Bull Pharm. Sci., Assiut University,
Vol. 13, Part 1, PP 51-58

PHYTOCHEMICAL STUDY OF EUPHORBIA HETEROPHYLLA L. CULTIVATED IN EGYPT

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ABSTRACT

A phytochemical study of Euphorbia heterophylla L. cultivated in Egypt is presented. β-amyrin, β-sitosterol taraxasterol acetate, β-sitosterol glucoside and hydrocarbon with ketonic group were isolated from pet. ether extract of the herb. While lupeol acetate, taraxasterol, β-sitosterol and β-sitosterol glucoside were isolated from ether extract of the root. In addition quercetin, 3-methyl quercetin, kaempferol-3-0-arabinoside, kaempferol-3-0-glucoside and kaempferol-7-0-glucoside were isolated from the ethyl acetate extract of the herb. The identification of these compounds was based on physical, chemical and spectral analysis.

INTRODUCTION

Euphorbia heterophylla L. is an annual herb belonging to family Euphorbiaceae. It was used as an antidote for the irritation produced by other species of Euphorbia. The flower and leaf gave positive antibiotic tests against T.B. [1]. The aqueous extract of the leaves was investigated for the purgative effect in animals possibly due to the increase in intestinal motility [2]. The plants of the genus *Euphorbia* have been reported to contain terpenoids (di- and tri-), alcohol, sterols, hydrocarbons and flavonoids.

On the other hand several other substances, viz, alkaloids, coumarins, tannins and acids were reported [3-7].

Current literature on *E. heterophylla* L. revealed the presence of triterpenes euphyl acetate and mortenone as well as 10,10-dimethylhexacosane-7-one [8]. The present work is directed for studying the lipids, terpenes and flavonoids of this plant.

EXPERIMENTAL

The plant material consisting of the aerial parts and roots collected during the flowering stage from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Assiut University. The plant was identified through "Garden Plants of the World in Colours", and kindly confirmed by Prof. Dr. N.E. El-Keltawi, Professor of Horticulture, Faculty of Agriculture Assiut University.

Melting points were determined using a koffler hot stage microscope. ^{1}H-NMR spectra were recorded in $CDCl_3$, $CDCl_3$+pyridine-d_5 and DMSO-d_6 at 400 MHz from Bruker WH 400. Mass spectra were measured using MS-50, Kratos, A.E.I. 70 ev. Unicam infra-red spectrophotometer SP-1025 for recording infrared spectra and Unicam SP-1750 recording Ultra-violet spectrophotometer for UV measurements.

Extraction:

a-The powdered herb of *E. heterophylla* L. (3 Kg) was extracted several times with 70% ethanol by percolation till exhaustion. The dried alcoholic extract (150 g) was mixed with 600 ml warm distilled water, and fractionated into pet. ether (Fr. I) and ethyl acetate (Fr. II). The pet. ether fraction was evaporated, the residue (70 g) was extracted with methanol (500 ml) (Fr. Ia) and then evaporated to yield a brownish residue (20 g) (Fr. Ib).

b-The powdered roots (700 g) was extracted with 70% ethanol as mentioned before. The dried alcoholic extract (25 g) was mixed with 200 ml warm distilled water and partitioned between ether (Fr. III) and n-butanol.

Investigation of the Pet. Ether Fraction of the Powdered herb:

A few spots of (Fr. Ia) was chromatographed on silica gel-coated plates using benzene-ethyl acetate (9:1) system I and chloroform-methanol (9:1) system II. The chromatograms were sprayed with 50% H_2SO_4, followed by heating at 110°C for few minutes. Eight spots were obtained having R_f values 0.96, 0.85, 0.74, 0.55, 0.38, by system I and 0.99, 0.98, 0.97, 0.87, 0.80, 0.40, 0.36 and 0.24 by system II.

