, β 1.12
13	41.85	–
14	56.16	0.96
15	23.85	α 1.53, β 1.03
16	27.77	α 1.78, β 1.24
17	55.40	1.09
18	11.78	0.61
19	19.20	0.80
20	35.60	1.35
21	18.60	0.88
22	33.34	α 1.34, β 0.99
23	25.45	1.15
24	45.70	0.91
25	29.20	1.61
26	19.60	0.82
27	18.90	0.95
28	22.60	α 1.26, β 1.17
29	11.80	0.65
1′	100.77	4.21
2′	73.46	2.91
3′	76.77	3.14
4′	70.09	3.06
5′	76.73	3.04
6′a	61.06	α 3.63, β 3.42

stems (15.8%) and the leaves (13.57%). It was proved that eicosane is present only in the leaves (15.24%) (Table 1).

GLC of the unsaponifiable fractions of the investigated parts of *C. capsularis* showed that the sterols found in the seeds are composed mainly of cholesterol (28.52%), whereas those found in the vegetative part are composed of cholesterol (12.46%), campasterol (24.98%), stigmasterol (4.38%) and β-sitosterol (10.39%). Hydrocarbon contents of the examined parts of *C. capsularis* showed that octadecane is the major compound in the seeds (45.13%) and then in the vegetative part (20.66%). Heptadecane was present in the vegetative part only (Table 2).

Fatty acids

The total fatty acids were found to be 1.4, 0.2, 0.8 and 0.4% (dry weight basis) in the seeds, roots, leaves and stems of *C. olitorius*; respectively.

The nature of fatty acids was determined by a comparative study of their methyl esters in GLC with authentic samples. The fatty acids of the seeds, roots, leaves and stems are composed of 90.08, 87.64, 96.46 and 93.30% saturated fatty acids, and 9.92, 12.36, 3.54 and 6.70% unsaturated fatty acids, respectively. Stearic acid represented the major compound in the case of the seeds (49.48%), followed by the roots (68.80%) and the leaves (58.67%), whereas palmitic acid (59.94%) was the major compound in the stems (Table 2).

The total fatty acids of the examined parts of *C. capsularis* were found to be 0.02 and 0.03% (dry weight basis) in the seeds and the vegetative part, respectively. The seeds and the vegetative part of *C. capsularis* were composed of 87.70 and 79.62% saturated fatty acids and 12.30 and 22.38% unsaturated fatty acids, respectively. Palmitic acid represented the major fatty acid in the seeds (81.68%) and the vegetative part (54.10%), whereas stearic acid was present in the vegetative part only (Table 2). Cholesterol was the main sterol of the seeds and vegetative part of *C. capsularis*.

Essential oils of the leaves of *Corchorus olitorius* and *Corchorus capsularis*

The fresh leaves of *C. oiltorius* and *C. capsularis* were extracted with methylene chloride; the solvent was distilled off under vacuum at a temperature not exceeding 30°C, and the residue was re-extracted with diethyl ether. The ether-extracted components were analysed using GC-MS.

GC of the ether fraction of *C. oiltorius* showed that it contains 40 compounds. The mass spectrum of each compound allowed the determination of its molecular weight and base peak and comparison with the reported data [38]. Eleven compounds were identified with a total concentration of 24.76%, and 29 compounds were still unidentified with a total concentration of 75.24% (Table 3). It was also noticed that cedrane-5-one represents the major component (17.77%), followed by methyl tiglate (2.73%) and *trans*-phytol (0.99%).

GC of the ether fraction of *C. capsularis* showed that it contains 40 compounds. Each compound was subjected to MS to detect its molecular weight and base peak, followed by comparison with the reported data [38]. Twenty-one compounds were identified with a total concentration of 62.97% (Table 4). Nineteen compounds were still unidentified with a total concentration of 37.03% (Table 4). γ-Terpinene represents the major component

(12.19%), followed by carvacrol methyl ether (10.01%), umbellulone (8.86%), sabinene (7.51%), myrtenal (6.78%) and 1-terpineol (3.15%) (Table 4).

Phytochemicals from the seeds of *Corchorus olitorius*

The concentrated methanolic extract of the grounded seeds of *C. olitorius* yielded a white precipitate, which was recrystallized several times from methanol to give the compound **I**.

