International Journal of Modern Biology and Medicine, 2013, 4(2): 64-73

International Journal of Modern Biology and Medicine **ISSN: 2165-0136**

Journal homepage: www.ModernScientificPress.com/Journals/IJBioMed.aspx *Florida, USA*

Article

Quantitative Analyses of Total Polysaccharides and Total Carotenes from *Lycium barbarium* Fruits

Zainab Yaseen Mohammed [1,] *, Khulood W. Alsamarrae [2,] *, Subhi Jawad Hamza [3]

[1] *Biotechnology Research Center, Al-Nahrain University, Baghadad, Iraq*

[2] *College of Science, Al-Nahrain University, Baghadad, Iraq*

[3] *Biotechnology College, Al-Nahrain University Baghadad, Iraq*

* Author to whom correspondence should be addressed; E-Mail: zaimaby2003@yahoo.com (ZYM); Khulood_Whayeb@yahoo.com (KWA).

Article history: Received 29 May 2013, Received in revised form 12 July 2013, Accepted 18 July 2013, Published 25 July 2013.

Abstract: *Lycium barbarium* have been grown in Iraqi desert as wild plant, and its fruit contains many active constituents with important human biological activity. Polysaccharides give sweet taste for the fruit, and carotenes are responsible for the fruit color. The purpose of this study is qualitative and quantitative determination for both constituents, which had not been investigated in the country previously. The polysaccharides were estimated qualitatively by the Benedict's test, and quantitatively by the *Dubois* colorimetric assay. The total carotenes were estimated qualitatively in corresponding to *β*-carotene standard for detecting the λ_{max}, and quantitatively by two methods, HPLC method and *β*-carotene standard curve method. The results showed that the dried fruit contained about 3.4 mg/g of total polysaccharides and 0.3 mg/g of total carotenes. The conclusion of this study was that *Lycium barbarium* fruit possesses nutritional and medical benefits that give the plant special importance for more investigation in the country.

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

1. Introduction

Lycium barbarium is a Solanaceous herbal plant, which grows in China, other different parts of Asia, also in Europe and around the Mediterranean. Its fruit has been used in traditional herbal medicine and functional food (Bensky and Gamble, 1993; Bryan *et al.,* 2008). The main active constituents of the fruit are the water-soluble polysaccharides (glycoprotein), which give the fruit sweet taste, and the carotenes particularly physalein (zeaxanthin dipalmitate) that gives the fruit reddish-orange color (Thomas, 2002). The fruit is considered one of the richest sources of carotenes (Guihao and Yuli, 2008). Also the fruit contains 18 amino acids including the 8 essential amino acids and about 21 trace minerals, which give the fruit its importance as functional food (Duke *et al.,* 2002). Many *in vitro* and *in vivo* studies showed that the fruit 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 (Lu *et al.,* 2004; Xu and Lui, 2000; Yim and Ko, 2002), 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 type. The aim of this study is to investigate quantitatively the active constituents of *Lycium barbarium* fruits, specially the polysaccharides and carotenes the plant might be rich with.

2. Materials and Methods

2.1. Extraction the Polysaccharides from the Fruit

About 25 g of powdered *Lycium barbarum* fruits were mixed with 300 mL distilled water, then boiled for one hour, cooled, and filtered with piece of guise, finally centrifuged for 30 min at 1500 rpm (Wang et al., 2010). The filtrate was collected. A cold solution of 95% ethanol was added and allowed to stand for 24 h. The precipitated polysaccharide was collected and washed with cold absolute ethanol, then acetone. The precipitate was weighted after drying, and kept in refrigerator at 4 ºC.

2.2. Determination of Total Polysaccharides Content in the Fruit

For total polysaccharide determination, different glucose standard solutions (0.3, 0.25, 0.20, 0.15 and 0.1 mg/mL) were prepared from glucose stock solution of 1 mg/mL. About 250 mg of the precipitate was dissolved in 50 mL hot water to get the concentration of 5 mg/mL and subjected to the following methods for the determination (Chia et al., 2009).

2.2.1. Qualitative determination

A general Benedicts test was done as primary qualitative estimation for polysaccharide (Edwin *et al.*, 2009).

