ESTIMATION OF THE ANTIOXIDANT ACTIVITY OF LOCAL *CAPPARIS SPANOSA* LEAVES

S. R. H. Al Badri
Researcher
Dept Plant Production - Ministry Agric.
Agric. – Univ. Baghdad.

N. M. S. Al Janabi
Prof.
Dept Food Science and Biotechnology – Coll.
Samah_r2016@yahoo.com

ABSTRACT

The objective of this experiment was to estimate the antioxidant actitivity of the plant *Capparis spinosa* leaves were collected during (April- Sept,2017) from Baghdad. Water and alcohol extracts were prepared from them and more methods were used to estimate the antioxidant efficacy, the iron binding method, The hydrolysis of hydroxide peroxide and the effect of free radical inhibiting. The water extract of alkaline leaves for April and May samples was characterized by the correlation of iron ion (97.8 and 98.1%) respectively, as well as the alcoholic extract of the April,June,July (89.47, 95.5 and 96.9%) respectively at same concentration of 10 mg / ml, these samples were superior to EDTA-Na and citric acid with 94.73% and 81% respectively at same concentration. The water and alcohol extracts of April and May samples were superior to the water and alcohol extracts of the other samples , and the highest percentage of hydrogen Peroxide inhibitor in the water extracts of the months (April, June, July and September) of 4.3%, 4.2%, 4.3% and 4% respectively, and the alcohol extract for May, June, July and August (4.3, 4.2, 4.3 and 4.2%)respectively at same concentration 400 µg / mL, These percentages are higher than that of industrial oxidants than BHT (3.9%) in the same concentration. The water extract for August and Abstract samples (87%) and 85.74% respectively (1250 µg / ml) on the water and alcohol extracts of the rest of the samples in the manner of effective suppression of free radicals. However, these percentages were lower than the percentage of ascorbic acid of 87.56% for the same concentration.

Keywords: *Capparis spinosa*, antioxidant activity, reduction power, iron ion bonding, hydroxide peroxide inhibitor.

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مجلة العلوم الزراعية العراقية – 64- 70 :49(1)/ 2018 **البدري و الجنابي**

تقدير الفعالية المضادة للأكسدة لأوراق نبات الكبر (*Capparis spinosa*) العراقي

سماح راشد حمادي البدري
باحث
قسم الانتاج النباتي – وزارة الزراعة
Samah_r2016@yahoo.com

نضال محمد صالح الجنابي
استاذ
علوم الاغذية والتقنات الاحيائية – كلية الزراعة – جامعة بغداد

المستخلص

بهدف تقدير الفعالية المضادة للاكسدة لأوراق نبات نبات الكبر *Capparis spinosa* تم جمع الاوراق للأشهر (نيسان وايار وحزيران وتموز واب وايلول) من عام 2017 من منطقة الرضوانية الشرقية في بغداد وتم تحضير المستخلصات المائية والكحولية منها واعتماد اكثر من طريقة في تقدير الفعالية المضادة للاكسدة ،طريقة قابلية ربط ايون الحديدوز والقوة الاختزالية وفعالية الكبح بيروكسيد الهايدروجين وفعالية كبح الجذور الحرة، وقد تميز المستخلص المائي لأوراق نبات الكبر لعينات شهري (نيسان وايار) بقابلية ربط ايون الحديدوز والتي بلغت (97.8 و98.1)% على التوالي وكذلك المستخلص الكحولي لعينات شهر(نيسان وحزيران وتموز) بلغت قوة ربط ايون الحديدوز (89.47، 95.5 و96.9)% على التوالي عند تركيز 10 ملغم/ مل، تفوقت هذه العينات بقابلية الربط على المركب EDTA–Na وحامض الستريك البالغة لهما 94.73% و81% على التوالي عند التركيز نفسه، وتفوق المستخلص المائي والكحولي لعينات شهري (نيسان وايار) على المستخلصات المائية والكحولية لباقي العينات بطريقة القوة الاختزالية، وكانت القوة الاختزالية هذه اعلى لمضادات الاكسدة الصناعية BHT وPG، وكذلك كانت اعلى نسبة مئوية لفعالية الكبح لبيروكسيد الهايدروجين في المستخلص المائي لعينات اشهر (نيسان وحزيران وتموز وايلول) والبالغة (4.3%، 4.2%، 4.3% و4%) على التوالي والمستخلص الكحولي لعينات اشهر (ايار وحزيران وتموز واب) (4.3، 4، 4.3 و4.2)%على التوالي عند تركيز 400 مايكروغرام/ مل، وهذه النسب اعلى من قابلية الكبح لمضاد الاكسدة الصناعي BHT والبالغة 3.9% عند التركيز نفسه، كما تفوق ايضاً المستخلص المائي لعينة شهر اب والمستخلص الكحولي لعينة شهر نيسان بنسب تثبيط بلغت 87.33% و85.74% على التوالي عند تركيز 1250 مايكروغرام/ مل على المستخلصات المائية والكحولية لباقي العينات بطريقة فعالية كبح الجذور الحرة، الا ان هذه النسب قلت عن النسبة المئوية لحامض الاسكوربيك البالغة 87.56% لنفس التركيز.

