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In Vitro Antioxidant Properties of Gum Extract From The Carob (*Ceratonia silique* L.) Plant

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Abstract: The experiment was conducted in the laboratories of the College of Agriculture, University of Basra, for the period from 22/9/2019 to 25/ 11/2019. The current study aims to extract gum from the carob fruits, to determine the active substances and the possibility of using them as antioxidants. The results showed that the gum of the fruits of the carob plant contains all the active compounds under study, which included Alkaloids, Phenols, Flavonoids, Glycosides, Steroids, Tannins, Resins, Saponins and Coumarins. The results also showed that the gum of the fruits of the carob plant showed antioxidant activity of about 84.55 % when used at a concentration of 0.2 %, while the average antioxidant efficacy of the industrial compound Butylated Hydroxy Toluene (BHT) and Ascorbic acid was about 93.43 %, 91.12 % at the same concentration. The Reductive power were about 73.20 % and 83.30 % with a concentration of 0.1 % and 0.2 % respectively, which was comparable to the effect of industrial antioxidants (BHT) and ascorbic acid with a concentration of 0.2 %, which was 91.40 % and 90.70 %, respectively. In addition, the gum of the fruits of the carob plant has the potential to sweep hydrogen peroxide with a capacity similar to ascorbic acid and without significant difference ($p \leq 0.05$), reaching 41 % and 50 % for concentrations 0.1 % and 0.2 %, respectively, while ascorbic acid reached 45 % with the presence of moral differences with the industrial antioxidant (BHT) in its susceptibility to the sweep of hydrogen peroxide at 73 %. The results also showed that the gum of the fruits of the carob plant is able to bind to the Ferrous ion and this portability increased with the increased concentration of gum to reach its highest average of 43.19 % at the concentration of 0.2 % while the average Ferrous ion for antioxidant (EDTA 2Na) was 55.48 %.

Keywords: Antioxidants, Carob fruits, Gum carob.

Introduction

Carob was a well-known Shrub for its nutritional and health-promoting edible pods due to its high phenolic contents (Chait *et al.*, 2020). The carob tree (*Ceratonia siliqua* L.) mainly grows in mild and dry places with unproductive soils in Mediterranean countries including Greece and Turkey (Bernardo-Gil *et al*., 2011). It has a variety of Practices the food manufacturing, such as gum, syrup, powder, biofertilizer, ethanol, mannitol, lactic acid, and citric acid (Oziyci *et al*., 2014). Gum was

characterized by different physical characteristics, which was a multi-use substance it was an excellent reinforcement material and stabilizers for emulsions, and the absence of toxicity encourages it's used in the medical, pharmaceutical and food industries (Karababa & Coşkuner., 2013). Many commercial companies used industrial antioxidants frequently in food conservation and the most commonly used antioxidants were Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisole (BHA), Recently there has been widespread fears about the safety of the use of industrial antioxidants due to their health status to the consumer, so the FDA has expressed concerns about the trend of artificial antioxidants in foods (Manhiani *et al*., 2013). Consequently, current work aimed to use the gum from *C. siliqua* and determined it's active substances, antioxidant potential and activity to capture free radicals.

Materials & Methods

The experiment was conducted in the laboratories of the College of Agriculture, University of Basra, for the period from 22/9/2019 to 25/ 11/2019.

Collect and prepare samples

The fruits of the carob plant were collect from Al-Ashar market, after which the fruits were cleaned, dried and milled in the electric mill smoothly and then placed in bags of polyethylene and stored in the refrigerator at a temperature of 4C° until the tests are conduct.

Extracting gum from the fruits of carob:

Oil Removing:

The oil was extract from the fruits of the carob plant by following the cold extraction method using hexane (1: 5 w / v) for six hours. The hexane was replenishes every hour by filtration (Marambe *et al.,* 2008).

Chemically gum extracting:

The carob gum was extracted according to the optimal conditions mentioned by Al-Aubadi & Al-Ani (2017) by mixing the carob plant with distilled water at a ratio of 1: 65 (W / V) for 30 minutes with a pH 8 at 60 C°. The gum deposition and purification process was carried out by adding ethanol 96 % 1:2 filtrate: ethanol (V / V), the mixture was left overnight at 5 C° and filtered with a cotton sieve to separate the glue, a small percentage of distilled water was added and mixed it by using a magnetic motor, dry at 40 C° for 24 hours, Then, the gum was dried, then it's ground with a laboratory mill and the powder was stored in airtight ampoules.