Column Chromatography of the Pet. Ether Fraction of the Powdered herb:

The methanol-soluble part (20 g) of (Fr. Ib) was transferred to a silica gel column (E-Merck, 500 g, 1 m x 4.5 cm, i.d.) and gradiently eluted with hexane and ethyl acetate. Fractions, 500 ml each were collected and monitored by silica gel G. coated plates using system I and II. Identical fractions were pooled together. Five compounds labelled E_1, E_2, E_3, E_4, and E_5 were obtained.

Compound E_1:

Compound E_1 (20 mg) occurred as white waxy substance, ethyl acetate, m.p. 74-76°C, R_f 0.97 & 0.99 by systems I & II respectively. It did not respond to Salkowski's and Liebermann-Burchard's tests. IR spectrum (KBr ν) showed the following bands 2950, 1710, 1450 and 1380 cm^{-1}. ^{1}H-NMR ($CDCl_3$) showed signal at δ 0.88 (m, for terminal CH_3, groups) 1.27, 1.59, 1.62 and

2.41 (m. for CH_2 protons). MS showed M^+ at m/z 408, diagnostic peaks at 390, 362 and other peaks spaced 14 mass units (corresponding to a difference of CH_2).

From the aforementioned chemical, physical and spectral data of compound E_1 it can be concluded that it is a hydrocarbon containing a ketonic group. It is most probably 10, 10-dimethyl hexacosane-2-one, previously isolated from the plant under investigation [8,9].

Compound E_2:

Compound E_2 (1 g) is colourless hexagonal plates, (ethyl-acetate) m.p. 248-50°C. It responded to Salkowski's and Liebermann-Burchard's tests. It had R_f values 0.74 and 0.97 by systems I & II respectively. IR (KBr ν) showed the following bands 2960, 1725, 1460, 1375, 1250 and 1030 cm^{-1}. ^{1}H-NMR ($CDCl_3$) showed six singlets at δ 0.73, 0.844, 0.849, 0.86, 0.90 and 0.93 ppm (6, s, 3H each, for 6 Me): 0.94 (3H, d, J=6.1 Hz, Me-29); 2.04 (3H, s, OAc); 1.01-2.3 (m, for methylene protons): 4.5 (1H, dd, J=9.9, 6.7 Hz, H-3) 5.2 (2H, t like, J=3.3 Hz, for methylene protons at C-30).

It was identified as taraxasterol acetate from the previous mentioned physicochemical and spectral properties [10] as well as direct comparison (mmp & Co-chromatography) with an authentic sample.

Compound E_3:

Compound E_3 (80 mg) is colourless needles, m.p. 198-200°C (methanol). It responded to Salkowski's and Liebermann-Burchard's tests. It had R_f values 0.55 and 0.87 by systems I & II respectively. It was identified as β-amyrin by IR, co-chromatography and mmp with and authentic sample.

Compound E_4:

Compound E_4 (800 mg) is colourless needles, m.p. 135-37°C (methanol). It reponded to Salkowski's and Liebermann-Burchard's tests. It had R_f values 0.38, 0.80 by systems I & II respectively. It was identified as β-sitosterol by IR, co-chromatography and mmp with a reference material.

Compound E_5:

Compound E_5 (50 mg) is white amorphous powder, (MeOH) m.p. 275-8°C. It responded to Salkowski's and Liebermann-Burchard's tests. It had R_f value 0.24 by system II. IR spectrum of the compound showed bands at 3400, 2940, 1450, 1370, 1610, 1070, and 1020 cm^{-1}. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$+pyridine-d5) showed the following signals at δ 0.67 ppm (S, Me-18) & 0.92 ppm (S, Me-19) & 0.92 ppm (d, J=6.1 Hz, Me-21) & 0.82 ppm (d, J=6.9 Hz, Me-26) & 0.84 ppm (d, J=6.9 Hz, Me-27) & 0.84 ppm (t, J=7.3 Hz, Me-29) & 3.55 ppm (m, H-3) & 5.3 ppm (t, J=5.01 Hz, H-6) & 1-2.5 ppm (m, CH_2 and CH protons) & 4.58 ppm (d, J=7.7 Hz, H-1`) & 3.77 ppm (t, J=8.8 Hz, H-2`,3`,4`) & 3.65 ppm (m, H-5`) & 4.07 ppm (dd, J=11.5, 3.5 Hz, H-6`-a) & 3.94 ppm (dd, J=11.4, 5.4 Hz, H-6`b). Ms showed a molecular ion peak M^+ at m/z 414 and other diagnostic peaks at 399(M^+-Me), 396(M^+-H_2O) 100%, 382(M^+-Me-H_2O), 294, 275(M-R), 255, 241, 213, 171, 161, 145, 133, 121, 107, 95, 81, 73, 69, 67, 57.

