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Article

Quantitative Analysis of Total Polysaccharides and Total Carotene from *Lycium barbarum* Fruit

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Abstract: *Lycium barbarum* fruit that grows in Iraqi deserts as a wild plant contain many active constituents with important human biological activity. Among the constituents, polysaccharides give sweet tastes for the fruit, and carotenes are responsible for the fruit color. The purpose of this study is to qualitatively and quantitatively determine both constituents that have not been investigated in the country previously. Benedict's test method was used for qualitative estimation of Polysaccharides and *Dubois* colorimetric assay was used for quantitatively analysis. The total carotenes were estimated qualitatively in corresponding to *β*- Carotene standard for detecting the λ_{max}, and quantitatively by two methods: HPLC and *β*- Carotene standard curve methods. Results showed that each 1.00g of dried fruit contains about 3.40 mg of total polysaccharides and 0.30 mg total carotene.

Keywords: total carotenes; total polysaccharides; *Lycium barbarum*; fruit; analysis; plant.

1. Introduction

Lycium barbarum fruit is a solanaceous herbal plant grows in Tibet, China and other different parts of Asia, Europe and around the Mediterranean. The plant has been used in traditional herbal

medicine and functional food (Bensky and Gamble, 1993; Bryan, *et.al.* 2008). The main active constituents of the plant are the water-soluble polysaccharides (glycoprotein), which give the plant sweet taste and the carotenes particularly Physalein (zeaxanthin dipalmitate) that gives the fruit reddish-orange color (Thomas, 2002). The plant is considered one of the richest sources of carotenes (Guihao and Yuli, 2008). Also it contains 18 amino acids including the 8 essential amino acids and about 21 trace minerals to give the fruit its importance as functional food (Duke, *et.al.* 2002). Many *in vitro* and *in vivo* studies showed that the plant had cardiovascular benefits (Jia, *et.al.* 1998 Jing, *et.al.* 2009), eye health benefits (Cheng, *et.al.* 2005), potent antioxidant (Gong, *et.al*. 2005; Li, and Ma, 2007; Li, *et.al*. 2007), immune modulator (Xu and Lui, 2000; Yim and Ko, 2002; Lu, *et.al.* 2004) and anti-inflammation including skin protection from UV radiation (Reeve, *et.al*. 2010).

Little is known about this plant which grows naturally in many Iraqi deserts as wild fruit. The aim of this study is to investigate qualitatively and quantitatively the active constituents of *Lycium barbarum* fruit specially the polysaccharides and carotenes the plant riches with.

2. Materials and Methods

2.1. Extraction the Polysaccharides from the Fruit

25.00 g of powdered *Lycium barbarum* fruit was mixed with 300 mL distilled water, then boiled for one hour, cooled, then filtered with piece of guise, finally centrifuged for 30minutes at 1500 rpm(Wang et.al., 2010). The filtrate was collected. Aliquot of 500 ml from 95% ethanol was added to the cold solution and allowed to stand for 24 hours. The precipitated (polysaccharides) was collected and washed with cold absolute ethanol and then acetone. The precipitate was weighted after drying and kept in refrigerator at 4ºC.

2.2. Determination of Total Polysaccharides Content in the Fruit

For total polysaccharides determination, different glucose standard solutions (0.30, 0.25, 0.20, 0.15 and 0.10)mg/mL were prepared from glucose stock solution of 1.00mg/mL. A quantity of 250.00 mg from the precipitate was dissolved in 50.00 mL hot water to get concentration of 5mg/mL and subjected to the following methods for determination(Chia ,et , al. 2009).

2.2.1.Qualitative determination

A general Benedicts test was used as primary qualitative estimation met for polysaccharides (Edwin, *et.al.* 2009*).*

2.2.2.Quantitative determination

For quantitative determination, a phenol-sulfuric method by Dubois, *et.al* (1956) was applied as follows: A volume of 0.40 mL of each solution (standard solutions and the extracted polysaccharides) was transferred into glass test tubes separately, then 0.40 mL of 5%phenol solution and 2.00 mL concentrated sulfuric acid were added to all tubes, mixed well then shook for 30 minutes and finally the absorption for all tubes and the blank (distilled water with the reagents) were measured at 490 nm wavelength. A standard curve was plotted with concentrations verses absorption. From the linear equation the total polysaccharides concentration was calculated as glucose.

