ASSESSMENT OF ANTIOXIDANT ACTIVITY OF IRAQI *CAPPARIS SPINOSA* L. FLOWER EXTRACT

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ABSTRACT

This study was aimed to explain the effect of the crude extract and the flavonoid extract of *Capparis spinosa* L flowers as an antioxidant *in vitro* and the effect of the flavonoid extract as an antioxidant in the tissues of kidney cells. Different concentrations(7.5, 15, 30, 60, 150, 300, 600) µg/ml of the two extracts were selected to determine the antioxidant activity *in vitro* by using DPPH 1,1 Dyphenyl-2-picrylhydrazyl radical. The results showed the efficiency of the flavonoid extract on the crude extract, and it was the highest at concentrations of 150, 300, and 600µg/ml, as it gave 100% compared with the crude extract, which gave 40%, 67%, 75% and for the same concentrations. The flavonoid extract was also used at a concentration of 50 mg per kg, to estimate the antioxidant activity on kidney tissues after the creation of free radicals (reactive oxygen species ROS) inside them by mefenamic acid. The results showed that the used extract has an anti-oxidant effect. The flavonoid extract of the caper plant led to a statistically significant decrease in the concentration of malondialdehyde (MDA), in addition to a significant increase the level of glutathione (GSH). This study confirmed a possibility of using the flavonoid extract of the caper plant *C. Spinosa* in medical applications and in the treatment of different types of diseases caused by free radicals

Key wards: caper, ROS ,MDA , GSH,extract ,flavonoid

تقييم النشاط المضاد للأكسدة لمستخلص ازهار نبات الكبر *Capparis spinosa* L. العراقي

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المستخلص

هدفت الدراسة إلى تقدير تأثير المستخلص الخام والمستخلص الفلافونويدي لأزهار نبات الكبر *Capparis spinosa* L كمضاد للأكسدة في المختبر وتأثير المستخلص الفلافونويدي كمضاد للأكسدة في انسجة خلايا الكلية . انتخبت تراكيز مختلفة (600,300,150,60,30,15,7.5) مايكروغرام/ ملليتر من المستخلصين لتحديد الفعالية المضادة للأكسدة في المختبر باستخدام مادة DPPH 1,1 Dyphenyl-2-picrylhydrazyl radical . أظهرت النتائج كفاءة مستخلص الفلافونويدي على المستخلص الخام وكانت أعلى فعالية عند التراكيز 150و300و600 مايكرو جرام/ ملليتر حيث أعطت نسبة 100% مقارنة مع المستخلص الخام والذي اعطى (75، 40،67)% ولنفس التراكيز .كذلك تم استخدام المستخلص الفلافونويدي بتركيز 50 ملليجرام لكل كيلوجرام لتقدير الفعالية المضادة للأكسدة على انسجة الكلية بعد استحداث الجذور الحرة ROS داخلها بواسطة حمض الميفيناميك. بينت النتائج أن المستخلص المستعمل له تأثيرا مضادًا للأكسدة .اذ ادى المستخلص الفلافونويدي لنبات الكبر الى انخفاضًا ذا دلالة إحصائية في تركيز MDA، بالإضافة إلى زيادة معنوية في مستوى GSH .اكدت الدراسة الى امكانية استعمال المستخلص الفلافونويدي لنبات الكبر *C.Spinosa* في التطبيقات الطبية وفي معالجة أنواع مختلفة من الامراض التي تسببها الجذور الحرة

الكلمات المفتاحية: كبر, الجذور الحرة ,Glutathione, Malondialdehyde, مستخلص, فلافونويد.

