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Evaluation of the Phytochemical Contents of Two Iraqi Date Palm Fruit Extracts (*Phoenix dactylifera* L. cvs. Berhi and Bream) and Assessment for Their Antioxidant Capacities

Duha Z. Al-Basiri[1,*], Ausama A. Jaccob[2] & Muhsin S.Gh. AL-Moziel[3]

[1]Department of Basrah Health, AL-Mawani Teaching Hospital, Ministry of Health, Basrah, Iraq.
[2]Department of Pharmacology and Toxicology, College of Pharmacy, University of Basrah, Basrah, Iraq.
[3]Department of Pharmacology and Toxicology, College of Pharmacy, University of Basrah, Basrah, Iraq.

[*]**Corresponding author email**: **D.Z.A.:** pgs.duha.zaidoon@uob.iq; **A.A.J.:** ausama1979@yahoo.com; **M.S.G.A.:** muhsinghalib24@gmail.com

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Abstract: This paper aimed to measure the bioactive components in the extracts of two date palm cultivars (Berhi and Bream), including flavonoids, phenols, tannins, and carotene, and assess their antioxidant potential. This investigation was carried out in a laboratory at the pharmacognosy department of the College of Pharmacy on October 2023, at the University of Basrah, Iraq. Both qualitative and quantitative phytochemical analytical methods were used in the research, including an estimate of carotene, total polyphenols, flavonoids, tannins, and primary phytochemical assays. as well as evaluation of their scavenging capacity using DPPH to determine their antioxidant activity in extracts from two date palm cultivars. The phosphomolybdate solution was used to measure the total antioxidants. Results: These studies showed that the fruits of the Berhi and Bream date palms cultivars have a high phenolic content, presented in milligrams for each gram from the extract, including total flavonoid content for Berhi and Bream cultivars 0.450mg, 0.333mg, total phenolic content 2.71mg for both varieties, total tannin content 0.2mg, 0.1mg, total carotene content 38.3microgram, 59.3microgram, furthermore IC50 and p-value were estimated for two cultivars 0.16 with p-value 0.189 for Berhi, 0.19 with p-value 0.234 for Bream. Conclusion: our study improved the antioxidant activity of both date palm cultivars making them suitable natural alternatives for synthetic products and promising candidates for the nutraceutical industries.

Keywords: Berhi, Beta-carotene, Bream, Crude extracts, Tannin, DPPH.

Introduction

Significant demand has been for natural and herbal products as sustainable alternatives to synthetic chemicals (Bettaieb *et al*., 2023). In poor nations, 75-80% of individuals utilize traditional medicinal plants as natural or manufactured medicines, owing to their accessibility, compatibility, and acceptance (Sedighi-Khavidak *et al*., 2022). The date palm tree (*Phoenix dactylifera* L.) is one of the earliest cultivated species in Southwest

Asia, North Africa, Mexico, California, and Texas. It belongs to the Aceraceae family and primarily comprises approximately 200 genera and a further 2,500 species (Al-Mahmoudi *et al*.,2023). Date fruit is rich in natural sugars, proteins, minerals, vitamins, particularly B-complex, and other secondary metabolites including anthocyanins, carotenoids, flavonoids, phenolic acids, procyanidins, and sterols (Hinkaew *et al*., 2021).

In recent years, significant interest in the numerous health-promoting effects of date fruits has led to extensive pharmacological study. Both *in vivo* and *in vitro* studies, as well as the quantification and documentation of phytochemical constituents (Alhuzali *et al*., 2023).

The phytochemical constituents of date fruits can be categorized into two primary groups: primary and secondary metabolites. Primary metabolites encompass nucleic acids, specific carbohydrates, and amino acids, which play crucial roles in cellular reproduction and metabolism. Secondary metabolites, such as phenols and alkaloids, can exert biological effects on other organisms (Ali *et al*., 2022).

