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RESEARCH ARTICLE

Quantitative Analysis of Lutein from Iraqi *Tagetes erecta*

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Abstract

Tagetes erecta is a plant belongs to the genus *Tagetes* and family Asteraceae. Phytochemical studies show that *tagetes erecta* leafs and flowers are rich in alkaloids, flavonoids, tannins and essential oils. Lutein which is an oxycarotenoid and phenolic compounds was found to be the major secondary metabolite. [1]The name lutein comes from the Latin word, *lutea*, meaning yellow. At normal concentrations in food, it is a yellow pigment, but can appear orange or red at high concentration [2].The tetraterpene Lutein and zeaxanthin are found in high concentrations in the macula of the human eye, giving the macula its yellowish color. In fact, the macula also is called the "macula lutea" (from the Latin *macula*, meaning "spot," and *lutea,* meaning "yellow") have an important role in reducing the oxidation and reduce the incidence of cataracts. It is believed that lutein and zeaxanthin in the macula block blue light from reaching the underlying structures in the retina, thereby reducing the risk of light-induced oxidative damage that could lead to macular degeneration (AMD) [3]. In this study lutein was isolated from *Tagetes erecta* by column chromatography. The isolated lutein was identified by TLC, HPTLC. Structure elucidation was performed using UV, FTIR, ^{1}H-NMR and ^{13}C- NMR. The quantity of lutein was measured in the Iraqi plant.

Keywords: *Tagetes; Lutein; Isolation.*

Introduction

Tagetes erecta, known as African marigold a plant belongs to the genus *Tagetes* and family *Asteraceae*. Phytochemical studies show that *tagetes erecta* leafs and flowers are rich in alkaloids, flavonoids, tannins and essential oils. The oxycarotenoid lutein and phenolic compounds are the major secondary metabolite found in the plant. The plant has been used in medicine since prehistoric times. In India the juice of the flowers was occasionally used as blood purifier and as remedy for piles, while the leaves were used for pain, inflammation and bleeding. The decoction of plant and flowers was used in Mexico for colds, rheumatic pains, and bronchitis.

Also the plant was used for boils and eye infections. In Brazil the plant was used for joint pains, muscle spasms and allergic contact [4]. Lutein is a natural compound; it is belonging to xanthophyll's which is oxygenated carotenoids [5]. Lutein is one of the major components of the macular pigment of the retina.

Marigold flower (*Tagetes erecta L.*) represents a rich source of lutein. It is grown for business purposes in Mexico, Peru, Ecuador, Spain, India or China [6]. Lutein is a free-flowing orange-red powder. It is insoluble in water but it is soluble in hexane [7].The name lutein comes from the Latin word, *lutea*, meaning yellow. At normal concentrations in food, it is a yellow pigment, but can appear orange or red at high concentration [8].Several studies show that lutein help prevent age related macular degeneration or slow its progression. Multivitamin products such as Centrum® usually contain small doses of lutein, 2 mg or less.

So-called "eye vitamin" products such as PreserVision® Areds 2 or Ocuvite® contain lutein in the range of 5-20 mg per daily dose. Lutein can also be found as an individual supplement containing 10-20 mg per capsule [9].Lutein is biosynthesized in plant through the coupling of two geranyl geranyl pyrophosphate Figure 1.