2.2.2. Quantitative determination

For quantitative determination, a phenol-sulfuric acid method by Dubois *et al.* (1956) was applied as follows: An amount of 0.2 mL from each solutions (standard solutions and the extracted polysaccharide) was transferred into glass test tubes separately, then 0.2 mL of 5% phenol solution and 1 mL concentrated sulfuric acid were added to all tubes, mixed well, then shacked for 30 min, and finally the absorption for all tubes and the blank (distilled water with the reagents) were measured at 490 nm. A standard curve was plotted between the concentrations verses absorption, then from straight line equation the total polysaccharides concentration was calculated as glucose.

2.3. Extraction of Total Carotenes from the Fruit

About 1 g of *Lycium barbarum* dried fruit powder was homogenized well with 3 mL distilled water with aid of porcelain mortar, then a quantity of 2 mL absolute ethanol was added, and mixed by vortex to denaturized proteins in the fruit (Kwok and Paul, 1999). The homogenized mixture was transferred to separate 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.

2.4. Determination of Total Carotenes Content in the Fruit

For total carotenes determination, 0.09 mg/mL of *β*-carotene stock standard solution was prepared, from which a serial dilution were made, including 0.0009, 0.0018, 0.0027, 0.0036, 0.0046, 0.0055, and 0.009 mg/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 matched.

2.4.2. Quantitative determination of total carotenes

The final volume and the absorbance at 490 nm of the collected hexane layers (step 2.3) were measured, and then the quantitative assay for total carotenes was done in two methods.

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 axes) verses their absorption (Y axes) to determine the concentration of *L. barbarum* fruit extract from the equation of the straight line (Kwok and Paul, 1999).

2.4.2.2. The HPLC Method

HPLC application for both *β-c*arotene standard and the fruit extract was used to measure the concentration of the extract with the following conditions: Instrument: Shimatzu LC-2010A (Japan); Column: C18; Mobile phase: methanol - acetonitrile - water (81:14:5); Flow rate: 1 mL/min; Injected volume: 10 µL; Detection wavelength: 450 nm (Wang *et al.,* 2010).

3. Results

3.1. Total Polysaccharides of the Fruit

The precipitate yielded from the extraction of 25 g of dried powdered fruit was about 1 g, which then was applied for a polysaccharide qualitative general test. Only 250 mg had been dissolved in 50 mL hot distilled water for quantitative determination of total polysaccharide, calculated as glucose.

A red precipitate was formed as a result of the Benedict's general test, which qualitatively showed the presence of polysaccharide in the fruit.

The absorbance at 490 nm for the glucose standard solutions by the phenol-sulfuric acid analysis method (Dubois, 1956), were recorded, and then plotted as standard curve, from which the concentration of the extracted total polysaccharides was estimated (Table 1 and Fig. 1). The results showed that the 25 g of dried powered fruit contained about 85 mg total polysaccharides, i.e. 3.4 mg/g dry weight of fruit.

3.2. Total Carotenes of the Fruit

The total carotenes extracted from 1 g of dried powdered fruit with n-hexane were determined qualitatively and quantitatively.

Fig. 1 showed the λ_{max} value for the standard *β*-carotene solution, while Fig. 2 showed the matching diagram of λ_{max} values for both the extracted hexane layer and the standard *β*-carotene solution. The results showed the presence of carotenes in the fruit qualitatively.