Calculating the yield of gum (Y):

The percentage of the sum of the gum extracted according to Razavi *et al.* (2009), was calculated from the following equation:

$$\text{yield} = \frac{\text{Weight of extracted gum (g)}}{\text{weight of fruit carob (g)}} \times 100$$

$$\text{yield} = \frac{140}{250} \times 100 \rightarrow \text{yield} = 56(\text{g})$$

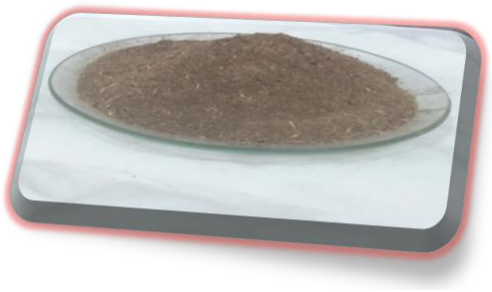

Fig.1 Gum of the fruits of the carob plant

Chemical detection of active substances:

Glycosides test

The method used by Malayaman *et al.* (2019), mixing equal parts of carob plant with Benedict's reagent, red precipitation appears, indicating the presence of glycosides. The result of the confirmation depends on the reaction between the reagent and the sample, therefore, an equal amount of it's taken with the carob plant and the reaction was left in a boiling water bath for 10 minutes. The appearance of the red precipitate indicates positive detection of sugars.

Alkaloids test

Alkaloids were quantified according to Al-Daihan *et al*. (2013), in which 100 mg of the carob plant was soaked in 5 ml of methanol, the mixture was filtered, 2 ml of the nomination was taken and mixed with 5 ml of hydrochloric acid at a concentration of 1 %, a few drops of Mayer's reagent and Draknov's reagent were added. The appearance of a reddish-orange precipitate indicates the presence of alkaloids.

Saponins test

The Saponins were detect in the manner mentioned by Sabreena (2019) by shaking the water extract to the gum of the carob in a test tube, if the detection was positive, the back of a thick foam stays for a long time.

Coumarins test

The Coumarins were detect in the manner mentioned before Malik *et al.* (1985). by taking 5 ml of the gum extract of the fruits of the carob plant and put it in a test tube covered with filter paper moisturizing by sodium hydroxide (2n), put the tube in a boiled bath and the filtration sheet was presented to ultraviolet light using the UV-Scan device, a greenish yellow colour indicates the presence of the coumarins.

Phenolic compounds test

Phenols were detected using the method used by Samejo *et al.* (2013), 0.5 gm of the carob plant was mixed with 10 ml of distilled water, added to the filtered mixture, a few drops of $FeCl_3$ to the juice at a concentration of 0.1 %. The appearance of a dark blue colour indicates the presence of phenols.

Tannins test

Tannins were reveal rendering to the method mentioned in Choudhary *et al.* (2013) As 0.5 g of carob plant was mixed in 20 ml distilled water, the mixture was filtered and $FeCl_3$ added 0.1 % concentration. The appearance of a dark blue colour to indicate the presence of tannins

Resins test

The method used by Ehrnford *et al*. (1980) to detect resins:

Add 10 ml of ethyl alcohol with a concentration of 95 % to 1 g dry weight of carob plant, leave in boiling water bath for two minutes and leach sweetened, add to the filter 20 ml distilled water acidified with drops of HCl acid concentration 4 %. The arrival of turbidity in the solution designates the presence of mastics.

Flavonoids test

Flavonoids were detect according to Samejo *et al*. (2013), mixing 0.5 g of carob with 10 ml of ethanol, the filtrate was added to the filtrate some Mg and drops of concentrated HCl acid, the appearance of a red color indicating the presence of flavonoids.

