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Gel Extraction from Caper Fruits (*Capparies spinosa* L.) and Assess its Effectiveness as Antioxidants

Anwar R.T. Al-Birawee* & Amera K. Nasser

Department of Animal Production, College of Agriculture, University of Basrah, Iraq.

*Corresponding author e-mail: noorraadtalib@gmail.com

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Abstract: The results of the current study showed that the caper plant contains active compounds such as phenols and flavonoids. Caper gel exhibited the highest antioxidant activity about 92.31 % when used at concentration 100 %, when was comparable to that shown by the industrial antioxidant Butylated Hydroxy Toluene (BHT), which reached to 93.41%. The results also indicated that the alcohol extract of the caper gel was able to sweep the hydrogen peroxide amounted to 81.05 % compared to that shown by the industrial antioxidant and without significant differences. It also showed a reduction of 2.25 % which was not significantly different from standard ascorbic antioxidant which had the highest average of 2.84 %. This ability increased with an increase in gel concentration with no significant differences.

Keywords: Antioxidants, Caper fruits (*Capparies spinosa*), Gel extraction.

Introduction

Recent studies have pointed to the role of medicinal plants as antioxidants as a substitute for industrial antioxidants, medicines, and chemotherapies (Comai *et al.*, 2007). Recent studies and scientific research have proven the pharmacological effects of many plant compounds in the treatment of many diseases as studies mention the positive role of plants in reducing the incidence of diseases (Kim *et al.*, 2006). Many industrial antioxidants are currently used commercially and largely include Butylated Hydroxy Toluene (BHT) and Butylated Toluene Anisole (BHA). In recent years, there have

been many doubts about the safety of these antibiotics because of their negative effects on consumer health, as it is known that industrial antioxidants are dealt with strictly by the US Food and Drug Administration. This is due to concerns about its toxicity above certain limits (Manhiani *et al.*, 2013). Phenolic compounds are the most prominent natural antioxidants. They have the ability to capture free radicals, bind mineral ions and stimulate antioxidants to break down the chain of oxidation reactions. They thus participate in the first line of defense against free radicals (Chaudhary *et al.*, 2010). There are many

substances involved in manufacturing and pose a high risk such as sodium nitrate and monosodium glutamate (Khaled, 2006).

The caper plant was one of the most important plants with a high content of antioxidants and is perennial spiny shrubs grow naturally and bloom fully during the summer (Pascual *et al.*, 2006). Caper is the most common aromatic plants in Mediterranean kitchens, where they are used as a spice (Tesoriere *et al.*, 2007). Therefore, this study aimed to extract the gel from caper plants and detect its active substances, identify its antioxidant potential and its ability to capture free radicals.

Materials & Methods

Collect and prepare samples

The caper fruits were collected from shrubs in July, selected carefully and cleaned of dirt, dried at 40 ° C by oven, the dry fruits were ground using a laboratory mill, put the fruit powder in polyethylene bags, store at 4 ° C until testing.

Gel powder Preparation:

Fat remove

The fat was removed from the dried ground of caper fruits by the intermittent extraction by soxhlet (Ashour *et al.*, 2005).

Chemically gel extracting

Gel extraction according to the method mentioned by Singer *et al.* (2011) with some modification; Blend caper deodorized powder with distilled water by (1: 40)(w/v). Adjust the pH to 9 by adding a sodium hydroxide solution at a concentration of 0.5 N. Mix for 30 minutes at room temperature. Put the mixture to a centrifuge under the cooling speed 4500 Revolution for 30 minutes. Adjust the pH of the filtrate to 4.8 by adding hydrochloric acid HCl at a concentration of 0.5 N. Place the filter with a centrifuge at a

speed of 4500 cycles for 30 minutes at 5 ° C. To increase the precipitation, two volumes of 95 % (1: 2)(v/v) ethanol were added, the extract was left for 24 hours at 5 ° C. Dissolve the precipitate with distilled water and adjust the pH to 7 by adding a 0.5 N NaOH solution. Dry at 40 ° C for 24 hours. Grind the dried gel with a laboratory mill. Keep the dried gel in airtight containers.

