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Phytochemical analysis of Portulaca oleracea leaves extract and study the role in protecting genomic human DNA from UV damage

Zainab T. Al-Dallee[1], Kawther T. Khalaf[*2], Yassar A. Dawood, Usama H. Ramadhan

[1] Department of Pharmacognosay, College of Pharmacy, University of Basrah, Basrah, Iraq
[2] Department of Clinical laboratory science, College of Pharmacy, University of Basrah, Basrah, Iraq.

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A B S T R A C T

Purslane which is the common name for Portulaca oleracea L. is a weed plant distributed all over the world and it has significant medicinal uses due to its present high percentage of phytochemical compounds. However, previous studies by researchers confirmed possession of Purslane leaves extracts anti-oxidant efficacy. Therefore, our study aims to determine the phytochemical composition of the ethanolic leaves extract of purslane and radiation-stimulated DNA damage protecting. Gas chromatography-mass spectrometry (GC-MS) analyses in the present research explore active constituents for P. oleracea, which are 17 active compounds most they are terpenoids and alkaloids. The extract showed considerable antioxidant activity and the highest inhibition-percentage (44.45 %) belongs to (20mg/ml) of an extract with an IC50 value of 4.6 mg/ml in H2O2 scavenging test and prevented DNA oxidative damage stimulated by hydrogen peroxide and ultraviolet light (UV/H2O2) at concentrations of (2-20 mg/mL). These findings suggest that the ethanolic leaves extract of P. oleracea, could be used as skin-care products to prevent UV-induced damage to the skin..

1. Introduction

The DNA in the cells of living organisms is exposed to many damages, some of these damages cause mutations if they are not repaired immediately by the body [1]. Ultraviolet (UV) rays are one of the causes of this DNA damage, in addition to reactive oxygen species (ROS) and hydroxyl radicals (OH) [2]. All of which can lead to aging, cancer, different other diseases [3]. The most harmful external agents to human skin are UV rays due to their ability to stimulate the production of free radicals/reactive oxygen species. [4]. Free radicals in the skin are mainly produced by solar UV rays

[*]**Corresponding author email :** kawther.khalf@uobasrah.edu.iq

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line at: https://bjrs.uobasrah.edu.iq

[5]. Free radicals lead to DNA damage [6], which leads to the elimination of various biological effects on the skin; therefore, it is possible to protect the skin from cancers and other diseases caused by the formation of free radicals as a result of exposure to ultraviolet rays by protecting DNA from those rays [7]. Certain compounds found in vegetables, fruits, and medicinal plants can act as natural antioxidants that prevent DNA damage to these compounds, including flavonoid polyphenols and carotenoids [8]. Using of medicinal plants in folk medicine has led to the discovery of many biologically active compounds that are effective tools in the treatment and prevention of diseases [9,10]. Portulaca oleracea L., is a weed belonging to the family Portulacaceae, widespread throughout the world [11]. It is considered one of the important plants because it contains a high percentage of omega-3 fatty acids, in addition to containing the essential minerals, and ascorbic carotenoids, research has shown the effectiveness of leaf extract as antioxidants [12].

2. Material and methods

2.1. Sample Preparation and Extraction procedure

P. oleracea L., plant was collected from local field at Abu Al- Khaseeb district in Basrah city (Iraq) in June and authenticated by Prof. D. Abd-Ridha AlMayah, College of Science /University of Basrah /Iraq. Leaves were separated manually from the stems and dried then powdered. Four grams of powdered samples were macerated with (500 mL of 95% ethanol) under- stirring for 3 days at - room temperature as, [13] with-some modification. The extractor was filtrated and ethanol was evaporated, concentrated extract was stored at -20℃.

