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Flavonoids and Lipoidal Matters from *Olea europaea.*

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ABSTRACT

The flavonoid compounds apigenin-7-*O*-β-D- glucopyranoside, luteolin-7-*O*-(2" -*O*-*p*-hydroxybenzoyl)-α-D- glucopyranoside, quercetin-7-*O*-β-D- glucopyranoside, luteolin-7-*O*-β-D- glucuronopyranoside, chrysoriol-7-*O*-β-D- glucuronopyranoside and quercetin, were isolated from the butanol and ethyl acetate fractions of *Olea europaea* using chromatographic methods. The isolated compounds identified by ^{1}H NMR, ^{13}C NMR and UV. Triacontane is the main hydrocarbon and cholesterol is the main sterol where identified from *Olea europaea*. Palmitic acid and oleic acid were detected as the main saturated and unsaturated fatty acids from *Olea europaea*.

Keywords: *Olea europaea L.,* oleaceae, flavonoids, fatty acids, lipoidal matters.

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INTRODUCTION

*Olea europaea L.*is valued for its fruit and oil which are used in food science and agroindustry. Also olive leaves are used since long time in traditional medicine as hypoglycemic [1], hypertensive [2,3], anti-inflammatory, antirhumatic, diuretic, antipyretic and vasodilatatory [4]. The leaves are taken for stomach and intestinal diseases [5]. Also it's used in treatment of respiratory and urinary tract infections [6]. *Olea europaea L.* is one of the most important species belonging to family oleaceae. It is native to the Mediterranean region, tropical and central Asia and various parts of Africa. *Olea europaea L* is also grown in California, Australia and South Africa(4).

MATERIALS AND METHODS

Plant Material

Leaves of *Olea europeae L.* were collected from trees growing in Horticulture Research Institute, Giza, Egypt and identified by Prof. Dr. Mamdouh Eisa, Professor of Fruits Diseases Research Section, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt. The leaves were air dried then powdered and kept in tightly closed containers.

Extraction and isolation

The air dried powder of the leaves of *Olea europaea L.* was found to contain flavonoids, tannins, glycosides and sterols in addition to traces of alkaloids and saponins [7,8]. Air dry powder of *Olea europaea L.* leaves (2 Kg) is extracting with petroleum ether and then with 80% methanol. The methanol extract was drying under reduce pressure. The residue was fractionated with chloroform, ethyl acetate and n-butanol. The n-butanol extract was fractionated on cellulose column chromatography with mobile phase water, water / methanol with decreasing polarity. The isolation and purification of fractions collected were carried out by using successive sephadex LH-20 column chromatography with n-butanol / water to isolate five compounds (I - V). The ethyl acetate extract fractionated on column chromatography using sephadex LH -20 with mobile phase n-butanol / water to yield compound VI.

Compound I: It was isolated as a yellow amorphous powder and its UV spectral data are λmax MeOH: 268, 333; NaOAc: 256, 267, 355, 387; H3BO3: 267, 340; AlCl3: 276, 300, 348, 386; HCl: 277, 299, 341, 382; NaOMe: 245, 269, 301, 386. 1H NMR spectra are δ ppm 7.79 (2H, *d*, J = 8.7 Hz, H-2`/6`), 6.86 (2H, *d*, J = 8.7 Hz, H-3`/5`),6.68 (1H, *d*, J = 2.1 Hz, H-8),6.56 (1H, *s*, H-3),6.43 (1H, *d*, J = 2.1 Hz, H-6), 5.1 (1H, *d*, J = 7.2 Hz, H-1``), 3.1 – 3.7 (m, sugar protons).

Compound II: It was isolated as a yellow amorphous powder and its UV spectral data are λmax MeOH: 255,267, 348; NaOAc: 259, 266, 365, 405; H3BO3: 259, 372; AlCl3: 274, 298, 329, 432; HCl: 273, 299, 358, 387; NaOMe: 263, 300, 394. 1H NMR spectra are δ ppm 7.65 (2H, *d*, J = 8.4 Hz, H-2```/6```), 7.32 (2H, *m*, H-2`/6`), 7.12 (2H, *d*, J = 8.4 Hz, H-3```/5```), 6.83 (1H, *d*, J = 7.5 Hz, H-5`), 6.71 (1H, *d*, J = 2.1 Hz, H-8),6.51 (1H, *s*, H-3),6.41 (1H, *d*, J = 2.1 Hz, H-6), 5.3 (1H, *d*, J = 3.6 Hz, H-1``), 4.98 (1H, *m*, H-2``), 3.2 – 3.6 (m, remaining of sugar protons).

