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HPLC AND MS ANALYSES OF LUTEIN-ESTERS FROM *TAGETES PATULA L.*

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كروماتوجرافيا السائل ذات الأداء العالي والطيف الكتلي لاسترات الليوتين من نبات التاجيتس باتيولا لين

محمد سعد الدين كراويه و فايزه محمد محمود
شمس الدين امبابي اسماعيل و عادل كمال زكي و نجلاء محمد نطيف

اسفرت دراسة المحتوى الكاروتينيدي لنبات القطيفة عن فصل وتعريف كل من زانثوفيلات الليوتين الحر وثنائي ميريسينات الليوتين وميريسيتات باليتات الليوتين وثنائي باليتات اليوتين وباليتات استيارات الليوتين كما تم التعرف عليها بواسطة الطيف الكتلي لكل منها . بالإضافة إلى تحضير لون أصفر طبيعي يستخدم كمكسب لون في الصناعات الغذائية والدوائية .

Key words: *Tagetes patula, Asterceae, lutein, xanthophyll, lutein dimyristate, lutein myristate palmitate, lutein dipalmitate, lutein palmitate stearate.*

ABSTRACT

Investigation of the xanthophyll esters of *Tagetes patula L.* flowers resulted in the isolation and identification of lutein, lutein dimyristate, lutein myristate palmitate, lutein dipalmitate and lutein palmitate stearate. In addition the preparation of natural yellow colour additive has been described.

INTRODUCTION

The flowers of the genus *Tagetes* are known to contain carotenoid pigments occurring as xanthophyll esters. Philip and Berry [1] identified several Lutein esters in *Tagetes erecta* (Marigold flowers). Gomez et. al. [2] revealed that the main carotenoid of *Tagetes erecta* is lutein which may be found either free or estrified to one or two fatty acids and could be separated chromatographically. Philip and Berry [3] found that lutein fatty acids esters from Marigold petals (*T. erecta*) could be purified by precipitation with alcohol. These partially purified lutein esters can be used to impact yellow to orange colour for foods. A purified lutein dipalmitate was prepared [4] and marketed under the name of adaptinol to be used as ophthalmological agent. Javier [5] separated lutein and its fatty acid esters from *T. erecta* by reversed phase HPLC on a Lichrosorb RP-18 or Zorbax ODS column. Gregory [6] quantified lutein esters in Marigold flowers and reported that their content in fresh petals varied from 4 µg/g in green yellow flowers to 800 µg/g in orange brown flowers.

Tagetes patula is widely cultivated in Egypt as an ornamental plant. The present work deals with the isolation and identification of lutein esters from the flowers. Moreover, the study aims to prepare stable and cheap natural colour additive to be used in foods, drugs and cosmetics as substituent for the synthetic dyestuffs*.

This study is a part of the project entitled "Industrialization of Natural Colour Additives from Local Plants" financed by the Academy of Scientific Research and Technology of Egypt.

EXPERIMENTAL

Plant material

The flowering heads of *Tagetes patula L.* (*Asteraceae*) were collected from the experimental station farm, Faculty of Agriculture at Moshtohor in August/September. It was kindly identified by Prof. Dr. Hassan A. Hassan, Professor of Horticulture. The dark orange red petals were detached from the green calyx and the receptacle, then dried in subdue light in a cool place.

Apparatus

Pye-Unicam SP 8-100 UV spectrophotometer, England; Knauer HPLC, pump type 364 version no. 0391, variable wavelength monitor with digital analysis version A0293; Knauer Degasser automatic for HPLC; Computer system, Faset software, Germany; Finnigan 4510 mass spectrophotometer, San Jose CA. Employing an ion source of 70 e.v.; and Varian unit - ^{1}H - NMR (500 Mhz), Austin, TX.