Chemical detection of active substances:

Glycosides test:

Glycosides were detected using a Benedict reagent, the reagent was prepared by dissolving 173 g of sodium citrate with 100 g of anhydrous sodium carbonate with 600 ml distilled water with quiet heating, dissolve 17.3 g copper sulfate in 150 ml distilled water, mix the two solutions with constant stirring, complete the volume to 1 liter, mix two equal parts of Benedict's reagent and plant extract, leave in a water bath for 10 minutes, the appearance of a red precipitate is indicative of the presence of glycosides according to the method (Yasser, 2011).

Alkaloids test

Alkaloids were detected according to the method (Harborne, 1984). As follows:

1. Mayer's Reagent: Added 13.5 g HgCl₂ mercury chloride and 50 g potassium iodide to 100 ml distilled water.
2. Wagner's Reagent: Added 100 ml of distilled water to 1.3 g of iodine and 20 g of potassium iodide.
3. Margus Reagent: Mix 1 ml of formaldehyde with 10 ml of concentrated sulfuric acid.

some drops of each reagent were added to 1 ml of alcoholic extract of gel, a yellow-purple precipitate is used when using the Margus reagent, the emergence of turbidity when using Mayer's reagent, the appearance of a

reddish-brown precipitate when using the Wagner's reagent. Indication of the presence

Phenolic compounds test:

Phenols were detected using ferric chloride 1 %, Added 1 ml of the solution to 1 ml of plant extract, appearance of green color, an indication of the presence of phenols according to the method (Harborne, 1984).

Tannins test

Boil 10 g plant powder with 50 ml distilled water, the solution was filtered, let the filtrate cool down, then divide into two and add to the first 1 % acetate lead and when a precipitate appears, white gelatinous textures indicate the presence of tannins, a solution of 1 % ferrous chloride was added to the second section, the bluish-green color indicates the presence of tannins (Yasser, 2011).

Resins test

5 g of plant powder, added 50 ml of ethyl alcohol, left for two minutes in a water bath, the solution was filtered, 100 ml of 4 % hydrochloric acid distilled water was added to the filtrate, the appearance of turbidity in the solution indicates the presence of resins (Yasser, 2011).

Flavonoids test

Use of potassium hydroxide 5 N Ethanolic KOH, added 1 ml of the reagent to 1 ml of alcohol extract, the yellow precipitate is an indication of the presence of flavonoids (Al-Khazaraji, 1991).

1. Shinoda's test: Add 1 cm³ of the extract a few drops of concentrated hydrochloric acid, added a small piece of magnesium tape or powder, the color is red indicating the presence of flavonoids.

2. Ferric chloride test: Mix 1 cm³ of the extract with a few drops of neutral (7.5 % ferric chloride) solution (pH=7). black or

of alkaloids.

black-red indicating the presence of flavonoids.

3. Zinc-HCl reduction: The appearance of the scarlet color when adding a little zinc powder and a few drops of hydrochloric acid to 1 cm³ of the extract indicates the presence of flavonoids.

4. Lead acetate test: A few drops of the reagent were added to 1 cm³ of the extract. Reddish-Brown color with the presence of a precipitating amount of flavonoids.

5. Sodium hydroxide test: A few drops of sodium hydroxide solution were added to 1 cm³ of the extract. A yellow color disappears when drops of dilute acid are indicated indicating the presence of flavonoids.

Chemical tests

Moisture and ash content were estimated according to A.O.A.C (2000). Protein content was determined by the Semi-micro Kjeldahl method as described in A.O.A.C (2000). Carbohydrates were estimated by difference according to A.O.A.C (2000).

Measuring antioxidant effectiveness

The antioxidant activity was determined using the linoleic acid system according to the method reported by Al-Moussawi and Al-Halfi (2012). 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 measured at a wavelength of 500 nm, BHT and tocopherol were used for comparison, the control sample

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

Measurement of Reducing Power

Followed the method Osawa and Namiki. (1981), included mixing 2.5 ml of caper alcohol extract and ascorbic acid, With 2.5 mM phosphate buffer solution, pH 6.6 and 2.5 mL of Potassium Ferricyanide solution 1 %, incubate the mixture at 50 ° C for 20 minutes, the reaction was then terminated by adding 2.5 ml of Trichloroacetic acid 10 %, the centrifugation of the mixture was carried out at a speed of 2000 r / min for 10 minutes,

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:

separate the top layer of the solution and add 5 ml distilled water and 1 ml of 0.1 % ferric chloride.