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INTRODUCTION

Over many generations and in several different cultures and traditions, medicinal and aromatic plants had attracted interest have been and used primarily for therapeutic purposes. They are regarded as herbal medicines with considerable value, particularly after numerous investigations have revealed that their secondary metabolites are in charge of a number of biological functions(10,20,30). *Capparis spinosa* L is a very important medicinal plant in the Capparaceae family. The plant, which is a perennial winter deciduous species, is a shrub with a height of between 30 and 50 cm, large white to pinkish flowers, and ellipsoid or ovoid-shaped caper the berries. Dry heat and strong sunlight are the ideal environments for caper plants (7,24,34,39). It is a native of the Mediterranean region and is found all throughout, from Morocco to Iran, Armenia, and Crimea. A number of countries including Greece, Italy, Spain, and Turkey, have a large production of *C. spinosa* (40). For a very long time, *C. spinosa* L. has been used in traditional phytomedicine to treat a wide range of ailments . In ancient Egypt and Arab cultures, the roots were taken to heal illnesses of the liver and kidney (25). Fruits, roots, and bark are used in Iran as diuretics and tonics against malaria and hemorrhoids .In Pakistan, the plant's leaves are used as an analgesic, an aperient, and a depurative (8). Caper dried fruits are meant to treat hypertension and diabetes problems when consumed orally with a glass of water in Morocco, whereas unopened buds are used topically to treat eye infections and prevent cataracts .Traditional Chinese medicine has employed the stem-leaves, fruits, and roots to treat gout and rheumatoid arthritis(14). Recent research' s revealed that this plant has a variety of biological benefits, including antioxidant, anticancer, antibacterial ,antifungal and antidiabetic actions (9,12,35). These biological activities are related to the presence of numerous bioactive substances from various chemical families, including phenolic acids, flavonoids, alkaloids, fatty acids, vitamins, and glucosinolates. Numerous studies have revealed the bioactive components of various parts of *C. spinosa*, including the leaves (8). Oxidative stress is an imbalance between oxidant production and antioxidant defenses that could harm biological systems, numerous diseases, including atherosclerosis, chronic obstructive pulmonary disease (COPD), Alzheimer's disease, and cancer, have been linked to oxidative stress(17).The body's natural antioxidant defenses can be strengthened or antioxidants can be added to the diet as a supplement to minimize the risk of chronic diseases or stop disease development . Studies have focused on natural (non-toxic) natural antioxidants because the most common industrial antioxidants used in food products are butyl hydroxy toluene (BHT), butylated hydroxyl toluene (BHA), and butylated hydroxyanisole (BHA). These antioxidants are very effective but have side effects similar to how they promote carcinogenesis (22). However, because of this, investigations of natural additions as potential antioxidants are becoming more popular. Numerous research groups have shown interest in the antioxidant capabilities of numerous herbs and spices(6). The natural antioxidants of plant origin (flavonoids, tannins, coumarins, curcuminoids, xxanthons, phenolics, lignans, etc.) which presences in fruits, leaves, seeds, and oils are known to protect from oxidation were therefore the focus of many researchers looking for a natural antioxidant to replace synthetic antioxidant (36).The polyphenols, phenolics, and flavonoids in the species *C. spinosa* have been shown to have potent antioxidant activity and effective free radical scavenging properties(3). Natural antioxidants play an important role in preventing degenerative, chronic diseases including high cholesterol, cardiovascular disease, and aging by suppressing reactive oxygen species and scavenging free radicals(11). This study was aimed to explain the effect of the crude and the flavonoid extract of *Capparis spinosa* L flowers as an antioxidant *in vitro* and *in vivo* in the tissues of kidney cells.

MATERIALS AND METHODS

Capparis spinosa flowers were collected from Rawa, classified in the college of Science for Women, Department of Biology . Extraction and analysis were done as mentioned in (4) . (100 g) of dry extract Flowers' powder was

extracted for 8 hours with 85% methanol using a thimble of soxhlet as the container. The extracts were purified and concentrated using a rotary evaporator that ran at a reduced pressure and temperature of around 40 °C. fractionated by adding ethyl acetate and filtered and dry evaporated for additional analysis.

Antioxidant activity: The flower extract was assessed by quantifying the scavenging ability of the free radical by using 1,1 Dyphenyl-2 picrylhydrazyl (DPPH) and ascorbic acid was used as a positive control. Equal volumes (0.5 ml) of DPPH solution (0.4 µM) and 1 ml of each concentration (7.5, 15, 30, 60, 150, 300, and 600 µg/ml) from extract were mixed and allowed to start for 30 min at room temperature in the dark 518 nm, by a spectrophotometer. Ascorbic acid was used as standard. The scavenging activity was calculated according to the formula:

$$\textbf{\textit{Scavenging activity}}\ \% = \frac{\textbf{\textit{A518 of control}} - \textbf{\textit{A518 of sample}}}{\textbf{\textit{A518 control}}}\ \textbf{\textit{x 100}}$$

Where A518 of control is the absorbance of DPPH prepared in methanol, A518 of sample is the absorbance of DPPH with a sample of extracted crude and flavonoid (19).