Preparation of oleoresin

About 100 g of air dried ground petals of *Tagetes patula* were mixed well with α - tocophyeryl acetate (0.01g%), and extracted by percolation with successive portions of methylene chloride to exhaustion. The combined extracts was evaporated *in vacuo* at 40° till dryness, yielding 7.8 g of brownish orange oleoresin.

Isolation of lutein esters

Lutein esters were prepared from oleoresin according to the method reported by Philip and Berry [3]. Ten grams of the oleoresin were dissolved in 50 ml hot isopropyl alchol, then kept over-night in a refrigerator. An intense brownish orange precipitate was deposited and separated by filtration through sintered glass funnel G.3., washed several times

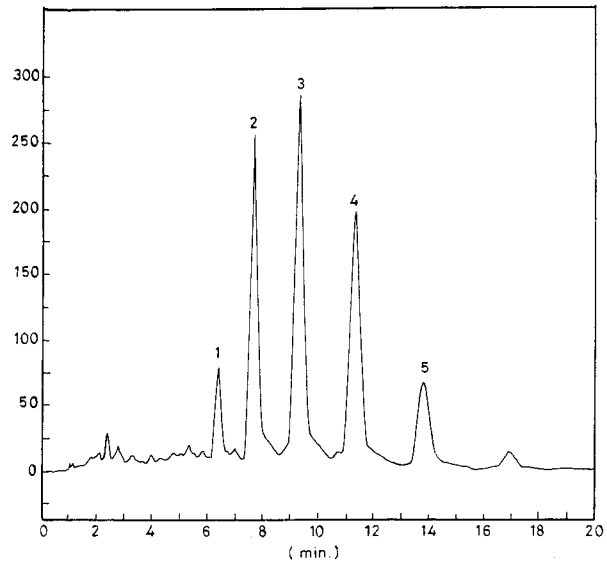
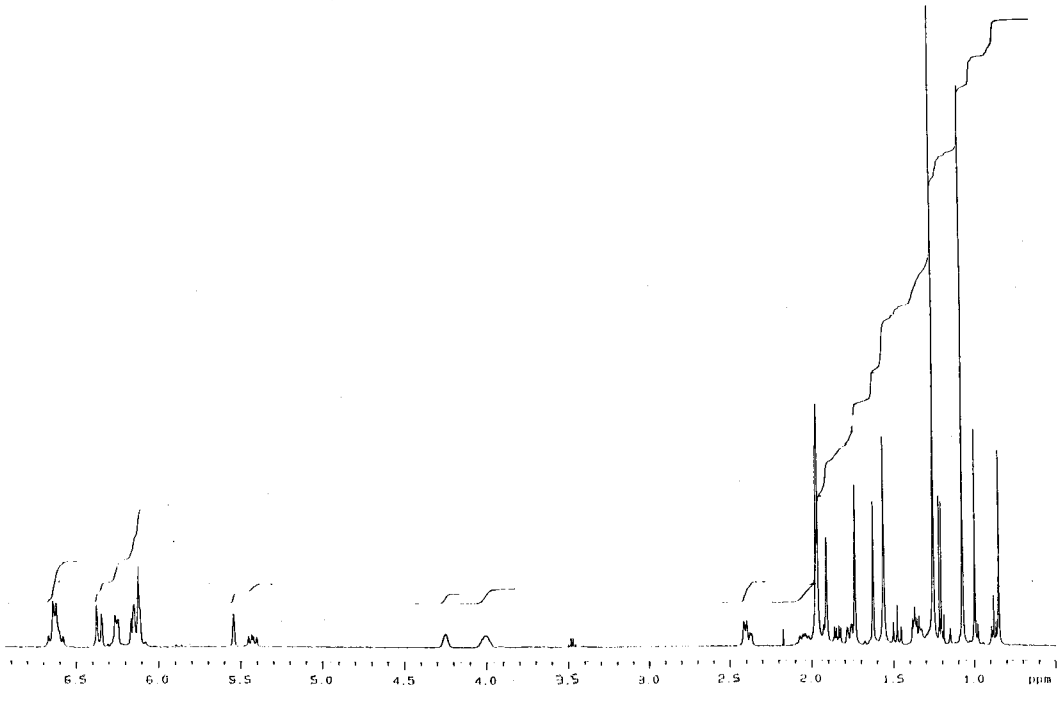


Fig. (1): HPLC-chromatogram of *Tagetes patula* extract.



