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Investigating the Antioxidant Potency of Ethanol Extracts of Chia Seeds

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ABSTRACT

Chia (*Salvia hispanica* L.) is a plant classified as a member of the Lamiaceae family. From a nutritional perspective, it is a considerable source of carbohydrates, proteins, fats, and dietary fiber with a very high number of antioxidants. The aim of this work is to evaluate the antioxidant activity of the alcoholic extract of chia seeds (*Salvia hispanica* L.) using multiple assays, including linoleic acid peroxidation inhibition, hydrogen peroxide scavenging, reducing power, and ferrous ion chelation, and to compare these activities against the synthetic antioxidant butylated hydroxytoluene (BHT) and the natural antioxidant tocopherol. Chia seeds were collected from local markets. After alcoholic extraction, the chia extract was evaluated using several tests including glycosides test, Keller-Kiliani test, sodium hydroxide test, alkaloids test, phenolic compounds test, tannins test, resins test, and flavonoids test. It also involved the measurement of antioxidant activity using the linoleic acid system, reducing power, scavenging of hydrogen peroxide, and ferrous ion binding activity. The results showed that the alcoholic chia extract demonstrated significantly higher antioxidant activity ($p < 0.05$) than the natural antioxidant tocopherol at 100% concentration. The alcoholic extract also showed the ability to scavenge hydrogen peroxide at 86.7% at 100% concentration, which was statistically superior to BHT (84.70%, $p = 0.031$).

Our study demonstrated satisfying antioxidant activity of the alcoholic chia plant extract compared to the synthetic antioxidant BHT and tocopherol. In addition, the alcoholic extract of the chia plant showed significant ability to scavenge hydrogen peroxide, superior to BHT.

Keywords: Chia (*Salvia hispanica* L.), antioxidants, butylated hydroxytoluene (BHT), tocopherol

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Introduction:

Medicinal plants have shown potential biomedical and pharmaceutical applications [1,2]. Chia (*Salvia hispanica* L.) is a plant that belongs to the Lamiaceae family. About 900 species of the genus *Salvia* are found. Chia height is about one meter, with leaf width of 3 to 5 cm and length of 4 to 8 cm [3]. Leaves are elongated, serrated, and placed in opposition. Bisexual flowers are white or purple, with a flower size of 3 to 4 mm. Seeds are oval, 1 to 2 mm in length and white or black in color (depending on specific species and cultivation area) [3]. White chia seeds contain higher omega-3 fatty acids in comparison with darker ones. Southern Mexico and Guatemala are chia's origin, but it is grown in Egypt and throughout the world [4]. Chia is a promising plant approved by the European Food Safety Authority in 2019 [5]. Chia seeds approval permitted its inclusion in various foods as well as cosmetics [6]. They hold many nutritional and active components [7,8]. Chia seeds are rich in carbohydrate (26–41%), proteins (15–25%), fats (30–33%), dietary fiber (18–30%), balanced vitamins and other minerals, with high antioxidant properties [9]. Polyunsaturated fatty acids (PUFAs) constitute about 68% n-3 (α-linolenic acid mainly) and 19% n-6 fatty acids [9,10].

Chia seed has been promoted as a nutritional supplement and a nutraceutical food for which the health benefits cannot be overstated [11,12]. Chia seeds supply fiber (30–34 g of dietary fiber), which consists of an insoluble fraction (85%–93%) and a soluble fraction (7%–15%) [13]. Additionally, chia seed is rich in polyphenolics such as rosmarinic acid, kaempferol, myricetin, quercetin, and flavonol glycosides alongside caffeic acid and chlorogenic acid [14]. Bioactive compounds have been known to play a significant role in the context of weight loss, inflammation, oxidative stress, and blood pressure [15–17]. Biochemical activity of flavonoids includes anti-inflammatory, antibacterial, hepatoprotective, antioxidant, anti-cancer, and antiviral properties, and they are therefore included as supplements in the management of various disease conditions [18]. Taga and colleagues reported that quercetin, kaempferol, and myricetin are present in methanol hydrolyzed extracts of chia seeds and evaluated their antioxidant activities [19]. In a study by Reyes-Caudillo and colleagues [20], the natural and hydrolyzed isolates of chia seeds were obtained from two locations in Mexico.