Animal and experimental design: From the Iraqi center for drug monitoring in Baghdad, Iraq, fifteen male mice weighing 20–25 g and aged 7-9 weeks were brought in. The mice models were kept in the Division of Biotechnology's animal home under controlled temperature conditions (23 °C± 5) and were screened for any unintentional infections before being injected. According to the guidelines of the U.S. National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, revised in 1996), the animal experimental procedure and care were approved by the animal ethical committee at the Division of Biotechnology, Department of Applied Sciences, University of Technology, Baghdad, Iraq (Approval ID: ASDB 2020-UOT-7). The mice were first randomly separated into three groups of five mice each, and then they were housed in different cages in accordance with predefined guidelines. Phosphate-buffered saline (PBS) in the amount of 250 µL was injected into the first group as a control. The second group of animals underwent intraperitoneal injections of mefenamic acid at a dose of 100 milligrams per kilogram (daily for 10 days). The third group was given an intraperitoneal injection of mefenamic acid at a level of 100 mg\kg over the course of 10 days while also being exposed to *Capparis spinosa* extract at a concentration of 50 mg\kg .Animals were anesthetized using anesthesia (chloroform) to be anesthetized by breathing, Kidney tissues were collected for further investigation.

Measurement of total intracellular reactive oxygen species ROS: The H2DCFDA Probe (Sigma, USA) was dissolved in DMSO at a concentration of 50 mg/ml in accordance with the accepted technique(15). The kidneys were inspected after being removed, sectioned, stained, and dissected. under a 40x fluorescence microscope.

Preparation of tissue cell suspensions and homogenates: For the purpose of determining the biochemical parameters, kidney organs were maintained in ice-cold PBS. Using surgical scissors, the kidneys were removed. The samples were subsequently chilled in PBS. Following the standard procedure for measuring malondialdehyde (MDA) and glutathione (GSH) detection. Each organ's was follow left half was weighed after being dried out. The following step involved homogenizing the samples with 10 mL/g of ice-cold PBS (pH 7.5) in a glass homogenizer that was set on ice. After this, the homogenate was centrifuged at 10,000 g for ten minutes at 4 degrees Celsius with the remaining 25% of the homogenate, and the supernatants were then gathered for MDA and GSH detection assays in accordance with the manufacturing procedure (MDA kits) and (GSH kits) from (linear,Spain).

Statistical analysis: To identify the impact of various factors on study parameters, SAS (2018) was utilized as the statistical analysis application. In this study, a significant comparison of means was made using the least significant difference (LSD) test (Analysis of Variation—ANOVA). (33).

RESULTS AND DISSCUSION

Antioxidant activity *in vitro*: The antioxidant activity was measured and represented as a percentage of inhibition. The antioxidant activity was then compared to the value of ascorbic acid, which served as the standard.

The results in Table (1) show the different results of flavonoid and crude extracts in antioxidant activity from (25-100)% in flavonoid extraction whereas crude extract yields (10-75)% in antioxidant activity. These findings concur with (37), who discovered that fraction extract (ethyl acetate solvent) is better at scavenging free radical activity than crude extract due to the high concentration of phenolic compounds present. Antioxidant activity was also found to be significantly dose-dependent, meaning that as extract concentration increased, the presence of inhibition also increased significantly. Total flavonoids had the highest antioxidant activity, and flavonoid extracts had higher levels of free phenolic and total carotenoids (9,4).Flavonoids are phytonutrients with chelating abilities that function as antioxidants to limit the oxidation of lipids, chelate redox-active metals, and reduce the harm caused by reactive oxygen species (ROS) (37).

Table1. Effect of crud and flavonoid Concentration (µg\ml) on Antioxidant Activity

Concentration (µg /ml)	Mean ± SE Flavonoid microgram	*Capparis spinosa* extract	Ascorbic acid	LSD value
	100.00 ±000	75.70 ±0.06	100.00 ±0.00	6.08 **
600	A a	B a	A a	
	100.00 ±0.00	67.26 ±0.12	100.00 ±0.00	5.02 **
300	A a	B b	A a	
	100.00 ±0.00	40.13 ±0.08	99.70 ±0.30	5.57 **
150	A a	B c	A a	
	99.40 ±0.30	31.83 ±0.03	96.20 ±0.10	5.84 **
60	A a	B d	A b	
	86.50 ±0.10	23.46 ±0.03	83.53 ±0.22	6.17 **
30	A b	B e	A c	
	64.46 ±0.13	15.30 ±0.11	77.43 ±0.24	4.61 **
15	B c	C f	A d	
	25.46 ±0.18	10.53 ±0.22	47.63 ±0.14	4.47 **
7.5	B d	C g	A e	
LSD value	0.447 **	10.61 **	0.545 **	---

Means with different big letters in the same row and small letters in the same column are significantly different. ** (P≤0.01).

