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Al Sultani & Ajeena

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ESTAMATION OF THE ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS OF PURSLANE (*Portulaca oleraceae L.*) PLANT EXTRACTS

Hind Abdul Abbas Faris Al Sultani [1] **,** ***Saba J Ajeena***[2]

[1]Department of Food Science, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq, hind.Abd2102m@coagri.uobaghdad.edu.iq
[2]Department of Food Science, College of Agricultural Engineering Sciences, *University of Baghdad*, Baghdad, Iraq, saba.jaafer@coagri.uobaghdad.edu.iq

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ABSTRACT

The chemical composition of both the leaves and stems of the *Portulaca oleraceae L* was evaluated the protein content of the leaves was slightly higher than the stems in *Portulaca oleraceael,* content of leaves were 4.025%, was slightly higher than stems 3.952%, as for the percentage of total fat, it turned out that the leaves contain a higher fat percentage than the stems and was as follows: The proportion of total fat content in leaves 0.4998% was higher than stems 0.3 68%.

the moisture content in the leaves and stem was 71.6% and 82.7 % In addition, the fibers, ash and carbohydrates in leaves and stems as estimated at 3.0183% and 2.6613%, 6.1 %, and 4.4 %, 14.7 and 5.9 respectively.

The total phenolic compounds and total flavonoids evaluated, it was noted that the alcoholic extract of the leaves had highest content of total phenols and total flavonoids, followed by the alcoholic extract of the stems and then the aqueous extract of the leaves, and the lowest content of total phenols were 98.448, 88.909, 70.689, 25.286 ppm respectively, and the flavonoids were 22.26, 18.22, 16.99, 13.45 mg / 100 respectively.

The Antioxidant activity was evaluated by DPPH method, where the antioxidant activity was measured and several concentrations were taken (50, 100, 150, 200 and 250) mg/ml and the removal rate of free radicals was calculated, the best removal rate was foe the leaves alcoholic extract at 150 mg/ mL with 100% of removal rate, followed by the stems alcoholic extract with 98.32% removal rate as well as leaves equeous exract in removal rate was 96.65% Finally, the stems aqueous extract had removal rate 84.10% in the same concentration. 150 mg/ml, which is the best concentration that gave a 100% removal rate.

Keywords. Phenolic Compounds, *Portulaca Oleraceae L*, Ash, Total flavonoid, plants

تقدير النشاط المضاد للأكسدة والمركبات الفينولية لمستخلصات نبات البقلة *portulaca oleraceae L.*

هند عبد العباس فارس ا لسطلاني[1] ، ***صبا جعفرمحسن***[2]

1قسم علوم الاغذية، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق. hind.Abd2102m@coagri.uobaghdad.edu.iq
2الاستاذ المساعد الدكتور، قسم علوم الاغذية، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق، saba.jaafer@coagri.uobaghdad.edu.iq,

تم تقدير التركيب الكيميائي لكل من أوراق وسيقان نبات البقلة *Portulaca Oleraceae L* حيث كان محتوى البروتين في الأوراق أعلى قليلاً من نسبة ما موجود في سيقان في نبات *Portulaca Oleraceae L.* حيث بلغت نسبته في الأوراق 4.025% وفي السيقان 3.952 %. أما نسبة الدهن الكلي فتبين أن الأوراق تحتوي على نسبة دهون أعلى من السيقان اذ كانت في الأوراق (0.4998%) والسيقان (0.368%).

أما نسبة الرطوبة في الأوراق فقد بلغت 71.6%، وفي السيقان 82.7%، كما قدرت الألياف والرماد والكربوهيدرات في الأوراق والسيقان 3.0183% و2.6613% و6.1% و4.4% و14.7 و5.9 على التوالي.

