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# In Vitro Evaluation of Antioxidant Capacity and DNA Protective Potential of Phenolic-Rich Extracts from Selected Brassicaceae Species

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# التقييم المختبري للقدرة المضادة للأكسدة وإمكانية حماية الحمض النووي لمستخلصات غنية بالفينولات من أنواع مختارة من الفصيلة الصليبية

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**Abstract:**
This study presents the first comprehensive characterization of the phytochemical composition, antioxidant activity, and genotoxicity of five wild species of the Iraqi cruciferous family. Rich in bioactive compounds, these plants offer promising natural resources for health and nutritional applications, particularly for the prevention of oxidative DNA damage. This study systematically evaluates the phytochemical profile, DPPH antioxidant potential, and genotoxicity of five Iraqi Brassicaceae species, identifying key bioactive compounds and their protective efficacy on human lymphocyte DNA against hydrogen peroxide damage. Five species of the Iraqi cruciferous family were extracted Antioxidant activity was compared using the DPPH assay with vitamin C. The extracts were also tested for their ability to protect human lymphocyte DNA from oxidative damage and assessed for genotoxicity. E. vesicaria demonstrated superior efficacy in removing DPPH free radicals (>85%), surpassing vitamin C (~80%), followed by D. harra (~70%). E. vesicaria extract showed remarkable protective efficacy against hydrogen peroxide-induced oxidative DNA damage in human lymphocytes, completely preventing DNA fragmentation and preserving strand integrity (at a level of 1.2 base pairs of destructive activity compared to 1000 base pairs in the hydrogen peroxide-damaged group). E. vesicaria and D. harra are potent natural antioxidants rich in phenolics, their strong DNA protection and high antioxidant activity make them promising candidates for Iraq's functional food and health sector.

**Keywords**: Antioxidants Activity, Brassicaceae, DNA Protective , Phenolic-Rich.

**الملخص**
تقدم هذه الدراسة أول توصيف شامل للتركيب الكيميائي النباتي، والنشاط المضاد للأكسدة، والسمية الجينية لخمسة أنواع برية من الفصيلة الصليبية العراقية. تُعد هذه النباتات، الغنية بالمركبات النشطة بيولوجيًا، موارد طبيعية واعدة للتطبيقات الصحية والغذائية، ولا سيما للوقاية من تلف الحمض النووي التأكسدي. تُقيّم هذه الدراسة بشكل منهجي التركيب الكيميائي

النباتي، وإمكانية مضادات الأكسدة باستخدام اختبار DPPH، والسمية الجينية لخمسة أنواع من الفصيلة الصليبية العراقية، مُحددةً المركبات النشطة بيولوجيًا الرئيسية وفعاليتها الوقائية على الحمض النووي للخلايا الليمفاوية البشرية ضد تلف بيروكسيد الهيدروجين. تم استخلاص خمسة أنواع من الفصيلة الصليبية العراقية، وقورن النشاط المضاد للأكسدة باستخدام اختبار DPPH مع فيتامين C. كما تم اختبار المستخلصات لقدرتها على حماية الحمض النووي للخلايا الليمفاوية البشرية من التلف التأكسدي، وتقييم سميتها الجينية. أظهر نبات E. vesicaria فعالية فائقة في إزالة جذور DPPH الحرة (<85%)، متفوقًا على فيتامين C (~80%)، يليه نبات D. harra (~70%). أظهر مستخلص نبات E. vesicaria فعالية وقائية ملحوظة ضد تلف الحمض النووي التأكسدي الناجم عن بيروكسيد الهيدروجين في الخلايا الليمفاوية البشرية، حيث منع تمامًا تفتت الحمض النووي وحافظ على سلامة السلسلة (بمستوى 1.2 زوج قاعدي من النشاط التخريبي مقارنةً بـ 1000 زوج قاعدي في المجموعة المتضررة ببيروكسيد الهيدروجين). يُعدّ كل من E. vesicaria و D. harra من مضادات الأكسدة الطبيعية القوية الغنية بالمركبات الفينولية، وتجعلهما قدرتهما الفائقة على حماية الحمض النووي ونشاطهما العالي كمضادات للأكسدة مرشحين واعدين لقطاع الأغذية الوظيفية والصحة في العراق.