Compound III: It was isolated as a yellow amorphous powder and its UV spectral data are λmax MeOH: 256,269sh, 372; NaOAc: 286, 378, 428; H3BO3: 261, 289,386; AlCl3: 259sh, 273, 339, 458; + HCl: 268, 303, 365, 426; NaOMe: 241sh, 291, 367. 457. 1H NMR spectra are δ ppm 7.66 (1H, *d*, J = 2.5 Hz, H-2`), 7.5 (1H, *dd*, j=8.4, 205 Hz, H- 6`), 6.8 (1H, *d*, J = 8.4Hz, H- 5`), 6.66 (1H, *d*, J = 2.1 Hz, H-8), 6.37 (1H, *d*, J = 2.1 Hz, H-6), 4.96 (1H, *d*, J = 7 Hz, H-1``), 3.2 – 3.9 (m, sugar protons).

Compound IV: It was isolated as a yellow amorphous powder and its UV spectral data are λmax MeOH: 215,253, 267; NaOAc: 225, 254, 372; H3BO3: 229, 260,364; AlCl3: 222, 274, 295, 398; + HCl: 272, 294, 355, 398; NaOMe: 223, 264, 398. 1H NMR spectra are δ ppm 7.35 (2H, *m*, H-2`/6`), 6.85 (1H, *d*, J = 9Hz, H- 5`), 6.71 (1H, *d*, J = 2.5 Hz, H-8), 6.64 (1H, *s*, H-3), `), 6.37 (1H, *d*, J = 2.5 Hz, H-6), 5.15 (1H, *d*, J = 7 Hz, H-1``), 3.81 (1H, *d*, J = 8.1 Hz, H-5``).

13C NMR spectra are: 181.9 (C-4), 170.8 (C-6``), 164.5 (C-2), 162.7 (C-7), 161.2 (C-5), 157.0 (C-9), 150.03 (C-4`), 145,9 (C-3`), 121.4 (C-1`), 119.2 (C-6`), 116.1 (C-5`), 113.6 (C-2`), 105.5 (C-10), 103.2 (C-3), 99.5 (C-1``), 99.5 (C-6), 94.6 (C-8), 75.8 (C-5``), 75.05 (C-3``), 72.9 (C-2``), 71.5 (C-4``).

Compound V: It was isolated as a yellow amorphous powder and its UV spectral data are λmax MeOH: 268,344; NaOAc: 262, 390; H3BO3: 268, 346; AlCl3: 274, 300, 351, 382; + HCl: 275, 300, 348, 382; NaOMe: 272, 305, 398. 1H NMR spectra are δ ppm 7.43 (2H, *m*, H-2`/6`), 6.86 (1H, *d*, J = 9Hz, H- 5`), 6.77 (1H, *d*, J = 2.5 Hz, H-8), 6.6 (1H, *s*, H-3), `), 6.4 (1H, *d*, J = 2.5 Hz, H-6), 5.00 (1H, *d*, J = 7 Hz, H-1``), 4.1 (1H, *d*, J = 8.1 Hz, H-5``), 3.87 (3H, *s*, OCH3).

Compound VI: It was isolated as a yellow amorphous powder and its UV spectral data are λmax MeOH: 255,269 sh, 301, 370; NaOAc: 257, 274, 329, 399; H3BO3: 261, 303 sh, 388; AlCl3: 272, 304 sh, 333, 458; + HCl: 265, 301 sh, 358, 428; NaOMe: 297, 321. 1H NMR spectra are δ ppm 7.70 (1H, *d*, J = 2.5 Hz, H-2`), 7.5 (1H,*d d*, J = 8.4, 2.5 Hz, H- 6`), 6.9 (1H, *d*, J = 8.4 Hz, H-5`), 6.45 (1H, *d*, J = 2.1 Hz, H-8), 6.20 (1H, *d*, J = 2.1 Hz, H-6).

Investigation of lipoidal matter

The air dried powder of *Olea europaea L.* leaves was extracted with petroleum ether (60-80). The petroleum ether extract was saponified by 10% alcoholic potassium hydroxide under reflux. After distillation of alcohol and dilution with water, the unsaponifiable fraction was extracted with ether and washed with water. The residue left after evaporation of ether was investigated for unsaponifiable matter by GLC using 3% OV-17 packed column, with detector flame ionization. The total fatty acid was investigated on the aqueous mother liquor after acidification with 10 % hydrochloric acid and the liberated fatty acids were extracted with ether [9]. The fatty acid methyl esters were identified on a GC-14A Shimadzu with C-R4A chromatopac integrator using GP 10% sp-2330 on 100/120 chromosorb WAW column, with detector flame ionization. Lipoidal matters are formed 3 % and its composition 95.5 % of unsaponifiable matter and 1.5 % total fatty acids.