كلمات مفتاحية: *Capparis spinosa*، الفعالية المضادة للأكسدة، القوة الاختزالية، قابلية ربط ايون الحديدوز، فعالية لكبح بيروكسيد الهايدروجين، فعالية كبح الجذور الحرة

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INTRODUCTION

Capparis spinosa An evergreen wild plants belonging to the *Capparidaceae.* This family includes 46 species, and *Capparis spinosa* is one of the main cultivars of this species(7, 14, 15). It is known in Iraq as Shafallah and kabar in Basra, kifri in the northern regions of Iraq. (13, 9). The plant did not used sufficiently, and all species of generosity It has been widely used in folk medicine by many cultures since antiquity, especially in the Mediterranean countries (Morocco, Spain, Tunisia, Italy and Turkey), and in the West as well as Central Asia (26). The commercial aspect is important therefore its of the candidate plants for agriculture and is considered a vegetable and seasoning in India (20,37). Plant adapts to soil diversity and climatic conditions such as drought, high temperature and salinity (16). It grows in poor soils especially in dry areas, thus playing a role in the environment by reducing erosion (4). *C. spinosa* contains many biologically active chemical compounds including alkaloids, clicosides, tannins, phenolics, flavonoids, sterols, terpenes, carbohydrates, and a wide range of metals and trace elements (3). It has a number of biological agents such as antifungal, Anti-oxidant and anti-toxicity of the liver (23). Various parts of *C. spinosa* including roots, floral shoots, fruits, leaves and seeds have been used in medicines, food and cosmetics (4). Although oxidative reactions are necessary for life, they can be harmful because they result in the formation of free radicals that begin with serial reactions that lead to cell damage (12) . It is possible to reduce the risk of chronic diseases or to prevent the progression of disease either by strengthening the body with natural antioxidant defenses or by using it as a supplement in the diet (31). The most industrial antioxidants used in food products are Butyl Hydroxy Toluene or (BHT) butylated hydroxyl toluene and butylated hydroxyanisole (BHA) and are very effective, but have side effects, similar to their work as promoters of carcinogenesis, so studies have been concerned with natural (non-toxic) natural antioxidants (36). Proved that the ethanolic extract of *C. spinosa* fruit has significant effectiveness in antioxidant protection and immunological efficacy in various laboratory tests (5, 29). Natural antioxidants in the aloe vera extract can penetrate harmful free radicals in vivo and reduce the risk of chronic diseases by enhancing the body's natural antioxidant defenses or supplementing them with dietary antioxidants (31). Large amounts of antioxidant compounds, such as redflavonoids, help prevent oxidation of vitamin C, and new research suggests that *C.spinosa* is used as a natural antioxidant and antimicrobial agent (21). The *C.spinosa* is a 4-hydroxy-5-methylfuran-3-carboxylic acid, 1 (33). Phenolic compounds in leaves and flower buds of *C.spinosa* showed the routine, tocopherol, carotenoids, vitamin C, α and--tocopherol, antioxidant activity (34,35) also demonstrated that the hydrolytic and ethanolic extract of *C.spinosa* leaves had antioxidant efficacy in both chemical and biological tests conducted for them (18)

MATERIALS AND METHODS

Extraction

The water and alcohol extracts of the samples of April, August, June, July, September and were collected during September from the Eastern Radwaniyah area in Baghdad, as reported in (25,39).

Antioxidant effectiveness

1-Ferrous ion Chelating: The ability to bind iron ion for water and alcohol extracts was determined according to the method described by (32). The method included mixing 1 ml of different concentrations of each extract (water and alcohol) ranging from 2-10 mg / ml with 0.2 ml ferric chloride concentration of 2 mM and added 0.2 ml of 8-hydroxy quinoline substitute for ferrozine at a concentration of 5 mM, the mixture was mixed for 10 min left at room. The photoluminescence of the models was read along at 562 nm wavelength , And the ability to bind the iron ion to the EDTA-2Na and ascorbic acid used as reference. inkability was calculated according to the following equation: The ability to link% = 1-(read of absorbance model / read absorbance control sample) × 100.