قدرت المركبات الفينولية الكلية والفلافونويدات الكلية حيث لوحظ أن المستخلص الكحولي للأوراق يحتوي أعلى محتوى من الفينولات الكلية والفلافونويدات الكلية، يليه المستخلص الكحولي للسيقان ثم المستخلص المائي للأوراق، و أقل محتوى كان المستخلص المائي للسيقان وكانت على النحو التالي 98.448و 88.909و 70.689و 25.286 حزء في المليون مجموع الفينولات الكلية وكان مجموع الفلافونويدات الكلية كالاتي 22.26و 18.22، 16.99، 13.45 ملغم/ 100 وعلى التوالي.

قدر النشاط المضاد للأكسدة بطريقة ال DPPH ، حيث قيس النشاط المضاد للأكسدة بأخذ عدة تراكيز 50و 100و 150و 200و 250 ملغم/ مل واحتسبت نسبة إزالة الجذور الحرة، حيث كانت أفضل نسبة إزالة للمستخلص الكحولي للأوراق بتركيز 150 ملغم/ مل وبنسبة إزالة 100%، يليه المستخلص الكحولي للسيقان وكانت نسبة الإزالة 98.32%، والمستخلص المائي للأوراق وبنسبة إزالة 98.32%. 96.65 وأخيراً وصل المستخلص المائي للسيقان إلى نسبة ازالة 84.10% وبنفس التركيز.

الكلمات المفتاحية: المركبات الفينولية، البقلة ، الرماد، الفلافونويد الكلي، النباتات.

INTRODUCTION

Plants were used as a remedy in the Sumerian and Akkadian civilizations around the third millennium BC. In the pas, it has been used in natural healing systems by humans. It has also become an urgent need to conduct the scientific and clinical research and studies to reveal the effectiveness of plant-based drugs. It is responsible for the medical properties of those plants dueo It contains various chemical compounds such as flavonoids, tannins and steroids. **(Ibrahim ,2023)** stated that it is preferable to use natural antioxidants instead of using artificial antioxidants. The reason is attributed to the fact that phenols are effective antioxidants because they work to break the chain of oxidation reactions. It refers to the H atom of the phenolic hydroxyl group to the peroxyl radical ROO is responsible for Propagation of the oxidative radical chain.

Researchers in the field of food technology seek to benefit from the treasures that plants contain Effective ingredients that have different biological effects, including antimicrobial activity, antioxidant activity, and others**(Nidhal *et al*., 2015).**

All food antioxidants have a common goal, that prolong the food preservation for along possible period without taste, color or smell changing. Therefore the consumers were motivated to look for some natural antioxidants which are utilized in food preservation, although the industrial additives have negative effects such us they turn into carcinogenic compounds, but natural antioxidants had most attention **(Carocho *et al*.,2017) , (Shamurad *et al*., 2019)** sesame wrapper extract from roasted and unroasted sesame seeds could be be used as a natural antioxidant to protect oil-rich food through avoid the potential risks caused by the use of artificial antioxidants. **(Hamad *et al*., 2021)** stated that the high content of linoleic acid contributes to the antioxidant activity of L. angustifolia (lavandula) oil and can be considered a source of highly valuable natural antioxidants.

(**Iman *et al*., 2019**) indicated that the volatile oils found within the internal components of rosemary are excellent natural antioxidants and are more effective than the synthetic antioxidants BHT and BHA. the Antioxidants are compounds that have the ability to interact with free radicals and end the reaction continuation before biomolecules were damaged several mechanisms, including the free radical inhibiting, then the oxidation process begins that makes it enables to generate reactive species and peroxides decomposition or may prevent the peroxides

formationin addition, , it might break the chain reaction of endogenous oxidation and reduce local oxygen concentrations.