From the aforementioned physicochemical and spectral studies the compound E_5 was identified as β-sitosterol glucoside according to the published data for β-sitosterol [11,12]. This was confirmed by completed acid

hydrolysis of the compound and the chromatographic study of both the aglycone and the sugar.

Investigation of the Ethyl Acetate Fraction (Fr. II) of the Powdered herb:

TLC investigation of (Fr. II) was performed using the following systems:

-For silica gel G plates:

System III: ethyl acetate-formic acid-water (10:2:3).

System IV : chloroform-MeOH (8:2).

-For cellulose plates:

System V : chloroform-acetic acid-water (50:45:5).

The chromatograms showed 7 flavonoidal compounds that were revealed by UV, ammonia vapour and 1% $AlCl_3$ spraying reagent.

Column Chromatography:

The ethyl acetate fraction (Fr. II) (15 g) was chromatographed on silica gel column (E-Merck, 300 g. 120X2.5 cm) and eluted with chloroform then chloroform-methanol gradient. Fractions 250 ml each collectd and monitored by TLC and PC and pooled according to similar R_f. Six groups were obtained and five flavonoids labelled F_1. F_2, F_3, F_4, and F_5 were isolated.

Compound F_1:

It was obtained as yellowish powder (MeOH) (50 mg), m.p. 315-17°C. It had R_f values 0.98, 0.63 by systems III & IV successively. From the UV spectrophotometric data with shift reagents Table 1, Co-chromatography and mmp with a reference sample it was concluded that compound F_1 is quercetin.

Compound F_2:

Compound F_2 (80 mg) was obtained as yellow needles, m.p. 268-70°C (methanol). It had R_f values 0.98, 0.50 by systems III & IV. The UV spectrophotometric data were recorded in Table 1. ^{1}H-NMR (400 MHz, DMSO-d6) showed the following signals at δ 3.78 ppm (3H, S, OCH3 at C_3) & 6.19 ppm (1H, d, J=1.9 Hz, H-6) & 6.41 ppm (1H, d, J=1.7 Hz, H-8) & 6.90 ppm (1H, d, J=3.5, H-5`) & 7.45 ppm (1H, dd, J=2.1 and 8.5 Hz, H-6`) 7.54 ppm (1H, d, J=2.1 Hz, H-2`).

Compound F_2 was identified as 3-methyl quercetin.

Compound F3:

It is yellowish powder, (39 mg), m.p. 275°C (decomp.) (methanol). It had R_f values 0.90, 0.87 by system III and V. The UV spectrophotometric data Table 1 proved that this substance is a flavonol glycoside. Partial and complete acid hydrolysis indicated that it is a monoside giving kaempferol aglycone (AF_3) that its UV spectrophotometric data were recorded in Table 1. The sugar was identified as arabinose. From the aforementioned data compound F_3 was found to be kaempferol-3-0-arabinoside.

Çompound F_4:

It was obtained as yellowish powder (30 mg) and decomposed at 265°C (methanol). It had R_f values 0.85, 0.81 by systems II, V. The UV spectrophotometric data Table 1 proved that this compound is a flavonol glycoside.

Partial and complete acid hydrolysis indicated that it is a monoside giving kaempferol aglycone on complete acid hydrolysis.