The remaining aqueous extract was extracted with chloroform, and the solvent was distilled off. The residue was chromatographed on Silica gel 60 using benzene, followed by benzene/methanol mixture. The fractions that gave a positive test with Kedde ?s reagent [36] were selected and separately rechromatographed on Silica gel 60 using chloroform/methanol mixture. The fractions containing the main compounds were concentrated, and the residue from each fraction was further purified by crystallization or column chromatography (Sephadex LH-20) to give rise to the compounds **II**–**VI**.

Compound I

It was crystallized from methanol/ethyl acetate mixture to give 0.5 g, melting point 90–92°C. It gave positive Molish ?s test for sugar compounds [39]. It was identified as the trisaccharide raffinose (galactose–glucose–fructose) (Fig. 1) from its spectroscopic measurements (^{1}H-NMR and ^{13}C-NMR, DEPT, ^{1}H–^{1}H-COSY, TOCSY, nuclear overhauser spectroscopy, HMQC and HMBC).

^{13}C-NMR and DEPT spectra of compound **I** (Table 5) showed the existence of 18 carbon signals arising from four methylene and 13 methine groups and one quaternary carbon atom, indicating that the compound is comprised of three sugar units. Irradiation of the anomeric proton at δ equal to 5.41 ppm gave rise to signal protons at δ equal to 3.32, 3.45, 3.72, 3.75, 3.90 and 4.07 ppm, which represent the proton signals of the glucose unit (Table 5). The sequence of the proton signals was determined by cross-peaks in its COSY spectrum. The β-configuration of the glucose unit was confirmed from the coupling constant of its anomeric proton at δ equal to 5.41 ppm (d, J=4 Hz). Inspection of the TOCSY and nuclear overhauser spectroscopy spectra was carried out, and, following the same method, the proton signals of the galactose and fructose units were determined (Table 5). The assignments of the carbon signals were determined from drawing cross-peaks between the assigned protons and their carbon analogue in its HMQC measurements. The attachment of the glucose unit with the fructose unit at C-2 was confirmed by the cross peak H-1′/C-2 in its HMBC spectrum. Moreover, the attachment of the galactose unit with the glucose unit at C-6 was confirmed by the cross-peaks H-1′/C-6′ and H-6′ (a and b)/C-1′ in its HMBC spectrum.

Compounds II and III

They were precipitated from methanol/diethyl ether as a mixture (10 mg), which gave a positive cardiac glycoside test using Kedde ?s reagent [36]. UV measurement showed the absorption maximum of the butenolide ring at λ_{max} 220 nm [21].

^{13}C-NMR and DEPT spectra showed that the mixture is composed of two compounds, *ca* 2 : 1, having the same aglycone and differing only in the stereochemistry at C-4 of the first sugar. The mixture could not be detected by using the available laboratory chromatographic tools. The two compounds were identified as coroloside [digitoxigenin 3-*O*-β-glucopyranosyl-(1→4)-β-boiviopyranoside] (**II**) (Fig. 1) and glucoevatromonoside [digitoxiginin 3-*O*-β-glucopyranosyl-(1→4)-β-digitoxopyranosyl] (**III**) (Fig. 1) by comparison with published data [12,40] and the spectroscopic measurements (one-dimensional ^{1}H-NMR and ^{13}C-NMR, DEPT, z-TOCSY and two-dimensional ^{1}H–^{1}H-COSY, HSQC and ^{1}H–^{13}C-HMBC).

The aglycone part was identified as digitoxigenin. The carbon signals of the α, β-unsaturated-γ-lactone ring were detected at the expected chemical shifts (Table 6). The absence of a quaternary carbon signal at δ equal to 73–76 ppm suggested the absence of –OH group at C-5 and, instead, a signal at 38.09 ppm of a methine group was detected. C-14 was detected as a quaternary carbon signal at δ equal to 86.56 ppm. The methyl groups' 18 and 19 signals were traced at δ equal to 16.4 and 24.0 ppm. The other carbon signals were found to be at the expected chemical shifts. Some proton signals (Table 6) were assigned on the basis of cross-peaks between the identified carbon signal and its corresponding proton signals in the HSQC spectrum.