2.2. Gas chromatography-mass spectrometry (GC-MS) analysis:

Analysis GC mass for P. oleracea L., ethanol extract was done in the College of Education for Pure Sciences/ Basrah in chemistry laboratories. Agilent 7890A gas chromatography and mass 5975C spectrometer detector with computer control at 700 Ev . The capillary column type was Hewlett - Packard HP-5MSsilica, with a diameter of 250 µm × 30 m in length×0.25µm in thickness. The carrier gas was Helium at a stable flow rate of 2ml/min and an injection volume of 1 µl with an injector temperature of 250°C, 18 min was the scanning time. The initial oven temperature was 60°C for 1 min. National Institute of Standards and Technology (N.I.S.T) 2008 mass spectral library was used for further identification [14].

2.3. Hydrogen peroxide scavenging test

2 mL of each ethanol extract concentration 2, 5, 10, and 20 mg/Ml were incubated for 10 min, with 1.2 mL H2O2 (40 mol/L in phosphate buffer, pH 7.4), and the absorbance of the solution was done at 230nm in a UV visible spectrophotometer. Plant extract without H2O2 was used as a blank solution and ascorbic acid was used as a control. The procedure was carried out in triplicate for each concentration [15].
The percentage of H2O2 -scavenging was calculated from:

$$\left[\frac{A_c - A_s}{A_c}\right] \times 100 \quad (1)$$

Where *A*c is the control absorbance and *A*s is the plant-extract absorbance.

2.4. Protection DNA-damage assay

The ability of P. oleracea L. leaves ethanolic extract to inhibit DNA damage was evaluated using "genomic DNA from human blood ''. DNA was extracted by using the Geneaid DNA extraction kit, and steps were followed according to the attached instructions by the company. The experiment was accomplished by, Adinortey et al. [16]. The total volume of the experiment was 30 µl in a microfuge tube contain 10 µl of DNA, 10 µl of ethanol extract in different concentrations (2-20mg /ml), and 10 µl of 30% H2O2. The internal control tube contains only DNA whilst the negative control tube contains DNA and H2O2 without adding extract. UV transilluminator (UVP Upland, CA 91786, USA)

was used to irradiate the tubes at 230 nm a wavelength for 13 h at room temperature. All tubes were run on agarose gel 1% and were photographed [17].

2.5. Statistical analysis

The result for H2O2 scavenging activity, of the ethanolic P. oleracea leaves extract was expressed as means ± standard deviations-of the replies-of three-replicates per sample. Statistical-analysis was performed by using SPSS version 42. ANOVA one-way was used under probability level $P \leqslant 0.05$ to compare between means.

3. Results

GC-MS chromatography test of P.aoleracea leaves ethanolicc extract: the result obtained from the GC-MS test are existed in Table 1, Fig. 1, Fig. 2, Fig. 3.

Table 1. Phytochemicals of P. oleracea leaves identified by GC- MS

peak	Phytochemical compound	Molecular formula	Molecular weight	Peak area %	Chemical Nature	Biological activity	Reference
A	Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid	$C_7H_{10}O_6$	190	40.65	Terpenoid	Acidifier, inhibit production of uric acid, urinary acidulant	18
B	4-Fluorohistamine	$C_5H_8FN_3$	129	14.82	Alkaloid	Not non	
C	Actinobolin	$C_{13}H_{20}N_2O_6$	300	5.49	Flavonoid	Antibiotic, antineoplastic	19
D	2-Formylhistamine	$C_6H_9N_3O$	139	3.53	Alkaloid	Not non	
E	Thiophene-3-ol, tetrahydro-, 1,1-dioxide	$C_4H_8O_3S$	136	3.39	Sulfone	antimalarial, antimicrobial, antimycobacterial, antidepressant, anticonvulsant, antiviral, anticancer, antihypertensive, anti-inflammatory and antioxidant	20
F	1,2-Hydrazinedicarboxamide	$C_2H_6N_4O_2$	118	24.39	Alkaloid	anticancer and antioxidant	21
G	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207	2.13	Alkaloid (Pteridine)	Antipsychotic, Moodstabilizer and Antiparasite	22
H	Cycloserine	$C_3H_6N_2O_2$	102	1.63	Alkaloid (cyclic amino acid)	Anti-tuberculosis	23