The sugar moiety was identified as glucose. From the above mentioned data compound F_4 was identified as kaempferol-7-0-glucoside.

Compound F_5:

Compound F_5 was obtained as yellowish powder (60 mg) m.p. 242-44°C (methanol). It had R_f values 0.81, 0.75 by systems III, V. The UV spectrophotometric data were recorded in Table 1. It shows that this substance is flavonol glycoside.

Partial and complete acid hydrolysis of this substance indicated that it is a monoside giving kaempferol aglycone on complete acid hydrolysis.

The sugar moiety was identified as glucose. From aforementioned data compound F_5 was identified as kaempferol-3-0-glucoside.

Investigation of Ether Fraction (Fr. III) of Powdered Roots:

TLC on silica gel G of (Fr. III) using benzene-ethyl acetate (9.5:0.5) system VI and system I showed 8 spots that were visualized by spraying with 50% H_2SO_4 followed by heating at 110°C. The spots had R_f values 0.98, 0.96, 0.95, 0.91, 0.88, 0.85, 0.80 and 0.24 by system II & 0.97, 0.95, 0.78, 0.60, 0.67, 0.44 and 0.31 by system VI.

Column Chromatography:

Fr. III (7 g) was chromatographed on silica gel (E.Merck, 280 g, 1 m X 2.5 cm) using hexane and hexane-ethyl acetate gradient. Fractions 500 ml each were collected and monitored by TLC using systems II and VI. They were pooled according to similar R_f. Six groups were obtained and four compounds labelled R_1, R_2, R_3 and R_4 were isolated.

Compound R_1:

Colourless needles (600) m.p. 211-13°C (methanol). It responded to Salkowski's and Liebermann-Burchard's tests. It had R_f values 0.96 and 0.99 by systems II & VI respectively. IR spectrum showed bands at 2930, 1730, 1640, 1460, 1380, 1250 and 1130 cm^{-1}. ^1H-NMR spectrum ($CDCl_3$) showed six singlets at δ 0.73, 0.85, 0.86, 0.93, 0.94 and 1.01 ppm (each for Me group) & 1.63 ppm (3H, s, C=C-CH_3), 2.04 ppm (3H, s, O-C-CH_3) & 4.46 ppm (2H, m, CH_2) & 5.2 ppm (2H, broad singlet, H-3). MS showed a molecular ion peak M^+ at m/z 468, peaks at 453(M^+-Me), 408(M^+-HOAc), 393(M^+-HOAc-Me) and other peaks at 257, 249, 231, 218, 204(100%), 189, 177, 161, 147, 135, 121, 109, 95, 84, 81, 69, 66, 43, 28. This pattern of fragmentation is characteristic for lupane series [13].

The aforementioned physicochemical and spectral data of this compound superimpose those reported for lupeol acetate [14].

Compound R_2:

Colourless needles (800 mg), m.p. 223-25°C (methanol). It responded to Salkowski's and Liebermann-Burchard's tests. It had R_f values 0.85 and 0.44

by systems II & VI respectively. IR spectrum showed bands at 3420, 2950, 1610, 1470, 1390, 1190, 1040 and 980 cm^{-1}. ^1H-NMR (400 MHz, $CDCl_3$) showed six singlets at δ 0.79, 0.87, 0.88, 0.95, 0.99 and 1.04 ppm (each for Me group) & 1.05 ppm (3H, d, Me at C_{29}) & 3.19 ppm (1H, dd, J=10.5, 6.5 Hz, H-3) & 4.60 ppm (2H, dd, methylene protons at C-30). MS showed a molecular ion peak M^+ at m/z 426 and other diagnostic peaks at 411(M^+-Me), 408(M^+-H_2O), 393(M^+-H_2O-Me), 357, 344, 315, 272, 257, 229, 218, 307, 189(100%), 175, 161, 147, 135, 121 and 109.

The above mentioned data superimpose those reported for taraxasterol [16].