Measuring antioxidant effectiveness

Estimated according to the method mentioned in Al-Moussawi & Al-Halfi (2012). The antioxidant activity was determined using the linoleic acid system, the reaction mixture consists of 4.1 ml of linoleic acid at a concentration of 2.5 % in ethanol, 4 ml of alcohol extract, 8 ml of 0.05 molar phosphate buffer solution 7 and 3.9 ml distilled water, 1ml of 80 Tween at a concentration of 0.05 % ethanol, the mixture was incubated at 40 C° for 24 hours. The degree of oxidation was determined by thiocyanate method, mix 0.1 ml of the mixture, added 9.7 ml of 75 % ethanol and 0.1 ml of ammonium thiocyanate at a concentration of 30 %, three minutes later, 0.1 ml ferrous chloride of 0.02 molar concentration prepared in 3.5 % hydrochloric acid was added. The absorbance was measure at a wavelength of 500 nm, Butylated Hydroxy Toluene (BHT) and ascorbic acid were used for comparison, and the control sample was prepared in the same way above except for mixing 4 ml ethanol instead of plant extract. The percentage inhibition of linoleic fatty acid peroxides was calculated according to the following formula:

$$\% \text{ Antioxidant Effectiveness} = \frac{\text{Sample absorbance reading}}{\text{The absorbance reading of the control}} \times 100$$

Measurement of Reducing Power

The method mentioned in Al-Moussawi & Al-Halfi (2012) was followed which included mixing 2.5 ml of alcoholic extract of carob gum with 2.5 phosphate buffer solution 200 mM and pH 6.6 and 2.5 ml of potassium ferricyanide solution (1 %), bosom. The mixture at a temperature of 50 C° for 20 minutes after that the reaction was terminated by adding 2.5 ml of Trichloroacetic acid (10 %). The central centrifugation of the mixture was perform at a speed of 2000 rpm for 10 minutes. Separate the top layer of solution and add 5 ml distilled water and 1 ml ferric chloride (0.1 %). The absorption measurement was at a wavelength of 700 nm. The control sample was prepared by adding all the previous materials except adding 2.5 ml ethanol instead of the alcohol extract of the carob seeds. The following formula was apply to calculate the amount of the reducing strength of the extract:

$$\% \text{ Reducing Power} = 100 - \frac{\text{Sample absorbance reading}}{\text{The absorbance reading of the control}} \times 100$$

Scavenging of Hydrogen Peroxide:

Determined according to Türkoğlu *et al*. (2010) by taking 1-5 $mg.ml^{-1}$ of gum and 0.6 ml of 0.002 molar H_2O_2 prepared in 0.1 mP phosphate buffer at pH 7.4, left for 10 minutes at laboratory temperature, measured Absorbance along 230 nm, the equation was used below:

$$\% \text{ Capabilty of capture} = \frac{\text{absorbance of the control sample} - \text{Sample reading}}{\text{absorbance of the control}} \times 100$$

Chelating ability of ferrous ion

The method described in by Gülçın *et al*. (2003), mixed 0.4 ml of alcoholic extract with 0.4 ml of ferric chloride 2 ml molar with 0.4 ml 8-Hydroxyquinoline at a concentration of 5 molar (ethanol prepared), incubate the mixture for 10 minutes at room temperature in a dark place. The absorbance was measured along a 562 nm wavelength, the results were compared

with (EDTA 2Na); the control sample was prepared in the same way except for the sample, the ability of the plant extract to chelate ferrous ion was calculated according to the following formula:

$$\% \text{ Chelating ability of Ferrous ion} = \frac{\text{Sample absorbance reading}}{\text{the absorbance reading of the control sample}} \times 100$$

Statistical analysis

Data were statistically analysed using the SPSS statistical program (SPSS, 2006), and the data were compared using a Revised least significant difference (RLSD) at $p \leq 0.05$ probability level. Using three replicates for each treatment.

Results & Discussions

Active compounds in the gum of the fruits of the carob plant:

Chemical detection of active compounds in the gum of the fruits of the carob plant. Containing the alcohol extract on most of the active compounds under study, which included alkaloid, phenolic, flavonoid, glycoside, tannin, resins, saponins and coumarins, all of which gave positive results when inferred through the interactions shown in the table (1). These results came with what he arrived at Papagiannopoulos *et al*. (2004). Where he indicated that the fruits of the carob plant contain glycoside, tannin phenolic and flavonoid. The results also agreed with Ydjedd *et al*. (2017) and Correia *et al*. (2018); where they indicated that the fruits of the carob plant contain tannin phenolic and flavonoid.