2.3. Extraction of Total Carotenes from the Fruit (Kwok and Paul,1999).

1.00 g *of* dried *Lycium barbarum* fruit powder was homogenized well with 3mL distilled water with aid of porcelain mortar, then a quantity of 2mL absolute ethanol was added and mixed by vortex to denaturized proteins in the fruit. The homogenized mixture was transferred to separator funnel and gently mixed with 10 mL *n*-hexane. After the separation of the two layers, the organic hexane layer that contained carotenes was collected. The extraction was repeated many times until no color appeared in the hexane layer. The final volume of the collected *n*-hexane layers represented the total carotenes extract solution obtained from one gm *L.barbarum* fruit.

2.4. Determination of the Total Carotene Content in the Fruit

For total carotenes determination, *β*–Carotene 90 µg/mL stock standard solution was prepared from which a serial dilution were made, including (0.9, 1.8, 2.7, 3.6, 4.5, 5.4, and 9.0) µg/mL, all were prepared in *n*-hexane.

2.4.1.Qualitative determination

This assay was carried out to detect λ_{max} value, using general spectral scan for the *β*-carotene standard solution and for the extracted carotene which was collected from *n*-hexane layer (Kwok and Paul,1999). The spectrum range was between 350 - 500 nm, and then both curves were overlaid.

2.4.2.Quantitative determination of Total β-Carotene

The final volume and the absorbance at 490nm of the collected hexane layers (step 2.3.) were measured, and then the quantitative assay for total *β*- Carotene was done in two methods as shown below.

2.4.2.1. Standard curve for different concentrations of the β -Carotene standard solutions

The *β*-Carotene standard curve was applied between different concentrations (X axe) verses their absorption (Y axe) to determine the concentration of *L. barbarum* fruit extracts from the equation of the straight line (Kwok and Paul, 1999).

2.4.2.2. The HPLC method(Wang,et.al.2010)

HPLC application for both *β-C*arotene standard and the fruit extract was used to measure the concentration of the extracts with the following conditions:

Mobile phase : Methanol : Acetonitrile: Water in ratio of 81:14:5

Column : C18

Flow rate : 1mL/min. Injection volume:10µL.

Wave length : 450nm.

Instrument : LC-2010A Shimatzu/Japan

Standard solution concentration: 0.0018 mg/ml

3. Results

3.1. Total Polysaccharides Plant Extract

25.00g of dried powder *Lycium*, the extraction yielded 1.00 g precipitate, which then was applied for a polysaccharides qualitative general test. 250.00mg was dissolved in 50.00ml hot distilled water for quantitative determination of total polysaccharide, calculated as Glucose.

3.2.Determination of Total Polysaccharides

3.2.1. Qualitative determination

A red precipitate was formed as a result of the Benedict's general test.

3.2.2. Quantitative determination

The absorbance at 490nm for the glucose standard solutions by Dubois (1956, phenol-sulfuric analysis) method, were recorded then plotted as standard curve from which the concentration of the extracted total polysaccharides was determined as shown in Table 1 and Figure 1.

Table1: The absorption of Glucose standard solutions in different concentrations at 490nm

Final concentration (mg/mL)	Absorption at 490 nm
0	0
0.10	0.783
0.15	1.016
0.20	1.270
0.30	2.200
Unknown Solution in final conc.	1.200

