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COMPARATIVE STUDIES OF THE OIL FROM *SILYBUM MARIANUM* CULTIVATED IN EGYPT USING GLC

By

F. M. HAMMOUDA, S. I. ISMAIL, N. M. HASSAN AND A. K. ZAKI
Pharmaceutical Sciences Dept., National Research Centre, Dokki, 12311, Cairo, Egypt.

الدراسه المقارنة لمكونات زيت شوك الجمل (السلبيم) المنزرع في مصر بواسطة كروماتوجرافيا الغـاز

فايزه محمـد حمـوده و شمس الدين امبابي إسماعيل
ناهد محمد حسن و عادل أحمد كمال زكي

تتضمن الدراسه لكمية المقارنة لمكونات الجزء الزيتي لبذور نبات شوك الجمل المنزرعه بالأراضي المستصلحه حديثاً مع بذور النبات البري بنوعيه ذو الزهرة البيضاء والزهرة البنفسجية والذي ينمو في مصر .

وأوضحت نتائج التحليل بواسطة كروماتوجرافيا الغاز للشق الغير متصبن وجود بيتاسيتوستيرول كمركب أساسي في جميع العينات بالإضافة إلى وجود الكلوستيرول والاستجماستيرول . وأن نسبة البيتاستيوستيرول بلغت (٢٢,١٪ ، ١٩,٩٪ ، ١٣,٨٪) بالنسبة للشق الغير متصبن في النبات المنزرع والبري ذو الزهرة البنفسجية والزهرة البيضاء على التوالي .

كما أوضح التحليل الكروماتوجرافي الغازي وجود شق الهيدروكربونات المشبعة بنسب متفاوته . كما تبين أن نسبة الاحماض الدهنية الغير مشبعه في النبات المنزرع تمثل ٩٦٪ بالنسبة لباقي الاحماض الدهنية وهي أعلى عن مثيلتها في النبات البري ذو الأزهار البنفسجية ٩٠٪ وذو الأزهار البيضاء ٨٣٪ كما تبين أن حمض الأوليك يمثل المكون الأساسي لمجموعة الأحماض الدهنية .

كما تمت الدراسه على شق الأحماض الدهنية للنبات المنزرع بمحطة التجارب بكلية الزراعة جامعة الزقازيق تحت ظروف ومعاملات زراعية من حيث التسميد النيتروجيني لكل فدان والري حيث أوضح التحليل الكروماتوجرافي الغازي لشق الأحماض الدهنية ارتفاع نسبتها إلى ٩٨٪ في حالة النبات المنزرع تحت ظروف التسميد النيتروجيني بنسبة ١٠٠ كجم لكل فدان والري بنسبة ٦٠٪ لكل فدان وأن نسبة المحتوى الزيتي ارتفعت لتصل إلى ٣٤٪ .

Key Words: *Silybum marianum* , Asteraceae, GLC analyses, Hydrocarbons, Sterols, Fatty acids.

ABSTRACT

Quantitative comparative study of the oil fraction constituents in the fruits of *Silybum marianum* successfully cultivated in new reclaimed land with those of the two wild types *viz.*, purple flower and white flower bearing plants growing in Egypt were presented. GLC analysis of the unsaponifiable fraction of each sample showed the presence of sitosterol as the major constituent in all samples in addition to cholesterol and stigmasterol. The highest content of sitosterol was found in the cultivated plant (22.1%) followed by the wild purple plant relative to the total unsaponifiable fraction. Also, GLC analysis showed the presence of 15 alkanes from *n*-C_{12} to *n*-C_{32} in addition to an unsaturated n-alkene $C_{30}H_{60}$ in variable ratios. GLC analysis of the fatty acids fraction of each sample revealed that the highest amount of the unsaturated fatty acids was found in the cultivated plant (96%) followed by the wild purple plant (90%) while the lowest content was found in the wild white plant (83%). Moreover, oleic acid was found to be the major component in all samples. These comparative studies have been also carried out on the fatty acids content of the cultivated plant using different water regimes of 75, 60 and 45% per field capacity and nitrogen fertilization levels of 0, 50, 100 and 150 Kg per feddan. GLC analyses showed that the cultivated plant under water treatment of 60% per field gave the highest yield of the total oil content (34%).