The absorbance was measured at a wavelength of 700 nm, the control sample was prepared by adding all the previous materials except the addition of 2.5 ml ethanol instead of the caper alcohol extract, the following formula was applied to calculate the amount of reducing the power of the extract:

$$\text{Reduction power} = 100 - \frac{\text{Sample absorbance reading}}{\text{The absorbance reading of the control sample}} \times 100$$

Scavenging of Hydrogen Peroxide

The alcoholic extract of caper gel was estimated to Scavenging hydrogen peroxide root according to the method described in Turkoglu *et al.* (2010), take 1 ml of alcohol extract for caper gel and 0.6 ml of 0.002 molar hydrogen peroxide, prepared in

phosphate buffer 0.1 molar at pH 7.4, the samples were then left for 10 minutes at laboratory temperature. The absorbance was measured at a wavelength of 230 nm. The results were compared with BHT. The ability to Scavenging hydrogen peroxide root was calculated according to the following formula:

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

Chelating ability of Ferrous ion

The ability of the caper gel alcohol extract to chelate ferrous ion to the method described in Gülçin *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, 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 analyzed statistically using Complete Randomized Design (CRD), using the statistical software SPSS (SPSS, 2012), the results were compared using the Revised Least Significant Difference (RLSD) at the probability level $p < 0.05$ (Al-Rawi and Khalaf Allah, 2000).

Results and Discussions

Chemical composition of caper gel

Table (1): shows the results of the chemical composition of the local caper gel, which is an estimate of moisture, protein, fat, ash and total carbohydrate levels. The moisture mean was 11.5%, which is close to the humidity found by Hosseini-Parvar *et al.* (2010) in the basil seed gel which reached 11.57 % and more than the moisture content in the Iranian basil seed gel which reached 5.79 (Razavi *et al.*, 2009). The difference in humidity is due to different extraction and sedimentation methods. The average ash content in the caper gel was 4.5 %, which is lower than the ash levels found by Fekri *et al.* (2008)

in quince seed gel and flax gel, which were 8.20 and 5.80, respectively. The fat percentage was 0.35 %, which is comparable to the percentage of fat found by Al-Jubouri *et al.*, (2014) in flaxseed gel which was 0.34 and 0.36 for two models (A and B) respectively. Protein content in caper gel was 1.4 %, which is comparable to the protein content in fenugreek seed gel 1.56 % found by Razavi *et al.* (2009). The presence of protein at these low levels in the gel is of great importance in activating the process of water absorption (Garti and Leser, 2001). It also helps in the formation of emulsions and their stability in the food systems and pharmaceutical industries (Dickinson, 2003). The percentage of carbohydrates in the caper gel was 82.75 %, which is higher than the proportion of carbohydrates found by Al-Jubouri *et al.*, (2014) in the flaxseed gel which reached 66.35 %. These findings are in agreement with those of Goycoolea and Caedenas (2003) that gel extracted from plants contain mostly carbohydrates.

Table (1): The chemical composition of the local caper gel

Chemical components	%
Moisture	11.50
Ash	4.50
Fat	0.35
Protein	1.40
Carbohydrates	82.75

Effective compounds in Caper gel

The results of the qualitative data revealed that the alcohol extract of caper gel contains most of the active compounds, which included glycosides, alkaloids, phenols, tannins, resins, and flavonoids, all statements have yielded positive results, inferred from

the reactions shown in Table (2). These findings were agreed with other studies that have shown that the alcoholic extract of caper plants contains biologically active compounds, which were found in different parts of the caper plant (Ma and Zhang, 2017).