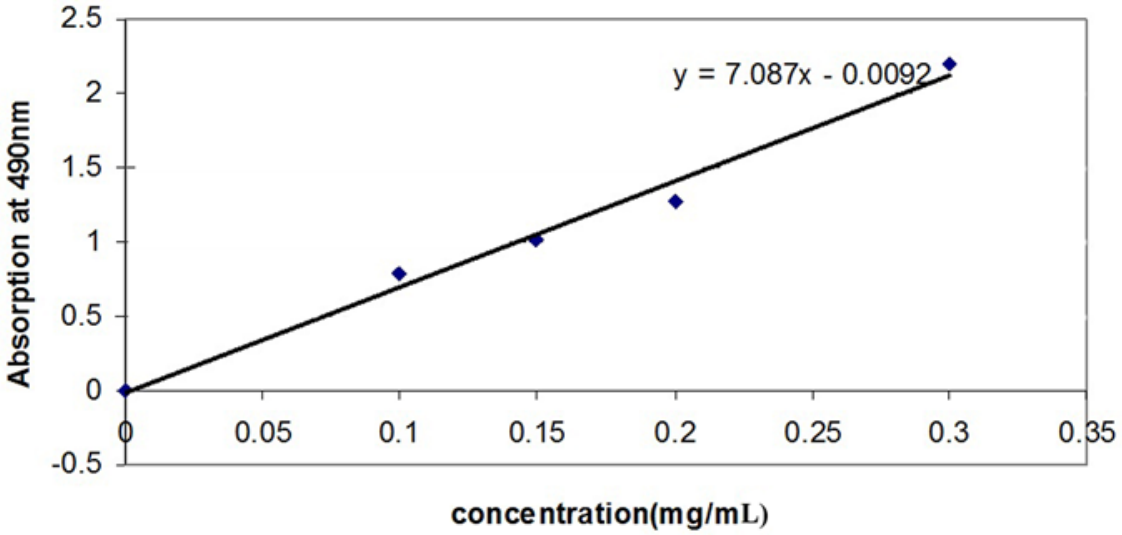


Figure 1: Glucose standard curve and the straight line equation

The standard curve linear equation was determined as:

Y=7.087X -0.0092

This straight line equation was used to determine the concentration of the extracted precipitate, as shown below:

1.20=7.087X-0.0092

X=0.171mg / 0.4mL from the extracted sample

So in the 50 ml solution, there are 0.171 x 50/ 0.4= 21.375 mg total polysaccharides (as glucose). This 50 ml solution was prepared from 250.00 mg precipitate, and for 1.00 g precipitate there will be

4 x 21.375 mg = 85.5mg / 25 g *Lycium* powdered fruits.

So each 1.00 g *Lycium* powdered fruits contains 3.432 mg.

3.3. Total Carotene Plant Extract

The total carotene extracted from 1 g dried powdered fruit with (165mL) n-hexane was determined qualitatively and quantitatively as followings.

3.3.1.Qualitative determination of total carotene

Figure 2 shows the value of λ_{max} for the standard β-Carotene solution, while Figure 3 shows the value of λ_{max} overlay diagram for both the extracted hexane layer and the standard β-Carotene solution.