(Sarah *et al.*, 2023) indicated that the variation in phenolic content in plants can be explained by differences in extraction conditions and polarity of phenolic compounds. Flavonoids are also found in green plants in very abundant quantities,As in fruits and seeds, it is responsible for the color, aroma and flavor characteristics of plants The high amount of phenols in aqueous extraction is due to it being high Polar compounds**.** The interest in phenolic compounds incrsuch us phenols, salsalic acid , Calic , thymol and ect had to used by researchers dueeased their and they have been introduced into the pharmaceutical industry, the food industry and the pesticide industry, due to their ability to change enzymatic and chemical reactions and their antioxidant effectiveness, One study revealed that the content of total phenols and flavonoids Its quantity varies according to the type of solvent used in the extraction method **(AL-Janabi ,2015).**

The recesnt studies were indicated that the leaves of the bulbous plant had multiple health benefits due to they contained the natural antioxidants which had the role in free radicals inhibition produced bration or by the food oxidation such as breathing and energy generation or by environmental contaminations such as smoke, polluted air and midicines **(Wang *et al.*, 2007)** due to their role in curbing free radicals produced by the oxidation of foodstuffs such as breathing and energy generation or by environmental pollutants such as smoke, polluted air, medicines.

The leaves of the *portulaca oleraceae L* plant, which is widespread in Iraq, as the compounds isolated from *Portulaca oleracea L*. such as flavonoids, alkaloids, sugars, fatty acids, terpenoids, sterols, proteins, vitamins and minerals. Possesses *Portulaca oleracea L*. Widely used pharmacological properties such as neuroprotective **(Wang *et al.*, 2017)**, antidiabetic **(Firouzeh *et al.*,2016)**, antioxidant**,)** and anti-cancer activities **(Cui *et al.*, 2017)**. As well as polyunsaturated fatty acids such as linolenic acid linolenic acid (Omega-3) **(Zhao *et al.*, 2017)** essential for growth and for protection against diseases.

Portulaca oleracea L. was also used to treat dysentery intestinal worms by the ancient Romans, head pain and stomach dysentery **(Yan *et al.*, 2009)** and there are many studies on *Portulaca oleracea L*. because of its biologically active ingredients, including alkaloids, coumarins, flavonoids, cardiac glucosides.

Recent studies have shown the role of medicinal plants as antioxidants instead of antioxidants Medicinal plants and medicinal herbs have a great place in pharmacology and medicine because they are a safe source of herbs.

That anise seeds have shown the ability against free radicals used, it can be considered a source antimicrobial and anti-biofilm drugs **(Al- wendawi *et al.*; 2021)**. One study showed a clear significant superiority of the aqueous extract of some fruits in terms of their high content of phenolic compounds. **(Ibtehaj & Malik 2021)**.

The aim of this study is:

- Identification of the chemical composition of the leaves and stems of the purslane plant in Iraq (protein, carbohydrates, moisture, fat, fiber and ash) .
- Evaluation effective compounds (total phenolic content and total flavonoids present in the plant).

- Evaluation of the antioxidant effectiveness by the DPPH method of aqueous and alcoholic extracts of the stems and leaves of the purslane plant.

MATERIALS AND METHODS

Plant collection and preparation of extracts

The plant was diagnosed by the Ministry of Agriculture/ Seed Testing and Certification Department as *Portulaca oleraceae L*., belonging to the Portulacaceae.

The *portulaca oleraceae L.* plant was taken and dried, then both the leaves and stems were grinded after separating and cleaning, where four extracts (water and alcohol extracts) were prepared from each of the stems and leaves, and that was based on what I mentioned **(Al-Halfi *et al*., 2011)** and the final dry extract of these extracts was taken and a study was conducted on them.

Evaluation of the chemical composition of the p*ortulaca oleraceae l.*

Evaluation of proteins

The protein was evaluated according to the kjeldahl method to evaluate the percentage of nitrogen in the sample **(A.O.A.C. 2000)** (the kjeldahl device was used in the Evaluation using Approximately 2 g of each of the stalks and leaves of the *portulaca oleraceae L,* powder was weighed The ratio for protein was calculated from the following law

$$\text{Protein ratio} = \frac{\text{V. Acid (ml)} \times \text{Standard} \times \text{constant nitrogen} \times \text{constant protein}}{\text{Weight of the origina (g)}} \times 100$$