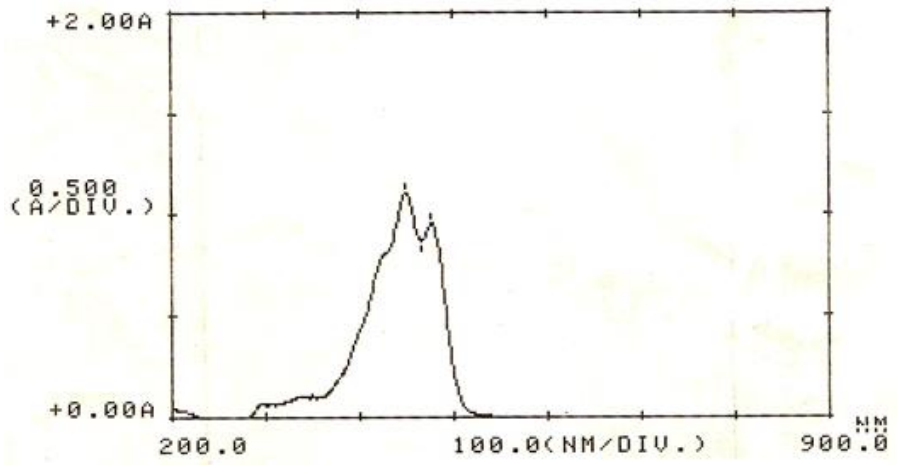


Fig. 1. The λ_{max} value of standard β-carotene

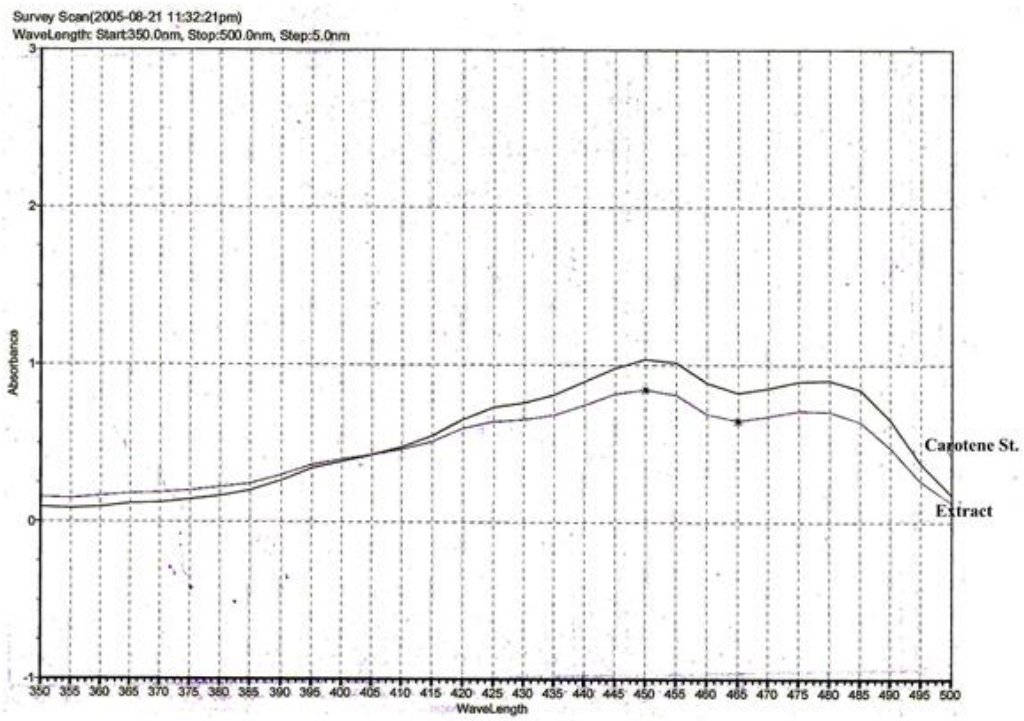


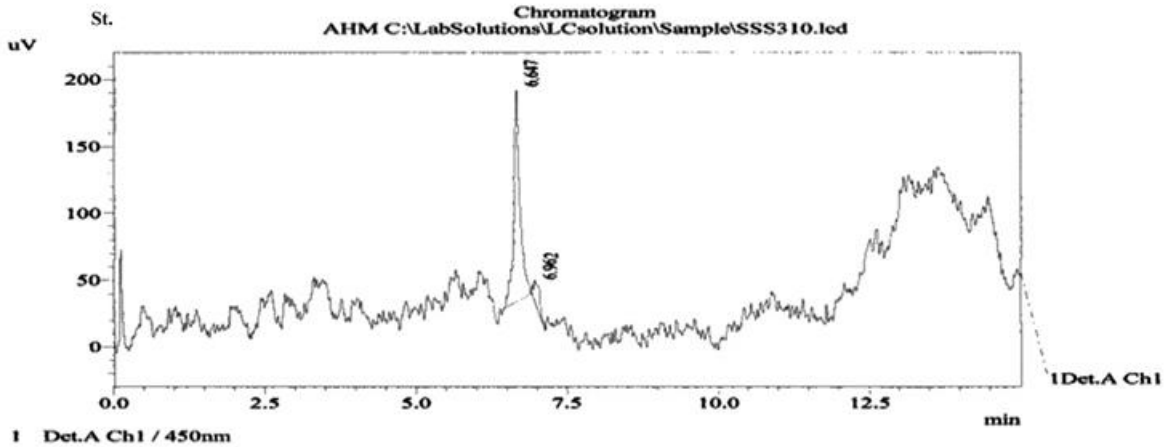
Fig. 2. The matching diagram of λ_{max} values for both the extracted hexane layer and the standard β-carotene

Quantitative determination of total carotenes in the fruit was carried out by two methods, standard curve method of β-carotene, and HPLC method.