Dates' antioxidant potential is attributed to their rich phytochemical constituents, particularly carotenoids and polyphenols, including gallic acid, ferulic acid, syringic acid, caffeic acid, and coumaric acid, along with isoflavones, flavonoids, and tannins. These compounds can neutralize free radicals due to their conjugated cyclic structure and hydroxyl groups, which interact with superoxide anions, oxygen radicals, and lipid peroxyl radicals (Ouamnina *et al*., 2024).

Polyphenols are essential natural antioxidants commonly found in date fruits, serving as potential agents with preventive properties against various ailments. They exhibit a superior antioxidant capacity, hence greatly contributing to the antioxidant potential of date fruits. A strong robust correlation exists between the antioxidant potential of date fruits and their overall phenolic content. Phenolic compounds are recognized for their multifaceted roles in various conditions, including the protection against and prevention of diseases such as atherosclerosis, diabetes, and neurodegenerative disorders like Parkinson's and Alzheimer's diseases, inflammation reduction, and potential mitigation of specific cardiovascular issues (AlFaris *et al*., 2021).

Flavonoids are a crucial category of common crops classified as plant secondary metabolites, characterized by a polyphenolic structure, and are prevalent in fruits, vegetables, and traditional beverages (Panche *et al*., 2016). Flavonoid molecules, characterized by their yellow pigmentation, possess significant antioxidant properties and confer several health advantages, including anti-aging, neuroprotection, anticancer effects, and anti-inflammatory actions (Agrawal, 2011).

In recent years, flavonoids have emerged as a focal area of research across various fields due to their potent antioxidant properties and diverse effects. Simultaneously, they are essential commodities in numerous applications such as nutraceuticals, medicines, and cosmetics (Lu *et al*., 2021).

The anti-inflammatory properties of flavonoids are mediated through various mechanisms, including the inhibition of cyclooxygenase and lipoxygenase enzymes, eicosanoid pathway inhibition, and the suppression of neutrophil degradation. These

mechanisms prevent the release of arachidonic acid and other prostaglandins, which are pivotal in the inflammatory process (Jamshidi-Kia *et al*., 2020).

Plant tannins constitute a significant category of antioxidants characterized by polyphenolic structures in food and beverages, offering many health benefits for humans. It is recognized as a phenolic compound possessing adequate hydroxyl groups and other appropriate groups to establish robust complexes with proteins and macromolecules under specific environmental conditions. They primarily consist of two principal categories: hydrolyzable tannins and condensed tannins, recognized for their advantageous benefits, including the reduction of blood glucose, cardio-protection, anticancer properties, and anti-inflammatory activities (Iylia Arina & Harisun, 2019).

Aim: as our study aimed to determine and characterize the total concentration of flavonoids, phenols, tannins, and carotene in extracts of two date palm cultivars (Berhi and Bream) and assess their antioxidant activity in the laboratory setting.

Materials & Methods

Plant collection

Two cultivars of date palm (*Phoenix dactylifera* L. cvs. Berhi and Bream) were obtained from orchards in Basrah City, southern Iraq. The authenticity of the cultivar was confirmed by (Dr. Abdul Ridha Alwan Al-Mayah) at the University of Basrah (Al-Mayah, 2016). The fruit was collected at the Tamar stage, and the seeds were removed, and the fruit pulps were cleaned with tap water and they were subjected to a 10-day shade-drying process at room temperature. Following this step, the dried pulps were cut into small pieces and prepared for extraction .

Extraction

The extraction was conducted at the University of Basrah, College of Pharmacy, Department of Pharmacognosy to get ethanolic extract using 10% ehanol. The hot extraction method (reflux) was employed as the extraction method; Thirty grams of the dried pulp from each date palm cultivar was separately soaked with 400 mL of 10% (v/v) ethanol solution. The mixture was heated at 60 °C for 3 hours using a reflex apparatus (Al-Rifai *et al*., 2017), then filtered using filter paper, and the extract was stored at 4 °C.