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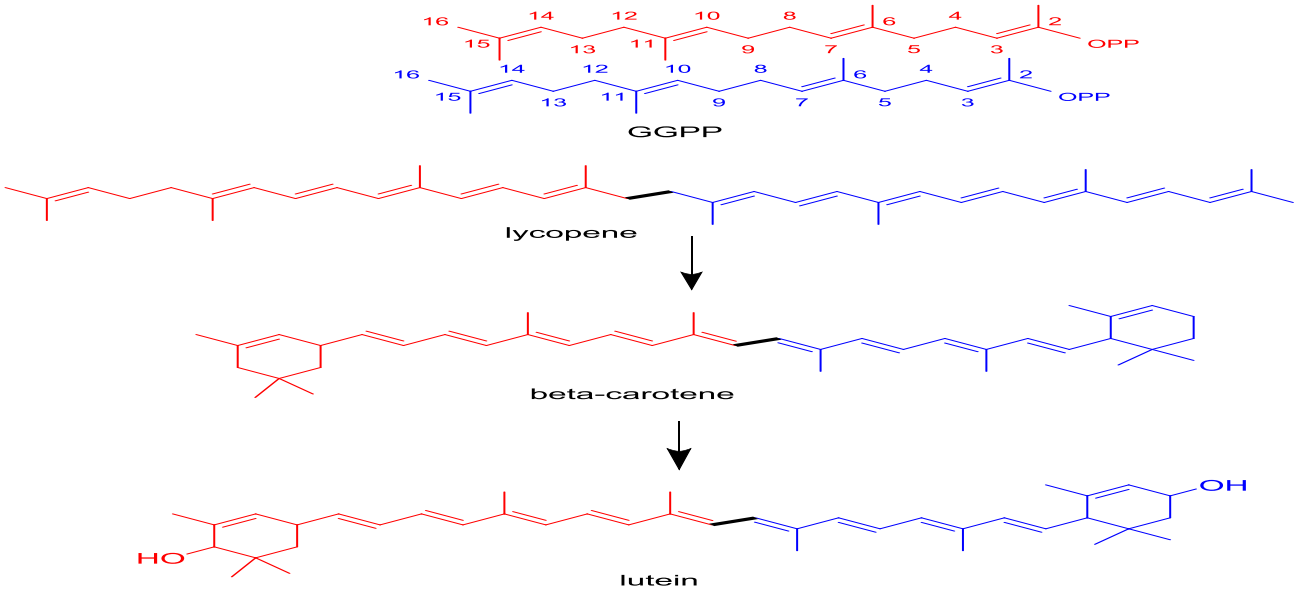

Figure 1: Biosynthesis of lutein from geranyl granyl pyrophosphate

Material & Methods

Preparative thin layer chromatography HPTLC (CAMAG), IR, UV spectroscopy, ^{1}H-NMR and ^{13}C- NMR All chemicals and solvents are of analytical grade. Standard lutein was obtained from Sigma Aldrich Company.

Plant Material

The flowers of *Tagetes erecta* were collected from AL-Itifia nursery during March and April of 2018 in Baghdad and authenticated by the National Herbarium at Abu-Graib, they were dried in shade for several days at room temperature and then grinded as powder and weighed. Extraction and isolation of lutein from *Tagetes erecta* flower 100 gram of powdered plant flowers extracted with 1500 mL of hexane using soxhlet apparatus. The extracts filtered and evaporated to dryness under reduced pressure using rotary evaporator. The hexane extract (5.4 gram) subjected to column chromatography (1M X 4cm) over silica gel (80-100mesh) and eluted with hexane. The fractions (20-30) were collected by monitoring on thin layer chromatography (TLC) using (petroleum ether: diethyl ether: glacial acetic acid, 8:2:1) as mobile phase and the spot was detected by eye and under UV 365 nm.

Preparative TLC

The combined fraction from column chromatography was further purified by preparative TLC performed on silica gel GF254 pre-coated plates (0.5mm thickness) and developed with (petroleum ether: diethyl ether: glacial acetic acid, 5:4:1), the separated bands were visualized by eye (445 nm), bands at Rf= 0.66 were scrapped off and eluted with acetone. Solvent was evaporated by rotary evaporator.

Results and Discussion

TLC of isolated lutein was compared with standard lutein using (petroleum ether: diethyl ether: glacial acetic acid, 8: 2: 1) as mobile phase, Figure 2.