RESULTS AND DISCUSSIONS

Compound I: the UV absorption in methanol showed characteristic absorption bands of an apigenin. The absence of bathochromic shift in band II with NaOAc referred to substituted 7-OH(10). The bathochromic shift of other shift reagents indicates that compound I is apigenin -7- *O*-glycoside. Acid hydrolysis showed the presence of apigenin and glucose. 1H NMR spectrum showed an AX system of two ortho – doublets in the aromatic region of δ 7.79 and 6.86 assigned to four protons at 2`, 6`, 3` and 5`. The downfield location of H-6 and H-8 resonances at δ 6.43 and 6.68 as an AM system of two meta-doublets (J = 2.1 Hz), detected the O-substitution at 7-OH. Presence of a O-β- glucopyranose moiety on position 7 was deduced from the characteristic δ- and J- values of its anomeric proton signal (δ 5.1 and J=7.2Hz). Compound I is identified as apigenin-7-*O*-β-D- glucopyranoside

Depending on the chromatographic properties, **compound II** is expected to be luteolin -7- mono -O-hexoside. UV spectral data in methanol showed characteristic absorption bands I (348 λmax) and II (255 λmax) of an luteolin like structure. Absence of bathochromic shift in band II on addition of NaOAc referred to substituted 7-OH (10). This evidence together with bathochromic shift observsd on addition of other shift reagents supported the structure of compound II as luteolin 7-O- glycoside. The complete acid hydrolysis revealed the presence of p- hydroxybenzoic acid, luteolin and glucose. 1H NMR spectrum showed an AAX system of H-2', H-6' and ortho coupled H-5' signals which diagnose the presence of 3',4'- dihydroxy B – ring. Characteristic downfield shift of both H-6 and H-8, as in case of compound I referred to the O- glycosidation on 7- OH. The above assigned resonances together with that of H- 3 elucidated the aglycone moiety as luteolin. Additionally, the presence of a p-hydroxybenzoyl moiety was deduced from the two ortho doublets at δ 7.65 and 7.12 assignable to H-2```/ H-6``` and H- 3```/ H-5``` respectively. The presence of an α- glucoside moiety was interpreted according to the δ and *J* values (5.3 and J = 3.6 Hz) of anomeric proton signal. The relative and strong downfield shift o both H- 1`` and H- 2`` resonances confirmed the acylation at O- 2`` of the glucoside moiety. Accordingly compound II is, luteolin-7-*O*-(2" -*O*-*p*-hydroxybenzoyl)-α-D- glucopyranoside.

The UV spectral data of **compound III** in methanol showed characteristic absorption bands at λmax 372, 256 of an quercetin like structure. The absence of bathochromic shift in band II on addition of NaOAc referred to substituted 7-OH(10). This evidence together with the bathochromic shifts which observed on addition of other shift reagents supported the substitution at 7- OH. Acid hydrolysis showed the presence of

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quercetin and glucose. 1H NMR spectrum showed an ABX system of H-2`, H-6` and ortho coupled H-5` signals was observed to diagnose the presence of 3`, 4` dihydroxy β – ring. Characteristic downfield shift of both H-6 and H-8, as in compound I referred to *O* – glycosidation on 7-OH. With the absence of H-3 signal elucidated the aglycone moity is quercetin. Thus compound III was identified a, querectin-7-*O*- β -D- glucopyranoside.

From UV spectral data of **compound IV** was expected to be luteolin - 7- O - glycoside. Acid hydrolysis showed the presence of luteolin and glucuronic acid.

The aromatic region of 1H NMR spectrum showed the characteristic δ- values and splitting pattern of a luteolin aglycone was assigned to indicate the presence of 7-*O*- glucuronide moiety from two characteristic resonances of H-1`` and H-5`` at δ 5.15 (1H, *d*, J = 7 Hz, H-1``) and 3.81 (1H, *d*, J = 8.1 Hz, H-5``) respectively.

Thus compound IV was identified as luteolin-7-*O*- β -D- glucuronopyranoside.

UV spectral data of **compound V** was expected to be chrysoriol - 7- O - glycoside. Acid hydrolysis showed the presence of glucuronic acid. Due to the downfield shift of H-2' prove that there is a substitution on 3` – OH. According to 1H NMR spectrum show a singlet signal at δ 3.87 of (OCH3) group. Thus compound V was identified as chrysoriol -7-*O*- β -D- glucuronopyranoside.

From UV spectral data and 1H NMR spectrum of **compound VI**, identified as quercetin.

The GLC analysis of hydrocarbon and sterol (table 1) revealed the presence of triacontane as the main hydrocarbon (11.64%) and cholesterol as the main sterol (9.86%).