I	8,9,9,10,10,11-Hexafluoro-4,4-dimethyl-3,5-dioxatetracyclo[5.4.1.0(2,6).0(8,11)]dodecane	$C_{12}H_{12}F_6O_2$	302	1.57	Alicyclic	Antimicrobial	24
J	Pteridine-8-oxide, 6-aldoximino-2-amino-4(3H)-oxo	$C_7H_6N_6O_3$	222	1.32	Alkaloid (Pteridine)	Not non	
K	Ethanediamide	$C_2H_4N_2O_2$	88	2.89	Oxamide	antitumour activity	25
L	Carbohydrazide	CH_6N_4O	90	26.3	Hydrazine Alkaloid	oxygen scavenger	26
M	1,2,5-Oxadiazole-3-carboxamide, 4-amino-N-(2-aminoethyl)	$C_5H_9N_5O_2$	171	3.99	Alkaloid	Not non	
N	Cyacetacide	$C_3H_5N_3O$	99	1.67	Alkaloid	Antibacterial	27
O	Hydroxyurea	$CH_4N_2O_2$	76	0.64	Alkaloid	antineoplastic drug	28

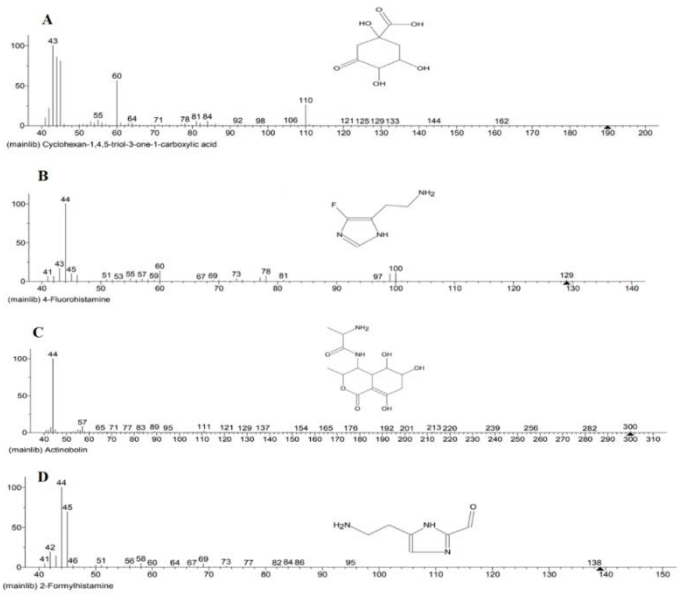

Fig. 1. Mass spectra from full scan analysis of ethanol extract: A. Cyclohexan-1, 4, 5-triol-3-one1-carboxylic acid B. 4-Fluorohistamine; C. Actinobolin; D. 2-Formylhistamine.