Phytochemical tests

Represented general tests for primary detection of flavonoids and polyphenols that include :

1. Shinoda Test: general test for flavonoids, A few milligrams of magnesium metal and a few drops of concentrated HCl were added to 1ml of the extract until a pink color formation. The pink color indicated the presence of flavonoids (Makhawi & Hamadnalla, 2019).
2. Ferric chloride Test (5%): A few drops of 5% ferric chloride solution were added to 1mL of the extract. The observation of green, blue, or violet tint formation indicates the presence of phenolic compounds, including some flavonoids.
3. Lead acetate Test (1%): A few drops of 1% lead acetate solution were added to 1mL of the extract. The formation of a yellow or white precipitate indicated a positive result.

4. Sodium hydroxide Test (10%): A few drops of 10% sodium hydroxide solution were added to 1mL of the extract. The observation of an intense yellow tint followed by its disappearance upon the addition of dilute hydrochloric acid indicated a shift in the conjugation pattern of certain compounds in the extracts.
5. Ferric chloride Test (5%): A few drops of ferric chloride solution were added to 1mL of the extract. The formation of green, blue, or violet tint indicated the presence of phenolic compounds.

Total flavonoid estimation

The aluminum chloride method was obtained to quantify the extract's total flavonoid content (TFC). A 0.5mL of the extract was mixed with 0.3mL of 5% sodium nitrite. After 5 minutes, 0.3mL of 10% aluminum chloride was added and incubated for 6 minutes. Subsequently, 2 mL of 1M sodium hydroxide was added, and the final volume was 5.0 mL with distilled water. The absorbance of the mixture was measured at 510nm against a reagent blank. Quercetin was used as a standard to express the flavonoid content as milligrams of quercetin equivalence (QE) per gram of extract (Chang *et al*., 2002).

Total polyphenols estimation

The plant extracts' total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Sasikumar, 2014). A 1mL of the sample was mixed with 1mL of Folin-Ciocalteu reagent. After 3 minutes, 1 mL of saturated NaCO3 solution (35%) was added, and the final volume was adjusted to 10 mL with distilled water. The mixture was incubated in the dark for 90 minutes. The absorbance was measured at 725 nm against a blank, Gallic acid was used as a standard, and the results were expressed as milligrams of Gallic Acid Equivalents (mg GAE) 0.3991X+0.285Y=0.8921, R2 (Daoud *et al*., 2019).

Total tannin content estimation

The measurable tannin content is estimated using the price and bustle method. 0.5mL from the extract was mixed with 1mL of potassium ferric cyanide and 1mL of ferric chloride 1% then the volume was brought to 10mL distal water, kept at room temperature for 5 minutes, The absorbance was measured at 720nm (Sasikumar, 2014).

Total carotene content analysis

The carotene content estimation was obtained by mixing 0.1g from the viscous extract with 25 mL of hexane in a dark volumetric flask. Absorbance was measured at 446nm using a UV-visible spectrophotometer, then the total carotene content was calculated using the formula: Carotene (microgram/ gram) = 25* A* 383/100 * sample weight * 100, A refers to the sample absorbance (Rini *et al*., 2022).

***In vitro*-Antioxidant activity estimation**

The antioxidant capacity of the date palm varieties was determined by measuring their free radical scavenging capacity using the DPPH assay, with slight modification to the method described by Erenler A solution of 0.8 mM DPPH in methanol was added to numerous concentrations [0.5-0.005 mg mL^{-1}] of the date extract (1 mL each). The tubes were covered tightly and kept in the shadowy for 30 minutes. The absorbance of the reaction combinations was then measured at 517 nm against a blank sample. Ascorbic acid was used as a standard to prepare a calibration curve (Daoud *et al*., 2019).

Total antioxidant activity estimation

Total antioxidant was estimated using the phosphor-molybdenum method described by Prieto. A 1mL from the extract was mixed with 1mL of standard reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate) the tube was closed and kept at 95 C for 90minutes, then left to cool at room temperature and the absorbance measured at 695nm against blank reagent. The total antioxidant capacity was expressed as a milligram of Ascorbic Acid Equivalence (AAE) per gram of the extract (Sasikumar, 2014).

