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A PHARMACOGNOSTICAL STUDY OF CERTAIN PAPAVER SPECIES GROWING IN EGYPT. PART I: INVESTIGATION OF THE LIPID CONTENT

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تم فحص المحتوى الدهنى لجذور وسيقان وأوراق وأزهار وثمار البابافير رواس المصرى وأمكن فص كل من الاستيرولات الغير مشبعة ، ألفا أميرين ، بيتا أميرين وكذلك مادتين مجهولتين من الجزء الغير متصبن باستخدام كروماتوجرافيا الطبقة الرقيقة والعمود وتم تنقية المواد المفصولة وتعيين ثوابتها الطبيعة والكيميائية باستخدام الامتصاص الطيفى وأمكن التعرف على المادتين المجهولتين باجراء التجارب الكيميائية الأولية ، التحليل الكيميائى الدقيق ، الامتصاص الطيفى والتذبذب النووى الجزئى ، وأثبتت هذه الدراسة انها كحولات الفاتية مشبعة أحدهما احادة وتركيبه الكيميائى الأولى هو ك٢٢يد٤٦أ ، والأخر ثنائى وتركيبه الكيميائى الأولى هو ك٢٩يد٦٠أ.

كذلك تم فحص الاستيرولات المفصولة بعد استرتها إلى خلات وفحصها بواسطة كروماتوجرافيا الطبقة الرقيقة ، واثبتت هذه الدراسة أن الاستيرولات الموجودة تتكون من البيتا سيتوستيرول وكامبيستيرول واستجماستيرول. كما تم التعرف على الأحماض الأمينية من الجزء المتصبن من المادة الدهنية بعد استرتها إلى خلات باستخدام كروماتوجرافيا الغاز ، وأثبتت هذه الدراسة وجود حمض اللوريك ، الميريستيك ، البالميتك ، الاستياريك ، الأراشيديك والبهينيك (كأحماض دهنية مشبعة) ، اللرى أوليك ، الميرست أوليك ، البالميت أوليك ، الأوليك ، اللين أوليك ، اللينولنيك والأراشيدونيك (كأحماض دهنية غير مشبعة).

تم فحص الزيت الثابت لبذور كل نبات على حدة وتم التعرف على الأحماض الدهنية من الجزء المتصبن من الزيت بعد أسترتها إلى خلات وفحصها باستخدام كروماتوجرافيا الغاز وأثبتت هذه الدراسة وجود حمض اللوريك ، الميريستيك ، البالميتيك ، الاستياريك والاراشيديك (كأحماض دهنية مشبعة) اللورى أوليك الميرست أوليك ، الاوليك ، اللين–أوليك ، اللينولنيك وكذلك الأراشيدونيك (كأحماض دهنية غير مشبعة) بالإضافة إلى وجود حامض البالميت أوليك فى زيوت بذور النباتات المذكورة ماعدا بذور البابافير براكنياتوم ووجود حامض البهينيك فى زيت بذور البابافير رواس فقط.

A phytochemical study of the lipid content of the leaves, flowers, fruits, stems and roots of Papaver somniferum L. (Family: Papavaraceae), as well as a comparative study of the fixed oil of the seeds of Papaver somniferum L., P. bracteatum Lindl. and P. rhoeas L. growing in Egypt were carried out, using TLC, column chromatography and GLC. The study of the unsaponifiable matters of the different investigated parts of Papaver rhoeas L., revealed the presence of α-and ß-amyrins, ß-sitosterol, campesterol (not present in seeds) stigmasterol and two saturated aliphatic alcohols. The first is a primary alcohol with an empirical formula $C_{22}H_{46}O$, while the second is a secondary alcohol with an empirical formula $C_{29}H_{60}O$.

INTRODUCTION

Genus Papaver represents one of the most important genera belonging to the family Papaveraceae and well represented in Egypt[1,2]. The economic importance of *Papaver rhoeas* L., resides in its use for colouring medicines and food stuffs[3]. It was reported[4-7] to contain alkaloids belonging to the rhoeadine and papaverrubine groups (7-ring expanded tetrahydroisoquinoline system)[7]. Papaver seeds were used as a diet owing to its high content of

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fixed oils and proteins[8]. It was reported that the saponifiable fraction of the oils of *Papaver somniferum* L. and *P. rhoeas* L. seeds contain stearic, palmitic and linoleic fatty acids[9].

Reviewing the current literature, very little was reported concerning the physical characters and the chemical composition of the saponifiable and unsaponifiable fractions of the different parts of *Papaver rhoeas* L. as well as the fixed oils of *Papaver somniferum* L., *P. bracteatum* Lindl. and *P. rhoeas* L. seeds[10-13]. Therefore, it was found of interest of cover this shortage.