Table (1): Detection results of active compounds in carob gum.

Active compounds	Reagent uses	Regent results
Glycoside	Benedict reagent	+
Alkaloid	Mayer's Reagent	+
Saponins	appearance of foam	+
Coumarins	UV-Scan	+
Resins	HCl acid	+
Flavonoid	Alkaline potassium hydroxide	+
Phenolic	Ferric chloride	+
Tannin	Lead Acetate	+

Measurement of antioxidant activity

It's clear from the table (2) that the alcohol extract of carob plants showed significantly approach antioxidant effectiveness compared to industrial antioxidant BHT, The effectiveness of carob gum fruit was about 47.12 % at a concentration of 0.1 % and 84.55 % at a concentration of 0.2 %, whereas the average anti-oxidant efficacy of BHT was about 93.43 %, and the average oxidative effectiveness of ascorbic acid was 91.12 %, at a concentration of 0.2, respectively.

Table. (2): Antioxidant activity of alcoholic extract of carob gum (mean ± standard error)

Treatments	Antioxidant activity %
BHT	93.43 ± 0.0631^{a}
Ascorbic acid	91.12 ± 0.0073^{a}
Gum 0.2	84.55 ± 0.1404^{b}
Gum 0.1	47.12 ± 0.2311^{c}

Means in the same column with different letters show significant differences ($p \leq 0.05$).

The reason for the high efficacy of the gum extract and the carob plant as an antioxidant is due to the effective compounds of the carob plant, which are either hydrogen-or electron donors or have the ability to capture free radicals (Makris & Kefalas, 2004).

Measurement of Reducing Power

Table (3) shows the reductive power of alcohol extract to glue of the fruits of the carob plant compared to ascorbic acid and industrial antioxidant (BHT), where the results showed that extract of gum of carob fruits possesses a reducing strength that increases to increase the concentration, as it averaged 73.20 % at a concentration of 0.1 % and 83.30 % at a concentration of 0.2 %, It is an approach to industrial antioxidant and ascorbic acid at a concentration of 0.2 as it reached 91.40% and 90.70%, respectively.

Table (3): Reducing power of alcohol extract for carob gum (Mean ± standard error).

Treatments	Reducing power %
BHT	91.40 ± 0.1400[a]
Ascorbic acid	90.70 ± 0.2333[a]
Gum 0.2	83.30 ± 0.1644[b]
Gum 0.1	73.20 ± 0.0221[c]

Means in the same column with different letters show significant differences ($p \leq 0.05$).

As the intensity of the apparent colour indicates the reductive strength of the extract of gum fruits of the vegetable plant to give or release hydrogen ions or increase the hydroxyl root and thus increase the reducing strength, which was a significant indicator that reflects the antioxidant effectiveness (Al-Birawee & Nasser, 2019).

Measurement of scavenging hydrogen peroxide

Table (4) shows that carob gum has the ability to sweep the hydrogen peroxide with the ability to resemble ascorbic acid and non-significant differences at $p \leq 0.05$. As the averages of the alcoholic extract for carob plant fruits 41 % and 50 % for concentrations of 0.1 % and 0.2 %, respectively. In addition to the significant differences between the alcoholic extract of the carrot gum and the industrial antioxidant (BHT) in its susceptibility to sweep the hydrogen peroxide, this process was very important to protect cellular systems, as hydrogen peroxide was a highly important compound because of its ability to penetrate cell membranes inside cells. Moreover, it's a toxic substance for cells because it raises the root of hydroxyl which has toxic effects.

Table. (4): Scavenging hydrogen peroxide of alcohol extract for carob gum. (Mean ± standard error).

Treatments	**Scavenging hydrogen peroxide %**
BHT	73.00 ± 0.2140[a]
Ascorbic acid	45.00 ± 0.1006[b]
Gum 0.2	50.00 ± 0.1222[b]
Gum 0.1	41.00 ± 0.2115[b]

Means in the same column with different letters show significant differences ($p \leq 0.05$).

In addition, it could inhibited number of enzymes are oxidized by the primary tiol groups (SH-), which have a biological advantage for cells, to control the amount of hydrogen peroxide inside the cell. (Sikha, 2016).

