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-sitosterol, δ-taraxasterol acetate, β-sitosterol glucoside and hydrocarbon with ketonic group were isolated from pet. ether extract of the herb. While lupeol acetate, taraxasterol, δ-sitosterol and β-sitosterol glucoside were isolated from ether extract of the root. In addition quercetin, 3-methyl quercetin, kaempferol-3-α-arabinoside, kaempferol-3-α-glucoside and kaempferol-7-α-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 norteronone 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. M.E. El-Keltawi, Professor of Horticulture, Faculty of Agriculture Assiut University.

Melting points were determined using a keffier hot stage microscope. 1H-NMR spectra were recorded in CDCl₃, CDC₁₃-pyridine-d₅ and DMSO-d₆ at 400 MHz from Bruker WH 202. Mass spectra were measured using MS-80, Eratos, A.E.I. 70 ev. Unicam infra-red spectrophotometer SF-1025 for recording infrared spectra and Unicam SF-1750 recording Ultra-violet spectrophotometer for UV measurements.

Extraction:

a-The powdered herb of E. heterophylla L. (3 E₅) 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 (78 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% H₂SO₄, followed by heating at 110°C for few minutes. Eight spots were obtained having Rf values 0.96, 0.65, 0.74, 0.55, 0.36, by system I and 0.99, 0.98, 0.97, 0.87, 0.80, 0.48, 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 E₁, E₂, E₃, E₄, and E₅ were obtained.

Compound E₁:

Compound E₁ (10 mg) occurred as white waxy substance, ethyl acetate, m.p. 74–76°C, Rf 0.97 & 0.99 by systems I & II respectively. It did not respond to Salkowski's and Liebermann-Burchard’s tests. IR spectrum (KBr disc) showed the following bands 2930, 1710, 1450 and 1360 cm⁻¹. 1H-NMR (CDCl₃) showed signal at δ 6 8.88 (m, for terminal CH₃, groups) 1.27, 1.59, 1.62 and
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2.41 (m. for CH₂ protons). MS showed M⁺ at m/z 408, diagnostic peaks at 390, 361 and other peaks spaced 14 mass units (corresponding to a difference of CH₂).

From the aforementioned chemical, physical and spectral data of compound E₁, 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 E₂:**

Compound E₂ (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 Rₖ values 0.74 and 0.97 by systems I & II respectively. 1H (δBr) showed the following bands 306. 1725. 1460. 1375. 1250 and 1030 cm⁻¹. 1H-NMR (CDCl₃) 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 MeH): 0.94 (3H, d, J=6.1 Hz, Me-29); 2.84 (2H, s, OAc): 1.85-2.3 (m. for methylene protons): 4.5 (1H, dd, J=9.3, 6.7 Hz, H-3) 5.2 (2H, t like, J=3.3 Hz, for methylene protons at C-29).

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

**Compound E₃:**

Compound E₃ (30 mg) is colourless needles, m.p. 198-208°C (methanol). It responded to Salkowski’s and Liebermann-Burchard’s tests. It had Rₖ values 0.55 and 0.87 by systems I & II respectively. It was identified as β-arnynin by IR, co-chromatography and mp with an authentic sample.

**Compound E₄:**

Compound E₄ (800 mg) is colourless needles, m.p. 135-37°C (methanol). It responded to Salkowski’s and Liebermann-Burchard’s tests. It had Rₖ values 0.38, 0.30 by systems I & II respectively. It was identified as β-sitosterol by IR, co-chromatography and mp with a reference material.