**الكلمات المفتاحية:** نشاط مضادات الأكسدة، الفصيلة الكرنبية، حماية الحمض النووي، غني بالمركبات الفينولية.

## 1. Introduction

Plant biotechnology is a cornerstone of contemporary scientific research, particularly in its pursuit of exploring natural resources with therapeutic and preventative potential [1]. This research falls within this framework, focusing on the fundamental relationship between the phytochemical composition and bioactivity of Brassicaceae plants prevalent in the Iraqi environment [2]. The Brassicaceae family (which includes mustard, rocket, and wild species such as *Z. spinosa* and *E. vesicaria*) is characterized by its rich repository of secondary metabolites with pharmaceutical value [3,4]. This complex composition encompasses a wide spectrum of bioactive compounds. Phytochemical studies of Brassicaceae species in Iraq have revealed a rich chemical composition [5], primarily characterized by phenolic compounds, flavonoids, alkaloids, glucosinolates, and other bioactive phytochemicals [6]. These compounds are mainly responsible for the prominent antioxidants and wide spectrum of biological activities shown by these plants, and their tannins that potentiate to plant therapeutic properties [7]. These plants have numerous phytochemicals including glucosinolates, ITCs, carotenoids and phenolics and vitamins which might contribute to the unique taste and indispensable bio-function[8] . Notably, the glucosinolates are precursors of biologically active ITCs, indoles and nitriles that have been linked with cancer preventative effects. Plant derived from natural antioxidants, nontoxic and effective for human use have recently attracted global interest [9] GC-MS, and High-performance liquid chromatography (HPLC) of the species such as Lepidium sativum; *C. draba* were rich in bioactive compounds mainly isothiocyanates, benzyl nitriles, phenolic derivatives responsible for the antioxidant and antimicrobial activity [10]. The antioxidant activity was found to be attributes of their high content of total phenolics and flavonoids, which are good free radical scavengers that chelate the metal ions, in this manner inhibiting lipid peroxidation. Various assays, such as DPPH (1, 1-diphenyl-2-picryl-hydrazyl) free radical scavenging method, FRAP technique (ferric oxidative antioxidant power), and hemolysis test disclosed strong anticancer activity in Iraq's cabbage extracts based on the IC50 value which were effective given that" it neutralizes strong of the free radical for DPPH action; hence several can get to a low concentration about 19 µg/ml these indicated that they could be good energy gain related their effect other than it thought to be good antioxidants[11]. Plant extracts have been not only as antioxidant agents, but also as antihaemolytic activity,anti-inflammatory or antiproliferative activity, these effects during therapy process could be utilized to prevent the damaging effect of oxidative stress [12]. Phytochemicals act as stabilizers of membrane, against oxidative damage and increase stress tolerance such as UV radiation or due to chemical action [13,14]. Despite the incredible biodiversity and plant diversity in Iraq yet however, a full academic documentation on many local plants has not been accomplished so far (one of which is some teachers from family Brassicaceae [15,16]. This may allow us to understand more about the chemical expression in these plants in their natural environment and build data base that helps as realize how important this source can be for discovering novel compounds with diverse activities.

This study aims to measure the antioxidant activity of these extracts using a reliable biotechnological technique (such as DPPH assay) to establish a clear and documented correlation between phytochemical content and bioactive activity. This represents a first step towards the biostandardization of these species and their potential applications in the health and food industries in Iraq.

## 2. Methodology

**Plant Collection and Identification**

The plants were collected from various regions of western Iraq and identified at the Natural History Museum of the University of Baghdad. The plants were dried at room temperature and away from sunlight.

**Preparation of plant extracts**

Extracts of Z. spinosa, E. vesicaria, D. harra, L. didymium and Capsula sp. were used. Stock solutions were prepared at a concentration of 100 mg $ml^{-1}$ by adding 1 g of the dried plant powder to 9 ml of distilled or deionized water. The solution was thoroughly mixed (using an ultrasonic or rotary mixer) and then centrifuged at 3000 rpm for 10 minutes to separate the precipitate. The filtrate was collected and filtered twice using Whatman filter paper to remove fine impurities(El Jery et al., 2018), and the specimens were kept dry until they were extracted and prepared for chemical and biological analysis.

**Preparation of Plant Extract and Its Fraction**

The evaluation of different parameters, bitterness value ,loss of drying, foaming index, crude fiber content, total ash, foreign organic matter, swelling index were studied[17]. Phytochemical screening was carried out on the crude extracts to detect the presence of plant secondary metabolites: Alkaloids, Steroidal nucleus, Flavonoids, Glycosides, Tannins, Phenolics Compounds, terpenoids, and carbohydrates using standard procedures as described in the literature[18].