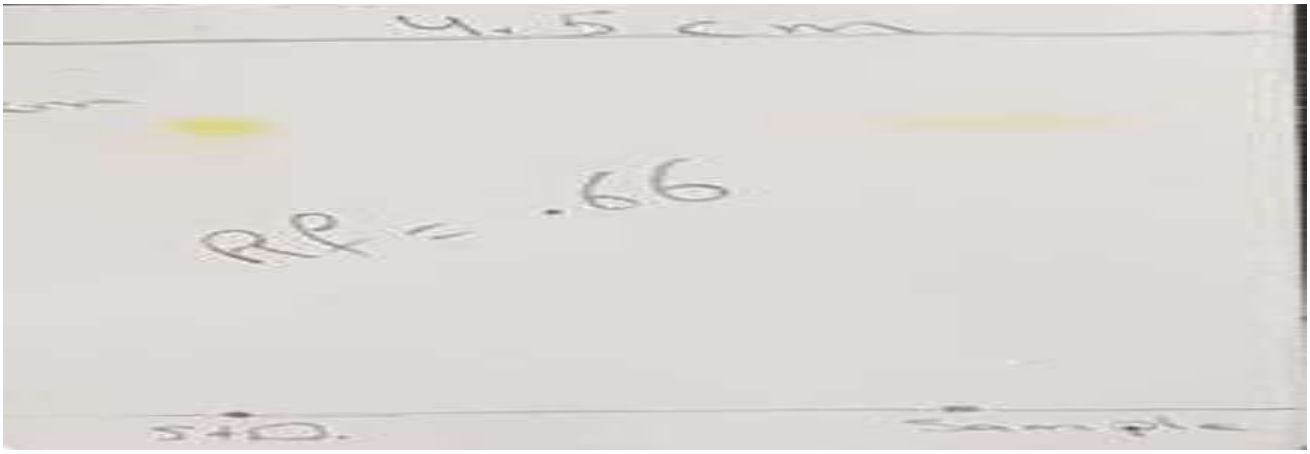

Fig 2: TLC of isolated lutein compared with standard

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Table 1: Rf of standard and isolated lutein

Solvent system	Rf value of standard	Rf value of isolated
Petroleum ether: Diethyl ether: Glacial acetic acid (8:2:1)	0.66	0.65

HPTLC also support the occurrence of lutein in *Tagetes erecta* of Iraq, Figure3 and 4.