INTRODUCTION

Silybum marianum (L.) Gaertn (family Asteraceae) is a plant growing wild in Egypt in the Nile valley. It occurs in two types, the most abundant one has purple flowers while the less abundant type has white flowers[1].

The oil from *Silybum marianum* fruits is known to possess therapeutic as well as nutritive value due to its high content of unsaturated fatty acids. Also it was proved that the oil can be used in food and as a lubricant[2].

The fruits of the plant have been used since antiquity for liver diseases. Many workers pointed out the importance of silymarin group as the active constituent which is used as remedy for hepatic diseases[3]. In our previous study we reported the evaluation of the silymarin content in the fruits of *Silybum marianum* cultivated under different agriculture treatments together with those of the two wild types[4].

The present work deals with the evaluation and comparative studies of oil content of the plant cultivated in the new reclaimed area as well as in the experimental station farm under different agriculture treatments including the effect of water regime and nitrogen fertilization levels with those of the two types growing wild in Egypt.

EXPERIMENTAL

(I) Comparative studies between the wild plant and cultivated plant in New Reclaimed land

The fruits of both types of the wild plant with purple flowers and white flowers were separately collected from the Cairo-Zagazig Road, Egypt and the seeds were cultivated, for the first time, in new reclaimed area (Salyhaya project). 200 G of seeds of the cultivated plant, wild purple plant and wild white plant were separately crushed and extracted with petroleum ether in Soxhlet apparatus till exhaution and the solvent was removed in *vacuo* (Table 1).

Table 1
Percentages of the Oil.

Samples	Seed's wt (gms.)	Oil %
The wild white flower	200	27.80
The wild purple flower	200	30.10
The cultivated purple flower	200	34.00

Five ml of the oil of each sample was separately saponified[5]. The unsaponifiable fraction of each sample was extracted with diethyl ether, dried over anhydrous sodium sulphate and evaporated under reduced pressure. These samples were subjected to GLC 304 GC Pye Unicam using coiled glass column (2.8 m x 4 mm) packed with diatomite C (100-120 mesh) coated with 1% OV-17, temperature program was gradient from 70°C to 27°C (10°C/min.), then isothermally for 25 min., injector temperature 300 °C, carrier gas N_2 30 ml/min., H_2 33 ml/min, air 330 ml/min and applying flame ionization detector.

The peak identification was performed by comparing the relative retention time of each component with those of the standard hydrocarbons and sterols (Table 2).

Table 2
Retention Times and Relative Percentages of the Hydrocarbon Fraction Components.

Alkane	Rentention Times			Relative Times		
	White (wild)	Purple (wild)	Purple (cultivated)	White (wild)	Purple (wild)	Purple (cultivated)
C_{12}	1.83	1.79	1.72	1.752	1.273	0.833
C_{14}	4.76	4.77	4.79	0.442	0.435	0.288
C_{16}	7.41	7.40	7.43	2.736	2.525	1.894
C_{18}	9.91	9.88	9.91	3.645	3.272	3.198
C_{20}	12.07	12.02	12.28	1.583	1.308	3.312
C_{21}	13.03	13.01	13.06	1.558	0.752	0.635

Table 2, Contd.

Alkane	Rentention Times			Relative Times		
	White (wild)	Purple (wild)	Purple (cultivated)	White (wild)	Purple (wild)	Purple (cultivated)
C_{22}	14.23	14.11	14.25	6.551	2.456	8.296
C_{23}	14.87	14.83	14.86	1.962	9.869	0.668
C_{24}	15.76	15.71	15.99	1.277	1.748	1.962
C_{25}	16.59	16.54	16.57	0.980	0.856	0.232
C_{26}	17.41	17.37	17.61	0.532	0.555	0.559
C_{27}	18.21	18.16	18.19	1.484	0.805	0.273
C_{28}	18.94	-	18.92	0.841	-	0.229
C_{30}-unsat.	19.76	19.67	19.68	2.545	2.534	0.558
C_{30}	20.44	20.93	20.75	1.013	2.407	0.999
C_{32}	21.99	22.45	22.45	0.050	1.884	0.256

The saponifiable fraction of each sample was rendered acidic, extracted with diethyl ether, dried over anhydrous sodium sulphate, then evaporated. One gram of the total fatty acids of each sample was esterified with methanol and subjected to GLC using the following conditions: GCV Pye Unicam using glass column (1.5 m x 4 mm) packed with diatomite C(100-120 mesh) coated with 10% PEGA, temperature program was gradient from 70 °C to 190°C (8°C/min) then isothermally for 45 min.; injector temp. 300 °C; carrier gas N_2 30 ml/min. and applying flame ionization detector.