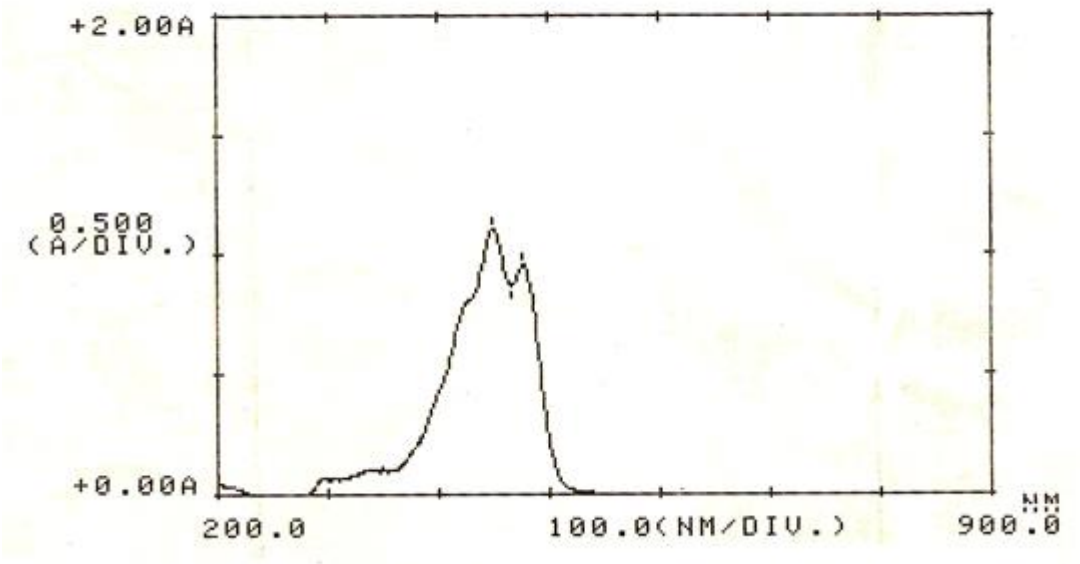


Figure 2: Standard β Carotene λ_{max} Value

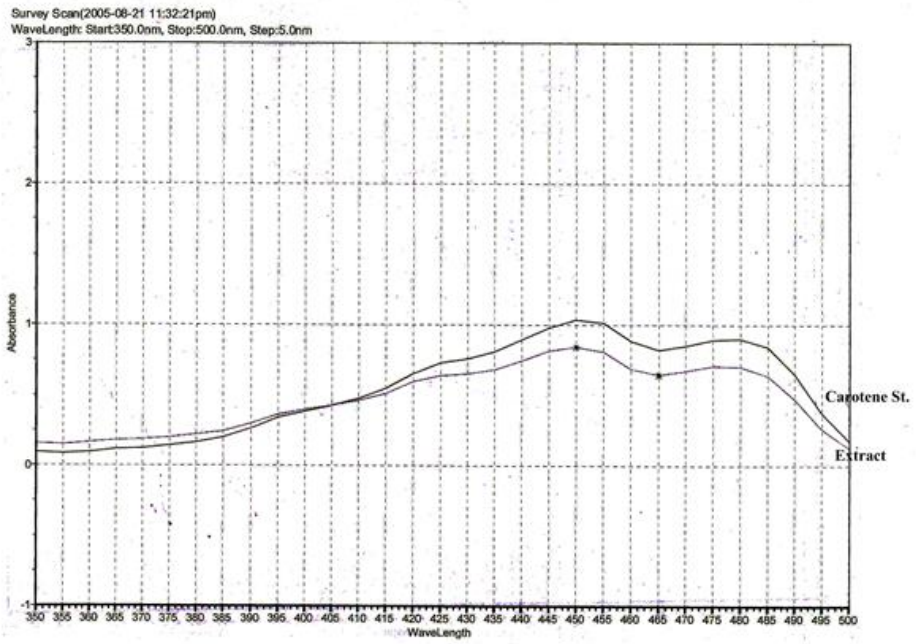


Figure 3: The overlay graphs both the extracted hexane layer and the standard β Carotene

3.3.2.Quantitative determination

Two methods were used for the quantitative analysis.

3.2.2.1. Standard curve for β -Carotene

The absorption of different concentrations of standard *β-C*arotene and the extracted carotene at 490nm were shown in Table 2. Figure 4 shows the standard curve application and the straight line equation.

Table 2: The absorbance of different concentrations of *β-C*arotene standard solutions

Concentration(mg/mL)	Absorption at 490nm
0.0009	0.169
0.0018	0.342
0.0027	0.453
0.0036	0.646
0.0045	0.726
0.0054	0.940
0.0090	1.357
Extracted Carotene	0.352