Due to their antioxidant characteristics, flavonoids have attracted a great deal of attention for their beneficial effects on health. Numerous studies examine the antioxidant properties of various plants that contain flavonoids like rutin, which safeguards cytosol, and curcumin, which safeguards red blood cell membranes. Iron and free radical scavenging are both bindable by these components (16). The bioactive components rutin, kaempferol, and derivatives of cinnamoylquinic acid may be responsible for this activity of *C. spinosa*, according to (29).

Effect of *C.spinosa* extract in Oxidative stress in kidney: According to the results of immunofluorescent ROS, groups who received mefenamic acid injections compared to the healthy control group had considerably higher ROS levels after kidney tissue had been stained with the H2DCFDA probe. As appears in Figure (3). While the group that received mefenamic acid injections while also being exposed to *Capparis spinosa* extract displayed a significant reduction in ROS generation.

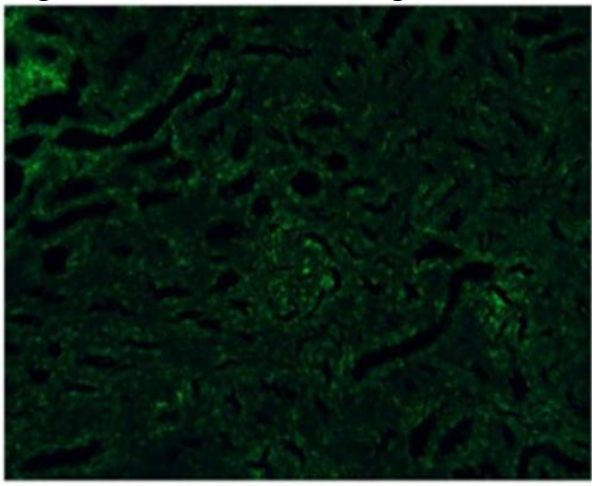

Figure 1. show normal cells of kidney cells of mice that received nutrient solution 250 µL for 10 days (control group).

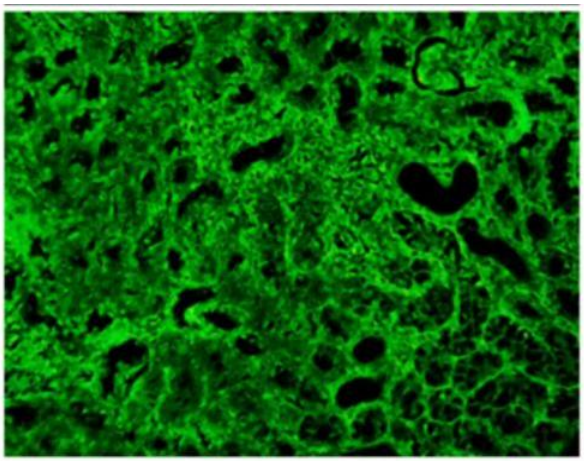

Figure 2. show a significant increase in the level of free radicals (ROS) of kidney cells in the group that received mefenamic acid 100 mg\kg for 10 days

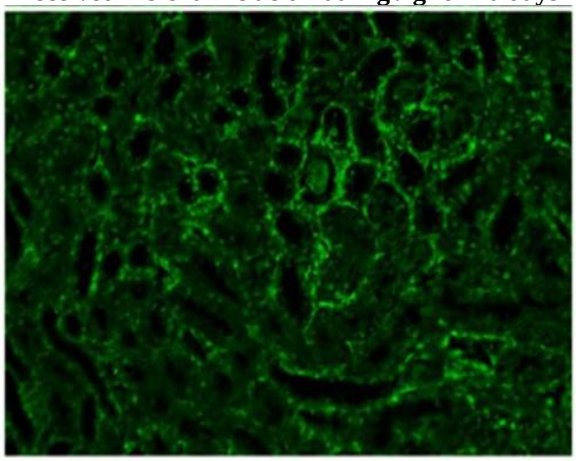

Figure 3. show the decrease in the level of ROS in the kidney cells of the group that received mefenamic acid 100mg\kg in the presence of the flavonoid extract of the caper plant 50mg\ kg for 10days