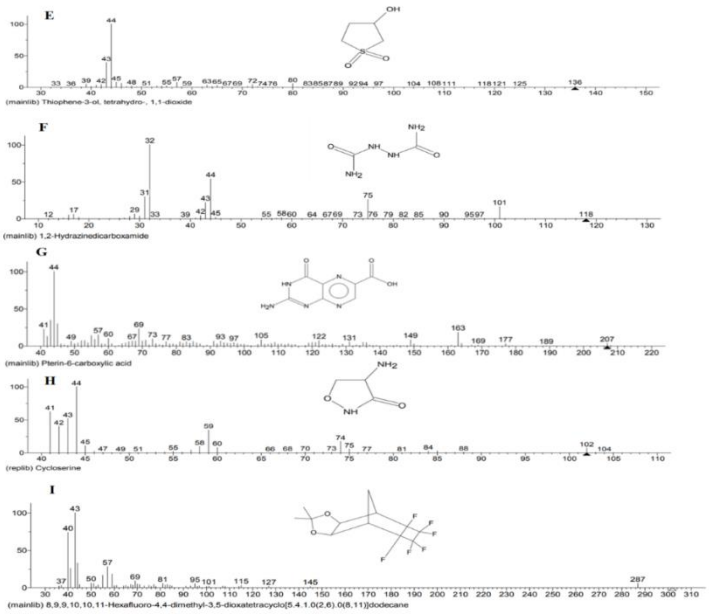

Fig. 2. Mass spectra from full scan analysis of ethanol extract: E. Thiophene-3-ol, tetrahydro-, 1,1-dioxide F. 1, 2-Hydrazinedicarboxamide; G. Pterin-6-carboxylic acid-2-amino-4(3H)-oxo; H. Cycloserine; I. 8, 9, 9, 10, 10, 11-Hexafluoro-4, 4-dimethy l-3, dioxatetracyclo [5.4.1.0 (2,6) .0 (8,11)] dodecane.

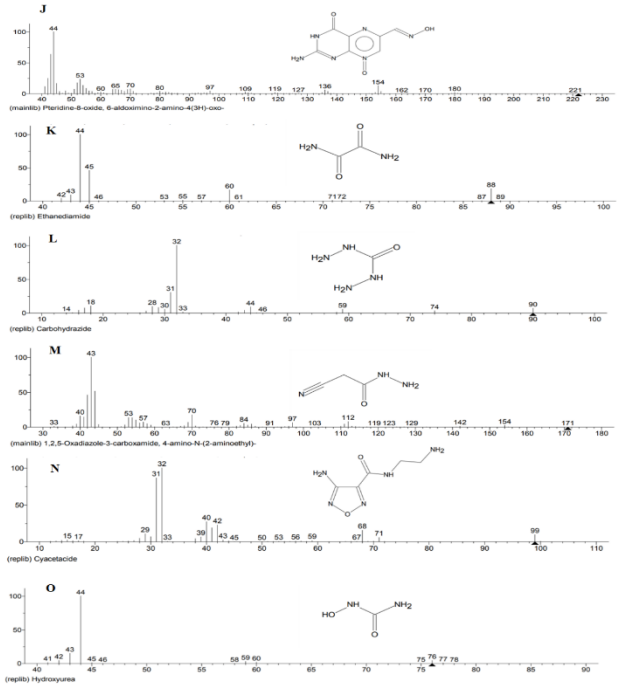

Fig. 3. Mass spectra from full scan analysis of ethanol extract: J. Pteridine-8-oxide, 6-aldoximino-2-amino-4(3H)-oxo; K. Ethanediamide; L. Carbohydrazide; M. 1, 2, 5-Oxadiazole-3-carboxamide, 4-amino-N-(2-aminoethyl); N. Cyacetacide; O. Hydroxyurea.

3.1. Hydrogen peroxide scavenging assay

The scavenging ability of ethanolic extract of P. oleracea leaves on hydrogen peroxide is shown in Fig.4 which is compared with ascorbic-acid as-standards. The result showed the highest inhibition rate percent was (44.45 %) belongs to (20 mg/ml) of extract compared with (47.26 %) for ascorbic acid. The IC50 of P. oleracea extract was 4.6 mg/ml.

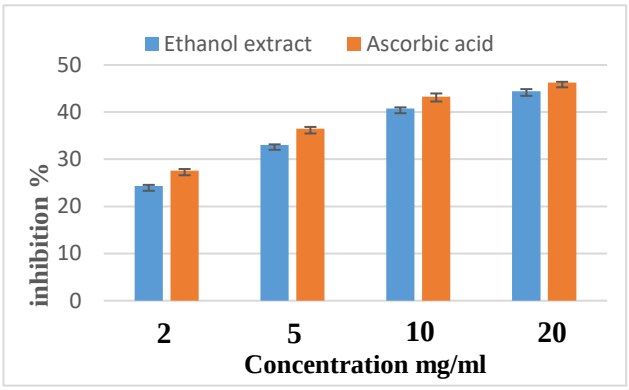

Fig. 4. Hydrogen peroxide radical scavenging activity of *P. oleracea* ethanol extract.