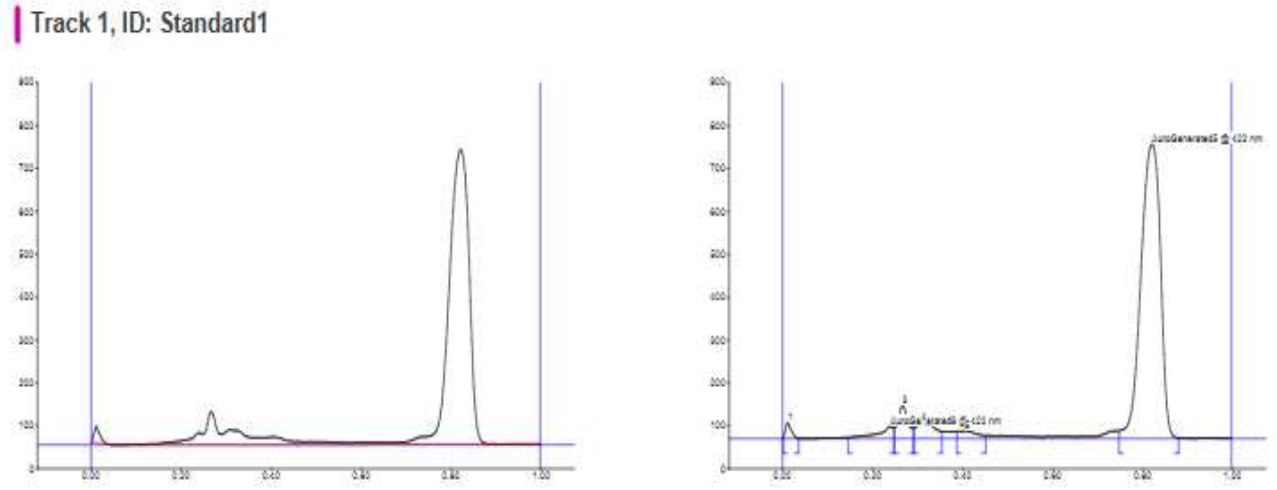

Fig 3: HPTLC chromatogram of standard lutein

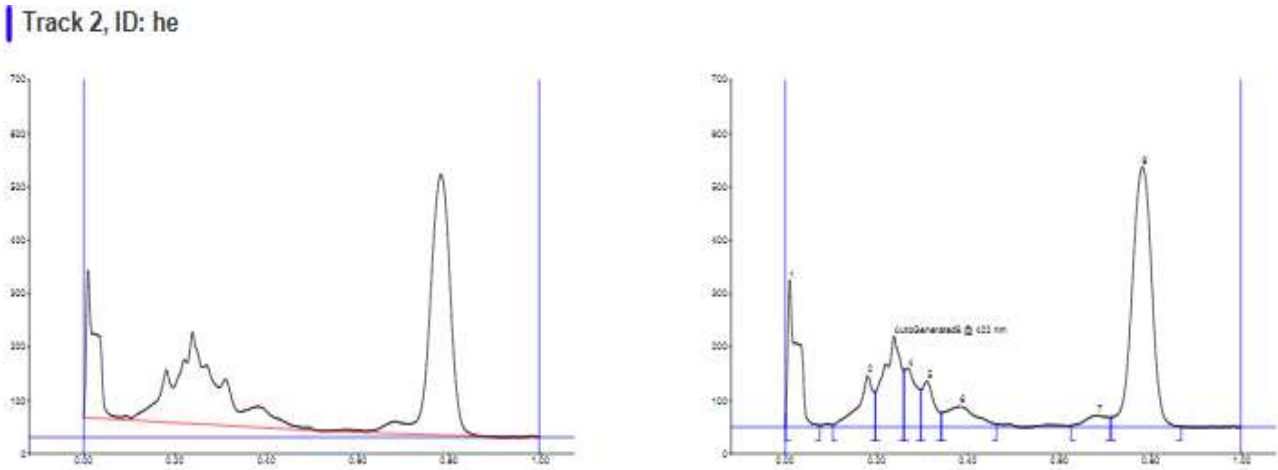

Fig 4: HPTLC chromatogram of isolated lutein

Preparative TLC with the same solvent system gives of 855 mg of isolated lutein from 100 grams of the plant (.855%)

Isolated lutein and standard lutein data UV and IR are shown in Figure 5, 6, 7 and 8 respectively.