2-Measurement of Reducing Power

By reducing the concentration of the extract according to the method (6) by mixing 1 ml of the extract (water and alcohol) and with different concentrations ranging from 10-50

mg / ml with 2.5 ml of Potassium Ferricyanide solution, 2.5 mL of solution Phosphate concentration was 0.2 M at pH 6.6, followed by incubation of mixture at 50 ° C for 20 min, 2.5 mL trichloro acetic acid 10% supplemented and then centrifugation at 4000 rpm Mix 2.5 ml of leachate with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride and was left for 30 minutes. Absorption measured at a wavelength of 700 nm, PG (Propyl gallate) and citric acid used as reference(Control). The following formula was applied to calculate the reduction force.

Reduction force = 100 - (Absorption reading of the model / absorption reading of the control sample × 100).

3- Hydrogen Peroxide Scavenging Activity

The hydroxide inhibitor activity determined by (10,27), prepared the water and alcohol extract of the leaves of the grown plant for the six months tested with a concentration of 50-400 µg / mL and added 0.6 ml of hydrogen peroxide and left 10 minutes. The absorbance was then studied along a 230 nm wavelength.) Butylated Hydroxyl Toluine for comparison (Control), and applied the following equation to calculate the effectiveness of braking.

Braking efficiency% = (absorption of the control sample - absorption of the model) × 100.

4- Effectiveness of free radicals

Followed by (17,19)to estimate the antioxidant efficacy of vitreous plant leaves using DPPH (1-diphenyl-2-picrylhydrazyl) and ethanol concen -tration at 0.1 molar concentration, with conc -entrations(50,100,150,200,250,500,1000, And 1,250) µg / mL for the water and alcohol extract, where 1 ml of the leaf extract is taken and 300 µL of DPPH and the annealed mixture are added. The mixture is left in the dark for 30 minutes at room temperature. The absorbance is measured at 517 nm, Industrial oxidation Ascorbic acid for comparison, DPPH and ethanol mixture was considered as a control model, plant extract with alta Well it is the solution Zeroing for each model and applied the following equation to calculate the percentage of inhibition.

Inhibition ratio% =[(Absorption of the control model - absorption of the model) / Absorption of the control model]× 100.

RESULTS AND DISCUSSION

Effectiveness of antioxidant

1 - Cheating Ferrous Ion: The Tables 1,2 showes water extracts of the leaves of al-Kabir plant for months (April, May, July , and August) had a binding capacity of 97.8, 98.1, 84.21 and 98% respectively, As well as the alcoholic extracts of leaves (April, June and July) had high binding capacity compared with citric acid (89.47, 95.5 and 96.9%), respectively, The lowest inhibitory rate for the month of August and September was with an inhibition ratio of 60.52%, at a concentration of 10 mg / ml. The extracts were superior to the citric acid, which was 81% but less than EDTA-Na. EDTA-Na high correlation with iron ion Because it is the prey of ions which inhibits the melting of a high percentage of iron, which delays electron yield and impedes the generation of free radicals (11).

Table 1. Percentage of iron-ion bond ability of water extracts kapar leaves in tested months compared with EDTA-Na and citric acid in different concentrations

Aquatic extracts of the tested months	Concentrate mg / ml				
	2	4	6	8	10
	Bond ability% of water extract				
April	12.63	28.94	68.42	97.36	97.8
May	42.1	52.63	71.05	81.57	98.1
June	15.26	31.57	39.47	42.1	52.63
July	15.26	26.31	31.57	52.63	84.21
August	23.68	31.57	36.86	92.1	98
September	40.1	55.26	57.89	60.52	68.42
citric acid	17.92	32.1	47.36	65.26	81.00
EDTA-2Na	34.21	63.15	78.94	84.21	94.73

Table 2. Percentage of iron-ion bond ability of alcoholic extracts kapar leaves in tested months compared with EDTA-Na and citric acid in different concentrations

Alcohol extracts of the tested months	Concentrate mg / ml				
	2	4	6	8	10
	Bond ability% of alcoholic extract				
April	28.94	39.47	47.36	73.6	89.47
May	21.05	36.89	50	55.26	68.4
June	10.26	15.78	47.36	95.18	95.5
July	23.68	28.94	65.78	84.21	96.9
August	26.31	39.47	47.36	52.63	60.52
September	18.42	21.05	44.74	52.63	60.52
citric acid	17.92	32.1	47.36	65.26	81.00
EDTA-2Na	34.21	63.15	78.94	84.21	94.73