Hydroxycinnamic acid binds quinic acid at various positions to form caffeoylquinic acids, the most abundant in the polar isolation of chia seeds being chlorogenic acid. Martínez-Cruz and Paredes-López [11] described the monomeric compounds from chia seeds isolated by ultra-high-performance liquid chromatography in Colima, Mexico. Polyphenolic compounds are reported to be up to ten times more effective as radical scavengers than simple antioxidants, neutralizing ROS-mediated oxidative stress [21]. Recent studies have reported antioxidant properties whereby bioactive compounds comprising various phenolic compounds and n-3 fatty acids are capable of prompting antioxidant function and could be prophylactic for hepatotoxicity [22]. Accordingly, the antioxidant effects of polyphenolic compounds in addition to α-linolenic acid can protect the liver against free radicals produced during oxidative stress induced by chemical toxins [23].

Many studies have demonstrated that chia seeds contain natural antioxidants. Sargi et al. demonstrated that chia seeds can quench 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic

acid) (ABTS) cation radicals [24]. According to that investigation, chia seeds also showed the ability to scavenge synthetic 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and reduce iron ions, with antioxidant activity significantly greater than flaxseed. Segura-Campos and colleagues confirmed the antioxidant activity of chia seeds [25], showing that chia seed extracts could scavenge DPPH radicals and achieve more than 70% neutralization against them.

The aim of this work is to evaluate the antioxidant activity of the alcoholic extract of chia seeds (*Salvia hispanica* L.) using multiple assays, including linoleic acid peroxidation inhibition, hydrogen peroxide scavenging, reducing power, and ferrous ion chelation, and to compare these activities against the synthetic antioxidant butylated hydroxytoluene (BHT) and the natural antioxidant tocopherol.

Materials and Methods

Plant collection and sample preparation

Chia seeds were collected from local markets (Figure 1) and the samples were finely ground in an electric grinder, sieved, then placed in labeled polyethylene bags and stored in the refrigerator at a temperature of 4°C until use.

Figure 1: Chia plant flowers (A) and seeds (B).

Preparation of the alcoholic extract

The method of alcoholic extraction was adopted according to the method of Harborne [26]. In preparing the plant extract, 100 g of chia seed powder was added to 500 ml of ethyl alcohol at a concentration of 98%, mixed well, and left for 24 hours at laboratory temperature (25°C), then filtered through filter paper (Whatman grade 1). The filtrate was concentrated using a rotary vacuum evaporator at a temperature of 40°C. The filtrate was left at room temperature to completely remove the solvent until a highly viscous concentrated substance was obtained. For every 2 g of this substance, 50 ml of distilled water were added, from which concentrations of 20%, 50%, and 100% were prepared and placed in sealed opaque bottles and kept at 4°C until use [7].

Chemical detection of active ingredients

Glycosides Test: Glycosides were detected by mixing 5 ml of Fahlink's reagent with 5 ml of the extract and leaving the mixture in a boiling water bath for 10 minutes. The appearance of a red precipitate is an indicator for glycoside yield [27].

Keller-Kiliani Test: This is a specific chemical test for detecting cardiac glycosides (particularly those containing deoxy sugars like digitoxose). It was performed by adding 1 ml of glacial acetic acid with 1 ml of the extract, allowing to cool in a water bath, and adding two drops of ferric chloride solution. The mixture was then transferred into another test tube containing 2 ml of concentrated sulfuric acid. A positive result is denoted by a brown ring at the interface and blue-green coloration in the upper acetic acid layer [28].

Detection of sodium hydroxide: This test is mainly used to detect glycosides hydrolyzed by sodium hydroxide reagent (e.g., anthraquinone glycosides). The reaction is based on color development from the aglycone moiety of specific glycosides, which are colored in alkaline medium. Anthraquinone glycosides are hydrolyzed by acid or enzyme to produce anthraquinone aglycones. The aglycones are the

active components of flavones and change to colored salts (red, pink, or purple) in alkaline medium (NaOH). The basis of the Borntäger's test [29] involves boiling powdered plant material with dilute HCl to liberate the aglycone. The product is then cooled, filtered, and extracted with organic solvent (chloroform). The chloroform layer is decanted and resuspended in half the original volume of dilute NaOH. A pink, red, or violet alkaline layer indicates the presence of anthraquinone aglycones.

Alkaloids Test: The detection of alkaloids was carried out according to the method of Harborne [26]. It was performed by adding 13.5 g of mercuric chloride ($HgCl_2$) and 50 g of potassium iodide to 100 ml of distilled water. A cream or pale-yellow precipitate indicates a positive yield.