Structure elucidation of Lutein

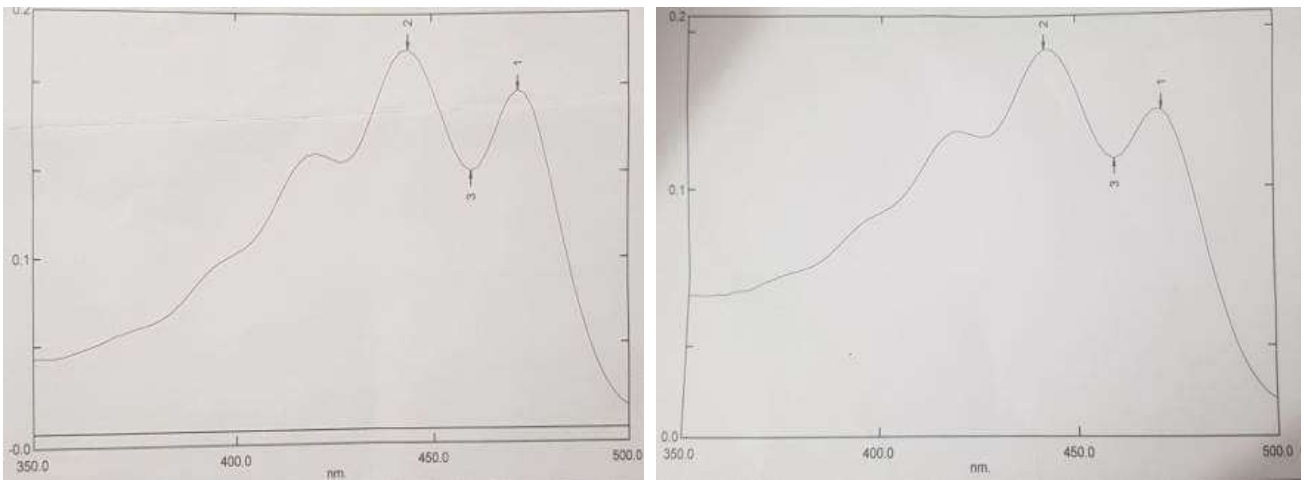

Fig 5 and 6: UV Spectra of standard and isolated lutien

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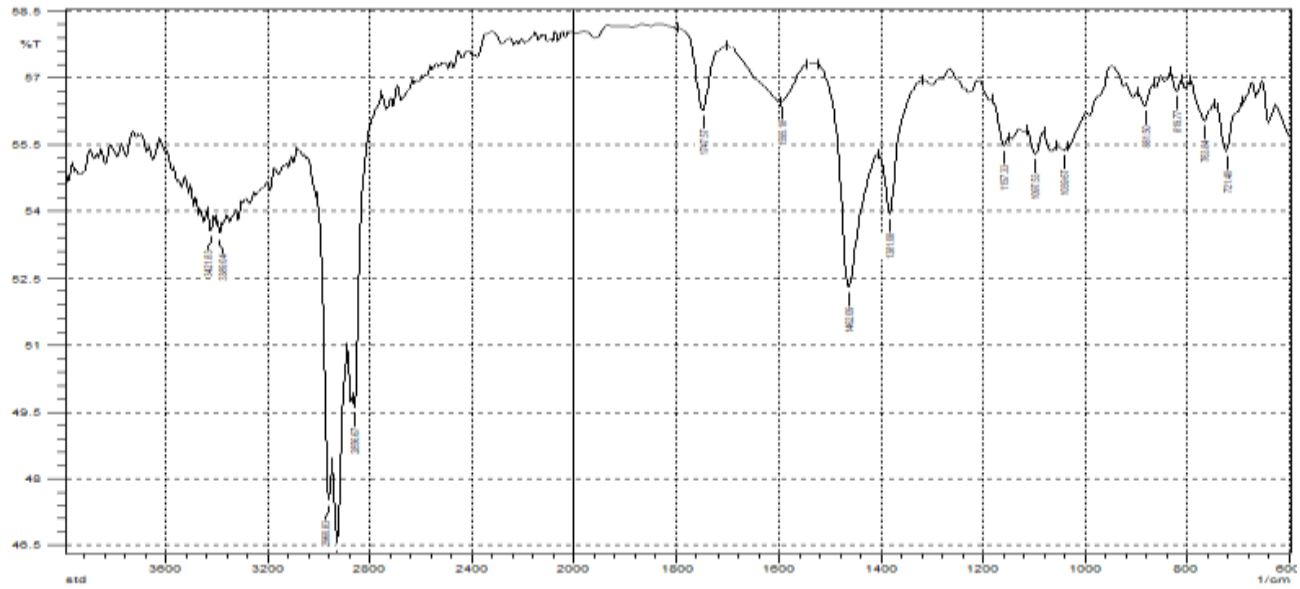