MATERIAL

Samples of the leaves, flowers, fruits, stems, roots and seeds of Egyptian *Papaver rhoeas* L. as well as the seeds of *Papaver somniferum* L. (identified by Prof. Dr. M. El-Hadidy, Prof. of Taxonomy, Botany Dept., Faculty of Science, Cairo University), were collected during the flowering and early fruiting stages of the cultivated plants in the Experimental Station of Medicinal Plants, Pharmacognosy Dept., Faculty of Pharmacy, Cairo University, at Giza. Each part was separately air-dried, powdered, sieved (sieve No. 10) and saved in brown bottles for this study. Seeds of *Papaver bracteatum* Lindl. were received from the Ministry of Public Health, Cairo, Egypt (in May 1987).

EXPERIMENTAL AND RESULTS

The lipid fraction of each part under investigation was prepared[14]. The physical percentage and physical constants viz. acid value[14], iodine value[14] and saponification value[14], for each lipid fraction were separately determined and recorded in Table I.

GLC analysis of the methyl ester of fatty acids

The fatty acids of each lipid fraction of each part under investigation were separately isolated[15]. The percentage of the total fatty acids, in each case, were determined and recorded in Table II.

The methyl ester of fatty acids, in each case, were separately prepared, their analysis was carried out using a column packed with 5% reoplex at 90°C and Pye Unicam Gas Chromatograph. Identification of the fatty acids was carried out by comparing the relative retention time of their corresponding peaks with those of pure available authentic, as well as, by referring to the published data on GLC analysis of fatty acids[17-19]. Results obtained were recorded in Table III.

Table I: Percentage and physical constants of the lipid fraction of each investigated parts of Egyptian *Papaver rhoeas* L.

	Papaver rhoeas L.						*Papaver somniferum* L. Seeds	*P. bracteatum* Lindl Seeds
	Leaves	Flowers	Fruits	Stems	Roots	Seeds		
Percentage (w/w)	4.20	2.80	2.83	2.40	2.75	22.43	38.89	31.71
Acid value[13]	38.13	46.17	105.00	38.79	46.67	15.10	15.30	14.91
Iodine value[13]	68.74	64.77	127.87	77.16	91.25	111.22	97.35	95.13
Saponification value[13]	98.18	122.94	162.69	98.18	115.98	192.25	201.02	195.73

Table II: Percentage of the total fatty acids and unsaponifiable matters of lipid fraction of the different parts of different Papaver species.

Plant	Part	Percentage of total fatty acids	Percentage of unsaponifiable matters
Papaver rhoeas L.			
	Leaf	2.10	0.70
	Flowers	1.90	0.90
	Fruits	2.30	0.21
	Stems	1.80	0.19
	Roots	1.60	0.10
	Seeds	21.51	---
P. bracteatum Lindl.	Seeds	30.25	---
P. somniferum L.	Seeds	37.34	---

Table III: Results of GLC analysis of the fatty acids methyl ester of lipid fraction of the different investigated parts of Egyptian *Papaver rhoeas* L.

Authentic fatty acids	R.R.T. to methyl pal- nitrate	*Papaver rhoeas* L.						*P. bracteatum* Lindl. Seeds	*P. somniferum* L. Seeds
		Leaf	Flowers	Fruits	Stems	Roots	Seeds		
m-Laureate	0.46	7.19	8.98	19.16	18.11	---	5.28	2.48	4.83
m-Laurioleate	0.60	---	9.11	---	13.93	---	6.91	4.38	5.92
m-Myristate	0.73	7.80	6.60	7.67	9.60	24.18	7.90	4.74	7.01
m-Myristoleate	0.86	5.30	2.93	11.38	5.57	27.20	4.47	5.62	8.70
m-Palmitate	1.00	16.33	19.40	17.42	16.10	13.10	19.87	10.94	11.06
m-Palmitoleate	1.66	5.68	1.71	---	9.91	10.48	2.26	---	4.60
m-Stearate	2.00	4.92	15.52	9.29	8.67	12.09	3.12	4.38	4.23
m-Oleate	2.20	6.63	16.55	30.66	8.67	8.06	6.86	8.17	6.53
m-Linoleate	2.80	20.54	9.86	2.79	6.50	3.88	28.89	20.13	23.57
Unknown-	3.00	17.23	---	---	---	---	---	---	---
m-Arachidate	3.66	4.02	8.32	0.81	2.32	---	4.66	11.09	7.61
m-Linolinoleate	4.20	4.35	---	0.81	0.62	1.01	4.06	9.19	8.70
m-Arachidoneate	6.66	---	0.11	---	---	---	2.94	18.89	7.25
m-Behineate	7.60	---	0.92	---	---	---	3.66	---	---

m., methyl ester.