2-Reduced Power

The results shows in Tables 3,4 that the water extracts of the leaves of the al-Kabir plant for April and May showed the highest reduction of BHT and PG, which reached (93.48 and 96.74) respectively at 50 mg / ml, 91.73 and 88.08% for BHT And PG respectively at the same concentration. The water extracts for the leaves of al-Kibar for (June, July, September and September) showed a lower strength compared with the industrial antioxidants BHT and PG, which reached (55.35, 76.57, 18.06 and 46.42%) respectively for the same concentration.

Table 3. reduced power Percentage of water extracts leaves of the grown plant in the tested months compared to BHT and PG at different concentrations.

Aquatic extracts of the tested months	Concentrate mg / ml				
	10	20	30	40	50
	Reducing power % of water extract				
April	10.52	13.86	53	78.2	93.48
May	15.14	27.66	36.86	67.96	96.74
June	16.19	21.34	39.81	44.74	55.35
July	18.5	57.56	68.69	71.32	76.57
August	10.57	13.18	14.8	15.75	18.06
September	12.2	15.57	16.71	21	46.42
BHT	51.12	53.00	74.01	82.7	91.73
PG	30.2	51.3	69.7	80.14	88.08

As for the alcoholic extracts of the leaves of the strong antioxidint for the months (April, May and September), it showed a higher reduction compared with the industrial antioxidants BHT and PG (97.58, 94.95 and 95.6%) respectively at 50 mg / ml concentration. This is reported by research on the parity between the reduced force and the concentration of motor compounds (24). The reduced capacity of the plant leaves may be due to the presence of phenolic compounds. The reduced capacity of phenolic compounds depends on the number of hydroxyl groups in these compounds and their ability to reduce the ferric ion by giving them a hydrogen atom that converts 3 [Fe (CN 6) ferricyanide to 4 [Fe (CN 6)] Ferrocyanide (30). The reason for increased reduction may be due to reductant agent (8).

Table 4. The percentage of the reduction alcoholic extracts of *Capparis spanose* leaves for the tested months compared with the BHT and PG complex with different concentrations

Alcohol extracts of the tested months	Concentrate mg / ml				
	10	20	30	40	50
	Reducing power % of alcoholic extract				
April	30.88	32.56	48.84	69.95	97.58
May	10	33.5	49.89	82.35	94.95
June	43.38	51.89	66.59	80.7	81.93
July	12.62	28.04	51.47	66.17	81.09
August	13.88	24.62	36.4	48.27	67.01
September	14.3	36.51	49.15	51.05	95.6
BHT	51.12	53.00	74.01	82.7	91.73
PG	30.2	51.3	69.7	80.14	88.08

3- Hydrogen Peroxide Scavenging Activity

Table 5 and 6 showes that the aquatic extracts of the leaves of al-Kabir plant for (April, June, July and September) have the highest ability to capture hydrogen peroxide compared with the industrial oxidative oxidation (BHT), which reached 3.9% at the highest concentration of 400 µg / ml. (4.3, 4.2, 4.3, and 4% respectively) at the same concentration**.**

Table 5. Percentage hydrogen peroxide Scavenging Activity for water extracts of Kapar leaves for tested months compared to BHT with different concentrations.

Aquatic extracts of the tested months	Concentrate µg / ml				
	50	100	200	300	400
	Hydrogen Peroxide capture potentid % for water extract				
April	1.7	1.8	2.8	4.1	4.3
May	1.2	1.6	3.3	3.7	3.9
June	1.1	2.2	2.5	3.3	4.2
July	1.6	2.1	2.7	3.6	4.3
August	2	2.6	3.1	3.4	3.9
September	1.7	2.2	2.6	3.5	4
BHT	1.3	2.2	2.5	3.3	3.9

Alcohol extracts showed the highest concentration of hydrogen peroxide compared to the industrial antioxidant BHT for (May, June, July and August), with May and July equating 4.3% and June and August(4 and 4.2%) respectively. With 400 µg / mL concentration. Alcoholic extracts for April and September showed an equal intake of hydrogen peroxide, which is lower than BHT at 3.9% at the same concentration.

Table 6. Percentage hydrogen peroxide Scavenging Activity for alcoholic extracts of Kapar leaves for tested months compared to BHT with different concentrations.