Fig 7: FT-IR spectroscopy for standard lutein

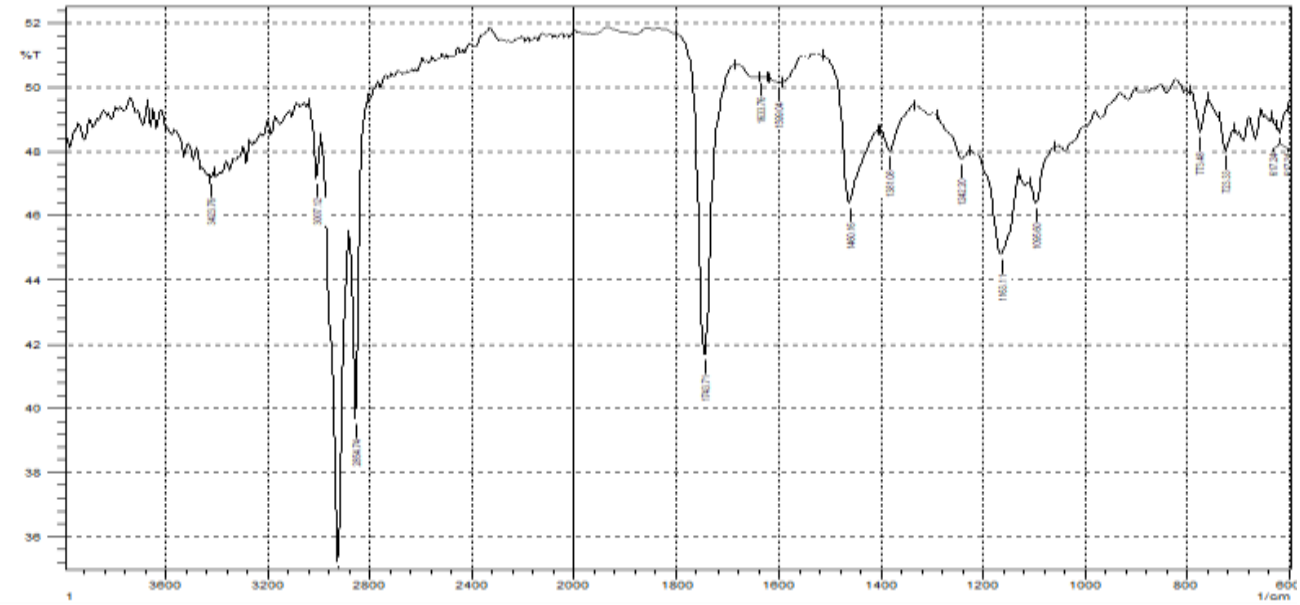

Fig 8 FT-IR spectroscopy for isolated lutein

^{1}H NMR and ^{13} C NMR. 30 mg of isolated lutein was subjected to ^{1}H and ^{13} C NMR to confirm the chemical structure of lutein Figure 9-11.