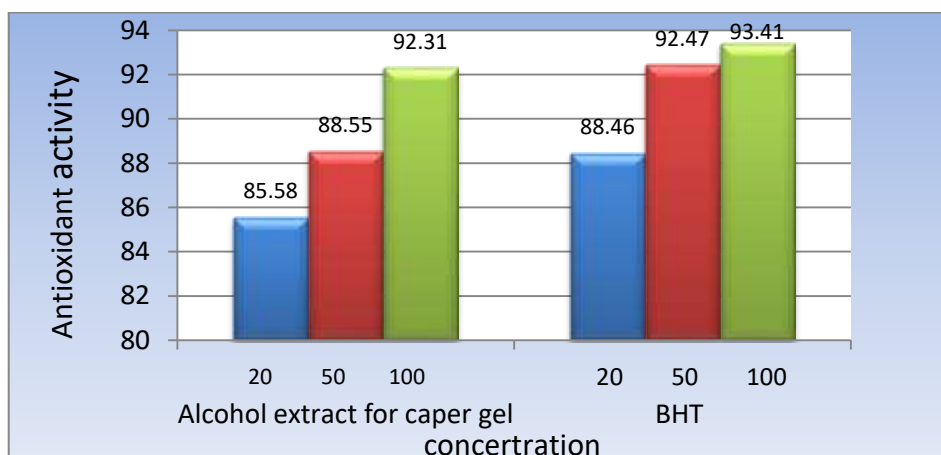
Table (2): Detection results of active compounds in alcohol extract for caper gel

Active compounds	Reagent uses	Regent results
Glycosides	Benedict reagent	+
Alkaloids	Mayer's Reagent	+
	Wagner's Reagent	+
	Margus Reagent	+
phenols	Ferric chloride	+
tannins	Lead Acetate	+
Resins	HCl acid	+
flavonoids	Alkaline potassium hydroxide	+

Measurement of antioxidant activity outside the body

The results of Fig. (1) showed that the alcoholic extract of adult gel possesses antioxidant activity of linoleic acid at different concentrations compared with synthetic antioxidants Butylated Hydroxy Toluene (BHT). It is clear from the figure that the alcoholic extract of the caper gel showed a nearly similar effect to that of the synthetic antioxidant BHT. Caper gel effectiveness was 85.58 %, 88.55 %, 92.31 % and 92.47 % when used as 20 %, 80 % and 100 %. Those of BHT was 88.46, 92.47 and 93.41 % respectively. The results of the study also

showed that the alcoholic extract of caper gel possesses antioxidant activity that was not significantly different from what the industrial antioxidant BHT showed at a concentration of 100 %. This result is encouraging the use of caper gel as a natural antioxidant as an alternative to industrial antioxidants and may be attributed to the high effectiveness of caper gel extract as an antioxidant due to effective compounds (Gunjan *et al.*, 2015). Phenols are the most abundant antioxidants in caper plants, which protect against oxidative damage, have the ability to degraded or capture free radicals or have chelating agents that have the ability to bind the catalytic minerals (Zia-Ul-Haq *et al.*, 2011)



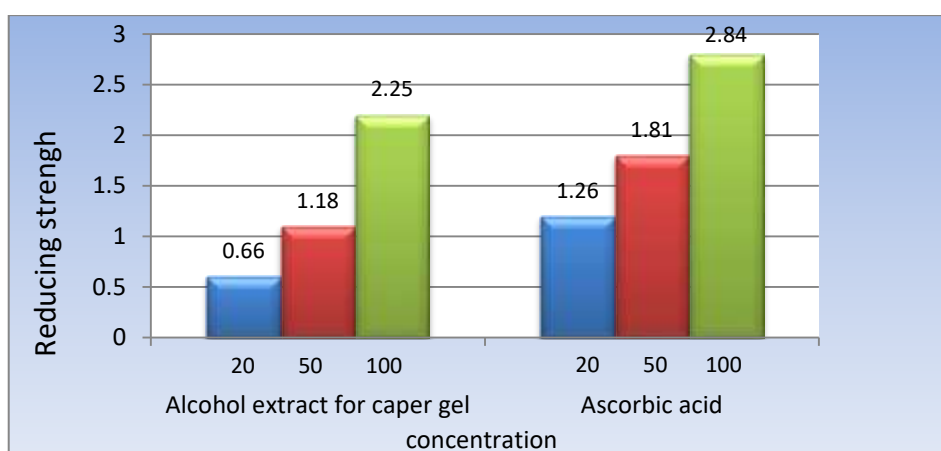
RLSD for concentration = 1.22

Fig (1): Antioxidant activity of alcoholic extract of caper gel.