Experimental design and Statistical analysis

All data were statistically analyzed using T-tests to achieve the consequences of the DPPH scavenging activity analysis in a completely randomized design using the statistical package SPSS software, p-value <0.05 to achieve a significant difference (Sabrina *et al*., 2022).

Results

Detection of flavonoids and polyphenols by phytochemical tests

The aqueous date extracts' findings indicate the presence of flavonoids and polyphenols presented in (Table 1). The Shinoda test showed the presence of flavonoids by producing a pinkish color that appeared after adding insufficient drops of concentrated HCl and a few milligrams of magnesium metal. Also, the presence of flavonoids was indicated throughout the ferric chloride test, lead acetate, and sodium hydroxide test all showed positive results confirmed by color changes following the addition of each solution. Furthermore, the presence of phenols was indicated by forming a green or bluish color after adding a few drops of 5% ferric chloride solution.

Table (1): Qualitative phytochemical analysis of Iraqi Date Palm Extracts (*Phoenix dactylifera* L. Berhi and Bream cultivars) with 10% (v/v) ethanol solution (+) detected (-) not detected. DE: date extract.

No.	Phytochemical tests	Color appearance	Berhi DE	Bream DE
1	Shinoda test	Red to pink	+	+
2	Ferric chloride 5%	Green to violet	+	+
3	Lead acetate 1%	Yellow ppt	+	+
4	Sodium hydroxide 10%	Yellow to colorless.	+	+
5	Ferric chloride 5% for polyphenols	Green to violet	+	+

Determination of TFC and TPC

Estimation of total flavonoid and phenolic content exhibited that the quantity of these products has some differences between the two extracts for both cultivars, as shown in (Table 2). Generally, the Berhi date extract contained a higher flavonoid content than the Bream date extract. Berhi and Bream cultivars contain a higher amount of phenolic content as shown in this table, and according to the sequence value that clearly explains the rise in concentration due to the increase in the bioactive ingredient.

Table (2): Determination of total flavonoid and phenolic content for Iraqi date palm extracts of the two cultivars (Berhi & Bream), the amount described in milligrams for each gram of the extract (total weight of the extract = 30g) SKEW: sequence, S.D: standard deviation.

No.	Phytochemical content	Berhi DE	Bream DE
1	Total flavonoid content	0.450mg/g	0.333mg/g
2	SKEW value	0.673	0.605
3	S. D	0.0147	0.0555
4	Total phenolic content	2.71mg/g	2.71mg/g
5	SKEW value	0.458	0.454
6	S. D	0.0181	0.0541
7	Percentage %	34.66%	58.86%

Quantitative tannin content

According to the research results shown in Table 3, by adding 1 ml of potassium ferric cyanide and 1 ml of ferric chloride, the Berhi date palm cultivar extract had a higher content of tannins than the Bream cultivar.

Table (3): Quantitively tannin content estimation for Iraqi date palm cultivars is presented as milligrams for each gram from the extract. DE: date extract.

No.	Date palm cultivars	Tannin content
1	Berhi DE	0.2mg $g^{=1}$
2	Bream DE	0.1mg $g^{=1}$

Total carotene analysis

According to the results shown in Table 4, both date palm cultivars contained carotenoids. According to the calculation used to estimate carotenoid content, the Bream cultivar extract had a higher carotene content than the Berhi cultivar extract.

Table (4): Total carotene analysis for Iraqi date palm fruits of two cultivars the amount expressed in micrograms per gram. DE: date extracts

No	Date palm cultivars	Carotene content
1	Berhi DE	38.3 µg /g
2	Bream DE	59.36 µg /g

Total antioxidant capacity

The total antioxidant abilities of the two date cultivars were calculated using different concentrations of vitamin C and the absorbance using a UV-visible spectrophotometer. The findings indicate total antioxidant activity equal to 0.005 AAE /g (5mg $g^{=1}$) for both Berhi and Bream.

