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Walnut Oil Extract and Its Ability to Scavenge Free Radicals, Phenolic Content and Effective Antioxidants

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ABSTRACT

Walnuts have been the oldest food for living things. It contains biologically active antioxidants, vitamin E, omega-3 fatty acids, moreover minerals such as sodium, calcium, iron, copper, magnesium and manganese, protein and fiber, etc making it a comprehensive healthy meal. This phenols in green walnut fruits have extracted in our study using methanol. Protocatechuic acid, sinapic acid, gallic acid, chlorogenic acid, and catechin have found among the individual and total phenols. Single-phenolic compound quantification is carried out using a SYKAMN chromatography system and reverse phase HPLC analysis. By using the Folin-Ciocâlteu technique, the total phenolic content (TPC) of the ethanolic extract has been calculated. The IC_{50} was measured, as well as the free radical scavenging of DPPH. The current research demonstrates the content of phenolic compounds in walnut oil found in the local markets of the city of Mosul, its activity as antioxidants, and its effectiveness in combating free radicals within the body when added to meals. This study may help clarify the overall health benefits of eating walnuts.

Keywords: Walnuts, Phenolic compounds, Antioxidants, Ethanol extract

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مستخلص زيت الجوز وقدرته على التخلص من الجذور الحرة والمحتوى الفينولي ومضادات الأكسدة الفعالة

شيماء عباس ايوب

قسم العلوم الاساسية، كلية طب الاسنان، جامعة الموصل، موصل، العراق

الملخص

الجوز أقدم غذاء للكائنات الحية. يحتوي على مضادات الأكسدة النشطة بيولوجيًا، وفيتامين E، وأحماض أوميجا 3 الدهنية، علاوة على المعادن مثل الصوديوم والكالسيوم والحديد والنحاس والمغنيسيوم والمنغنيز والبروتين والألياف وغيرها، مما يجعله وجبة صحية شاملة. تم استخلاص هذه الفينولات الموجودة في ثمار الجوز الأخضر في دراستنا باستخدام الميثانول. لوحظ وجود Protocatechuic acid, gallic, catechin, chlorogenic acid, sinapic acid, acid بين الفينولات الفردية والمشبعة. تم إجراء القياس الكمي للمركب أحادي الفينول باستخدام نظام كروماتوجرافيا SYKAMN وتحليل HPLC للمرحلة العكسية. باستخدام تقنية Folin-Ciocâlteu، تم حساب المحتوى الفينولي الكلي (TPC) للمستخلص. وقياس قيمة IC_{50} ، وقياس مسح الجذور الحرة لـ DPPH. يوضح البحث الحالي محتوى زيت الجوز الموجود في الاسواق المحلية لمدينة الموصل من الموكبات الفينولية ونشاطها كمضادات للاكسدة وفعاليته على مقاومة الجذور الحرة داخل الجسم عند اضافته الى وجبات الغذاء. قد تساعد هذا الدراسة في توضيح فوائد تناول الجوز على الصحة العامة.

1. INTRODUCTION

Walnuts are the most important and most consumed nuts since 1000 BC[1]. Its tree belongs to the Juglandaceae family. Walnuts, rich in dietary fiber, are a polymer of galacturonic acid and have a variety of physiological and nutritional effects[2]. Walnuts are suitable to be eaten and an important ingredient of a healthy food, and their consumption is on the rise all over the world as people become interested in a healthy lifestyle [3]. Walnuts, when added to breakfast, baked goods, appetizers, desserts, and soups, increase the feeling of fullness due to their nutritional value, and this leads to increased consumer demand for them[4]. The seed of the nut is a rich source of nutrients, such as proteins and essential fatty acids. It contains a high percentage oil (52-70 %). Its main constituents are triglycerides, such as unsaturated fatty acids (mainly oleic acid), polyunsaturated fatty acids, and omega-3 and omega-6. It is a good source of vitamins such as niacin and tocopherols and minerals, as it contains 14 elements, such as calcium, magnesium, potassium, iron, and selenium[5]. In recent years, screening plant fruits and identifying new antioxidants to improve health has become very important[6]. Antioxidants are substances present in a lower concentration compared to oxidizing agents, and they significantly delay or prevent oxidation [7]. Walnuts are among the sources of phenolic compounds that have health avails and distinguished by great pharmacological activity, such as anti-cancer, oxidative stress, inflammation, microbes and viruses[8, 9]. Regular consumption of polyphenols may reduce cardiovascular disease, neurodegeneration, diabetes risk and many other physiological diseases[10, 11]. As well as nuts; that contain antioxidants, play a major role in maintaining health and avoiding diseases, and in the past few years they have received great attention[12]. The aim of the research is to determine the total flavonoids and phenolic compounds in the ethanol extract of walnut oil

using an HPLC device, measure the ability of walnut oil to displace free radicals using the DPPH method, and measure the IC_{50} of the walnut oil extract, which represents its ability to inhibit a certain vital function.