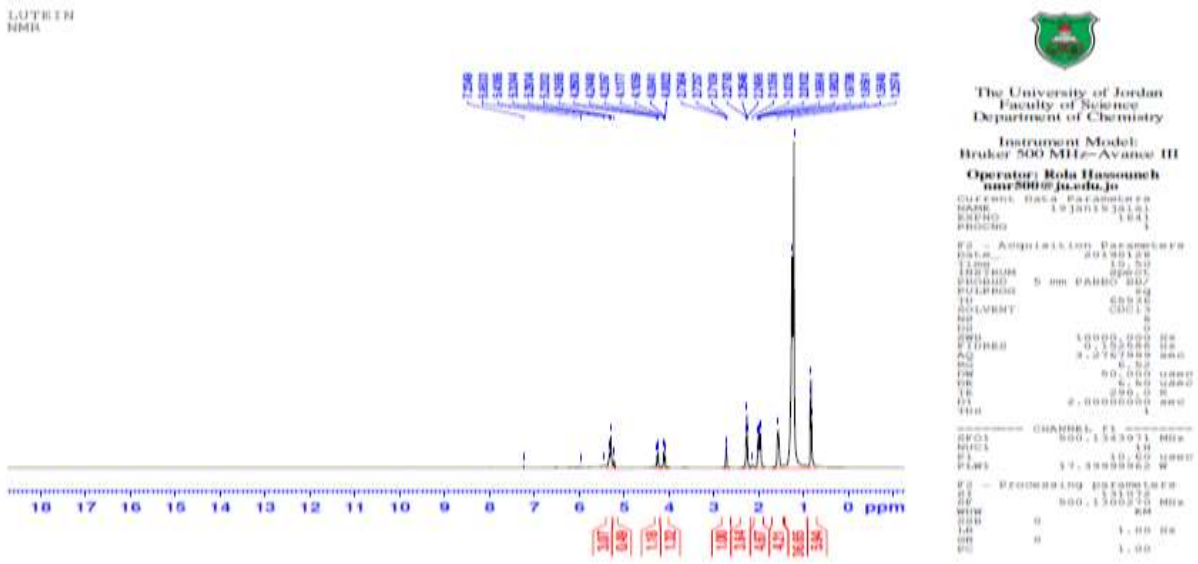

Figure 9: ^{1}H-NMR of Lutein in deuterated hexane

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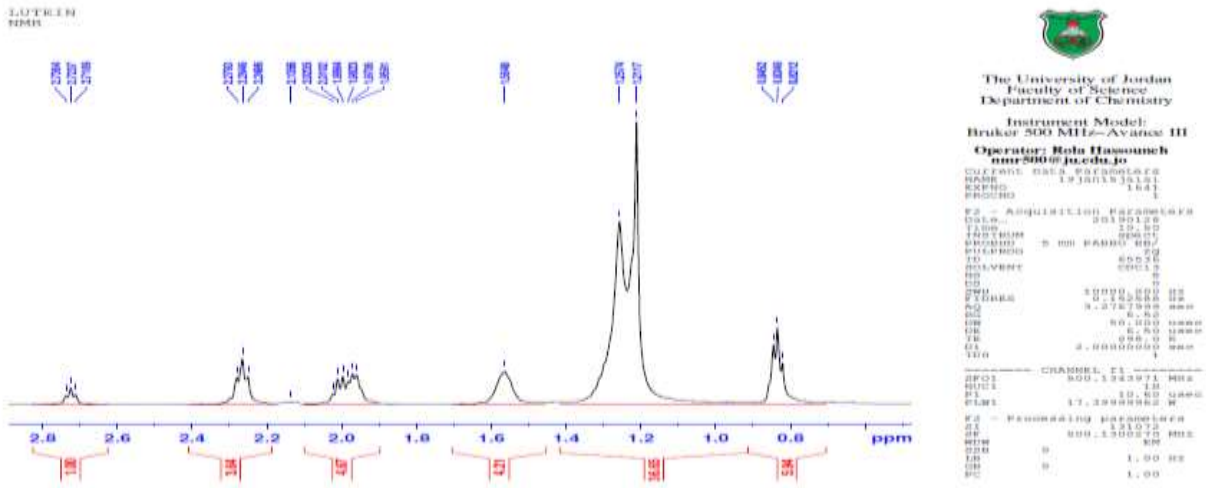

Figure 10: ^{1}H-NMR of Lutein in deuterated hexane

Table 2: ^{1}H-NMRData and their Interpretation of Lutein

Group	Chemical shift ppm	No. of H	Interpretation
H -1-CH_3, 4-CH_2, H-8	0.85-0.89	6	Multiplet
18,22,26-CH_3,H-17	1.24	10	Singlet
21-H, 30$(CH_3)_2$, 5-$(CH_3)_2$,13,9- CH_3,H 6,12,14	1.27	21	Duplet
2-$(OH)_2$,27-CH_2	1.59	4	Broad singlet
H-3, 10,19,29-CH_2	1.98-2.05	5	Multiplet
H-2, 16,20 H	2.29	3	Triplet
H-15	2.76	1	Triplet
H-11	4.13	1	Duplet of duplet
H-7	4.29	1	Duplet of duplet
H-28	5.26	1	Quartet
H-21,23,24	5.30-5.39	3	Multeplit