**Compound E₅:**

Compound E₅ (50 mg) is white amorphous powder, (H₂O) m.p. 275-8°C. It responded to Salkowski’s and Liebermann-Burchard’s tests. It had Rₖ value 0.24 by system II. IR spectrum of the compound showed bands at 3400, 2940, 1450, 1370, 1610, 1070, and 1920 cm⁻¹. 1H-NMR spectrum (400 MHz, CDCl₃+pyridine-d₅) showed the following signals at δ 0.67 ppm (5, Me-18) & 0.92 ppm (5, Me-19) & 0.92 ppm (δ, J=6.1 Hz, Me-21) & 0.82 ppm (δ, J=6.9 Hz, Me-26) & 0.84 ppm (δ, J=6.9 Hz, Me-27) & 0.84 ppm (δ, J=7.3 Hz, Me-29) & 3.55 ppm (m, H-3) & 5.3 ppm (t, J=5.8 Hz, H-6) & 1-2.5 ppm (m, CH₂ and CH protons) & 4.58 ppm (δ, J=7.7 Hz, H-1") & 3.77 ppm (t, J=6.8 Hz, H-2', 3', 4') & 3.65 ppm (m, H-5") & 4.07 ppm (δδ, J=11.5, 3.5 Hz, H-6'-a) & 3.94 ppm (δδ, 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⁺-Fe), 396(M⁺-H₂O) 198, 328(M⁺-Me₃-2H₂O), 294. 275(M⁺-H₃), 255, 241. 213, 171, 161, 145, 131, 121, 187, 95, 81, 73, 69, 67, 57.

From the aforementioned physicochemical and spectral studies the compound E₅ 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 (18:2:3).
  System IV: chloroform-MeOH (8:2).

-For cellulose plates:
  System V: chloroform-acetic acid-water (58:45:5).

The chromatograms showed 7 flavonoidal compounds that were revealed by UV, ammonia vapour and 1% AlCl₃ spraying reagent.

Column Chromatography:

The ethyl acetate fraction (Fr. II) (15 g) was chromatographed on silica gel column (E-Merck, 380 g, 120x2.5 cm) and eluted with chloroform then chloroform-methanol gradient. Fractions 250 ml each collected and monitored by TLC and PC and pooled according to similar Rf. Six groups were obtained and five flavonoids labelled F₁, F₂, F₃, F₄, and F₅ were isolated.

Compound F₁:

It was obtained as yellowish powder (MeOH) (58 mg), m.p. 315-17°C. It had Rf values 0.90, 0.63 by systems III & IV successively. From the UV spectrophotometric data with shift reagents Table 1, Co-chromatography and mp with a reference sample it was concluded that compound F₁ is quercetin.

Compound F₂:

Compound F₂ (80 mg) was obtained as yellow needles, m.p. 258-70°C (methanol). It had Rf values 0.98, 0.50 by systems III & IV. The UV spectrophotometric data were recorded in Table 1. 1H-NMR (400 MHz, CDCl₃-d₆) showed the following signals at 6.3 at ppm (H, S, OCH₃ at C₃) 6.19 ppm (H, d, J=1.9 Hz, H-6) 6.41 ppm (H, d, J=1.7 Hz, H-8) 6.30 ppm (H, d, J=3.5, H-5') 7.45 ppm (H, d, J=2.1 and 8.5 Hz, H-6') 7.54 ppm (H, d, J=2.1 Hz, H-2').

Compound F₂ was identified as 3-methyl quercetin.

Compound F₃:

It is yellowish powder, (19 mg), m.p. 275°C (decomp.) (methanol). It had Rf values 0.90, 0.87 by system III and IV. 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 (AF₃) that its UV spectrophotometric data were recorded in Table 1. The sugar was identified as arabinose. From the aforementioned data compound F₃ was found to be kaempferol-3-O-arabinoside.

Compound F₄:

It was obtained as yellowish powder (38 mg) and decomposed at 265°C (methanol). It had Rf 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 F₃ was identified as kaempferol-7-β-glucoside.

**Compound F₅:**

Compound F₅ was obtained as yellowish powder (60 mg) m.p. 242-4°C (methanol). It had Rₖ 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 F₅ was identified as kaempferol-3-β-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 3 spots that were visualized by spraying with 50% H₂SO₄ followed by heating at 110°C. The spots had Rₖ values 0.96, 0.95, 0.91, 0.85, 0.80 and 0.24 by system II & 0.37, 0.35, 0.78, 0.60, 0.47, 0.44 and 0.31 by system VI.

**Column Chromatography:**

Fr. III (7 g) was chromatographed on silica gel (E.Merck, 200 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 Rₖ. Six groups were obtained and four compounds labelled R₁, R₂, R₃ and R₄ were isolated.