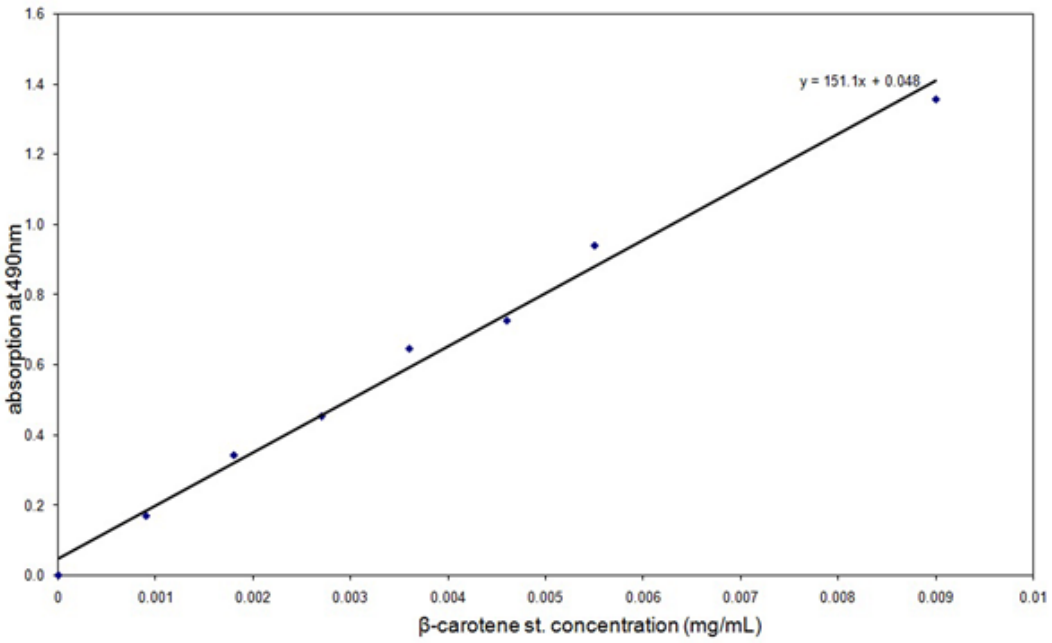


Figure 4: Standard Curve for different concentrations of *β-C*arotene

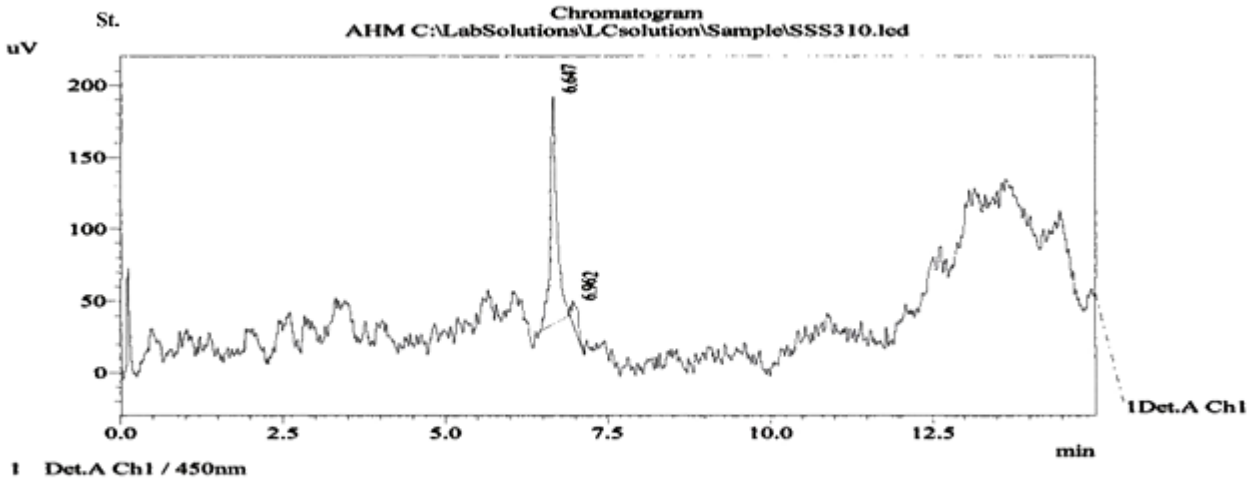
From the straight line equation, the concentration of Extracted total carotene can be calculated as follow:

$$0.352 = 151.11X - 0.0484$$

X =0.002 mg/mL, then total concentration for the extract Carotene in 165 mL as total volume =0.33mg.This is the total Carotene in 1 g dried fruit powder. The yield is 0.033%

3.2.2.2.The HPLC method

Figure 5 shows the peak height and retention time for *β-C*arotene standard. The retention time is 6.647 min. and the peak area is 159.