Phenolic Compounds Test: Phenolic compounds were evaluated using ferric chloride 1%; 1 ml of this solution was added to 1 ml of plant extract. A green color indicates the presence of phenolics [30].

Tannins Test: A 50 ml aliquot of distilled water was added to 10 g of alcoholic extract, followed by boiling and filtration. After cooling, one portion of the filtrate was treated with 1% lead acetate; a white gelatinous precipitate indicated the presence of tannins. A second portion was treated with 1% ferrous chloride; the formation of a bluish-green color confirms the existence of tannins [31].

Resins Test: 5 g of extract was dispersed in 50 ml of ethyl alcohol, left for three minutes in a water bath, filtered, and made up to 100 ml volume using acidified distilled water (4% hydrochloric acid). Turbidity of the solution suggests the presence of resins [32].

Flavonoids Test: It was performed using alcoholic potassium hydroxide 5 N (Ethanoic KOH). 1 ml of reagent was added to 1 ml of alcoholic extract; a yellow precipitate indicates the presence of flavonoids [33].

Measurement of antioxidant activity

The antioxidant activity of extracts was estimated by the linoleic acid system [7,34]. The percentage inhibition of linoleic acid peroxidation was calculated as follows: % Antioxidant Effectiveness = 100 − [(Sample absorbance reading / 1% absorbance reading of the control) × 100].

Measurement of reducing power

This method was described by Vijayalakshmi and Ruckmani [35]. It involves adding 2.5 ml of alcoholic extract of chia seeds to ascorbic acid, mixed with 2.5 ml of phosphate buffer solution (200 mM, pH 6.6), followed by potassium ferricyanide (1%) solution. The mixture was incubated at 50°C for 20 minutes. The reaction was terminated by adding trichloroacetic acid (10%), and the mixture was centrifuged at 2000 rpm for 10 minutes. After separation, 5 ml distilled water and 1 ml ferric chloride (0.1%) were added to the supernatant. Absorbance was measured at 700 nm. % Reducing Power = 100 − [(Sample absorbance reading / 1% absorbance reading of the control) × 100].

Scavenging of hydrogen peroxide

The hydrogen peroxide scavenging ability of alcoholic chia seed extract was expressed as the capacity to neutralize hydrogen peroxide [36]. It involves mixing 1 ml of alcoholic chia seed extract (1–5 mg/ml) with 6 ml of a 0.002 M prepared hydrogen peroxide phosphate buffer solution. Results were compared to BHT, alpha-tocopherol, ascorbic acid, and citric acid by monitoring absorbance at 230 nm after stirring the samples for 10 minutes at room temperature. % Scavenging Capacity = [(Absorbance of the control − Sample

absorbance reading) / Absorbance of the control] × 100.

Ferrous ion binding

Determination was carried out by mixing 0.4 ml (200 µg/ml) of alcoholic extract with 0.4 ml (1 mM) ferrous chloride, followed by 0.4 ml (5 mM) 8-hydroxyquinoline prepared with 98% ethanol. The mixture was incubated at room temperature for 10 minutes in the dark. Absorbance measurements were taken at 562 nm. The chelating ability was assessed in comparison with EDTA disodium and citric acid [30]. % Chelating ability of Ferrous ion = (Sample absorbance reading / 1% absorbance reading of the control) × 100.

Statistical analysis

Results were presented using Microsoft Excel and analyzed using GraphPad Prism 9 software (GraphPad Software Inc., San Diego, CA, USA). Data are shown as mean ± standard error of mean (SEM). Between-group comparisons were performed using the t-test, with exact p-values reported for each comparison. A p-value of < 0.05 was considered statistically significant.

Results and Discission

The present study aims to examine the phytochemical basis and antioxidant power of alcoholic extracts from chia seeds (*Salvia hispanica*). Qualitative analysis revealed the presence of phenols, tannins, flavonoids, alkaloids, resins, and glycosides as bioactive compounds (Table 1). These compounds are among those known to possess strong antioxidant properties and therefore contribute to the increased free radical quenching activity observed in the assays.

The results of the qualitative tests showed that the alcoholic extract contained most of the active phytochemical compounds, including phenols, alkaloids, flavonoids, resins, glycosides, and tannins (Table 1). The important content of phenolic compounds, with special emphasis on their powerful antioxidant capability [37–39], is consistent with data reported for other plant-based foods in the literature. Phenolic hydroxyl groups are able to donate hydrogen atoms to free radicals, thus preventing their formation and reducing oxidative damage. The presence of alkaloids and resins suggests a very complete phytochemical profile that may act synergistically to enhance the antioxidant activity.