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ct	80
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FLAGS	
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in	y
dp	y
DECOUPLING	
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dm	nnn
dmm	c
dmf	not used
dpwr	6
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vs	225
sc	45
wc	350
hzmm	9.28
is	600.00
ins	1.000
frl	303.4
rfp	0
th	2.9
nm ph	

(Fig. 2): ^{1}H-NMR spectrum of isolated Lutein

with cold isopropyl alcohol and then drived in vacuum oven at 40° when 2.3 g was obtained. The precipitate was subjected to TLC analysis using silica gel H plates developed with petroleum-ether (b.r. 40-60°)/acetone (90:10), and (95:5).

Quantitative evaluation of the isolated lutein esters

Lutein esters were subjected to HPLC analysis by injecting 0.1% solution in acetone (5µl) into C_{18}-RP (125x4mm) Merck Lichrocart (Lichrosphere 5µ) column, using methanol/ethyl acetate (60:40) as mobile phase, with flow rate of 1ml/min. and chart speed of 0.5 cm/min. and applying a variable wavelength detector at 446 nm.

The obtained HPLC chromatogram (Fig. 1) showed five peaks. Applying preparative HPLC using C_{18}-RP column (25 cm x 4 mm i.d.) with the same isocratic solvent system as mentioned before the five fractions were separated.

Each of the collected fractions was evaporated *in vacuo* then subjected to mass spectroscopic analysis. Table (2) shows mass numbers of the significant ions in the mass spectra of the lutein fatty acid esters.

Isolation of lutein

About 0.5 g of the purified lutein esters was subjected to saponification process following the same method reported by Baranyai [7]. An intense orange residue was obtained, dissolved in a mixture of petroleum ether/ether (2:1) and left overnight in a refrigerator (-4°) to give intense orange crystals.

The purity of the isolated substance was emphasized by co-chromatography with authentic lutein, where it gave a single spot of R_f 0.34 on silica gel G coated plates developed with petroleum ether/benzene/acetone (70:10:20).

UV/VIS spectral measurements of the isolated substance in *n*-hexane, (Fig. 1) showed the characteristic bands at λ_{max} 470, 445, and 418 (sh) which are identical to that of authentic lutein. Also identity of the isolated substance as lutein was verified by ^{1}H-NMR spectrum (Fig. 2), Table (2). The data are in agreement with that reported for lutein [8].

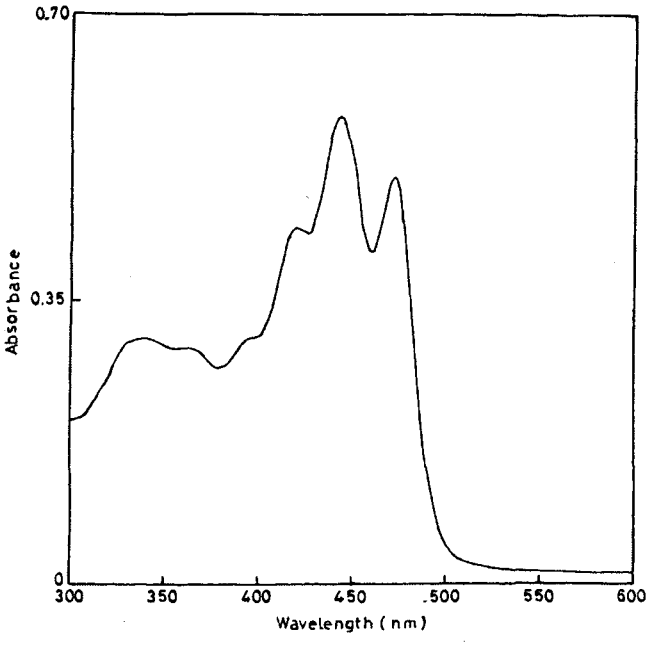


Fig (3): Absorption spectrum of *Tagetes patula* petals extract.