Unsaponifiable matters

The unsaponifiable matters of each part of Egyptian *Papaver rhoeas* L. was reported[15]. Their percentage were determined and recorded in Table II. The methanolic solutions of the unsaponifiable matters which failed to produce any crystals were subjected to the following studies:

a) TLC

The unsaponifiable matters of each part was examined by TLC, adopting the solvent system,

Benzene-Ethyl acetate (84:16)[20]. The study revealed the presence of α and ß-amyrins, ß-sitosterol as well as two unknowns (R_f 0.67 and 0.91) in all the investigated organs.

b) Column chromatography

The unsaponifiable matters of each part under investigation, was subjected to column chromatography (aluminum oxide, 2.5x30 cm), elution was carried out by light petroleum (b.r. 40-60°C); light petroleum-benzene; 9:1. 7:3, 1:1, benzene, benzene:chloroform, 1:1; chloroform and chloroform:ethanol, 1:1, successively.

Each eluate, in each case, was separately concentrated to a small volume (5 ml) and subjected to TLC investigation, using the solvent system mentioned before.

The study revealed, in all cases, the presence of two unknown materials in light petroleum:benzene (1:9) (R_f 0.67 & 0.91) and α-amyrin (R_f 0.55) in light petroleum:benzene (1:1), ß-amyrin (R_f 0.63) in benzene:chloroform (1:1) and sterol or sterols (R_f 0.46) in chloroform:ethanol (1:1) eluate.

Identification of the isolated crystals

1. The crystals obtained from light petroleum:benzene (1:1) eluate, in all cases, were found to melt at 185-187°C and showed no depression when mixed with authentic α-amyrin. They gave a violet colour which did not change to green with Liebermann-Burchard test[21]. The IR spectrum and chromatographic characters were found to be identical with those of α-amyrin.
2. The crystals obtained from benzene: chloroform (1:1) eluate, in all cases, were found to melt at 196-198°C and showed no depression when mixed with authentic ß-amyrin. They gave a violet colour with Liebermann-Burchard test[21]. The IR spectrum and chromatographic characters were found to be identical with those of ß-amyrin.
3. The crystals obtained from chloroform: ethanol (1:1), in all cases, were found to melt at 136-138°C and showed no depression when mixed with authentic ß-sitosterol. The crystals gave a blue colour with Liebermann-Burchard test[21]. The IR spectrum and chromatographic characters were found to be identical with those of ß-sitosterol.
4. The amorphous substance obtained from light petroleum:benzene (9:1) eluate, in all cases, was found to be a mixture of two unknown substances.

This mixture was separated by reparative TLC using the same solvent system mentioned before. The isolated materials were separated and recrystallized from benzene.

Identification of substances A and B

Both substances "A" and "B" are white amorphous substances, soluble in light petroleum (b.r. 60-80°C), ether, pyridine and hot benzene; slightly soluble in cold benzene, chloroform, methanol and ethanol. Insoluble in water, mineral acids and alkalies. Substance "A" melts at 73-75°C. Both substances were found to be saturated aliphatic organic compounds[22] and completely free from nitrogen[23], halogens, sulfur and phosphorous. They failed to give Molish's test and to reduce Fehling's solution before and after hydrolysis.

Microchemical analysis

The elemental analysis of both substances were carried and revealed the following results:
C (80.98%), H (14.11%), O (4.91%) for substance "A".
C (82.08%), H (14.15%), O (3.77%) for substance "B".

IR (KBr) of substance "A" (cm^{-1}): 3320 (OH); 2940 (C-H stretch of methylene groups); 1465 (C-H bending of methylene groups); 1370 (C-H bending of methyl group); 1065 (C-O stretch of primary alcohol) and 720 (C-H bending of aliphatic methylene groups).

IR (KBr) of substance "B" shows almost the same absorption bands as substance "A", but it shows stretching at 1124 cm^{-1} (C-O stretch of secondary alcoholic group).

Preparation of acetate ester of both substances "A" and "B"[25]

About 0.2 gm of substance "A" and "B" was separately esterified. The purified esters (from methanol) were found to melt at 54-56°C (sub. "A") and 42-44°C (sub. "B") respectively.

The esters of both substances were tested for their purity by TLC, using silica gel DG Rehidel impregnated with 0.1 M boric acid as adsorbent, benzene as a solvent system and 50% v/v alcoholic sulfuric acid[26] as spraying reagent.

IR (KBr) of the acetate ester of both substances were carried out and revealed absorption bands at 1745 cm^{-1} (C=O stretch of saturated aliphatic ester) and at 1240 cm^{-1} (C-O stretch of acetate ester). The absorption band at 3320 cm^{-1} (O-H stretch of free hydroxy group) disappeared as a result of the esterification[24].