Table 1: Results of the detection of active compounds in the alcoholic extract of the chia plant

Active Compounds	Detection Results (Chia Seed Extract)
Phenols	+
Tannins	+
Flavonoids	+
Alkaloids	+
Resins	+
Glycosides	+

+ indicates the presence of the active ingredient

Measuring antioxidant activity

The results in Figure 2 show the antioxidant activity using the linoleic acid system for the alcoholic chia plant extract compared to BHT and tocopherol at 100% concentration. The extract showed significantly higher antioxidant activity ($p = 0.019$) than the natural antioxidant tocopherol, whose average antioxidant effectiveness was 91.33% at 100% concentration. It possesses effective compounds that are either hydrogen and electron donors, have the ability to scavenge free radicals, or possess chelating agents capable of binding metals that catalyze oxidation processes. In evaluating antioxidant capacity, the alcoholic chia seed extract exhibited superior activity compared to natural tocopherol and comparable or better activity than synthetic antioxidants like BHT. Specifically, the extract demonstrated significantly higher radical scavenging activity against linoleic acid oxidation at 100% concentration (Figure 2), which is consistent with findings that plant phenolics can effectively inhibit lipid peroxidation [40–42]. The observed efficacy may be attributable to the high phenolic content, which can donate electrons or hydrogen atoms to free radicals, stabilizing them and preventing chain reactions of lipid oxidation.

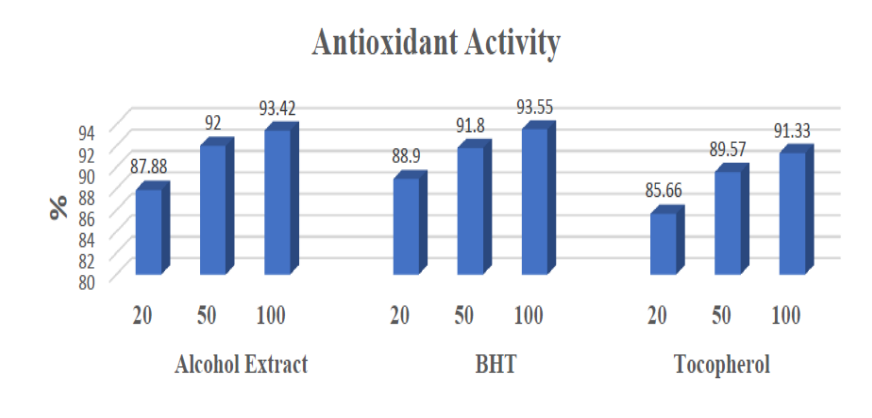

Figure 2: Antioxidant activity of alcoholic chia seed extract compared to BHT and tocopherol using the linoleic acid system.

The ability for scavenging hydrogen peroxide

Our data shown in Figure 3 demonstrate that the alcoholic extract of chia plant is capable of scavenging hydrogen peroxide, significantly more so than the synthetic antioxidant BHT ($p = 0.031$). At the highest tested concentration (100%), the alcoholic chia extract demonstrated a hydrogen peroxide scavenging efficiency of 86.7% which was statistically significantly higher than the synthetic antioxidant BHT, which achieved 84.70% at the same concentration. Hydrogen peroxide is a reactive oxygen species capable of crossing cell membranes and causing oxidative damage through Fenton reactions, generating even more harmful radicals [43]. The results indicate that the extract effectively neutralizes hydrogen peroxide, consistent with the identified protective effects of plant phenolics in biological systems [44].

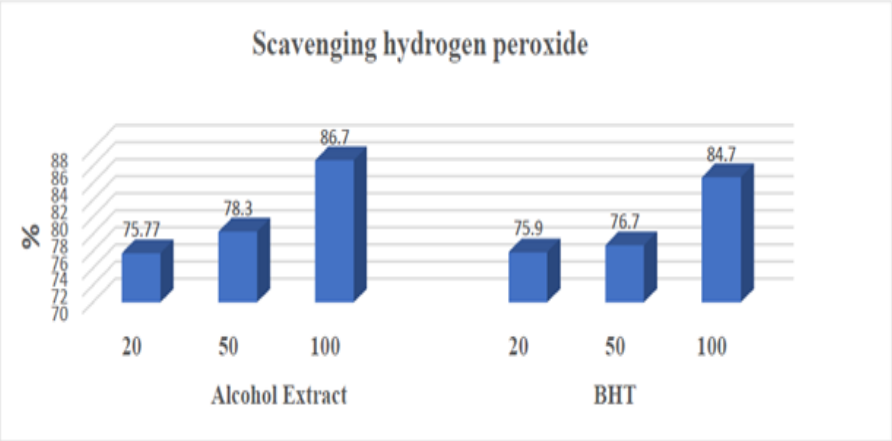

Figure 3: Hydrogen peroxide scavenging capacity of alcoholic chia seed extract compared to BHT.