Table 1

Retention times and area percent of lutein fatty acid esters of *Tagetes patula*

Peak no.	R.T. (min.)	Relative %	Peak identity
1	6.48	5.93	–
2	8.15	24.93	Lutein dimyristate
3	10.05	33.14	Lutein myristate palmitate
4	12.21	26.01	Lutein dipalmitate
5	15.12	9.99	Lutein palmitate stearate

Table 2

Mass numbers of the significant ions in mass spectra of the isolated lutein esters

	Peak No.			
	2	3	4	5
M	988	1016	1044	1071.5
a	760	760	788	788
b	760	788	788	816
c	532	532	532	532
d	228	256	256	284
e	228	228	256	256
Lutein esters	Dimyristate	Myristate palmitate	Dipalmitate	Palmitate stearate

Preparation of the total carotenoids as food colour additive either in solid form or in liquid form:

I - In Solid form (Absorption on carbohydrate carrier):

About 10 g of *Tagetes patula* carotenoids was supported separately on carbohydrate carriers *viz.* dextrin/lactose (1:1) and maltodextrin/lactose (2:7) as follows:

The oleoresin (*ca.* 10 g) was dissolved in 500 ml of methylene chloride in round bottom flask of rotary evaporator, followed by the addition of carbohydrate carrier, and Tween 80, thoroughly mixed by allowing to rotate for about two minutes. Rapid evaporation of methylene chloride was carried out, and the residual solvent was removed under vacuum in a desciccator. An orange yellow, homogeneous, water powder was obtained.

II - In Liquid form (Water soluble base):

A solution of the oleoresin 20% containing the total carotenoids was dissolved in polysorbate 80 as diluent.

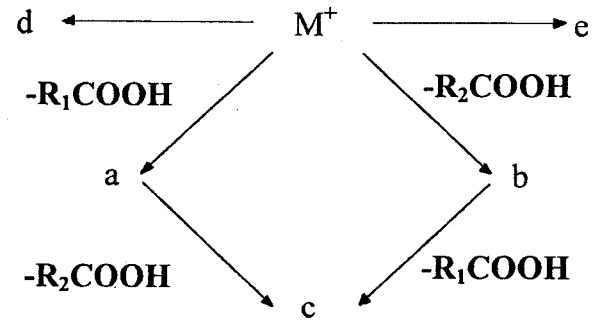


Fig. (4) Schematic representation of the fragmentation pattern of lutein esters in mass spectroscopic analysis.

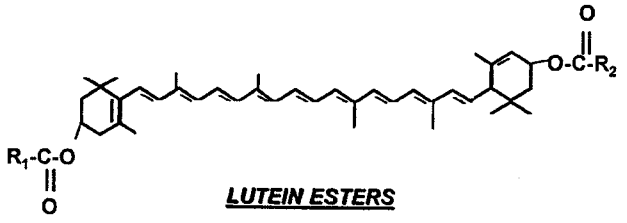
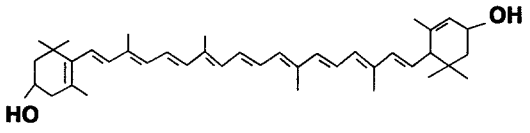