Acetylation of compound R_2 gave compound E_2 that was isolated from the pet. ether fraction of the powdered herb.

Compounds R_3 and R_4 were proved to be β-sitosterol and β-sitosterol glucoside following the same procedure mentioned under E_4 & E_5.

RESULTS AND DISCUSSION

From the aerial parts of *Euphorbia heterophylla* L. cultivated in Egypt, β-amyrin, β-sitosterol, β-sitosterol glucoside taraxasterol acetate and 10,10-dimethyl hexacosane-2-one were isolated from pet. ether extract. In addition quercetin, 3-methyl quercetin, kaempferol-3-0-arabinoside, kaempferol-3-0-glucoside and kaempferol-7-0-glucoside were isolated from ethyl acetate extract of the herb, while lupeol acetate, taraxasterol, β-sitosterol and β-sitosterol glucoside were isolated from the ether extract of the root.

The identity of the isolated compounds was confirmed through determining their physical and chemical characters, as well as their chromatographic and spectral analysis.

Referring to the literature euphyl acetate, 10,10-dimethyl hexacosane-7-one and mortenone were previously isolated from *E. heterophylla* L. while the other compounds are reported here for the first time.

Table 1: UV-spectra of isolated flavonoids F_1 F_2 F_3 F_4 F_5 and AF_3.

Compound	Band	MeOH λmax	NaOMe λmax	NaOMe Δ	$AlCl_3$ λmax	$AlCl_3$ Δ	$AlCl_3$/HCl λmax	$AlCl_3$/HCl Δ	NaOAc λmax	NaOAc Δ	NaOAc/H_3BO_3 λmax	NaOAc/H_3BO_3 Δ
F_1	II	254	270	+16	265	+12	266	+12	270	+16	270	+16
	I	370	390	+20	436	+66	422	+52	390	+20	390	+20
F_2	II	256	272	+16	276	+20	276	+20	274	+18	274	+18
	I	358	406	+48	434	+76	406	+48	378	+20	378	+20
F_3	II	264	288	+24	268	+4	268	+4	268	+4	264	-
	I	350	414	+64	398	+48	398	+48	360	+10	350	-
F_4	II	266	282	+16	270	+4	270	+4	266	-	266	-
	I	346	398	+52	356	+10	356	+10	346	-	346	-
F_5	II	264	278	+14	270	+6	270	+6	268	+4	264	-
	I	350	398	+48	398	+48	398	+48	358	+8	350	-
AF_3	II	268	278	+10	270	+2	270	+2	274	+6	268	
	I	366	414	+48	424	+58	424	+58	374	+8	366	

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ACKNOWLEDGEMENT

The authors are gratefull to Prof. Dr. H.Itokawa, Prof. of Pharmacognosy, Tokyo College of Pharmacy, Japan for carrying out the MS and ^{1}H-NMR spectral analysis.

دراسة الكيمياء العقاقيرية لنبات اليوفوروبيا هيتيروفيللا ل. المنزرع فى مصر

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من خلاصة البترول الايثيرى لمسحوق اعشاب النبات تم فصل والتعرف على خمسة مركبات هى ١٠و١٠-داى ميثيل هكساكوزان-٢-اون ، خلات التراكساستيرول ، بيتا سيتوستيرول وبيتا سيتوستيرول جلوكوزيد.

ومن خلاصة خلات الايثيل لمسحوق الاعشاب تم ايضا فصل والتعرف على كويرستين ٣-مثيل كويرستين ، كامبيوفرول-٣- أ- ارابينوزيد ، كامبيوفرول-٧- أ-جلوكوزيد ، كامبيوفرول-٣- أ-جلوكوزيد.

كذلك تم فصل والتعرف على خلات الليبيول ، تراكساستيرول ، بيتا سيتوسيترول ، بيتا سيتوسيترول جلوكوزيد من خلاصة الاثير لجذور النبات.

Received in 15/2/1990 & accepted in 16/5/1990.