Measurement ferrous ion chelating

Table (5) shows the ability carob gum to binding ferrous ion compared to the antioxidant. EDTA 2Na (Ethylene diamine tetraacetic acid).

Table (5): Ferrous ion chelating of alcohol extract for carob gum. (Mean ± standard error).

Treatments	Ferrous ion chelating %
EDTA 2Na	55.48 ± 5.0076[a]
Gum 0.2	43.19 ± 3.6541[a]
Gum 0.1	32.91 ± 4.0034[a]

Means in the same letters show non-significant differences ($p \leq 0.05$).

The results showed in the table a highly significant susceptibility to gum of carob fruits to bind ferrous ion compared to the anti-oxidant (EDTA 2Na) and this ability amplified with increasing the concentration of the extract until it reached its highest average of 43.19 % at a concentration of 0.2 %, while the average ferrous ion of the antioxidant reached 55.48 % when comparing with the anti-oxidant (EDTA 2Na), it was observed that the ability to bind ferrous ion by carob gum was almost as closed to that shown by the antioxidant, and the ability of the alcoholic extract for gum the carob to bind ferrous ion was due to the presence of a large number of active compounds which have a high ability to bind iron (Nagulendran *et al*., 2007).

Conclusions

The results showed that gum of carob fruits was rich in bioactive compounds, in addition, carob gum has antioxidant activity.

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Conflict of interest: The authors declare that they have no conflict of interest.

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خصائص مضادات الاكسدة لمستخلص صمغ نبات الخروب (*Ceratonia silique* L.) خارج جسم الكائن الحي

مروة ثامر غياض العامري واميرة كاظم ناصر

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المستخلص: أجريت التجربة في مختبرات كلية الزراعة جامعة البصرة للفترة من 2019/9/22 إلى 2019/11/25. تهدف الدراسة الحالي إلى استخلاص الصمغ من ثمار الخروب وتحديد المواد الفعالة وإمكانية استخدامه كمضادات اكسدة. أظهرت النتائج أن صمغ ثمار نبات الخرنوب يحتوي على جميع المركبات الفعالة قيد الدراسة، والتي شملت القلويات، الفينولات، الفلافونيدات، الكلايكوسيدات، التانينات، الراتنجات، الصابونيات والكومارينات. كما أظهرت النتائج أن صمغ ثمار نبات الخرنوب امتلك نشاطًا مضادًا للأكسدة بنسبة 84.55٪ عند استخدامها بتركيز 0.2٪، في حين كان متوسط الفعالية المضادة للأكسدة وحامض الاسكوربيك حوالي 93.43٪، 91.12٪ بنفس التركيز. والقوة الاختزالية بلغت حوالي 73.20% بتركيز 0.1% وحوالي 83.30% بتركيز 0.2%، وهي نتائج مقاربة لتأثير مضادات الأكسدة الصناعية (BHT) وحامض الاسكوربيك بتركيز 0.2% والتي بلغت 91.40% و90.70 % على التوالي. وبالإضافة إلى ذلك، فإن صمغ ثمار نبات الخرنوب لديه القدرة على اكتساح بيروكسيد الهيدروجين بقدرة مماثلة لحامض الاسكوربيك ودون اختلاف معنوي لتصل إلى 41٪ و 50٪ للتراكيز 0.1٪ و 0.2 ٪ على التوالي، في حين أن حامض الاسكوربيك وصل إلى 45٪ ، مع وجود اختلافات معنوية مع مضادات الأكسدة الصناعي (BHT) في قابليتها لاكتساح بيروكسيد الهيدروجين حيث بلغ 73٪.كما أظهرت النتائج أن صمغ ثمار نبات الخرنوب قادرة على ربط أيون الحديدوز وتزداد قابلية الربط هذه مع زيادة تركيز الصمغ لتصل إلى أعلى متوسط لها 43.19% عند تركيز 0.2% في حين أن متوسط ايون الحديدوز لمضادات الأكسدة (EDTA 2Na) بلغ حوالي 55.48%.

الكلمات المفتاحية: نبات الخرنوب، الصمغ، الخرنوب، مضاد اكسدة.