The peak identification was performed by comparing the relative retention time of each peak with those of standard fatty acids mixture (Table 3).

Table 3

Retention Times and Relative Percentages of the Sterol Fractions

Molecular Formula	Sterols	Retention times			Relative Percentages		
$C_{27}H_{46}O$	Cholesterol	23.65	23.72	23.79	0.610	2.782	3.41
$C_{29}H_{48}O$	Stigmasterol	25.77	25.95	25.98	1.975	3.400	4.04
$C_{29}H_{50}O$	Sitosterol	27.83	27.64	27.97	13.821	19.914	22.12

II Comparative Study of the Cultivated Plants Under Different Agriculture Treatments

The seeds of purple flowering type of *Silybum marianum* were cultivated under different agricultural treatments.

100 Grams of each sample were extracted with petroleum ether (Table 4). Following the same method as previously described, the oil fraction (5 ml) was saponified and the saponifiable fraction was esterified. The methyl esters of the total fatty acids of each sample were separately subjected to GLC using the above mentioned conditions.

Table 4

Retention Times and Relative Percentages of Fatty acids

Fatty acids		Retention times (min.)			Percentages		
	Rt	White (wild)	Purple (wild)	Cultivated	White (wild)	Purple (wild)	Cultivated
C_6: O	1.30	1.88	1.83	-	0.402	1.424	-
C_8: O	2.47	2.29	-	-	2.677	-	-
C_{10}:O	5.77	5.13	-	-	0.993	-	-
C15:O	12.92	13.75	-	-	0.919	-	-
C_{16}:O	14.11	14.13	14.14	14.13	2.161	0.784	1.746
C_{16}:1	14.36	14.77	14.78	14.75	30.930	29.550	27.690
C_{18}:O	16.69	16.68	16.66	-	3.378	1.545	-
C_{18}:1	17.39	17.48	17.46	17.41	46.310	52.580	58.550
C_{18}:2	18.50	18.08	18.08	18.07	6.000	8.168	9.718
C_{20}:O	21.50	21.65	21.41	21.55	6.235	5.178	2.306

RESULTS AND DISCUSSION

The GLC analyses of the unsaponifiable fraction showed the presence of a series of 15 saturated hydrocarbons of $C_{12}H_{26}$ to $C_{32}H_{66}$ in the cultivated plant in the new reclaimed land and the wild white plant, while the wild purple plant had only 14 saturated hydrocarbons with the absence of $C_{28}H_{58}$. In addition, there was only the unsaturated hydrocarbon $C_{30}H_{60}$ in all samples with different percentages 2.5, 2.5 and 0.5% in the wild white, wild purple and the cultivated plants, respectively. Also GLC analyses revealed the presence of sitosterol as the major constituent of the unsaponifiable fraction; 13.8, 19.9 and 22.1% in the wild white, the wild purple and the

cultivated plants, respectively, in addition to cholesterol and stigmasterol.

REFERENCES

[1] **Tackholm, V., 1974.** Students Flora of Egypt, Published by Cairo University, Printed by Cooperation Printing Co., Beirut.

[2] **Ivanov, S., A. R. Salchinkin** and **A. S. Vorobyev, 1931.** The vegetable oils of the USSR VI. Oil of the Crucifera in connection with the climatic conditions of their home, Chem. Umschau Fette, Oele, Wachse Harze, 37: 349-354.

[3] **Wagner, H., L. Hoerhammer** and **R. Muenster, 1968.** Chemistry of silymarin (silybin) the active component of *Silybum marianum* fruits (*Carduus marianus*), Arzneim. Forsch, 18: 688-696.

[4] **Hammouda, F. M., S. I. Ismail, N. M. Hassan, A. K. Zaki** and **A. Kamel, 1993.** Evaluation of the silymarin content in *Silybum marianum* (L.) Gaertn. cultivated under different agricultural conditions, Phytotherapy Research, 7: 90-91.

[5] **Klare, S. M., 1964.** Fatty acids and their Chemistry Properties, Production and Uses, Part 3, Interscience Publisher, New York.