Reducing Power

Our results in Figure 4 reveal the reducing power of the alcoholic chia extract compared to ascorbic acid (a natural antioxidant) and BHT (a synthetic antioxidant). The reducing power values for the chia extract were 1.20%, 1.69%, and 2.80% at concentrations of 20%, 50%, and 100%, respectively. At 100% concentration, the reducing power of the chia extract (2.80%) was slightly lower than that of ascorbic acid (2.85%), and the difference was not statistically significant ($p = 0.087$). These findings suggest that the alcoholic chia extract possesses comparable reducing power to ascorbic acid, particularly at higher concentrations. This electron-donating capacity is a hallmark of phenolic antioxidants and supports their proposed mechanism of action [45–47].

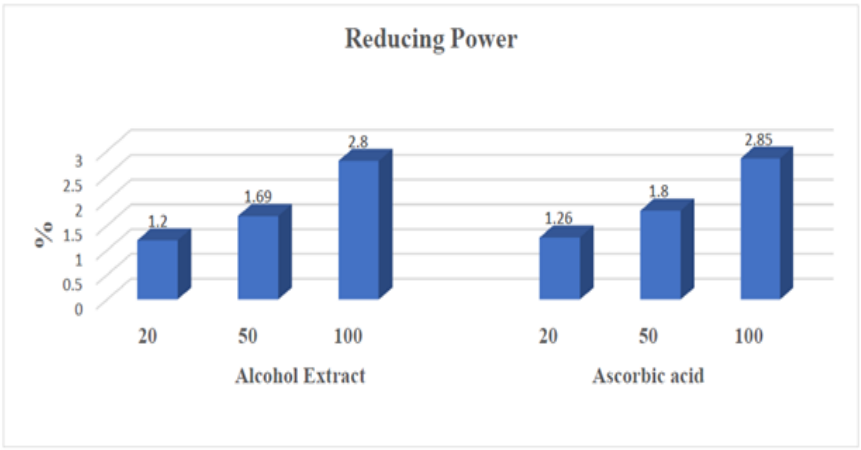

Figure 4: Reducing power of alcoholic chia seed extract compared to ascorbic acid and BHT.

Ferrous ion binding

As shown in Figure 5, the alcoholic chia seed extract demonstrated notable ferrous ion chelating capacity, comparable to the industrial antioxidant EDTA. A statistically significant difference was observed between the chelating capacity of the chia extract (93.98%) and EDTA (93.15%) at 100% concentration ($p = 0.032$). The ability of the alcoholic extract to chelate ferrous ions is most likely attributable to

phenolic compounds such as phenols, tannins, and flavonoids, which possess various organic hydroxyl groups capable of transferring hydrogen for binding these ions. The ability to chelate transition metals like Fe^{2+} is crucial in preventing the catalysis of free radical generation via Fenton reactions [48]. The phenolic compounds, particularly tannins and flavonoids, are known to possess hydroxyl groups capable of binding metal ions, which further enhances their antioxidant efficacy by inhibiting metal-catalyzed oxidation.

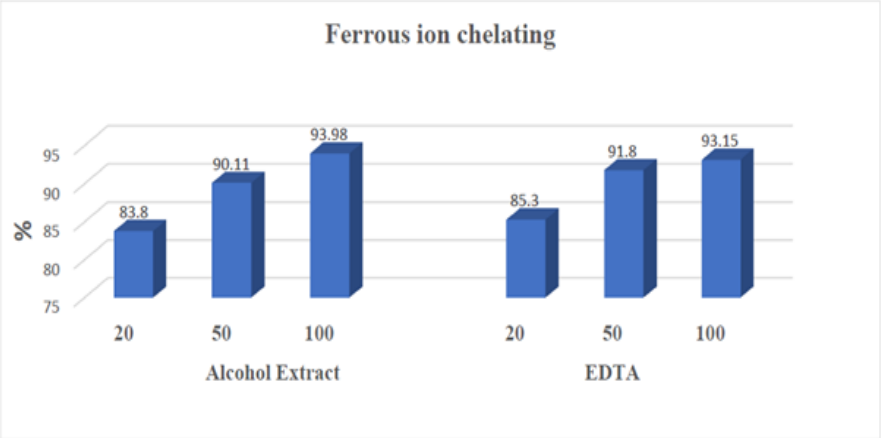

Figure 5: Ferrous ion chelating capacity of alcoholic chia seed extract compared to EDTA and citric acid.