Protein constant =6.25
Nitrogen constant =0.014

Fat percentage evaluation

Evaluation of fats using the soxhlet device, fat is Evaluated according to the method **(A.O.A.C. 1984),** by the method of intermittent extraction in the soxhlet device weighing 10 g of a dry matter sample using an organic solvent in the extraction process to obtain fat and evaporation of the solvent and calculate its percentage using the following equation :

$$\text{Fat percentage \%} = \frac{\text{W before extraction \_w after extraction}}{\text{sample wt(gm)}} \times 100$$

Fiber evaluation

followed the standard method given in **(A.O.A.C. 2000)**.
Accounts
The percentage of raw fiber can be calculated.

$$\frac{\text{Weight loss (the difference between the two weight) - the result of a photo examination}}{\text{Sample wt(gm)}} \times 100$$

Ash percentage evaluation:

The standard method was followed **(A.O.A.C. 2000)**, where the ash was Evaluated based on the dry weight directly, by taking 5 grams of dry matter, carefully weighed, placed in a porcelain lid and ashed at a temperature of 055 degrees Celsius for 5 hours, and after completion of incineration and obtaining a light gray ash, it is left in the glass dryer to calculate the weight stability and calculated according to the following equation *:*

$$\textit{Ashes \%} = \frac{\text{Sample weight after incineration}}{\textit{Original sample weight (gm)}} \times 100$$

Moisture rating:

I followed the standard method mentioned in **(A.O.A.C. 2000)** Take a weight of the ground sample for each of the plant's stems and leaves, place it in clean, dry weighed dishes, weigh it carefully, and place it in an oven at a temperature of 105 degrees Celsius for three hours, after which it is covered and left to cool (withdrawing moisture from the glass for an hour until the weight stabilizes, then
It is weighed with a sensitive scale. The moisture content is calculated as follows:

$$\textbf{Moisture \%} = \frac{\text{Weight of the sample before drying}}{\text{Weigh the sample}} - \frac{\text{Weight of the sample after drying}}{\text{before drying in grams}} \times 100$$

Evaluation of total carbohydrate percentage

The percentage of carbohydrates in the samples under study was Evaluated according to the previously mentioned method **(Eshun *et al.*; 2013).** By the difference between the total components represented by the percentage of moisture, ash, fat and protein subtracted from 100 and as follows: Total carbohydrates % 100 - moisture + ash + protein + fat

Determination of total phenolic compounds

The amount of total phenolic compounds in alcoholic and aqueous extracts of *Portulaca oleraceae L.* was determined using the standard Folin-Ciocalteu reagent **(Laouini &Ouahrani 2017).**

Use the mixture containing 100 µL of extract, 500 µL of Folin-Ciocalteu reagent (Merck, Germany) and 1.5 mL of 20% sodium carbonate. The sample was then mixed on an ester and diluted with distilled water to a final volume of 10 mL. after two hours, reaction,

the absorbance at 765 nm was determined and used to estimate the phenolic content using the calibration curve made with gallic acid (Sigma– Aldrich, Germany). The total amount of phenolic compounds was expressed in mg gallic acid equivalent (GAE) per g dry weight.

Determination of Total flavonoid content

The total flavonoid content of the crude extract was determined by the aluminum chloride colorimetric method **(Habibalni *et al.*; 2017)**. 50 µl each of the crude extract (for the four extracts) (1 mg/mL ethanol) was made into 1 mL of methanol, mixed with 4 mL of distilled water and then 0.3 mL of a 5% NaNO2 solution; 0.3 ml of 10% AlCl3 was added and left for 5 minutes, after that 2 ml of 1 mol/L NaOH solution was added The final volume of the mixture was supplemented to 10 mL with double distilled water. The mixture was left for 15 minutes, and the absorbance was measured at 510 nm. The total flavonoid content was calculated from the calibration curve, and the result was expressed as mg rutin equivalent per gram dry weight.