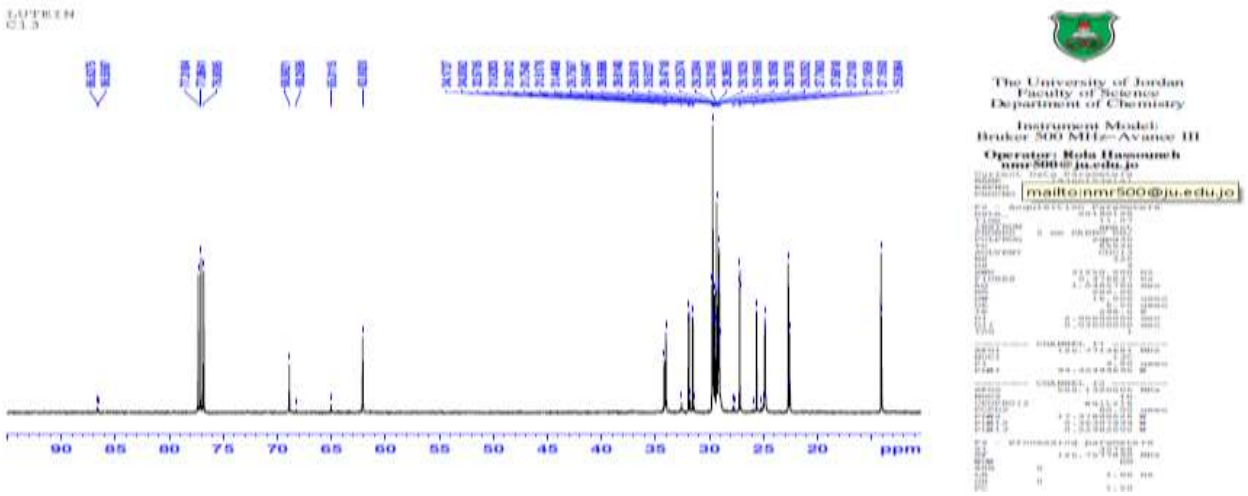

Figure 11: ^{13} C-NMR of Lutein in deuterated hexane

Table 3: ^{13} C-NMR Data and their Interpretation of Lutein

Group	Chemical shift ppm	No. of C
5-CH_3	13.7	1
5-CH_3	13.8	1
30-CH_3	22.2	1
30-CH_3	22.3	1
9-CH_3	24.5	1
13-CH_3	24.5	1
1-CH_3	25.2	1
C-4	26.8	1
C-18	28.7	1
C-19,20,27	28.8	3
C-5,30	28.9	2
C-,9,29,(26-CH_3)	29.0	3
C-8	29.1	1
18-CH_3	29.2	1
C-7,10,11	29.3	3
C-6,(22-CH_3)	29.4	2

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C-12	31.2	1
C-13,14	31.6	2
C-16	33.7	1
C-17	33.8	1
C-25	61.8	1
C-2	68.6	1
C-1	127.6	1
C-26	127.7	1
C-21	129.3	1
C-22	129.4	1
C-23	129.6	1
C-24	129.7	1
C-15	129.8	1
C-28	172.5	1
C-3	172.9	1

Conclusion

The valuable Iraqi medicinal plant Tagetes erecta, contains lutein as a major active constituent. Lutein is responsible for the treatment of cataract and age related macular degeneration. This percentage indicates that Tagetes erecta of Iraq is very rich source of lutein compared with the same plants from different countries since studies show that the percentage of lutein in other countries vary from 16.7 mg -57.5 mg per 100 grams [10] while the Iraqi plant lutein percentage is 855 mg per 100 grams. Also it is higher than lutein percentage in other plants since lutein is found in large concentration in green leafy vegetables, such as lettuce (between 171 and 3824 µg/100 g), basil (from 3650 to 8270 µg/100 g), parsley (4326 µg /100 g), garden rocket (7440 µg/ 100 g), and cilantro (7703 µg/100 g) [11].

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