2. MATERIALS AND METHODS

2.1. Methodology for making the alcoholic walnut pulp extract

Walnuts have been purchased from open markets in Iraqi governorate of Mosul. It has been cleaned, dried, and ground using coffee grinder. One kilogram of walnuts is soaked in five liters of ethanol. This immersion is carried out at room temperature for (48 hours). It should be filtered using a cloth and placed in a Pyrex container and heated for (24 hours) at (45 °C). According to the measurements made with a balance, one Kilogram of nuts has given seventy-five grams of oil and the mixture should be put in an airtight glass container, and keep in the refrigerator until use[13]. In this research, five-hundred grams of walnuts has been soaked in two liters of methanol as a methanol solution. A Soxolite device has been used as a device to separate the oil from the liquid. the methanol solvent is placed in the glass beaker and connected to it is a column in which the walnut powder is placed, which is connected to the condenser. It contains an opening for cold water to enter and another for hot water to exit, with a heat source installed at the bottom of the glass beaker according to the working method. When the appliance is turned on and the boiling continues, the solvent evaporates and the extracted walnut oil is formed instead. Forty grams of extract for every 500 grams of walnuts have obtained.

2.2. Phenolic compound determination using HPLC

Utilize a UV detector, Chemstation, and a (0.25 cm) through (4.6 ml) Zorbax Eclipse Plus-C18-OSD the column in conjunction with a SYKAMN high-performance liquid chromatographic system. In addition, the column's temperature is (30 °C). Using both eluents A (methanol) and B (1% formic acid in water (v/v), the gradient elution method is applied to each individual phenolic compound in order to obtain a quantitative measurement. (40 %) B for the first (0 – 4 minutes); (50 %) B for the next (4 – 10 minutes); and (0.7 ml/min) of flow. The autosampler is utilized to perform the automatic injection of (100 µl) of standards and (100 µl) of injected samples. Spectra at (280 nm) are obtained. But ethanol extracts are the only ones to which these guidelines can be applied; aqueous extracts may not be good to be used[14]. In this research, the column temperature is (25 °C), methanol is used in eluent A, and the measurement is at (300 nm).

2.3. Calculating the total content of phenolic compounds

Scientists Shahidi and Zhong is employed the Folin-Ciocalteu method to determine an ethanolic extract's total phenolic content. One and a half milliliters of twenty percent sodium carbonate, five hundred microliters of Folin-Ciocâlteu reagent (Merck, Germany), and one hundred microliters of walnut extract have been added to complete the work. The sample should be combined in the device's blender and diluted to a final volume of ten milliliters using distilled water. The mixture's absorbance at the wavelength of (765 nm) has to be measured after (2 hours) at lab temperature. Gallic acid standards (Aldrich, Germany) are utilized to create the calibration curve. The data has shown as gallic acid equivalent (GAE) for each dry weight gram[15]. In this research, methanol has been used as an extraction agent, and the absorbance is measured at a wavelength of (750 nm).

2.4. Antioxidant extract using DPPH assay

DPPH of (0.04 g) with just (100 ml) of methanol could be dissolved in this research. The concentration of DPPH is (400 µg/L) [16]. Half a gram of the above vitamin, (100 ml) of the above alcohol, and distilled water are combined to create the standard liquid (vitamin C) and sample. The dilution rule is used to prepare the remaining concentrations of vitamin C (50, 100, 200, 400,

and 600 µg/L) and the sample (50, 100, 200, 400, and 600 µg/L)[17]. The concentration of the standard solution is (5000 µg/L). After a thorough mixing of the mixture and a half-hour at room temperature, the absorbance at (517 nm) is measured with a UV-Vis Shimadzu spectrophotometer [18].

To determine the percentage of the DPPH scavenging effect, use the following equation: (A0 - A1 / A0 × 100) is the DPPH scavenging effect or inhibition percentage.

The absorbance of the blank is shown by A0, and the absorbance when the test sample is present is shown by A1.

A sigmoid dose-response curve is used to calculate an IC_{50} value, which is defined as the concentration of the test sample that reduces fifty percent of the free radical concentration and is expressed as µg/L[19]. In this part of the research, absorbance is measured at (500 nm).

3. RESULTS AND DISCUSSION

Table 1: lists all of the phenols and flavonoids found in walnut methanolic extracts. Age and eventually die as that clock strikes twelve. Nevertheless, there is a great deal of diversity in the timing of this genetic clock based on factors such as development and real lifestyle (quality of life, food, cleanliness, medical practices).