**Phenolic Compound Extraction**

Adhering to the methodology in [19], 1g of powdered plant material underwent hexane treatment for fat and oil removal. The defatted material was then mixed with 1 mL of juice and treated with 100 ml of an 80:20 methanol: water solution. Ultrasonication of the solution for 25 minutes at 25°C enhanced phenolic compound release. Post-centrifugation at 7500rpm for 15 minutes, the supernatant was treated with activated charcoal to remove impurities. The phenolic-rich extract was finally concentrated using a rotary evaporator under vacuum.

**Determining the Antioxidant Activity**

The2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent was used to prepare an extract solution at a concentration of 1 mg $ml^{-1}$ in methanol to measure the free radical scavenging capacity of the plant extracts. A volume of 3 mL of the DPPH solution at a concentration of 0.06 mM was kept in a dark container, to which 0.77 ml of the methanolic extract solution was added. The sample was placed in a thermostat and left there for 15 minutes. The CARY 50 UV-VIS spectrophotometer was used to measure the absorption at 515 nm. For the control, 0.77ml of methanol was added to the DPPH solution, after which the absorption was measured immediately. The antioxidant activity was calculated as the percentage of inhibition.

$$\text{Inhibition } \% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where: A-DPPH is the absorbance of the control sample (DPPH + methanol) Sample A is the absorbance of the sample [20].

**Evaluation of the protective properties of extracts against DNA fragmentation in human lymphocytes**

Human lymphocytes were isolated at the Biotechnology Center, University of Baghdad, in accordance with Ethical Approval 2344/GF/2025. A cytotoxicity assay was performed to determine the concentration range ( 5, 10, 25, 50 µg $ml^{-1}$) harmful to lymphocytes. This was done by exposing the cells to the extract for 1-2 hours to confirm its non-toxicity. Subsequently, the extracts (25 µg $ml^{-1}$) prepared in the preceding paragraphs were added to the lymphocytes for 2 hours in a suitable culture medium. After a protective incubation period, oxidative stress induction was induced by adding hydrogen peroxide ($H_2O_2$) at a concentration of 100 µmol/ $ml^{-1}$. There was a negative control group containing lymphocytes without extracts and without hydrogen peroxide, and a positive (damaged) control group containing lymphocytes treated with hydrogen peroxide only [21].

**DNA Fragmentation Assay via Agarose:**

DNA was extracted from each treatment group and analyzed using 1% agarose gel electrophoresis. The DNA was carried along with a DNA ladder (50–1000 base pairs). Electrophoresis was performed at 70 V and 20 mA for at least one hour. The gel was imaged under fluorescence. Ultraviolet light was used to measure the distance traveled (X) per strand of DNA in the samples and the marker . A standard curve was plotted using Microsoft Excel 2019. The molecular size (Y) of the DNA was calculated using a standard curve. The resulting linear regression equation was then used to calculate the molecular size per strand of DNA in the experimental samples.

The equation used is:

$$Y = -26.84X + 833.59$$

The lysis level is expressed as the percentage of fragmented DNA extracted from the well compared to the total DNA (fragmented and unfragmented). For classification of fragmentation based on the calculated molecular size (lysis level), the following criteria can be used: partial lysis: if the analyzer level is ≤ 200; moderate lysis: if the analyzer level is between 200 and 200. 700. Complete dissolution: if the analyzer level is ≥ 700 [22].

**Statistical Analysis**

The obtained antioxidant and antibacterial results were expressed in mean ± standard error with observation recorded in triplicates. Analysis of variance for individual parameters was performed based on mean values to determine the significance at $p < 0.05$ using SPPS v20. Regression analysis was deployed to calculate and obtain the $IC_{50}$ from the regression equation using Excel 2019.