Alcohol extracts of the tested months	Concentrate µg / ml				
	50	100	200	300	400
	Hydrogen Peroxide capture potentid % for alcoholic extract				
April	2	3	3.2	3.6	3.7
May	2.3	2.6	3.7	4	4.3
June	1.1	1.9	3.1	3.9	4
July	1.5	2.1	3.6	3.9	4.3
August	2.1	2.7	3	3.6	4.2
September	1.2	1.5	2.5	2.7	3.7
BHT	1.3	2.2	2.5	3.3	3.9

With increasing susceptibility to hydrogen peroxide with increased concentration. The capture of hydrogen peroxide by the water and alcohol extracts of the alkabir leaves for the six months tested can be attributed to the phenolic compounds, which were estimated in a previous study of the same researchers. The capture of hydrogen peroxide by phenolic compounds is due to its ability to give electrons (38).

4- Free Radical Scavengiun

The resnlts in the Table 7,8 showes the water and alcohol extracts of the leaves of the grown plant for the six months tested, except for August, showed a lower effect on free radicals compared with ascorbic acid, which amounted to 87.56% at a concentration of 1250 µg / ml. The inhibitory effect of the water extract for the month of August compared to the effectiveness of ascorbic acid and extracts 87.33 (April, May, June and July) was 69.45, 54.43, 76.3 and 50.98%, respectively, at the same concentration. Kapar is also a powerful antioxidant because it has the ability to inhibit free radicals and bind the transition metal ions (28).

Table 7. Percentage anti-oxidative efficacy using DPPH for water extracts of Kapar leaves for tested months compared with the ascorbic acid and different concentrations.

Aquatic extracts of the tested months	Concentrations µg / mL							
	50	100	150	200	250	500	1000	1250
	Antioxid. efficacy of DPPH of water extract							
April	62.68	64.4	64.53	64.53	64.9	67.85	68.10	69.45
May	28.57	30.04	31.38	31.4	32.89	37.8	41.25	54.43
June	67.48	68.71	69.95	70.07	71.79	73.6	73.64	76.3
July	47.9	48.15	48.27	48.39	48.51	48.64	49.5	50.98
August	66.1	67.5	77.83	81.89	82.14	82.63	83.25	87.33
September	75.24	76.97	79.55	79.8	80.29	81.65	82.14	85.01
Ascorbic acid	18.45	25.09	49.08	61.82	78.94	82.31	85.43	87.56

Alcohol extracts were the most effective inhibitors of free radicals for (April, June and August) of 85.74, 84.65 and 81.15% respectively, but less than the proportion of ascorbic acid.

Table 8. Percentage of anti-oxidative efficacy using DPPH for the alcoholic extracts of the leaves of the grown plant for the tested months compared to the ascorbic acid and different concentrations.

Alcohol extracts of the tested months	Concentrations µg / mL							
	50	100	150	200	250	500	1000	1250
	Antioxid. efficacy of DPPH of alcoholic extract							
April	82.75	83	83.12	83.49	83.74	84.6	84.72	85.74
May	24.87	25.1	25.24	25.36	25.73	25.86	25.98	27.21
June	78.07	78.81	78.94	79.55	80.29	80.54	80.91	84.65
July	64.65	65.02	65.64	65.76	65.88	66.13	66.25	66.62
August	78.57	79.06	79.18	79.67	80.04	80.54	80.66	81.15
September	75.98	76.23	76.35	76.47	76.6	76.72	76.84	77.7
Ascorbic acid	18.45	25.09	49.08	61.82	78.94	82.31	85.43	87.56

While the alcoholic extracts for (May, July and September) showed a lower antioxidant effect compared to the other tested months (27.21, 66.62 and 77.7%) respectively at the same concentration. These results are consistent with the findings of (2). that the antioxidant efficacy of DPPH in fresh leaves and flower buds of Kapar ranged from 10.4-63.2% and 25.77% respectively, compared to 34.4-87.5% Ascorbic acid. The difference in the content of active and phenolic compounds may be due to the six-month samples tested for the effect of their antioxidant effectiveness. Thus, phenolic compounds may act as a brake on free radicals based on their hydrogen capacity (22). Several studies have shown the association between antioxidant activity from alkazer and phenolic content (1, 4). The protective effect may be

attributed to the large amount of phenolic compounds, tocopherols and carotenoids. The leaves and flower buds of *C.spinosa* contain phenolic compounds such as rutin, tocopherols, carotenoids and vitamin C, which have demonstrated antioxidant efficacy (33,34).

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