PeakTable

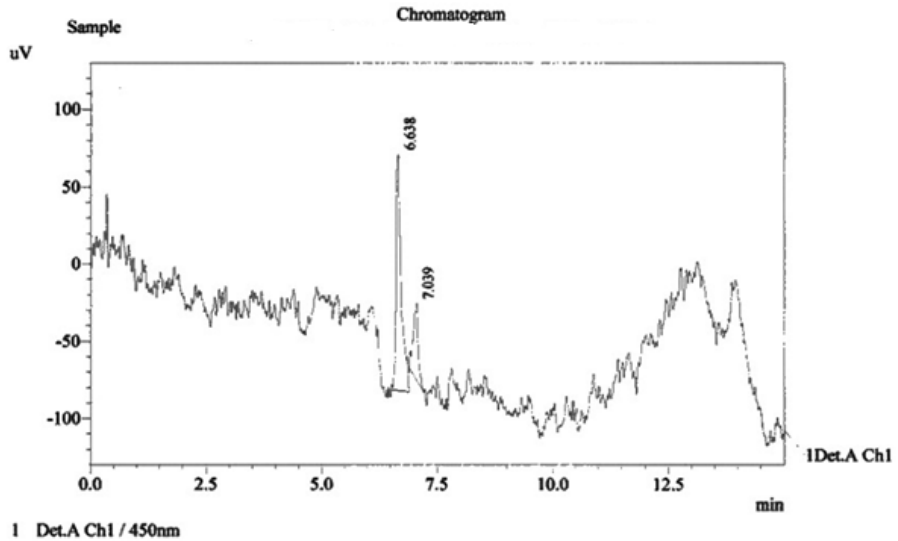
Detector A Ch1 450nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.647	1145	159	90.127	90.525
2	6.962	125	17	9.873	9.475
Total		1271	176	100.000	100.000

Figure 5: The peak height and retention time for the standard *β-C*arotene

Figure 6 is the chromatogram of the extracted Carotene. The retention time is 6.638 min. and the peak area is 154.

Figure 7 is the overlay graphs of standard and extracted Carotene. It can be seen that they have almost identical retention times.



PeakTable

Detector A Ch1 450nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.638	1162	154	76.226	75.752
2	7.039	362	49	23.774	24.248
Total		1524	203	100.000	100.000