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## 3. Results and Discussion

**DPPH Assay (In-Vitro Antioxidant Activity)**

The data shown **i**n **Fig. 2**. represent the results of an evaluation of the antioxidant activity of plant extracts from the cabbage family, specifically *Z. spinosa*, *L. didymium*, and *E. vesicaria* as well as an unspecified capsule sample, compared to two standards a topical concentration of 50 (a reference or standard concentration for comparison) and ascorbic acid, a well-known standard antioxidant. The results represent the percentage of inhibition (%) for the different species at an unspecified concentration. Ascorbic acid (vitamin C), the positive standard, showed the highest percentage of inhibition, approximately 80%. This result confirms the efficacy of this compound as a potent antioxidant and its high capacity to scavenge free radicals, consistent with its known role in biological and chemical systems. Regarding the plant samples, the extract of *E. vesicaria* emerged as the strongest inhibitor, achieving the highest inhibition rate among all the plant extracts studied, exceeding 85%—a rate significantly surpassing even the positive standard for ascorbic acid. This result indicates that the *E. vesicaria* extract contains a high concentration or potent combination of phenolic compounds, flavonoids, or glucosinolates (a characteristic of the cabbage family), which possess an exceptional ability to donate electrons and scavenge free radicals, making it a promising source of natural antioxidants. *D. harra* came in second with a 70% inhibition rate. In contrast, extracts of the *Z. spinosa*, *L. didymum*, and *Capsula* showed moderate to good antioxidant activity. *E. vesicaria* achieved slightly over 85% inhibition, while *Lepidium didymium* showed slightly less inhibition, around 45%. The capsule achieved nearly 64% inhibition. These results indicate inherent antioxidant properties in these species, although their activity is considerably lower than that of *E. vesicaria* and the control group. This variation in activity among different species within the same plant family (Brassicaceae) is expected and results from differences in the genetic, environmental, and secondary chemical composition of the extracts, particularly the diversity of types and quantities of secondary metabolites responsible for biological activity.

As for the $IC_{50}$ sample, which showed an inhibition rate of approximately 70%, if it represents the effective concentration that inhibits 50% of free radicals (the 50% inhibitory concentration), then its placement on the axis likely represents a reference value for comparing efficacy. Overall, these results, taken together, suggest that cruciferous plants are rich natural sources of antioxidants, with particular emphasis on the therapeutic and health potential of *E. vesicaria*, thus justifying further research to isolate and identify the active compounds responsible for this significant efficacy.

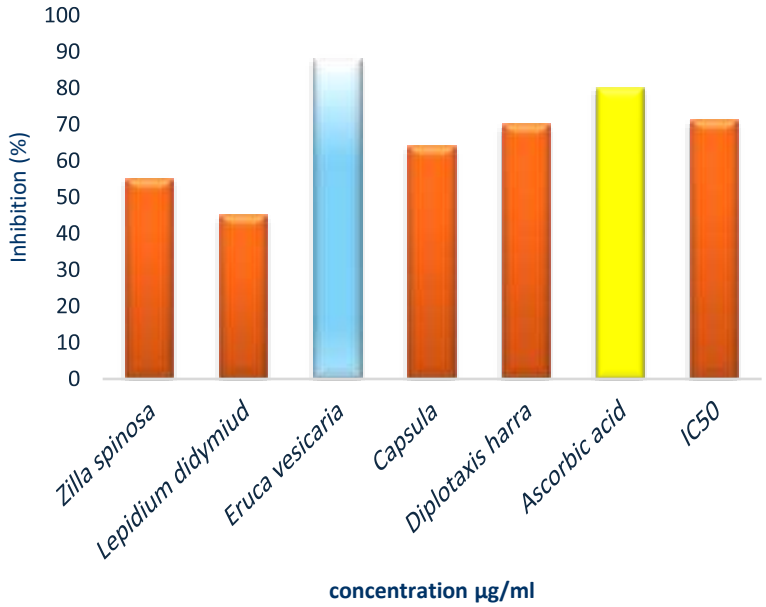


**Fig. 2 .** DPPH assay extracts of Brassicaceae (Cruciferous*)* species

Analysis of the presence of active compounds indicates a strong positive correlation between the presence of phytochemical classes, particularly phenolic compounds (including flavonoids and tannins), and overall antioxidant activity. However, the relationship is not simply linear, as overall potency depends primarily on the specific concentration of secondary metabolites within these classes. The superiority of *E. vesicaria* underscores the need for rigorous quantitative analysis to identify the specific phenolic compound (or group of compounds).