The absorption of different concentrations for standard β-carotene and the extracted carotene at 490 nm was measured. The result showed that the fruit contained total carotenes 0.33 mg/g dry weight of fruit.

Fig. 3 showed the peak height and retention time for β-carotene standard, while Fig. 4 showed the chromatogram of the extracted carotene. Peak height and retention time were very identical specially when matching both chromatograms (Fig. 5). The result showed that the fruit contained total

carotenes 0.287 mg/g dry weight of fruit calculated by peak height, or 0.3 mg/g dry weight of fruit calculated by peak area.

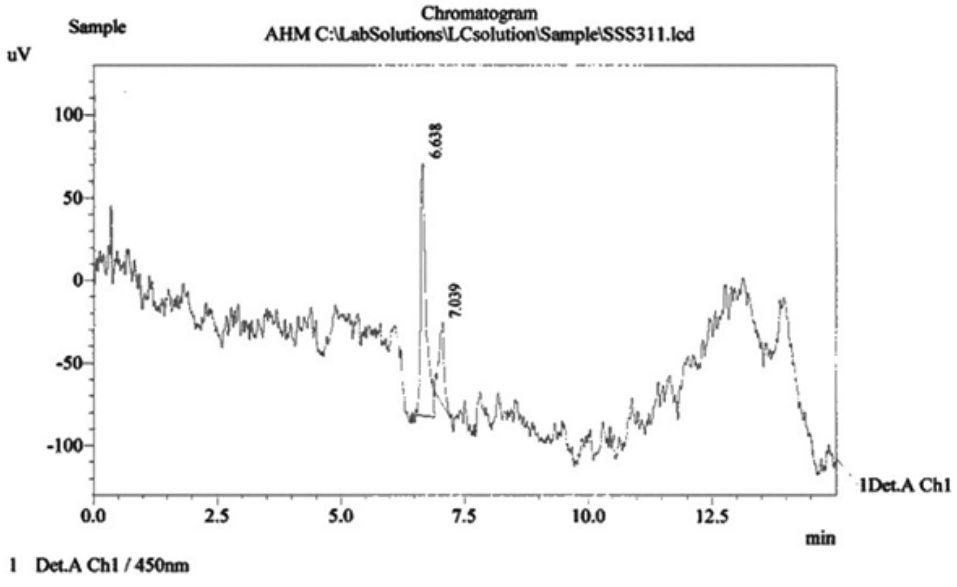


PeakTable

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

Fig. 3. The peak height and retention time for the standard β-carotene



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

Fig. 4. Chromatogram as well as the peak height and retention time of the extracted carotene