The ^{1}H-NMR spectrum of the mixture could be simplified by applying a selective irradiation of some signals (selective z-TOCSY). When the signal of H-22 at δ equal to 5.9 ppm was irradiated, the intensity of the signals of the two nonequivalent protons attached to C-21 at δ equal to 5.04 and 4.9 ppm were affected. When the signal of H-3′ in the first sugar moiety at δ equal to 4.16 ppm was irradiated, the signals H-1′, H-4′ and methyl –6′ and the methylene group at C-2′ were affected. Similarly, irradiation of the signal at 4.37 (H-1′) of glucoevatromonoside (**III**) led to change in

the signal of H-2′ and methylene-6′ of the second sugar moiety. Accordingly, the signals corresponding to the sugar moieties could be easily detected.

The attachment of the boivinose unit with digitoxigenin at C-3 in coroloside (**II**) was confirmed by the cross-peaks C-3/H-1′ and C-1′/H-3. Moreover, the attachment of the glucose unit with the boivinose unit at C-4 was confirmed by the cross-peaks C-4′/H-1′ and C-1′/H-4′. The attachment of the sugar part with the aglycone part in glucoevatromonoside (**III**) was also established by the cross-peaks in the HMBC spectrum. The signal assignment of the two compounds is illustrated in Tables 6–8.

Compounds IV and V

They were precipitated from methanol/ethyl acetate as a mixture (50 mg), which gave a positive cardiac glycoside test using Kedde ?s reagent [36]. The UV spectrum showed an absorption maximum of the butenolide ring at λ_{max} equal to 220 nm [21].

^{13}C-NMR and DEPT spectra showed that the mixture is composed of two compounds, *ca* 2 : 1, having the same aglycone and differing only in the sugar moiety. The difference between the two compounds was found to be only in the stereochemistry at C-4 of the first sugar. The mixture could not be detected by using the available laboratory chromatographic tools.

The two compounds were identified as erysimoside [strophanthidin 3-*O*-β-glucopyranosyl-(1→4)-β-digitoxopyranoside] (**IV**) and olitoriside [strophanthidin 3-*O*-β-glucopyranosyl-(1→4)-β-boiviopyranoside] (**V**) (Fig. 1), respectively, in comparison with published data: one-dimensional ^{1}H-NMR, ^{13}C-NMR and DEPT, two-dimensional HMQC and HMBC [5,41].

The aglycone part of the two compounds was identified as strophanthidin on the basis of the following: the carbon signals of the α,β-unsaturated lactone ring are clearly detected at their expected chemical shifts (Table 6). The presence of quaternary carbon signals at δ equal to 75.3 and 56.2 ppm suggested the presence of the OH group at C-5 and aldehydic group at C-10, correspondingly. The aldehydic C-19 was established by the presence of the signals at δ equal to 210 in ^{13}C-NMR and at δ equal to 10.1 ppm in its ^{1}H-NMR. The presence of the hydroxyl group at C-14 was established by the presence of a quaternary carbon signal at δ equal to 86.04 ppm. The methyl group (C-18) was found at δ equal to 16.25 ppm. The other carbon signals were found to be at their expected chemical shifts [12].

Some proton signals (Table 6) were assigned on the basis of the cross-peaks between the identified carbon signals and their corresponding proton signals in the HMQC spectrum.

The attachment of the digitoxose unit with strophanthidin at C-3 in erysimoside (**VI**) was confirmed by the cross-peaks C-3/H-1′ and C-1′/H-3. Attachment of the glucose unit with the digitoxose unit at C-4 was confirmed by the cross-peaks C-4′/H-1′ and C-1′/H-4′. The attachment of the sugar part in olitoriside (**V**) was established by the cross-peaks in the HMBC spectrum. The signal assignments of the two compounds is illustrated in Tables 6–8.

Compound VI

It was crystallized (15 mg) from methanol/diethyl ether mixture. It gave positive cardiac glycoside test using Kedde ?s reagent [36]. Its UV spectrum showed the absorption maximum of the butenolide ring at λ_{max} (MeOH) 220 nm [21]. It was identified as glucoolitoriside **VI** (Fig. 1) by comparison of its spectroscopic measurements with published data [5,12].