Table 3

^{1}H-NMR-spectral data of the isolated Lutein



H-Position	PPM	Reported data	Grade of multiplicity
H-2	1.56 1.63	1.47, 1.70	Triplet
H-3	4.00	4.00	
H-4	2.07 2.37	2.04, 2.39	multiplet
H-7	6.12	6.11	
H-8	6.11	6.11	
H-10	6.15	6.14	
H-11	6.63	6.63	
H-12	6.38	6.35	
H-14	6.26	6.24	
H-15	6.62	6.62	br. s.
H1, 1-Me's	1.07	1.07	
5-Me	1.74	1.74	
9-Me	1.97	1.97	
13-Me	1.97	1.97	
H1', 1'	0.84	0.85, 1.00	singlet
Me's	0.99		
-'5 Me	1.62	1.62	
-'9 Me	1.91	1.91	
'3 B-(H)	4.25	4.22	

RESULTS AND DISCUSSION

The absorption spectrum (Fig. 3) of *Tagetes patula* petals extract exhibits maxima typical of xanthophylls. It is similar to that reported in the literature [9]. Further study of the extract by HPLC showed that it was composed of five main components (Fig. 1), the structures of which were elucidated with of mass spectrometry. The mass spectra of the xanthophyll fatty acid esters are characterised by their simplicity. Six characteristic types of ions appear apart from the very intense alkyl and alkylene fragments below m/e 200 (Fig. 4). Table 2 shows mass numbers of the significant ions in the mass spectra of the lutein fatty acid esters [4].

From the obtained data it is clear that the major lutein ester in *Tagetes patula* is lutein myristate palmitate (*ca* 33% of the total carotenoids) followed by lutein dipalmitate (*ca* 26%), lutein dimyristate (*ca* 25%) and palmitate stearate (*ca* 10%). While free lutein does not exceed 1.5% of the total carotenoids. However, in case of *Tagetes erecta* the major component was lutein dipalmitate (35.5%) followed by lutein myrisitate palmitate (24.7%) [3]. ^{1}H-NMR spectrum (Fig. 2), Table (3). The data are in agreement with that reported for lutein [8].

The yellow food colour additive prepared from *Tagetes patula* carotenoids showed a high stability towards daylight, and room temperature. It was stable over a wide pH range (2.8-12). It can be safely used as a substituent for the commonly used synthetic dyestuffs like sunset yellow or other imported natural colour additive like Annatto.

REFERENCES

[1] **Philip, T.,** and **Berry, J.W., 1975**. Nature of lutein acylation in Marigold (*Tagetes erecta*). J Food Sci., **40**(5), 1089-1090.

[2] **Gomez, R., Goni, F.M.,** and **Macarulla, J.M., 1978**. Carotenoids from Marigold (*Tagetes erecta*). Rev. Esp. Fisiol **34**(3), 253-256.

[3] **Philip, T.,** and **Berry, J.W., 1976**. Process for the purification of lutein-fatty acid esters from Marigold petals. J. Food Sci., **41**(1), 163-164.

[4] **Wolfgung, G., Hans-Jurgen, P.,** and **Christian, W., 1983**. Mass spectrometric identification of xanthophyll fatty acid esters from Marigold flowers (*Tagetes erecta*) obtained by HPLC and Craig counter-current distribution. J. Chromatography, **262**, 277-284.

[5] **Javier, D.L.R., 1989**. Reversed-phase HPLC separation of lutein and lutein fatty acid esters from Marigold flowers. J. Chromatography, **464**(2), 442-447.

[6] **Gregory, G.K., Chen, T.S.,** and **Philip, T., 1986**. Quantitative analysis of lutein esters in Marigold flowers (*T. erecta*) by HPLC. J. Food Sci, **51**(4), 1093-4.

[7] **Baranyai, M., Matus, Z.,** and **Szabolcs, 1982**. Determinatio, by HPLC, of carotenoids in paprika products. Acta Aliment, **11**, 309-323.

[8] **Isler, O., 1971**. Carotenoids, Birkhause-Verlag, Basel.

[9] **Cholnoky, L., Szabolcs, J.,** and **Nagy, E., 1958**. Formation and transformation of carotenoids during the ripening of *Capsicum annum*. Justus Liebigs Ann. Chem., **616**, 207.