**Protective properties of some plant extracts against oxidative DNA damage**

The results in the **Fig.3,4, 5** and **Tab. 3** show the evaluation of the protective effect of five plant extracts (*Z. spinosa, E. vesicaria, D. harra, L. didymium,* and *Capsula sp*.) against oxidative damage to DNA in human lymphocytes stimulated by hydrogen peroxide. Damage was analyzed based on DNA laddering and lysis level determination using agarose gel electrophoresis. Untreated lymphocytes (Negative Control) showed no signs of DNA lysing (No Lysis), with no DNA fragmentation observed and the DNA remaining intact (DNA Molecular Size; DMS = 0 bp. The current study demonstrates a marked contrast in the protective efficacy of plant extracts against the oxidative effects of hydrogen peroxide on lymphocytes. Exposure to hydrogen peroxide at a concentration of 100 $mUmol^{-1}$ resulted in complete cell lysis, manifested as smears on electrophoresis with a lysis level of approximately 1000 base pairs, confirming hydrogen peroxide's ability to cause severe DNA damage via oxidative stress mechanisms. In contrast, extracts from the plants *E. vesicaria* and *D. harra* exhibited superior protective efficacy, almost completely preventing cell lysis and registering the lowest levels of DNA lysis (1.2 and 5.5 base pairs, respectively). These results suggest that these two extracts contain active compounds capable of neutralizing free radicals and preventing oxidative DNA damage. Other plant extracts *Z. spinosa, L. didymium,* and *Capsula* sp. showed moderate protective activity, with degradation levels ranging from 79.5 to 90.4 base pairs, along with partial cell lysis. This suggests that these extracts can reduce oxidative damage, but with less effectiveness than the two previously mentioned plants.

These protective effects are attributed to the presence of active compounds in these plant extracts that act as antioxidants through multiple mechanisms, including free hydroxyl radical scavenging, inhibition of the Fenton reaction, and preservation of cell membrane integrity. These findings open new avenues for developing medicinal preparations that protect against oxidative damage, highlighting the promising potential of *E. vesicaria and D. harra* extracts as natural sources of DNA-protective compounds.

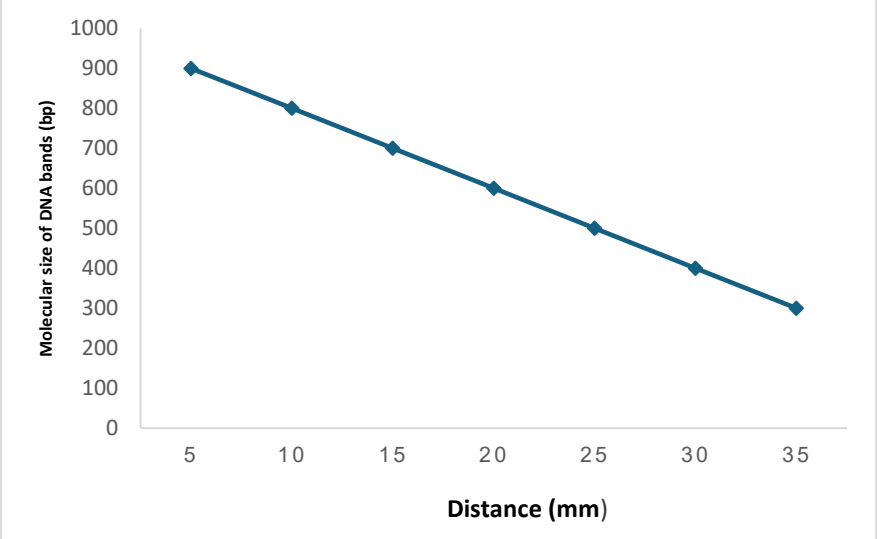


Figure 3. DNA standing curve (50-1000) bp in 1% agarose for 2 hours

**Table 3.** The lysis is the level of human lymphocyte DNA and types of lysis that are exposed to $H_2O_2$ and different plant extracts

| Number of sample | Type of lysis | DNA M.S | Lysis level bp |
|---|---|---|---|
| $H_2O_2$ (100 mUmol$^{-1}$) | Complete lysis | Smear | 1000 |
| *Z. spinosa* (25 µg ml$^{-1}$) + $H_2O_2$ | Partial lysis | 433 | 86.7 |
| *E. vesicaria* (25 µg ml$^{-1}$) + $H_2O_2$ | No lysis | 20 | 1.2 |
| *D. harra*(25 µg ml$^{-1}$) + $H_2O_2$ | No lysis | 44 | 5.5 |
| *L. didymium*(25 µg ml$^{-1}$) + $H_2O_2$ | Semi lysis | 300 | 90.4 |
| *Capsula* sp. (25 µg ml$^{-1}$) + $H_2O_2$ | Semi lysis | 360 | 79.5 |
| Lymphocytes only | No lysis | 10 | 7 |