The plant *C. spinosa* have unique ethnobotanical, pharmacological, and commercial relevance due to its important bioactive compounds (18) On the other hand, mefenamic acid has been utilized more frequently as a potent oxidizing/positive agent to examine the protective effects of various natural products both *in vitro* and *in vivo*(31). The present study's findings showed that mefenamic acid therapy significantly increases ROS creation, MDA production, accompanied with GSH. The different negative effects caused by therapy with mefenamic acid alone were greatly decreased by the co-administration of *C. spinosa* flowers extract. Due to extract treatment, kidney cells showed a significant restoration of the normal level of ROS. An increase in the GSH enzyme's activity. The findings are consistent with several investigations that found *C. spinosa* fruit extracts have nephroprotective properties against monosodium glutamate -induced kidney damage in rats(28). According to a recent study, *C. spinosa* hydroethanolic extracts reduced the nephrotoxicity that cyclophosphamide caused in mice (23). Similarly, Al-Anazi (2) have demonstrated that *C. spinosa* guards against oxidative stress in liver.A significant number of studies have also been published recently on the protective properties of *C. spinosa*, including its antioxidant, hepatoprotective, and nephroprotective activities in a variety of model species both *in vivo* and *in vitro* (1, 13, 23, 27). This is because the existence of numerous secondary metabolites in plants offers a different source to scavenge free radicals (21). The high concentrations of polyphenolic chemicals and flavonoids in the extract of *C. spinosa* may be the cause of the reported protective benefits (5,26). To evaluate the effect of *Capparis spinosa* extract in the MDA, and GSH levels induced by Mefenamic acid , The results of the investigation showed a statistically significant decrease in the MDA concentration, as well as a significant increase in the level of GSH in kidney homogenates as in Figures (7, 34). The obtained results agreement into several studies (32,38) which showed that the *C. spinosa* leave extract decrease the amount of (MDA). A recent study found that *C. spinosa* ethanolic extracts decreased the level of Malondialdehyde(MDA)(27).Kalantari demonstrated that the *Capparis spinosa* extract reduced MAD and raised (GSH) (23).

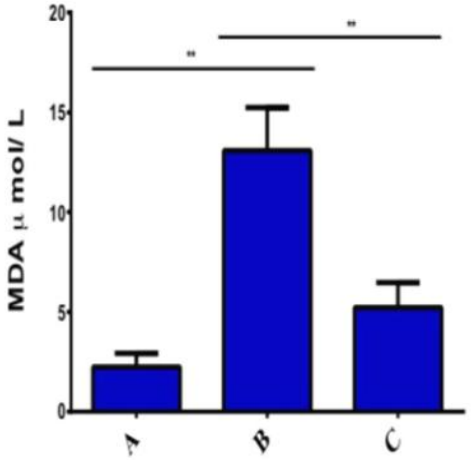

Figure 4. Effect of *Capparis spinosa* extract in the concertation of MDA(mol\L) A, Healthy normal control. B, Mefenamic acid injected group. C, Mefenamic acid in the presence of *Capparis spinosa* extract injected group.

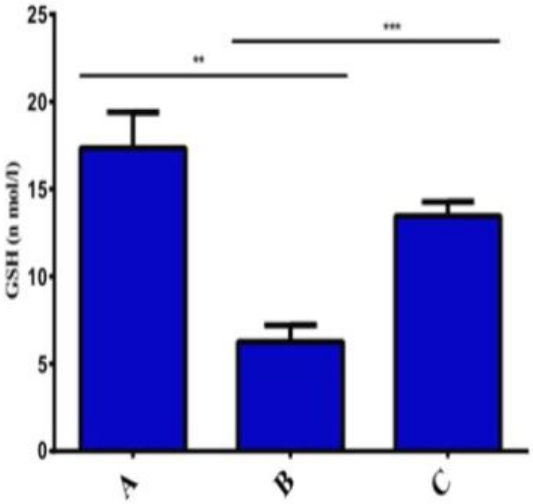

Figure 5. Effect of *Capparis spinosa* extract in the concertation of GSH(mol\L). A, Healthy normal control. B, Mefenamic acid injected group. C, Mefenamic acid in the presence of *Capparis spinosa* extract injected group.

Conclusion

The flavonoid extract of *Capparis spinosa* L. flowers showed superior antioxidant activity compared to the crude extract, achieving complete DPPH radical scavenging at higher concentrations. In vivo, it significantly reduced MDA levels and increased GSH in kidney tissues under oxidative stress. These results suggest that *C. spinosa* flavonoids may serve as a promising natural antioxidant for therapeutic applications**.**

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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