Antioxidant activity

The total antioxidant potential of the two cultivars was estimated in *vitro* using DPPH (2,2-diphenyl-1-picrylhydrazyl), and 50% inhibition concentration (IC50) was calculated to admission the different antioxidant capacities of the extracts in contrast with recognized standard usually, ascorbic acid as shown in Figure1. According to the IC50 results, both date cultivars showed DPPH scavenger activity. The IC50 for Berhi date and vitamin C was 0.16 with a p-value of 0.189. The potent antioxidant ability of Berhi date fruit extract as a natural substance has a higher antioxidant capacity than Bream date. The IC50 value for the Bream and vitamin C was 0.19 with a p-value of 0.234, also indicating the scavenging effect of the Bream date cultivar to inhibition concentration presented the minimum concentration required for inhibition of the DPPH activity, thus the material that requires the lowest concentration for inhibition is considered a potent antioxidant material.

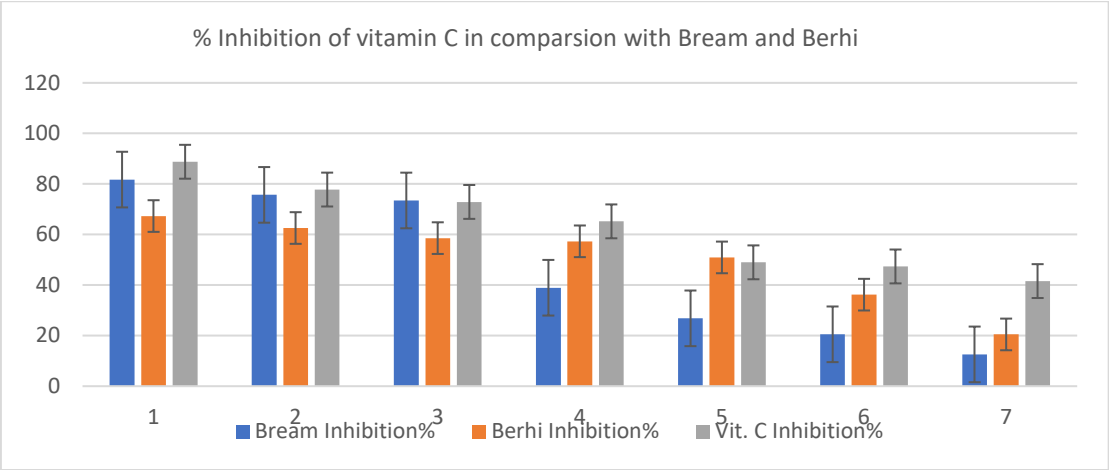

Fig. (1): Compared with vitamin C, the antioxidant capacity for Iraqi date palm fruit extracts, is IC50 for Berhi = 0.16, IC50 for Bream = 0.19, and IC50 for vitamin C = 0.02, Error bars in the Excel columns represent the degree of uncertainty or variability associated with the data points, they visually indicate how much the actual value might deviate from the displayed value.

Discussion

Flavonoids and phenols are promising substitutes for bioactive ingredients in pharmaceutical formulations that aim to improve human health and disease prevention. As a result, they are widely used nowadays; our study evaluates and measures total flavonoid content, total phenolic content, tannins, and carotene, as well as the in vitro assessment of their antioxidant potential. Numerous studies have shown a clear correlation between the content of phenolic and flavonoid compounds and their antioxidant and anti-inflammatory effects (Sun & Shahrajabian, 2023).

Phytochemical testing is the primary technique used to determine whether phytochemical components are present in crude extracts. The Shinoda test, a common technique for identifying flavonoids, turns pink when a few drops of concentrated HCl and magnesium ribbon are added. These color changes indicate that the date palm fruit extract is rich in phenolic and flavonoid compounds. Additionally, the presence of flavonoids in the aqueous extract was confirmed using reagents consisting of 10% sodium hydroxide, 5% ferric chloride, and 1% lead acetate. The presence of phenolic components in the aqueous extract was verified using 5% ferric chloride. To demonstrate this, a green-blue pattern was created (Makhawi and Hamadalla, 2019). Finally, phytochemical analyses validate the presence of the bioactive compounds in the crude aqueous extract due to these bioactive compounds were normally isolated using polar solvents such as ethanol or methanol (Al-Rifai *et al*., 2017).