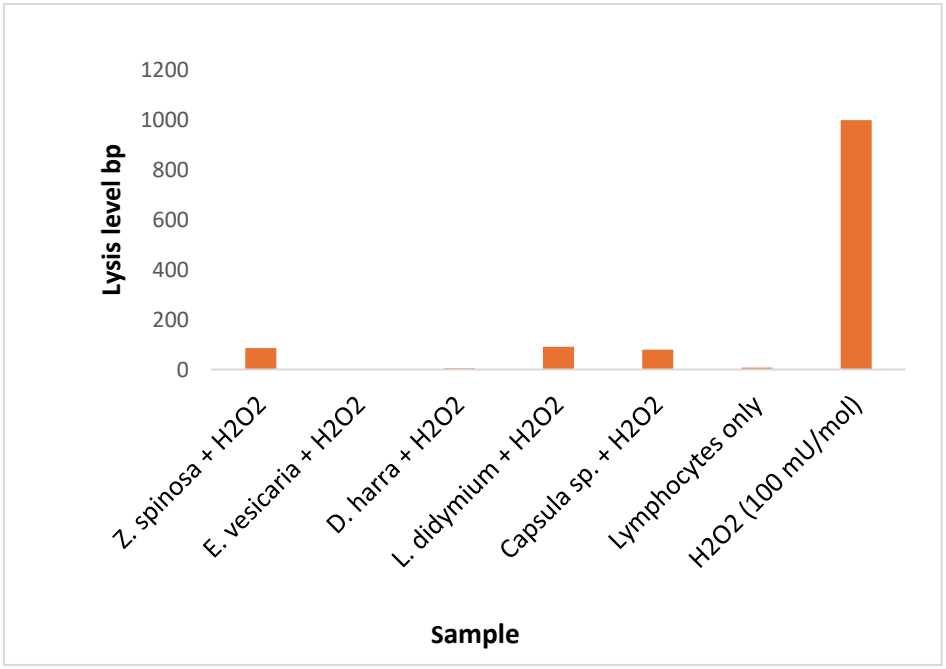


**Fig.** 4 . The lysis is the level of human lymphocyte DNA that is exposed to $H_2O_2$ and different plant extracts.

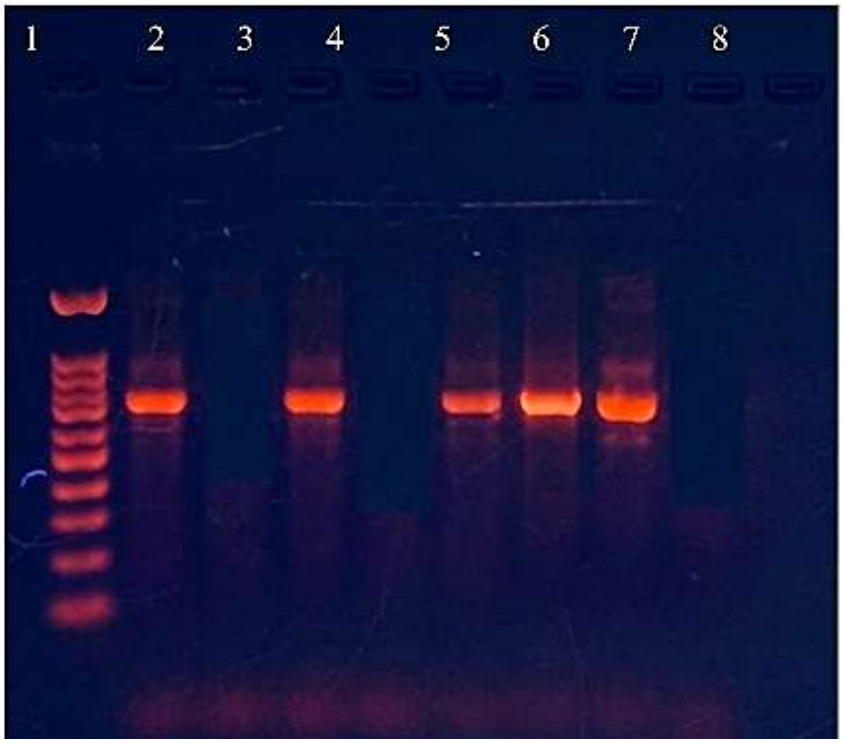


**Fig. 5.** The level of DNA analysis of human lymphocyte exposure to $H_2O_2$ and various plant extracts is shown in the table above.