3.2. DNA damage protection

The DNA electrophoresis pattern after exposure to UV-radiation and H2O2 in the presence and absence of various concentrations of the ethanolic extract is shown in Fig.5. The untreated DNA showed a bright band on agarose gel (lane 1), also observed in lanes 6 that DNA irradiated in the presence of H2O2 without adding extract resulted in the unclear band. In lanes 2 to 5 there was an addition of extract to DNA exposed UV in the presence of H2O2 there was considerable preservation from damage this depicted because these bands (2-5) appearance bright and have the same location as a band of standard DNA.

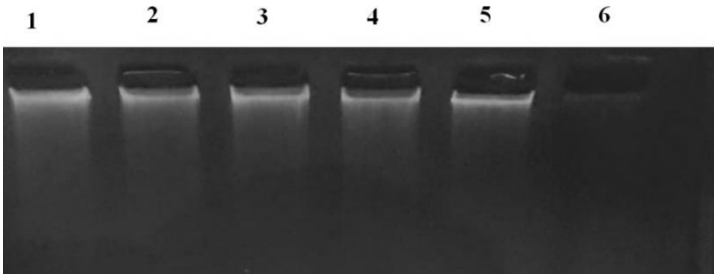

Fig. 6. Electrophoresis pattern of protect DNS from UV damage after ultraviolet-photolysis of H_2O_2 (30%) at 13h in the presence of the ethanolic leaf extract of P.oleracea. Lane1: DNA alone; Lane2: 2 mg/ml extract + UV + H_2O_2 + DNA; Lane3: 5 mg/ml extract + UV + H_2O_2 + DNA; Lane 4: 10 mg/ml extract + UV + H_2O_2 + DNA; Lane5: 20 mg/ml extract + UV + H_2O_2 + DNA; Lane 6: UV + H_2O_2 + DNA.

4. Discussion

The implication of free radicals in the development of different kinds of disease, research has focused on the discovery of new antioxidants; antioxidants face free radicals and protect the human body from various ailments. Numerous studies have proven that phenolic compounds in foods and medicinal plants have oxidative activity in addition to phenolic are flavonoids [29]. Moreover, alkaloids are phytochemicals that are playing an important role in maintaining human health and protecting it from diseases [30], as the study of both Alam et al. [31] and Al-Mosawi et al. [32] confirmed that the Purslane contains an oxidant as a result of the presence of these compounds, in addition to other active compounds. The current study aimed to reveal the ability of ethanol extract

of P. oleracea leaves to protect DNA from UV damage, and investigate the phytochemicals present in this extract GC-MS analysis of the current study proved the existence of phytochemicals such as alkaloids, terpenoid, flavonoid, and other compounds are present in Purslane extract, as summarized in Table1, the GC-MS test has also shown that terpenoid compounds have the highest percentage (61%), followed by alkaloids compounds (51.12%), and presence of other chemical compounds in different percentage Table1. Terpenoids and alkaloids have been widely researched due to their biological efficiency and many medicinal uses, like antimicrobial, antifungal, antiviral, antioxidant, anticancer, antihyperglycemic, analgesic,