^{13}C-NMR and DEPT spectra of compound **VI** showed the existence of 41 carbon signals. The sugar moiety showed 14 CH, three CH_2 and one CH_3 signals; indicating a trisaccharide. The aglycone part exhibited 23 carbon atom signals arising from one CH_3, 10 CH_2, six CH and six quaternary carbon atoms. The presence of three anomeric carbons at δ equal to 99.2, 102.5 and 105.2 ppm is an evidence for the presence of three sugar units (Table 7), which was confirmed by the presence of three anomeric protons at δ equal to 4.90, 4.30 and 4.41 ppm in its ^{1}H-NMR (Table 8).

The assignment of ^{13}C-and ^{1}H signals of the lactone ring was achieved by comparison with the published data [5,12]. The assignment of H-22, H-21a and H-21b could be achieved from the carbon, hydrogen cross-peaks in the HSQC spectrum. The signals of the other protons and carbons were assigned on applying one-dimensional ^{1}H-NMR, ^{13}C-NMR and selective TOCSY and two-dimensional ^{1}H–^{1}H-COSY, rotating-frame overhauser spectroscopy, HSQC–TOCSY, HSQC and HMBC, and on comparison with published data [42–44].

Isolation of a glucositosterol from *Corchorus capsularis*

The ground-air-dried vegetative part of *C. capsularis* was exhaustively extracted with 80% methanol. The solvent was distilled off, and the residue was re-extracted with

chloroform. The residue of chloroform was chromatographed on Silica gel 60, eluted with benzene, followed by the gradual increase of ethyl acetate proportions during the course of fractionation. The benzene/ethyl acetate fractions (1 : 9 v/v) were collected together and crystallized from methanol to give rise to 25 mg of compound **VII**.

Compound **VII**, melting point 265°C, gave a positive Liebermann test for steroids [45] and was identified as 3-*O*-β-d-glucopyranosyl-β-sitosterol (**VII**) (Fig. 1) from its MS, IR and NMR spectroscopic measurements: one-dimensional ^{1}H and APT and two-dimensional ^{1}H–^{1}H COSY, HMQC and HMBC in comparison with published data [37].

Its IR-spectrum showed the presence of hydroxyl group at 3422 cm^{-1}. Its MS showed the absence of an ion corresponding to the molecular weight and instead an ion corresponding to the loss of isopropyl group (–43) was detected at *m/z* 533. Moreover, the expulsion of the sugar moiety and one proton gave rise to an ion peak at *m/z* 396.

APT spectrum of compound **VII** (Table 9) showed the existence of 35 signals, the sugar moiety showed one methylene group and five methine groups, whereas the aglycone part exhibited 29 carbon signals, arising from six methyl groups, 11 methylene groups, nine methine groups and three quaternary carbon atoms.

The aglycone moiety was identified as β-sitosterol from its ^{1}H and APT spectra (Table 9). The sugar moiety was shown to be connected to C-3 atom, as indicated from its HMBC spectrum cross-peaks H-1′/C-3. The sugar moiety was identified as glucopyranose from its ^{1}H-NMR, APT, ^{1}H–^{1}H-COSY and HMQC spectra.

Conclusion

A study of the lipid content of different parts of *C. olitorius* and C. *capsularis*, which grow in Egypt, showed that cholesterol was the major compound. In contrast, stigmasterol was present only in the stems of *C. olitorius*. Stearic acid represented the major fatty acid in the seeds, roots and leaves, whereas palmitic acid was the major fatty acid in the stems of *C. olitorius* and the seeds and the vegetative part of *C. capsularis*. GC-MS of the essential oils of the leaves of *C. olitorius* and *C. capsularis* led to the identification of 11 and 21 compounds, respectively. Cedrane-5-one and γ-terpinene represented the major essential oil in each plant, respectively. Phytochemical investigation of *C. olitorius* led to the isolation of raffinose **I**, coroloside **II**, glucoevatromonoside **III**, erysimoside **IV**, olitoriside **V** and gluco-olitoriside **VI**. Meanwhile, 3-*O*-glucopyranosyl-β-sitosterol **VII** was isolated from the vegetative part of *C. capsularis*. The isolated compounds were identified by spectral tools (^{1}H-NMR, ^{13}C-NMR, electron ionization mass spectrometer).

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Conflicts of interest
There are no conflicts of interest.

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