Lane 1: DNA ladder ,Lane 2: Negative control, Lane 3: $H_2O_2$ (100 mU $mol^{-1}$), Lane 4: *E. vesicaria* + $H_2O_2$, Lane 5: *L. didymium* + $H_2O_2$, Lane 6: *D. harra* + $H_2O_2$, Lane 7: Lymphocytes only, Lane 8: *Capsula* sp. + $H_2O_2$

For years, plants of the Brassicaceae family have been a subject of fascinating research due to their rich chemical composition of bioactive compounds. Extracts from these plants exhibit a variety of beneficial effects with high biological value in treating diseases, thanks to their bioactive properties anti-obesity, anticancer, antimicrobial, antioxidant [23], hepatoprotective, cardioprotective, gastrointestinal protective, anti-inflammatory, anti-anemic, and immunomodulatory[24]. This review summarizes the chemical composition, describes the bioactive compounds isolated from plant extracts, and highlights their diverse biological activities, particularly their antimicrobial and antioxidant properties [25]. As a source of natural bioactive agents, Brassicaceae plants hold promising potential for improving human nutrition and health.

This widespread distribution suggests a shared biochemical base among these species, supporting their traditional use in folk medicine and reinforcing their credibility as sources of natural remedies [26].

The chemical constituents of secondary metabolites in *Brassicaceae* (Cruciferous*)* species include essential oils, flavonoids, coumarins, sesquiterpene coumarins, sulfur compounds, and various terpenes [127]. These compounds are abundantly present in leaves and flowers of this plant. Specific components identified in species include dimethyl trisulfide, dimethyl tetrasulfide, alpha-pinene, lavandulyl 2-methylbutanoate, alpha-terpinyl isobutyrate, alpha-terpinyl n-pentanoate, germacrene, himachalene, carotane, humulene, guaienes, ducane esters, farnesiferol A and B, sinkiangenin C and E, along with numerous monoterpenes, preformed coumarins, and plant estrogens [28]. These compounds have been found to exhibit various biological activities such as antimicrobial, insecticidal, antioxidant, and cytotoxic properties [29]. The diversity of these components contributes to the plant's pharmacological potential, making it a valuable resource for natural product research and drug development. The presence of plant estrogens and other bioactive compounds in of *Brassicaceae* (Cruciferous*)* species suggests potential therapeutic applications, including hormone-related treatments and antioxidant therapies [30]. The identification and characterization of these compounds are essential for understanding the plant's medicinal properties and for exploring its use in various pharmaceutical and therapeutic applications.

They reach their highest levels, which may indicate their high capacity to generate antioxidants to protect cells from oxidative stress[31]. Previous literature has pointed to another wild species of *Sinapis, S. arvensis* (wild mustard), as a potential source of antioxidant compounds due to its content of active compounds. Studies on extracts obtained from various wild species of the Brassicaceae family, using different extraction procedures [32],

have shown that the hydroalcoholic flower extracts have stronger free radical scavenging and reducing properties than those of the leaves and stems [33]. The flower extracts have also been found to contain higher amounts of total polyphenols. In contrast, our investigations highlighted a stronger primary antioxidant capacity in the leaf extract, while the flower extract exhibited superior secondary antioxidant properties [34.35].