Figure 6: Chromatogram of the extracted Carotene

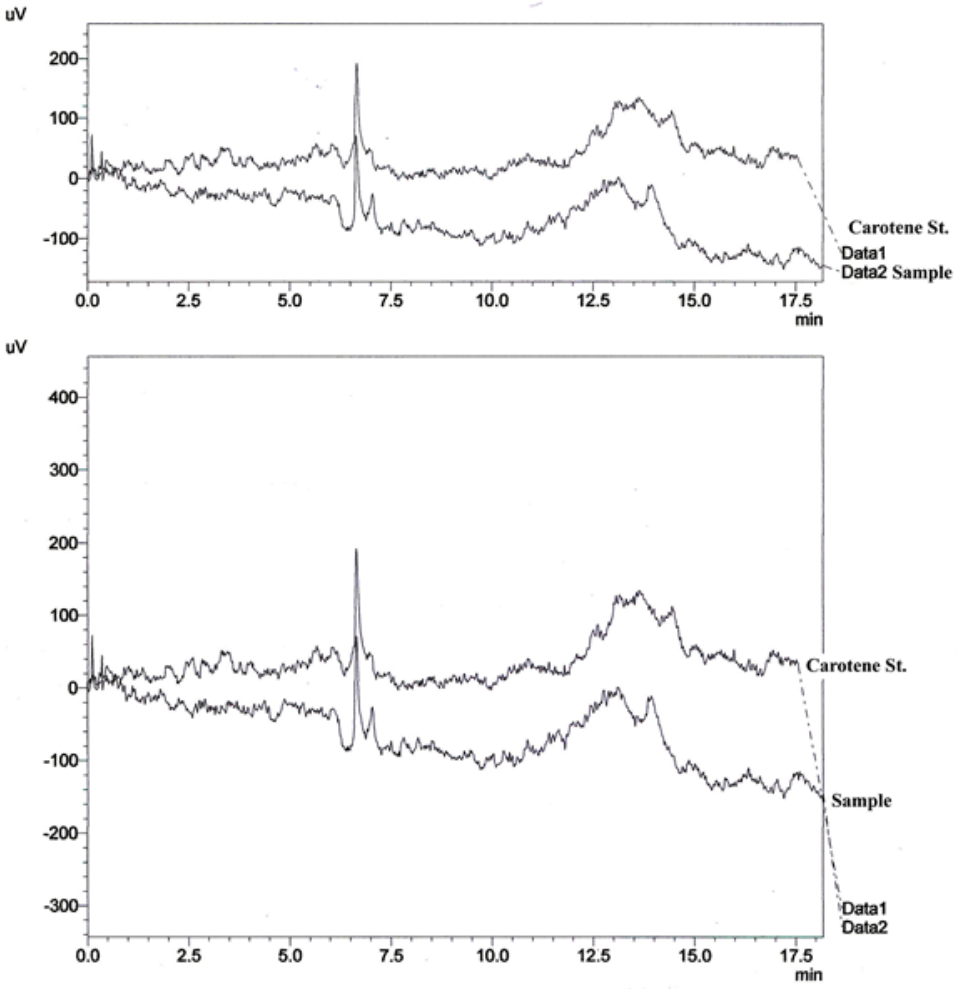


Figure 7: The overlay Chromatograms of the standard and the extracted Carotene

When data applied for peak height or area under the curve at retention time 6.647 and 6.638 of the standard and extracted carotene respectively the concentration of total carotene can be calculated as followings:

$$\frac{Peak\ area\ of\ extracts}{Peak\ area\ of\ standard} \times Standard\ solution\ concentration \times total\ volume\ of\ extract$$
$$= \text{total carotene (mg) in 1g dried fruit powder}$$

That is:

$$\frac{154}{159} \times 0.0018\frac{mg}{ml} \times 165ml = 0.287\ mg$$

total carotene in 1g dried powdered fruit.

So, 1 g of dried fruit contains 0.287 mg Total carotene, yield is 0.029%.

From these two methods, the average of (0.033% + 0.029%) gives average yield 0.031%.

4. Discussion

Lycium barbarum is a traditional Chinese herb possessing vital biological activities, such as cancer prevention and age-related macular degeneration, is widely used in Asian countries (Ke, *et. al.*, 2011). The main active components of this plant have been identified as flavonoids, caroteniods, zeaxanthin and polysaccharides. All of these components have been reported to be closely associated with the health-enhancing effect (Sheng, *et,al.*, 2007; Yang, *et,al.*, 2008).

Systematic characterization and identification of active compounds in medicinal herbs and their mechanisms of action for providing the rationale for their efficacy and for transforming herbal practices into evidence-based medicine, is an important goal of researcher to discover and identify a new potential drug. The present study investigates quantitatively the most important active constituents of *Lycium barbarum* fruit grown in Iraqi deserts. Little are known about the amount of active components polysaccharides and carotenes. The first component for *L. barbarum* fruit, polysaccharides, is a kind of proteoglycan composed from 6 kinds of Monosaccharids, they are Arabinose, Glucose, Galactose, Mannose, Rhamnose and Xylose (Harunobu and Norman, 2011).

Extraction and isolation of polysaccharides are simple, as they are soluble in hot water and the easiest method is first produce a hot water extract of herb using more than one extraction to get most of polysaccharides into solution, and then force the polysaccharides out of water solution by adding alcohol in which they are not soluble, then the liquid is separated off and the residue is dried to produce the finished polysaccharides (Harunobu and Norman, 2011). Quantitative measuring for the polysaccharides extracted from Iraqi *Lycium* fruit estimated as glucose is about 3.4mg/g dried fruits. The references showed 5-8% polysaccharide content in cultivated type as in Chinese desired and more content up to 10-15% can be obtained with optimized condition of extraction (Guihao and Yuli, 2008; Harunobu and Norman, 2011). The other major component of *Lycium* is the color components of the fruit which is a group of caroteniods that make up 0.03-0.5% of the dried fruit in cultivate Chinese type, while the Iraqi wild *lycium* fruit the total caroteniods content estimated as β –carotene is about 0.031% of the dried fruits as average results in both standard curve method and HPLC method.

5. Conclusion

The identification and evaluation of new bioactive compounds from herbs can help in the development of novel drugs, leading the way to discover interesting, possibly less harmful, and also clinically useful active components to support human health. *Lycium barbarum* is an interesting herb and food in the Chinese medicines, proved with*in vitro and in vivo* studies. While little is known, if not, about *Lycium* grown in Iraq. Even though less amounts of *lycium* active constituents in wild Iraqi plant than the cultivated Chinese fruit, the results still show that the plant is still a good source for both

constituents. Focusing should be given to improve the cultivating conditions of *Lycium barbarum*, and to use it an important medical purpose. In addition, more investigation should be done in the country to estimate quantitatively other constituents that might be toxic such as betaine alkaloid which is never estimated in the plant yet.

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