The quantitative assessment of total flavonoids and phenols reveals the presence of phenolic compounds in the crude extracts of date palm fruits from both cultivars. These were calculated using specific equations, expressed in milligrams per gram of the total extract weight, as illustrated in Table 2. The results closely align with the findings of the initial phytochemical analysis, indicating that date palm fruits are rich in flavonoids and polyphenols, which act as effective scavengers against oxidative stress and free radicals. This conclusion is corroborated by several studies that support our findings

(AlFaris *et al*., 2021). The quantitative assessment of total flavonoids and phenolic content in the aqueous extracts revealed variability between the two date cultivars; Berhi date extract is expected to contain a higher concentration of flavonoids about 0.450mg g^{-1}, followed by the Bream cultivar was equal to 0.333mg g^{-1}, as indicated by the results. According to Al-Mayahi (2021) both cultivars contain elevated levels of polyphenols equal to 2.71 mg and according to the sequential analysis that indicates a direct correlation between concentration and bioactive components. This implies that an increase in extract concentration leads to a rise in the plant's bioactive content, thereby enhancing the antioxidant activity of the aqueous extracts. Consequently, our findings elucidate this phenomenon. In conclusion, this part of our study tries to evaluate and calculate the concentration for each bioactive component to ensure their in vitro activity and their protective capacity before they are applied to other organisms (Nguyen *et al*., 2021).

The data obtained for total tannin content estimation indicated that both date palm extracts are rich in tannins; however, the Berhi date cultivar has a greater quantity of 0.2 mg g^{-1} than the Bream date cultivar 0.1mg/g from the extract, as illustrated in Table 3. Consequently, many researchers currently focus on tannins and their biological activity due to their diverse pharmacological properties, including cardio protective effects, anti-cancer, anti-inflammatory, enhanced glucose uptake, and gastric protective effects (Kumari & Jain, 2012). Tannins are highly reactive compounds characterized by multi-hydroxyl groups and aromatic structures that can mitigate oxidative stress and reactive oxygen species (Hoque *et al*., 2024). This elucidates why the Berhi date cultivar is preferred over other date varieties for treating gastric ulcers, as possesses a higher tannin content that alleviates inflammation and oxidative stress while providing gastric protection (Kadhim Hussein *et al*., 2023).

Table 4 demonstrates that both date cultivars contain a carotene component; additionally, the Bream date extract exhibits a greater concentration of carotene about 59.36 micrograms/gram than the Berhi date extract 38.3 micrograms for each gram of the extract, as indicated in the table below. These values illustrate the disparities in nutritional compounds among date palm fruit varieties, influenced by factors such as ripeness, variety, and preparation methods. According to (Hussain *et al*., 2020), carotenoid compounds are recognized as powerful antioxidants and constitute a significant category of phytochemicals found in the lipid fraction of various fruits and vegetables, such as date palms. They serve as precursors to vitamin A, which is a robust antioxidant that protects cells from free radicals, thereby playing a crucial role in mental performance and psychological well-being (Messina *et al*., 2023). All these data support our hypothesis that date palm fruits are rich in different bioactive compounds and can function as potent antioxidants .

The in vitro antioxidant capability of the two date varieties was assessed using DPPH material and IC50 concentration, as illustrated in Figure 1. The results indicated that both date varieties possess scavenging capacity against DPPH, demonstrating their ability to mitigate free radicals and reactive oxygen species, as evidenced by the solution's transition from colored to colorless. Conversely, the IC50 value for Berhi date and vitamin C is 0.16, with a p-value of 0.189, highlighting the superior antioxidant efficacy of the Berhi date fruit extract as a natural

substance compared to Bream date. The IC50 value for the Bream and vitamin C is 0.19, with a p-value of 0.234, showing that Bream exhibits reduced potency compared to Berhi and vitamin C. IC50 denotes the minimal concentration necessary to inhibit DPPH activity; therefore, a material that necessitates a lower concentration for inhibition is regarded as a powerful antioxidant.