The studies suggest that extracts of Brassicaceae family may possess in vitro antioxidant properties, which could be beneficial in preventing or treating various diseases. The activity may vary when using different solvents for traditional extraction. The DPPH assay is a commonly used in vitro method for assessing the antioxidant activity of wild cabbage species by measuring their ability to absorb 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH), Tannins are the most concentrated category in general in the extracts studied, hence the biological result of the extract *E. vesicaria* had the highest antioxidant activity (DPPH). Studies of wild cabbage species, including *B. incana*, *B. macrocarpa*, *B. villosa*, and *B. rupestris*, demonstrate a diversity of antioxidant activities associated with their phenolic and ascorbic acid content. Absorption activity in the DPPH assay is primarily related to the concentration of ascorbic acid and phenolic compounds, particularly flavanol derivatives such as kaempferol and quercetin (Kiefer et al., 2025). Different plant parts and extracts exhibit concentration-dependent antioxidant activity [36], although quantitative analyses showed that total tannins were the most concentrated class in the majority of the studied species, the superior biological performance of the *E. vesicaria* extract in the DPPH assay (inhibition exceeding 85%) suggests that the antioxidant activity is not necessarily directly and quantitatively related to the highest tannin concentration alone, The extract of the plant (*Eruca vesicaria*) showed strong antioxidant activity, achieving a free radical inhibition rate of 85.3% at a concentration of 100g/ml. This superiority is likely due to the synergistic effect of the complete chemical composition, particularly the contribution of flavonoids and low molecular weight phenolic acids. These compounds often exhibit greater efficiency in donating hydrogen atoms and stabilizing free radicals compared to high molecular weight tannins, thus increasing the overall free radical scavenging capacity of the extract and making the qualitative profile of the phenolic compounds more important than the quantitative concentration of any single class. Antioxidant potency is often expressed as $IC_{50}$ values (the concentration required to inhibit 50% of DPPH radicals). For example, extracts with high phenolic content tend to show lower $IC_{50}$ values, indicating stronger antioxidant potential, wild cabbage species possess remarkable antioxidant activity according to DPPH assessment , mainly due to their rich phenolic and ascorbic acid content. These findings support the genetic and biochemical diversity of wild abbage relatives as valuable sources of natural antioxidants [37,38.39].

The results obtained highlight the protective properties and activities of plant extracts against oxidative DNA damage in a human lymphocyte model. The results showed that peroxide hydroxin causes extensive DNA damage, manifested as complete lysis and agarose gel staining. It acts as a precursor to free radicals, particularly the free hydroxyl radical $OH^-$ via the Fenton reaction in the presence of transition metal ions such as iron and copper [40]. This is one of the most destructive forms of DNA oxidation, leading to chain breakage and the formation of oxidation products such as 8-hydroxy-2'-deoxyguanosine. Molecular Mechanisms Behind Complete Protection [41].

The exceptional performance of extracts from D. harra and E. vesicaria. (from the Brassicaceae family, or rich in their active compounds) in providing complete protection for DNA points to their exceptional content of potent antioxidants and active compounds such as phenols, flavonoids, and tannins [42], which act as free radical scavengers , neutralizing free hydroxyl radicals directly before they can attack DNA; and glucosinolates and their derivatives (such as isothiocyanates), compounds characteristic of the cruciferous vegetable family that may modulate enzymes [43], enhancing the cells' ability to detoxify oxidants internally (for example, by increasing the activity of catalase or glutathione enzymes, complete protection indicates that these extracts were able to almost completely prevent damage, making them promising candidates for chemoprevention applications [44]. The variation in partial protective efficacy reflects the differences in degradation levels between the extracts 1.2 bp for E. vesicaria extract versus 86.7 bp for Z. spinosa extract, and the differences in the quantitative antioxidant capacity and bioavailability of the active compounds. The *E. vesicaria* extract, which exhibited a low degradation value (208.9 bp), likely possesses an effective concentration of compounds that react rapidly with $H_2O_2$ or reduce its uptake by cells. This level of residual damage may represent damage that the extracts were unable to neutralize during the experimental period. The *Z. spinosa* extract (545.6 bp) suggests that the active compounds were less efficient or present at lower concentrations, allowing for greater oxidative damage [45.56]. This gradient in protection reinforces the structure-activity relationship of plant compounds in the face of oxidative stress.

## Conclusion

This study confirmed the pharmacological value of Brassicaceae plants in the Iraqi environment. The extract exhibited the highest free radical inhibition rate (over 85%), exceeding that of standard ascorbic acid (approximately 80%). This potent activity is attributed to the high concentration or specific synergistic effect of phenolic compounds and glucosinolates, a characteristic feature of the Brassicaceae family. In contrast, quantitative analysis revealed a predominance of tannins in most species, while *D. harra* exhibited the highest

levels of phenolic compounds and carotenoids. The species *D. harra* and *E. vesicaria* are promising candidates for the development of bio-chemo preventive agents or functional food supplements. The findings provide a solid scientific basis for studies to isolate and chemically characterize the compounds responsible for this superior genetic protection, opening the door to utilizing these native plant resources in strategies to prevent degenerative diseases associated with oxidative stress and DNA damage.


## ACKNOWLEDGMENTS

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### Conflict of Interest

The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

All procedures involving human blood samples were reviewed and approved by the Ethics Committee of the Biotechnology Center at the University of Baghdad (Approval No. 2344/GF/2025). Written informed consent was obtained from all healthy donors prior to blood collection.

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