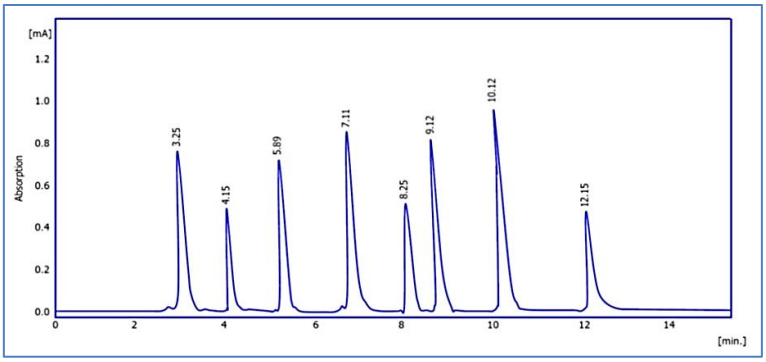

Fig. 1: HPLC of the Methanolic walnut extract.

Table 1: Total flavonoids and phenols in an methanolic extract of walnut oil.

No	Retention Time (min)	Area in mAUs	Elevation (mAU)	Area percentage	Height percentage	W05 (minutes)
1	3.25	4256.98	794.25	12.05	12.01	0.20
2	4.15	2564.89	520.11	10.11	10.14	0.15
3	5.89	5623.66	630.59	11.58	11.63	0.20
4	7.11	7854.15	840.22	13.65	13.45	0.25
5	8.25	2360.55	564.77	10.25	10.32	0.15
6	9.12	6985.47	812.44	14.22	14.20	0.27
7	10.12	7541.49	960.21	18.58	18.35	0.30
8	12.15	1266.59	420.11	9.25	9.33	0.15

Table 2: The total phenolic content.

No	Name	Conc (μgL^{-1})
1	Catechine	75.4
2	Ferulic acid	88.9
3	Gallic acid	95.4
4	Vanillic acid	41.5
5	Chlorogenic acid	52.4
6	Synirgic acid	35.9
7	Sinapic acid	41.5
8	Cinnamic acid	22.9

Table 3 displays the results for both the DPPH free radical scavenging activity, expressed in IC_{50} values, and the total antioxidant capacity, expressed as ascorbic acid (AA), which equivalent in ppm of dry extract. The outcomes demonstrate the scavenging of free radicals. Under the same circumstances, the standard compound ascorbic acid displayed an IC_{50} of (209.2 μgL^{-1}) Numerous studies have calculated walnuts' high total antioxidant and free radical-scavenging properties.

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Table 3: DPPH radical scavenging activity, IC_{50} (µg L^{-1}) of walnut oil extract.

AA (%)	30 (µgL^{-1})	60 (µgL^{-1})	120(µgL^{-1})	250 (µgL^{-1})	500 (µgL^{-1})
Vit C	17. 8	24.6	37.9	44.6	61.6
walnut oil	34.5	50.9	64.9	83.5	97.4

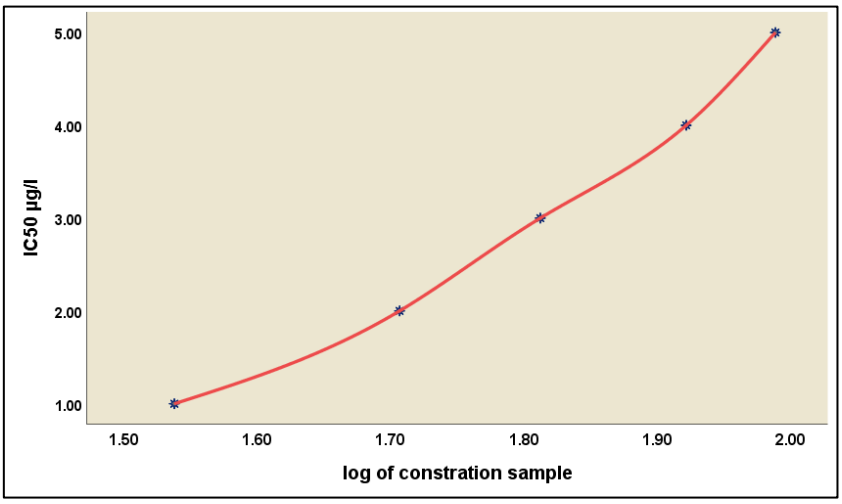

Fig. 2: IC_{50} and log conc walnut oil.