Conclusion

Date palm cultivars Berhi and Bream contained a variety of bioactive substances in their crude extracts. They exhibited significant in-vitro antioxidant activity, making them promising candidates for both pharmaceutical and nutraceutical industries as a source of natural components in the future. The resulting data suggests that aqueous extracts may serve as a valuable source of natural antioxidants with beneficial effects on health; furthermore, further separation is necessary to isolate bioactive components for the identification and classification of their pharmacological effect.

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Contributions of authors

D.Z.A: Collection of specimens and laboratory techniques, wrote and revised the manuscript.

A.A.J: Suggestion the proposal of the article, wrote and revised the manuscript, identification of the parasite.

M.S.G: Suggestion the proposal of the article, revised the manuscript.

ORCID

A.A. Jaccob: https://orcid.org/0000-0002-1433-6731

M.S.G.Al-Mozeil: https://orcid.org/0000-0002-6789-7188

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical approval

All ethical guidelines related to the national institutes of health (NIH), we got permission for these experiments from the ethics committee of college of pharmacy at the university of Basrah on 1/9/2023 EC34.

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تقييم المحتوى الكيميائي النباتي لمستخلصين من ثمار نخيل التمر العراقي (*Phoenix dactylifera* L. Berhi and bream) وتقييم قدراتهما المضادة للأكسدة

ضحى زيدون البسيري[1] و أسامة ايوب يعقوب[2] ومحسن صغير غالب المزعل[2]

[1]دائرة صحة البصرة، مستشفى الموانئ التعليمي، البصرة، العراق

[2]فرع الادوية والسموم، كلية الصيدلة، جامعة البصرة، العراق

المستخلص: هدفت هذه الورقة البحثية إلى قياس المكونات النشطة بيولوجيًا في مستخلصات صنفين من نخيل التمر العراقي (البرحي والبريم)، بما في ذلك الفلافونويدات والفينولات والتانين والكاروتين، وتقييم إمكاناتها كمضادات للأكسدة. تم إجراء هذا البحث في مختبر قسم العقاقير بكلية الصيدلة في أكتوبر 2023، بجامعة البصرة، العراق. تم استخدام كل من الأساليب التحليلية الكيميائية النباتية النوعية والكمية في البحث، بما في ذلك تقدير الكاروتين والبوليفينول الكلي الفلافونويدات والتانين والاختبارات الكيميائية النباتية الأولية. وكذلك تقييم قدرتها على العمل كمادة مضادة للأكسدة باستخدام DPPH لتحديد نشاطها وفعاليتها في مستخلصات لكلا الصنفين من نخيل التمر. تم استخدام محلول الفوسفومولبيدات لقياس إجمالي مضادات الأكسدة. النتائج: أظهرت هذه الدراسات أن ثمار صنفي نخيل التمر برحي وبريم تحتوي على نسبة عالية من الفينول، والتي تم تقديمها بالمليجرام لكل جرام من المستخلص، بما في ذلك إجمالي محتوى الفلافونويدات لصنفي برحي وبريم 0.450 مجم، 0.333 مجم، إجمالي محتوى الفينول 2.71 مجم لكلا الصنفين، إجمالي محتوى التانين 0.2 مجم، 0.1 مجم، إجمالي محتوى الكاروتين 38.3 ميكروجرام، 59.3 ميكروجرام، علاوة على ذلك تم تقدير IC50 والقيمة الاحتمالية لصنفين 0.16 مع قيمة احتمالية 0.189 لصنف برحي، 0.19 مع قيمة احتمالية 0.234 لصنف بريم. الاستنتاج: حسنت دراستنا نشاط مضادات الأكسدة لصنفي نخيل التمر مما جعلهما بدائل طبيعية مناسبة للمنتجات الاصطناعية ومرشحين واعدين لصناعة المستحضرات الدوائية والغذائية.

الكلمات المفتاحية: البرحي، البريم، التانين، البيتا كاروتين، DPPH، المستخلصات الخام.