Conclusion

Our study demonstrated satisfying antioxidant activity of the alcoholic chia plant extract compared to the synthetic antioxidant BHT and the natural antioxidant tocopherol. Using the linoleic acid system, the chia extract showed significantly higher antioxidant activity than tocopherol at 100% concentration ($p = 0.019$). In addition, the alcoholic extract showed a significant capacity to scavenge hydrogen peroxide (86.7% at 100% concentration), superior to BHT ($p = 0.031$). The reducing power of the chia extract was comparable to that of ascorbic acid, and its ferrous ion chelating capacity was comparable to EDTA, demonstrating its multifaceted antioxidant potential. These findings support the use of chia seed extracts as promising natural antioxidant agents.

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دراسة الفاعلية المضادة للأكسدة للمستخلصات الإيثانولية لبذور الشيا

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الخلاصة

: تُعدّ نبتة الشيا (Salvia hispanica L.) من النباتات المصنّفة ضمن الفصيلة الشفوية (Lamiaceae). ومن الناحية الغذائية، تُمثّل مصدراً غنياً للكربوهيدرات والبروتينات والدهون والألياف الغذائية، فضلاً عن احتوائها على نسبة مرتفعة جداً من المركبات المضادة للأكسدة. **:** تهدف هذه الدراسة إلى تقييم النشاط المضاد للأكسدة للمستخلص الكحولي لبذور الشيا (Salvia hispanica L.)، باستخدام مقايسات متعددة، تشمل: تثبيط أكسدة حمض اللينوليك، ومكافحة جذور بيروكسيد الهيدروجين، والقدرة الاختزالية ومضاد (BHT) وتثبيط خلب أيونات الحديدوز، مع مقارنة هذه الأنشطة بمضاد الأكسدة الاصطناعي بيوتيل هيدروكسي تولوين (BHT) والأكسدة الطبيعي التوكوفيرول.

جُمعت بذور الشيا من الأسواق المحلية، وبعد إجراء الاستخلاص الكحولي، خُضع المستخلص لسلسلة من الاختبارات النوعية الكيميائية، شملت: اختبار الغليكوزيدات، واختبار كيلر-كيلياني، واختبار هيدروكسيد الصوديوم، واختبار القلويدات، واختبار المركبات الفينولية، واختبار العفص (التانينات)، واختبار الراتنجات، واختبار الفلافونويدات. كما تضمّنت الدراسة قياس النشاط المضاد للأكسدة باستخدام منظومة حمض اللينوليك، والقدرة الاختزالية، ومكافحة بيروكسيد الهيدروجين، ونشاط ارتباط أيونات الحديدوز.

مقارنةً بمضاد ($p < 0.05$) كشفت النتائج أن المستخلص الكحولي لبذور الشيا أظهر نشاطاً مضاداً للأكسدة أعلى بصورة معنوية الأكسدة الطبيعي التوكوفيرول عند التركيز 100%. كما أبدى المستخلص الكحولي قدرةً على مكافحة جذور بيروكسيد الهيدروجين البالغة 84.70 (BHT) بنسبة 86.7% عند التركيز ذاته، وهي نسبة أعلى بدلالة إحصائية من تلك المسجّلة لمركب ($p = 0.031$).

أثبتت الدراسة أن المستخلص الكحولي لنبتة الشيا يمتلك نشاطاً مُرضياً مضاداً للأكسدة مقارنةً بمضاد الأكسدة الاصطناعي BHT والتوكوفيرول. علاوةً على ذلك، أظهر المستخلص الكحولي لنبتة الشيا قدرةً معنويةً ملحوظة على مكافحة جذور بيروكسيد الهيدروجين، تفوق تلك التي يُبديها مركب (BHT).

الكلمات المفتاحية: بذور الشيا (Salvia hispanica L.)، مضادات الأكسدة، بيوتيل هيدروكسي تولوين (BHT)، التوكوفيرول