NMR spectra of the acetate ester of both substances "A" and "B" were recorded in Varian 60 MHz Spectrometer with a 500 CPS sweep width and $CDCl_3$ solvent. Both substances revealed the following peaks[24,27]. At 0.9 ppm, indicating methyl proton (CH_3-CH_2-); at 1.3 ppm, indicating methylene proton (C-CH_2-C) and at 2.0 ppm, indicating methyl proton of an aliphatic ester (CH_3-C-C-OCH_3).

From NMR spectra, it was concluded that both substances "A" & "B" are saturated aliphatic esters which esters.

A study of the separated sterol fraction

The sterol fractions of different parts of *Papaver rhoeas* L., were separately acetylated[25,28]. The investigation of acetyl derivatives, using silica gel G impregnated with 10% w/v silver nitrate[26] as adsorbent, light petroleum:chloroform:acetic acid (75:25:0.5)[25] as solvent system and 50% sulfuric acid as spraying reagent revealed the probable presence of campesterol, stigmasterol and ß-sitosterol in all investigated parts.

In order to confirm these results, the acetylated sterol fractions of each part, was subjected to reparative TLC, adopting the same procedure previously mentioned. The band corresponding to each substance were separately scraped out, refluxed with alcoholic pot, hydroxide (5%) for three hours. The solution, in each case, was filtered and extracted with successive portions of ether (3X20 ml each). The combined ethereal extracts were distilled off, the residue remained was crystallized by dissolving in methanol, concentrated and left to stand for 24 hour in refrigerator, filtered and dried over anhydrous calcium oxide.

The determination of m.p. of the separated materials and their acetate esters showed that the sterol fraction of all parts under investigation consists of campesterol (m.p. 158°C, acetate, 138-140°C); stigmasterol (m.p. 171-172°C) and ß-sitosterol (m.p. 138-140°C, acetate, 127-129°C).

CONCLUSION

1. The leaves of *Papaver rhoeas* L. contains the highest percentage (4.2%) of the total lipid, while the stems contain the lowest one (2.4%). The lipid fractions of the flowers, fruits and roots of the same plant attain 2.8%, 2.83% and 2.75%, respectively.
2. The iodine values of the lipid fractions (Table I) of the leaves (68.74 I_2) flowers (64.77 I_2) and stems of *P. rhoeas* L. (77.16 I_2) indicate that they are of non-drying nature, while those of the fruits (124.87 I_2) and roots (91.25 I_2) are of semi-drying nature[15].
3. The fixed oil of *P. somniferum* L. seeds contains the highest percentage of total fatty acids (37.34%) while that of *P. rhoeas* L. contains the lowest (21.51%).

The study of the fatty acids of the lipid content of the different investigated parts of the Egyptian *Papaver rhoeas* L., which were not previously studied, revealed that:

- The lipid fractions of the different investigated parts contains myristic palmitic, stearic and arachidic (with exception of roots) as saturated fatty acids and myristoleic, oleic, linoleic and linolenic with the exceptions of flowers as unsaturated fatty acids.
- The lipid fractions of the leaves, fruits, seeds and roots of *P. rhoeas* L. contains the highest percentage of linoleic (20.54%), oleic (30.66%), palmitic (19.87%) and myristoleic (27.20%) acids, among the

different investigated parts.

- The lipid fraction of the fruit contains the highest percentage of lauric acid (19.16%) among the different investigated parts, while that of the stem contains the highest percentage of palmitoleic acid (9.107%) and the lowest percentage of linolenic acid (0.615%).
- In the lipid fraction of the fruits, oleic acid constitutes the highest percentage of fatty acids (30.66%) among the different investigated parts while in flowers arachidonic acid constitute the lowest percentage of fatty acids (0.11%).
- The lipid fraction of the flowers is the only fraction containing arachidonic and behenic acids.
- Lauric and arachidic acids are present in the lipid fractions of the different investigated parts except the roots, while laurioleic acid is present only in those of the flowers and stems.
- Palmitoleic acid is present in the lipid fractions of the different investigated parts except that of the fruits, while linolenic acid is present in all lipid fractions except that of the flower.

4. The fixed oils of the seeds of *Papaver rhoeas* L., *P. Somniferum* L. and *P. bracteatum* Lindl was found to contain lauric, myristic, palmitic, stearic, arachidic (as saturated fatty acids) and laurioleic, myristoleic, oleic, linoleic and arachidonic (as unsaturated fatty acids) as well as palmitoleic acid, with the exception of that of *Papaver bracteatum* Lindl.
5. The fixed oil of the seeds of *Papaver rhoeas* L. contain, in addition, behenic acid. The unsaponifiable matters (Table II) of the leaves, flowers, fruits, stems and roots of Egyptian *Papaver rhoeas* L. (not previously studied) were found to contain ß-sitosterol, stigmasterol, campesterol, α- and ß-amyrins as well as two saturated aliphatic alcohols. The first is primary with an empirical formula $C_{22}H_{46}O$, while the second is secondary with an empirical formula $C_{29}H_{60}O$.

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