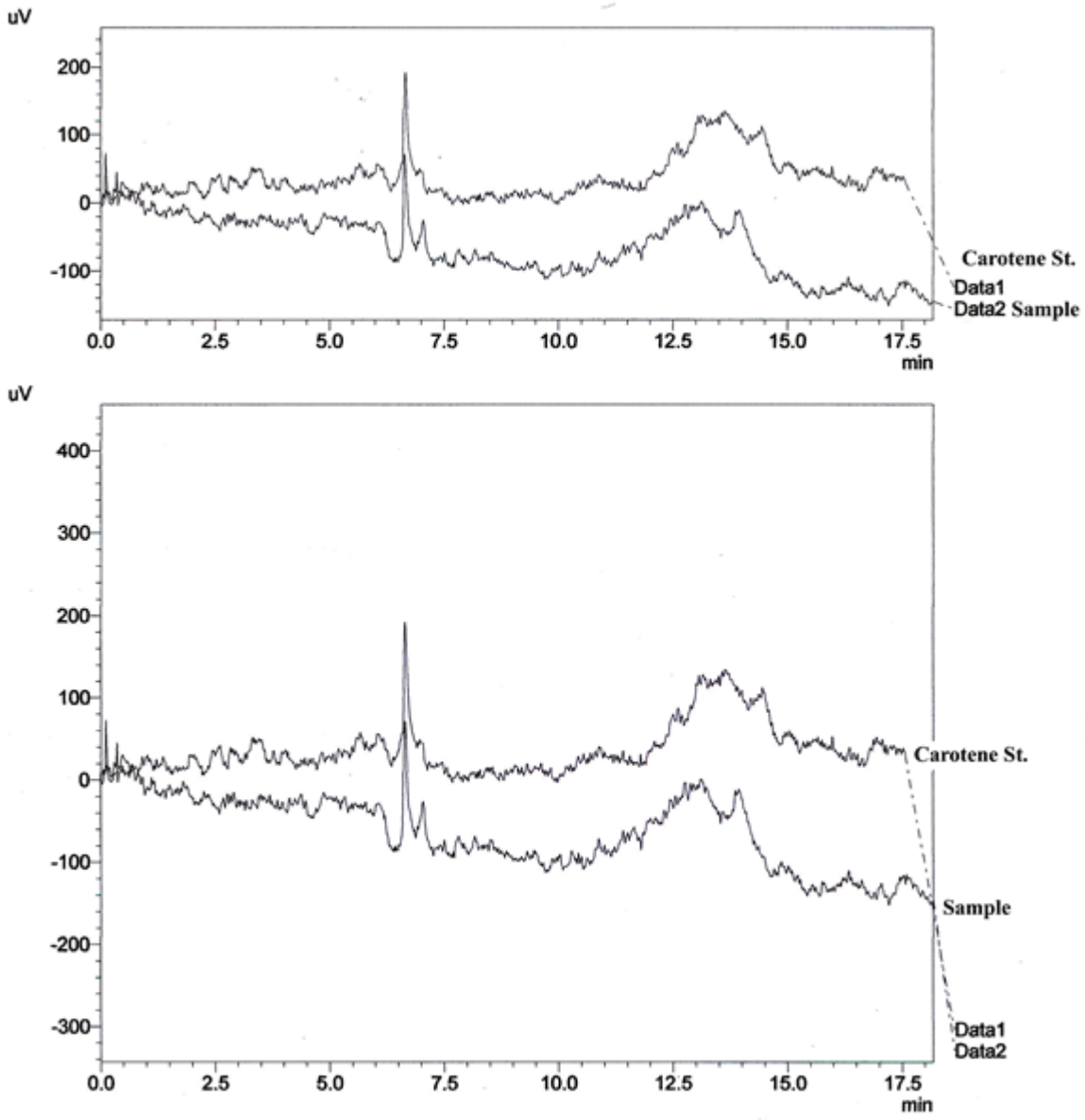


Fig. 5. Peak matching for the standard and the extracted carotene

4. Discussion

Lycium barbarum, a traditional Chinese herb possessing vital biological activities, such as prevention of cancer 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 (specially zeaxanthin) and polysaccharides, all of these component have been reported to be closely associated with the health-enhancing effect (Sheng *et al*., 2007; Yang *et al*., 2008).

Systemic 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 research to discover and identify a new

potent drug, so the current study investigated quantitatively the most important active constituents (polysaccharides and carotenes) in the fruit of *Lycium barbarum* grown in Iraqi deserts, since little is known about the amount of active components of this fruit. The polysaccharides from *Lycium barbarum* fruit is a kind of proteoglycan composed of 6 kinds of monosaccharids, which are arabinose, glucose, galactose, mannose, rhamnose and xylose (Harunobu and Norman, 2011).

Extraction and isolation of polysaccharides is 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 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 barbarum* fruit estimated as glucose is about 3.4 mg/g dry weight of fruit. The yield of *Lycium barbarum* polysaccharides in the present study is about 4% of the dried fruits from the wild Iraqi type, while references showed 5-8% polysaccharides 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). Another major component in fruit of *Lycium barbarum* is the color components which are a group of caroteniods that make up 0.03-0.5% of the dried fruit in cultivate Chinese type, while the Iraqi wild *Lycium barbarum* fruit the total caroteniods compound estimated as *β*-carotene is about 0.033% of the dried fruits. Two methods for quantitative estimation of carotene component in the fruit were used in the current study. The results obtained are nearly similar by both methods, i.e. the HPLC method and the *β*-carotene standard curve method.

5. Conclusions

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* fruit is an interesting herb and food in the Chinese medicines, improved with *in vitro and in vivo* studies, while little is known, if not, about *Lycium barbarum* grown in Iraq. Even if the less contents of active constituents in fruit of wild Iraqi plant than the cultivated Chinese fruit, the results showed that the fruit is still a good source for both constituents. This will give the plant an important focusing to improve the cultivated conditions, and to be used as an important medical plant and special importance for more investigation in the country to estimate quantitatively other constituents that may be toxic such as betaine alkaloid which is never estimated in the plant yet.

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