Measurement of antioxidant activity by the DPPH method

The DPPH: It is a simple and rapid method for testing antioxidants by spectrophotometric measurement, the DPPH radical is a chemical substance that represents an artificial free radical, stable at room temperature, used as a measure of the antioxidant activity of the sample, as its solution appears in a purple or purple color when it is dissolved in methanol or ethanol And the more the sample has high antioxidant activity, it leads to a decrease in the intensity of the violet color, and thus it is more transparent or yellowish due to stopping the inhibition chain of free radical interactions.

The examination was carried out in the University of Technology laboratories / the laboratory of tissue culture and immunochemistry.

The method mentioned in **(Brand *et al.*; 1995)** was followed in conducting an antioxidant test using the method (2,2-diphenyl -1-picryl-hydrazyldrate DPPH)

By adding 0.024 grams of DPPH to 50 milliliters of absolute ethyl alcohol, and it is well dissolved on the magnetic mixer without heat, then the volume is completed to 100 milliliters of absolute ethyl alcohol to give a final concentration equal to 0.024 mg / milliliter, then we take half a milliliter of series concentrations of the extract Hot water and ethanol (50, 100, 150, 200, 250) mg/ml was added to the mixture of DPPH (0.5) mm and 3 ml of absolute ethanol.

The amount of color change was measured using a spectrophotometer at a length of (514) nanometers during 100 minutes of reaction at room temperature. As for the plank tube, it contained 3.3 ml of absolute ethanol and (0.5) ml of DPPH. The removal percentage was calculated according to the following equation shown below:

$$Removal\ rat\% = 100 - \frac{Sample\ absorbency}{Control\ absorbency} \times 100\%$$

RESULTS AND DISCUSSION

Chemical composition of leaves and stems of the *Portulaca oleraceae L.* Table (1) showed the chemical content of the leaves and stems of the plant, where the results showed content of protein in the leaves and the stems,of the plant as the protein percentage reached (3.952, 4.025). Where it was mentioned **(Ashraq *et al.*; 2021)** that the protein quality index that gives a visualization of the true protein efficiency ratio, the low value of protein.

The fat percentage of leaves was 0.4998 and stems 0.368, moisture of leaves 71.6 and stems 82.7, carbohydrates of leaves 14.7569 and stems a ratio of 5.9187, and ash for leaves 6.1 and stems 4.4, while the percentage of fibers in leaves was 3.0183 and in stems 2.6613. The quality of the *Portulaca oleraceae L.* depends largely on the chemical composition it contains, such as carbohydrates and proteins, the percentage of fat and ash, and the moisture to give flavor and taste. This is consistent with (Badawi and On, 2018) *Portulaca oleraceae L.* is a rich source of important nutrients such as fibre, ash, protein and agricultural carbohydrates such as calcium, copper, potassium, zinc, phosphorus, manganese, iron and sodium. It also contains vitamins such as A, C, E and B complex.**).**

Table (1): Chemical composition of stems and leaves purslane plant.

Chemical composition	Stems (%)	Leaves (%)
Protein	3.952	4.025
Fat	0.368	0.4998
Moisture	82.7	71.6
Carbohydrates	5.9187	14.7569
Ash	4.4	6.1
Fibers	2.6613	3.0183

Total phenolic content

Table (2) The total content of phenols at ppm concentration was revealed in the extracts of the stems and leaves (aqueous and alcoholic) of the portulaca oleraceae L. plant, as they showed the highest percentage in the alcoholic extract of the leaves of the portulaca oleraceae L. plant. of total phenolics compared to the rest of the extracts, which amounted to 98.448 parts per million, followed by the alcoholic extract of the stems of the portulaca oleraceae L. The

aqueous leaves extract of *portulaca oleraceae L*. Then Aqueous extract of the stems (88.909, 70. 689, 25.286) ppm, respectively, the high content of phenols in the plant gives it medical importance. This is indicated **(Jasim *et al* .; 2018)** that plants that contain effective compounds such as phenols and flavonoids have a number of vital activities, It was found **(Safaa & saba, 2023)** that aqueous and alcoholic extracts of kiwi fruits contain phenolic and flavonoid compounds that increase their activity as antioxidants and lead to a decrease in triglyceride levels, total cholesterol levels, high-density lipoprotein levels, and very low-density lipoprotein levels.