The tables above show differences between the walnut cultivars sourced from different parts of the world. There are a lot of factors that could influence the differences, including genetic variations, soil, climate, harvesting season, and storage conditions. The most common species of tree nut in the world is the walnut tree (*Juglans regia* L). Different genotypes of walnuts have different physical and chemical properties, productivity levels, and relationships to forests. A few of them have been deemed promising and could serve as sources of germplasm for breeding[20]. Good compound separation is provided by HPLC technology, which also lowers routine analysis costs and management time. The active antioxidant components found in *M. oleifera* leaves are phenolics and flavonoids. Since the food and pharmaceutical industries use plant extracts, it's important to comprehend their chemical makeup and any potential biological properties[21]. Unsaturated fatty acids, primarily linoleic acid with lesser amounts of oleic and linolenic acid, make up a large portion of walnut oil. Walnut oil's secondary constituents include hydrocarbons, volatile compounds, phospholipids, and tocopherols. Researchers have found that phenolic compounds, which are extracted from nut oil poorly but present in high concentrations in the seed coat, have potent antioxidant qualities. Furthermore, the oil extract has been used in the formulation of numerous food products due to its high content of proteins (including arginine, glutamic, and aspartic acids) and its numerous health benefits[22, 23] Secondary products of the metabolic Secondary processes that take place inside the plant are phenolic compounds and antioxidants. They display the color of the plant's fruits and flowers and provide protection against fungi, bacteria, and UV rays[24]. A significant proportion of them can be found in fruit peels. Effective antioxidants phenols and flavonoids are present in the sample (*Juglans regia* L) extract used in this investigation. Research and studies already conducted a test to the high antioxidant content and potential of walnuts (*Juglans regia* L) to counteract the effects of free radicals. The walnut kernel is more beneficial because it contains a variety of antioxidants[25]. When it comes to antioxidant activity, walnuts have 40–50 times more than citrus fruits, with (2400 – 3700

mg) of vitamin C per (100 grams). Roughly (7.3 – 28.7 g) of tocopherols is present in walnut oil. There are between (100 and 436 mg/kg) of tocopherol in walnut oil. State that the quantity and quality of fats and other essential components are influenced by the extraction technique used to extract walnut oil. Essential ideas for its extraction include minimizing heat-induced damage to the oil, extracting as much of the oil as possible with the least amount of impurities, and maintaining the oil's flavor and antioxidant qualities. The most used method for extracting oils industrially is solvent extraction [(26)]. Table (2) shows the concentrations of phenols in the research sample, which is the concentration of gallic acid Higher than all the phenols present in the first place comes ferulic acid, catechin with a low concentration, then comes Chlorogenic acid, sinapic acid, and vanillic acid are at similar concentrations. As for the content of walnut oil of synergic acid, Cinnamic acid is less pronounced ‹As is clear in the chart in Figure (1), This is consistent with previous research[(2)]. When it comes to absorbing and dislodging free radicals, quenching single and triple oxygen, or dissolving peroxides, phenolic elements are highly helpful. Research indicates that flavonoids can help prevent chronic illnesses caused by oxidative stress, such as cancer and cardiovascular disease. Antioxidant-containing substances found in walnuts include polyphenols and tocopherols[(27)]. Juglans regia has therapeutic qualities due to its bioactive compounds with a large number of secondary metabolites, such as polyphenols, flavonoids and glycosides, in its leaves, peels, flowers and bark [(28)]. Gallic acid is the most abundant bioactive compound in the ethanolic extract in the present study[(14)]. Phenolic acids are derived either from benzoic acid such as gallic acid, vanillic acid, syringic acid and others, or from cinnamic acid such as sinapic and other types[(29)]. Foods with active, functional qualities are judula regia. The biological characteristics and the components that embedded in this crop are correlate favorably. These bioactive substances work by lowering free radicals (ROS), inducing inflammation, blocking the release and function of enzymes, raising insulin levels, and interfering with the body's cell cycle[(30, 31)]. Table (3) and Figure (2) shows an analysis of the evaluation of walnut oil as antioxidants using the DPPH radical method As well as measuring the IC_{50} (a measure of the concentration of a certain substance to inhibit a certain biological or biochemical process by 50 %) for walnut oil compared with vitamin C at different concentrations, which is considered a powerful antioxidant, as it was noted that DPPH and IC_{50} Walnut oil is higher than vitamin C, and this is confirmed by research[(32, 33)]

4. CONCLUSION

There are many types of walnuts spread around the world in different shapes and colors, and they are an important food and industrial source for human life. Walnut oil has effective benefits for improving general health, because it contains ingredients with high nutritional value. Different techniques have been used to extract the oil from the kernel while preserving its components such as phytosterols, tocopherols, polyphenols, squalene, and minerals, with the presence of secondary components in the oil such as hydrocarbons and volatile compounds and phospholipids. Due to the antioxidants that walnuts and their oil contain, such as arginine, glutamic acid, and aspartic acid, and their health benefits, people are consuming it well. As for the organic antioxidants found in the leaves, they are phenols and flavonoids. They have played a role in the food and pharmaceutical industries, which help in alleviating the severity of diseases.

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