Reducing strength

The results of Fig. (2) showed the reductive strength of the caper gel alcohol extract compared to the natural antioxidant ascorbic acid, which has the potential to reduce ferric ion Fe^{+3} in a complex $[KFe(CN)]$ to the ferrous ion Fe^{+2} by losing an electron. The results showed that the alcoholic extract of caper gel possesses a significant reductive strength which increased by increasing concentration (0.66 %, 1.18 % and 2.25 % when gel concentrations were 20 %, 50 %, and 100 % respectively).

The highest reductive strength of caper gel was found to be 2.25 % at 100% concentration. This approach is comparable to the effect of the standard ascorbic acid (2.84 %) at the same concentration. The apparent intensity of the color indicates the reductive power of the caper gel alcohol extract to give or release hydrogen ions or increase the hydroxyl root. Thus, the reduction of reductive power, which is a significant indicator reflecting the antioxidant activity, as well as the number and location of hydroxyl has a role in increasing the reduction force and thus increasing the antioxidant activity.



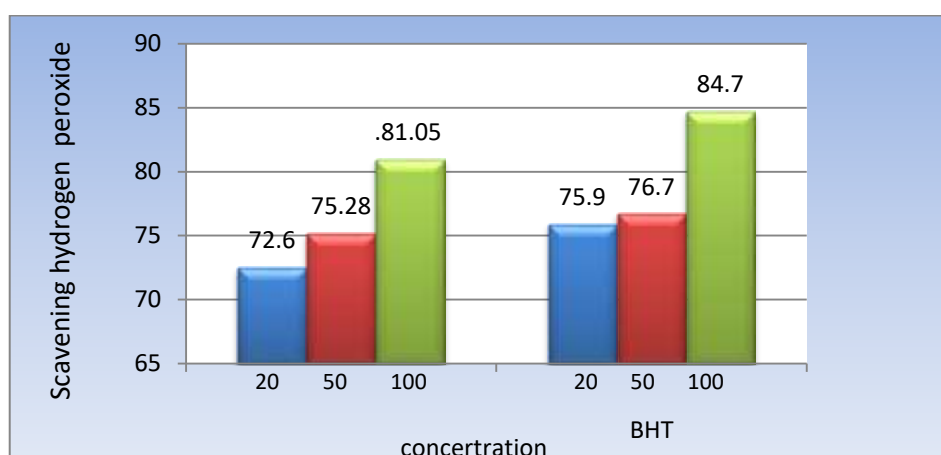
RLSD for concentration = 0.50

Fig. (2): Reducing strength of alcohol extract for caper gel

Scavenging hydrogen peroxide

The caper gel was able to scavenge hydrogen peroxide significantly ($P < 0.05$) compared to the industrial antioxidant BHT, the averages for caper gel extract were 72.6 %, 75.28 % and 81.05 % for concentrations of 20 %, 50 %, and 100 % respectively Fig.(3). However, explained there were no significant differences between the alcoholic extract and the BHT at 50% concentration (75.28 and

76.7 respectively). The ability of the caper gel alcohol extract to scavenging hydrogen peroxide may be due to active compounds. The active compounds in caper protect cellular systems from the toxic hydrogen peroxide compound, penetrate cell membranes, the process of active compounds was the scavenging of hydrogen peroxide (Bhaduri, 2016).