anti-inflammatory, and antiparasitic [33]. The potential of plant extracts as antioxidants depends on the number of total Terpenoids and Flavonoids contents, in additional to Alkaloids which have powerful anticancer activity [34,35]. The GC-MS results analysis of P. oleracea leaves extract showed a discrepancy from those of previous reports, this may be due to the difference in the environment as harvest time and growing conditions which play a considerable role in determine the type and amount of chemicals [36]. The extract of P. oleracea leaves is an effective scavenger of in-vitro free radicals (hydrogen peroxide) as mentioned in Fig.4. Ability to quench free radicals protection DNA strands from oxidative breaks. Oxidative DNA strands as a resulting to UV exposure and hydroxyl radicals produced causing relaxed forms or open circular-DNA, due to react these radicals with DNA bases and generate sugar and base radicals leading to breakdown of sugar-phosphate-backbone [37].The research results show that the ethanol extract of p. oleracea leaves is effective in protecting, DNA from damage. Protection DNA strands breaking from the oxidative induced by hydrogen peroxide and UV exposure Fig.5. Hydrocarbon free radicals have been known to cause damage to cellular DNA. UV photolysis of H2O2 creates OH radicals, which are accountable for damage most protein and DNA oxidation, accordance to Guha et al. [38]. DNA Oxidative damage is one of the most significant mechanisms in the prompting of cancer and this damage is usually caused by OH radicals [39]. In this study, the Purslane extract showed significant protective efficiency against DNA damage mediated by free radicals at all concentrations were used and thus could be used in cancer prevention. Different plants have been recording to protect against DNA damage mediated by free radicals [40,41].

5. Conclusion

This study demonstrated the presence of a high percentage of terpenoids and alkaloids in the ethanolic leaf extract of P. oleracea also reflected in the capacity of this extract protection against UV-induced oxidative DNA damage. It can be used in antioxidant therapy and cosmetic sunscreen industries.

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التحليل الكيميائي لمستخلص أوراق Portulaca oleracea L. ودراسة دوره في حماية الحمض النووي البشري من أضرار الأشعة فوق البنفسجية

زينب طعمة الدلي [١]، كوثر طعمة خلف*[٢]، يسار عبد الحسين داود [١] ، أسامة حامد رمضان [٢]

[1] قسم العقاقير، كلية الصيدلة، جامعة البصرة، العراق.
[2] قسم العلوم المختبرية السريرية، كلية الصيدلة، جامعة البصرة، العراق.

معلومات البحث

الاستلام ٨ كانون الاول ٢٠٢٢
القبول ١٢ شباط ٢٠٢٢
النشر ٣١ تموز ٢٠٢٢

الكلمات المفتاحية

تأثيرات حماية الحمض النووي، Portulaca oleracea L، تلف الحمض النووي.

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الملخص

البَقْلَة وهو الاسم الشائع لـ Portulaca oleracea L. هو نبات عشبي منتشر في جميع أنحاء العالم وله استخدامات طبية كبيرة نظرًا لوجود نسبة عالية من المركبات الكيميائية النباتية. ومع ذلك، أكدت الدراسات السابقة التي أجراها الباحثون أن حيازة خلاصة أوراق الرجلة فعالية مضادة للأكسدة. لذا ، هدفت دراستنا إلى تحديد التركيب الكيميائي لمستخلص الإيثانول لاوراق نبات الرجلة ودراسة دوره في حماية تلف الحمض النووي المحفز بالإشعاع. كشفت تحليلات كروماتوغرافيا الغاز - مطياف الكتلة (GC-MS) في البحث الحالي المكونات النشطة لـ P. oleracea ، وهي ١٧ مركبًا نشطًا معظمها من التربينويدات والقلويدات. أظهر المستخلص نشاطًا مضادًا للأكسدة وأعلى نسبة تثبيط (٤٤,٤٥٪) تعود إلى التركيز (٢٠ مجم / مل) من المستخلص وبقيمة تكيز مثبط نصف قصوى (4.6 IC50 مجم / مل) في اختبار الكسح H2O2 كما يحتوي المستخلص على تأثير وقائي للحمض النووي ضد الضرر الناتج عن الأشعة فوق البنفسجية بوجود بيروكسيد الهيدروجين التي تحفز التلف المؤكسد. تشير هذه النتائج إلى أنه يمكن استخدام مستخلص الأوراق الإيثانولية من P. oleracea كمنتجات للعناية بالبشرة لمنع تلف الجلد الناتج عن الأشعة فوق البنفسجية.

***Corresponding author email :** kawther.khalf@uobasrah.edu.iq

N: 1817-2695 (Print); 2411-524X (Online)
line at: https://bjrs.uobasrah.edu.iq