**Compound R₁:**

Colourless needles (600 mg) m.p. 211-13°C (methanol). It responded to Salkowski's and Liebermann-Burchard's tests. It had Rₖ values 0.96 and 0.99 by systems II & VI respectively. IR spectrum showed bands at 2950, 1730, 1640, 1460, 1300, 1250 and 1130 cm⁻¹. ¹H-NMR spectrum (CDCl₃) showed six singlets at δ 0.70, 0.85, 1.06, 2.03, 2.94 and 3.11 ppm (each for Me group) & 1.63 ppm (3H, s, O-C=CH₃), 2.84 ppm (3H, s, O-C=CH₃) & 4.46 ppm (2H, m, CH₂) & 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(10%), 189, 177, 161, 147, 135, 121, 109, 95, 84, 81, 69, 66, 43, 38.

This pattern of fragmentation is characteristic for lupeol series 13.

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

**Compound R₂:**

Colourless needles (800 mg), m.p. 223-25°C (methanol). It responded to Salkowski's and Liebermann-Burchard's tests. It had Rₖ values 0.85 and 0.44.
by systems II & VI respectively. IR spectra showed bands at 3426, 2950, 1610, 1470, 1390, 1190, 1040 and 980 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) showed six singlets at δ 0.79, 0.87, 0.88, 0.95, 0.99 and 1.04 ppm (each for Me group) & 1.85 ppm (3H, d, Me at C29) & 1.19 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), 386(M⁺-H₂O), 292(M⁺-H₂O-Me), 237, 229, 219, 187, 189(100%), 175, 161, 147, 135, 121 and 109.

The above-mentioned data superimpose those reported for taraxasterol.¹⁰

Acetylation of compound F₂ gave compound F₃ that was isolated from the pet. ether fraction of the powdered herb.

Compounds R₅ and R₆ were proved to be δ-sitosterol and 3-β-sitosterol glucoside following the same procedure mentioned under E₄ & E₅.

RESULTS AND DISCUSSION

From the aerial parts of Euphorbia heterophylla L. cultivated in Egypt, β-aminin, β-sitosterol, β-sitosterol glucoside, taraxasterol acetate and 10,18-dimethyl hexacosane-7-one were isolated from pet. ether extract. In addition quercetin, 3-methyl quercetin, kaempferol-3-O-arabinoside, kaempferol-3-O-glucoside and kaempferol-7-O-glucoside were isolated from ethyl acetate extract of the herb, while lupenol acetate, taraxasterol, β-sitosterol and 3-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, 10,18-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 F₁, F₂, F₃, F₄, F₅ and A₅, max and max

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<th>Band</th>
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<th>ACF₂/HCl</th>
<th>NaOMe</th>
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<td>246</td>
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<td>256</td>
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<tr>
<td>F₂</td>
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دراسة الكيمياء العفافية لنبات اليوپوروبا هيتيروفينلا ل. المزرع في مصر
نصر أحمد الجبر - مقبول أحمد مقبول - محمد عبد المطلب عبد الحافظ
سليم فاروق فرج
قسم العفافير - كلية الصيدلة - جامعة استوطة

من خلاصة البترويل البوتوري لمسموح افتتاح النبات تم فصل والتعثر
على خلاص بركات في 2,001-3، والبيتا-هيماكوزان-2-اوه، خلاص
البراسستينول، البيتا-سبوتريول وبيتا-سبوتريول جلوكوزيد.

وبين خلاصة خلاص البترويل لمسموح افتتاح النبات تم أيضا فصل والتعثر على
كبرستين-3،-البيتا-كويرستين، كابيروفرول-3-ا،-رايروزوزيد،
كابيروفرول-7-أ-جولوكوزيد، كابيروفرول-7-أ-جولوكوزيد.

كذلك تم فصل والتعثر على خلاص الليبيبول، تراكاستيرول، بيتا
سبوتريول، بيتا-سبوتريول جلوكوزيد من خلاصة الاخير لجزء النبات.