Table (2): Total phenolic content of *Portulaca oleraceaeL*

Extract	Total phenol (ppm)
Leaves alcohol	98.448
Leaves equeous	70.689
Stem alcohol	88.909
Stem equeous	25.286

Total flavonoid content

The results total flavonoids were as follows, from highest to lowest (alcoholic extract of the leaves, the aqueous extract of the leaves and the alcoholic extract of the stems, the aqueous extract of the stems) concentrations were as follows (22.26, 18.22,16.99, 13.45) mg/ 100 gm respectively. This gives the plant importance of antioxidant activity as it shown in Table (3). where it was mentioned **(AL-Janabi *et al*.; 2013)** that most of the phenolic or polyphenolic compounds in the nature have antioxidant activities, such as tocopherols, flavonoids and other organic acids.

Table (3): Total flavonoid content of *Portulaca oleraceae L.*

Extract	Total flavonoid (mg /100 gm)
Leaves alcohol	22.26
Leaves aqueous	18.22
stem alcohol	16.99
stem aqueous	13.45

Antioxidant activity

Table (4),for the antioxidant activity, the alcoholic extract of the leaves showed the best removal ratio at a concentration of 150 mg / ml, followed by the aqueous extract of the leaves, the alcoholic extract of the stems, and the aqueous extract of the stems at a concentration of 200 mg /ml , and alcoholic extract of the leaves of plant at concentration of 150 mg /ml extract gave a 100% removal rate, while the rest of the extracts gave a 100% removal rate at a concentration of 200 mg / ml. This ruselt corresponds to **(Ajeena *et al* 2019)** that the ethanoic extract obtained the highest percentage of reducing power than the aqueous extract of sage leaves, as well as the percentage of chelating power. Explain **(Sawsan & Sara, 2022)** that phenols appear superior as antioxidants at all concentrations studied on the comparison sample

of vitamin C in olive leaves. As the chemical composition of phenols (the number of hydrogen-donating hydroxyl groups that have a significant impact on the activity of removing free radicals), phenols are considered a strong source of antioxidants. Oxidative stress.

He also confirmed **(Al-Khafaji *et al*.; 2022)** the effect of factors and the accumulation of elements as natural antioxidants for the carrot plant. To scavenge the root of DPPH in the roots.

As mentioned (**Hind&Luma, 2023**) that natural antioxidants can enhance human health in terms of nutrition and shelf life. As mentioned **(AL-Janabi *et al*.; 2013)**, phenolic compounds have antioxidant activity due to their redox properties, which can play an important role in absorbing and neutralizing free radicals. The antioxidant activity of the group of phenolic compounds in SGR grape skin extracts was similar to that of synthetic antioxidants (BHT). Therefore, SGR is recommended as a natural antioxidant for use in diets.

Table (4): Antioxidant activity of purslane plant extracts using the DPPH method.

BY DPPH mg/ ml				
Stem equeous	**Stem alcohol**	**Leaves equeous**	**Leaves alcohol**	**Con. mg/ ml**
61.92	70.50	70.29	72.59	50
73.84	76.56	75.31	85.98	100
84.10	98.32	96.65	100	150
100	100	100	100	200
100	100	100	100	250

CONCLUSIONS

In this study, it was concluded that the alcoholic extract of the leaves has a high content of total phenolic compounds and total flavonoids.

It was also shown that the alcoholic extract of the leaves has antioxidant activity at a concentration of 150 mg/ml, with a removal rate of 100%, which was higher than o the alcoholic and aqueous extracts of the stems and the aqueous extract of the leaves.

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