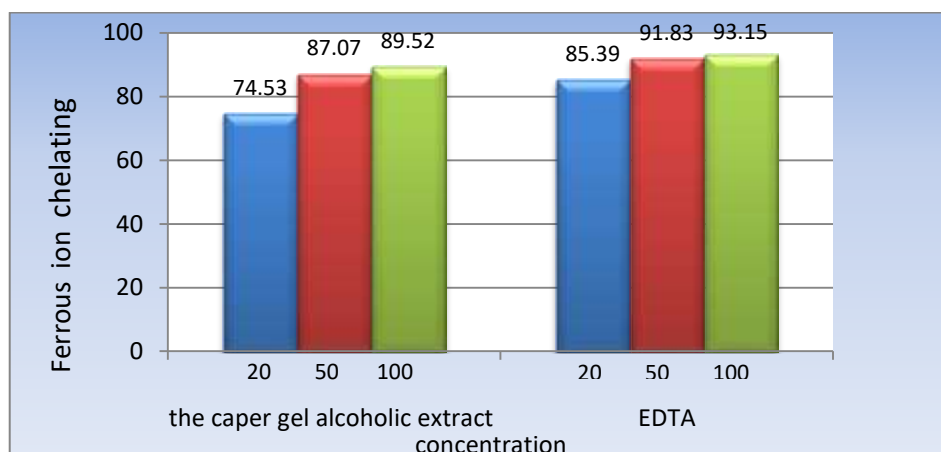
RLSD for concentrations = 2.05

Fig. (3): The ability of the caper alcoholic extract gel to scavenging hydrogen peroxide

Ferrous ion chelating

Fig. (4). showed that the ability of the caper gel alcoholic extract to chelate ferrous ion was highly significant ($P < 0.01$) compared to EDTA (Ethylene diaminetetraacetic acid). Increasing the extract concentration increased chelating ability (74.53 % at 20 % concentration, 87.07 % at 50 % concentration and 89.52 % at 100 concentrations).

The chelating capacity of EDTA was 85.39 %, 91.83 % and 93.15 % at the same concentrations, respectively. The ability of the alcoholic extract to chelate ferrous ion was the effective compounds that have a high ability to bind metals (Nagulendran *et al.* 2009).



RLSD for concentrations = 2.55

Fig. (4): The ability of alcohol extract of caper gel to ferrous ion chelating

Conclusions

The extraction of gel from caper fruits was possible. Caper gel contains active compounds such as phenols, alkaloids, resins, and flavonoids. No significant differences between caper gel and synthetic antioxidants were observed.

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Conflicts of interest

The authors declare that they have no conflict of interests.

Amera K. Nasser:

<https://orcid.org/0000-0001-9505-0120>

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استخلاص الهلام من ثمار الكبار (*Capparies spinosa*) وتقدير فعاليته كمضادات للأكسدة

انوار رعد طالب البيراوي و اميرة كاظم ناصر

قسم الانتاج الحيواني، كلية الزراعة، جامعة البصرة، العراق

المستخلص: أظهرت نتائج الدراسة الحالية احتواء ثمار الكبار على المركبات الفعالة مثل الفينولات والفلافونيدات، واطهر هلام الكبار اعلى فعالية مضادة للاكسدة عند التركيز 100%، اذ بلغت 92.31 % وهو مقارب لما اظهره مضاد الاكسدة الصناعي BHT الذي بلغت فعاليته 93.41 % عند التركيز 100 % . كما أوضحت النتائج ان للمستخلص الكحولي لهلام الكبار قابلية اكتساح بيروكسيد الهيدروجين بلغت 81.05% مقارنة لما ابداه مضاد الأكسدة الصناعي وبدون فروقات معنوية . واطهر كذلك قوة اختزالية عند التركيز 100% بلغت 2.25% والتي لم تختلف معنويًا عن مضاد الأكسدة الاسكوربيك القياسي التي بلغ اعلى متوسط له 2.84% و نفس التركيز، كما أظهرت النتائج قابلية عالية لهلام ثمار الكبار على ربط ايون الحديدوز مقارنة بمضاد الأكسدة EDTA وارتفعت هذه القابلية مع زيادة تركيز الهلام وبدون فروقات معنوية.

الكلمات المفتاحية: مضادات الاكسدة، ثمار الكبار (*Capparies spinosa*)، استخلاص الهلام.