The GLC analysis of saturated and unsaturated fatty acids (table 2) revealed the presence of palmitic acid as the main saturated fatty acids (34.5%) and oleic acid as the main unsaturated fatty acids (9.435%).

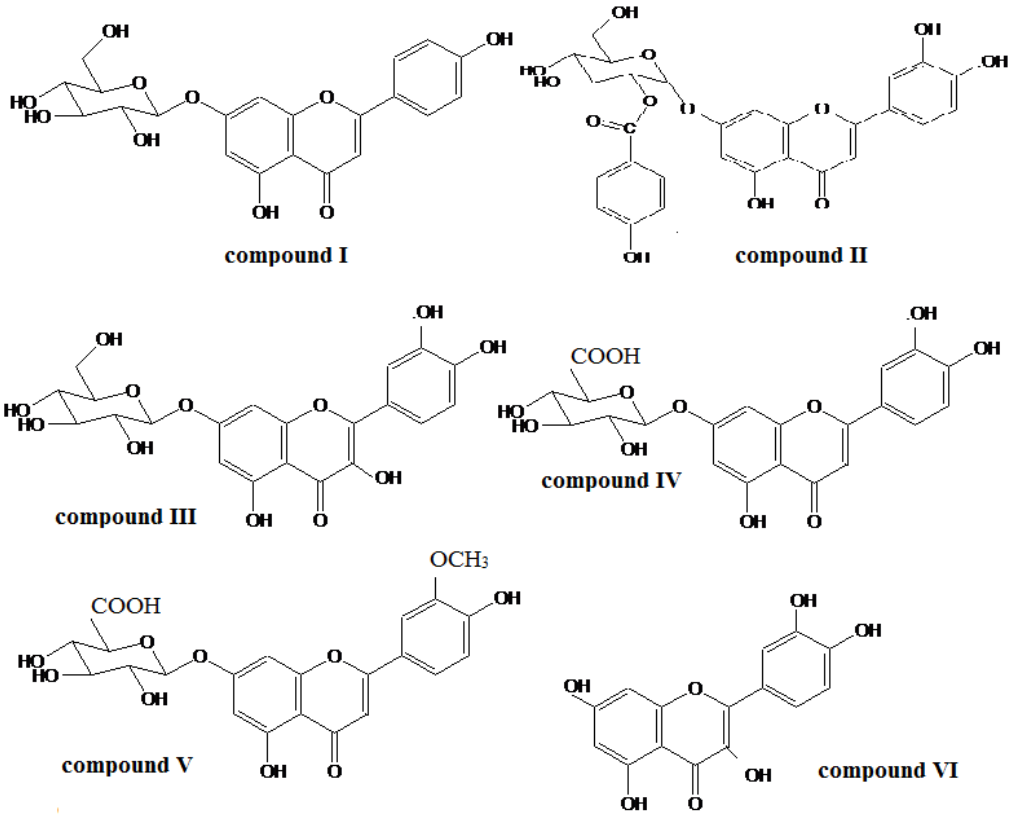

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Table 1: Gas liquid chromatography analysis of hydrocarbons and sterols in unsaponifiable matter of the leaves of *Olea europaea L*

Identified compounds	RRT*	% Concentration
Dodecane (C-12)	0.28	0.30
Tetradecane (C-14)	0.38	0.10
n-Octadecane (C-18)	0.47	0.27
Eicosane (C-20)	0.55	0.34
n-Docosane (C-22)	0.62	5.20
n-Tetracosane (C-24)	0.69	2.13
n-Hexacosane (C-26)	0.77	1.95
n-Octacosane (C-28)	0.80	4.89
Squalene	0.95	3.89
Triacontane	1.00	11.64
Cholesterol	1.20	9.86
Campsterol	1.32	0.78
Stigmasterol	1.38	-
β-Sitosterol	1.46	3.20
Hydrocarbons		30.71
Sterol mixture		13.84
Unidentified compounds		55.45

RRT* = relative retention time of triacontane = 1 with RT = 26.25

Table (2): Gas liquid chromatography analysis of fatty acid methyl esters of the leaves of *Olea europaea L*

Identified compounds	RRT*	% Concentration
Enanthic acid (C-7:0)	0.35	2.222
Capric acid (C-10:0)	0.49	7.273
Lauric acid (C-12:0)	0.69	0.604
Tridecanoic acid (C-13:0)	0.81	34.509
Myristic acid (C-14:0)	0.86	2.350
Palmitic acid (C-16:0)	1.00	34.500
Oleic acid (C-18:1)	1.29	9.435
Linolenic acid (C-18:3)	1.50	1.031
Saturated fatty acid		81.458
Unsaturated fatty acid		10.466
Unidentified fatty acids		8.076

RRT* = relative retention time of palmitic acid = 1 with RT = 17.00

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