Changes in anatomical features, phytochemical screening, and antibacterial activity of certain Brassicaceae genera in Iraq

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Abstract

This study investigates the phytochemical composition, anatomical features, and antibacterial potential of methanolic leaf extracts of three Brassicaceae species, *Diplotaxis harra, Eruca vesicaria*, and *Lepidium coronopus* collected from similar desert habitats in southern Iraq's Salman region. Anatomical analysis revealed distinctive epidermal cell morphologies, anisocytic stomata, and non-glandular trichomes between the species. Phytochemical characterization identified the presence of alkaloids (1.02–1.76%), glycosides (0.42–0.64%), tannins (4.0–10.0%), and coumarins (0.03–0.10%) while saponins were not detected in any species. Flavonoids were observed in *D. harra* and *E. vesicaria* but not in *L. coronopus*. The extracts were assayed for antibacterial activities against five clinically relevant bacterial strains by well diffusion assay: *Klebsiella pneumoniae*, *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris,* and *Staphylococcus aureus*. The highest antibacterial activity was demonstrated by the methanol extract of D. harra against S. aureus (17.0 mm), and the minimum inhibitory concentration (MIC) of 1.3 mg/mL was recorded for *L. coronopus* against the same pathogen. The lowest activity was exhibited with the methanol extract of *L. coronopus* and *D. harra* against *K. pneumoniae* (6.5 mm and 6.6 mm, respectively), where MIC assays were 8.0 mg/mL and 16.0 mg/mL, respectively. These findings emphasize the therapeutic promise of Iraq Brassicaceae species, particularly *D. harra*, as natural sources of antibacterial compounds against antibiotic-resistant pathogens. Also, this study recommends further isolation and characterization of bioactive compounds to harness their pharmaceutical applications.

Key words: epidermal cell, flavonoids, MIC assays, morphological attributes

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Introduction

Historically, herbal medicines had a significant role in the management of a wide range of health problems, and today they can be receiving a renaissance in modern medicine owing to the challenge of synthetic drugs (Li and Weng, 2017). Chemical drugs can be riddled with problems such as high side effects, low utility, and microbial resistance that can make them useless (Ventola, 2015). If this is the case, rediscovery of herbal drugs and the discovery of new bioactive compounds from natural products can be one

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possible approach that brings to market safer and better medicine (Fabricant and Farnsworth, 2001). This potentially solves issues with current prescribed drugs and allows for opportunity to promote less toxic, more efficacious therapies. The distribution of the Brassicaceae family is mainly throughout the Mediterranean region (Martínez-Sánchez et al., 2006). This family consists of more than 2,500 species in about 350 genera around the world. There are 177 species from 80 genera of this family in Iraq, making this family one of the top ten plant families of economic value in the country (Mathree and Aliwy, 2024). This family includes important vegetables such as cabbage, lettuce, broccoli, and kale (Almasoudi and Schlosser, 2021). The genera Diplotaxis DC. and Eruca Mill. have become more popular in recent years for their value as salad vegetables (Martínez-Sánchez et al., 2006; Pasini et al., 2011). Some species of this family have anticancer properties (Heimler et al., 2006). Of these, the genus *Lepidium* L. is notable for its high content of bioactive alkaloids with notable pharmacological usefulness for things such as antidiabetic and anticancer uses (Muzammil et al., 2022).

Species within this family of plants show common floral features such as having 4 sepals 4 petals that are arranged in cross shape, 6 stamens, and dehiscent fruits (Ahmed and Aliwy, 2023). Much of the research emphasis of this group of species focuses on their medicinal attributes because of the variation in shape and biological activity associated with their natural compounds (Jurinjak Tušek et al., 2022). Some of these species have been traditionally utilized to treat some extremely serious health conditions. For example, *Diplotaxis harra* (Forssk.) Boiss. species contains cardiac glycosides and other molecules utilized for cancer, heart disease, and asthma (Badalamenti et al., 2024). *Eruca vesicaria* (L) Cav. also contains bioactive molecules with potentially therapeutic outcomes, though with potential side effects of nausea or decreased heart rate (Al-Snafi, 2022). *Lepidium* coronopus (L.) Al-Shehbaz contains over 100 different types of alkaloids, some of which exhibit anticancer, antibacterial, and antidiabetic properties. (Muzammil et al., 2022). These plants are also a good source of important nutrients like iron, phosphorus, and potassium (Yuan et al., 2021). In addition, phytochemicals such as dithiolthiones inhibit cancer by improving enzyme activity (Fahey et al., 2001)while sterols, fatty acids, and glucosinolates aid in the identification and classification of species belonging to this group (Bell and Wagstaff, 2014). In this research, phytochemical content and antibacterial activity of some Brassicaceae species from southern Iraq are examined. By comparison of some species and their applicability, this research elucidates their significance in nature and their potential in the future to be utilized in medicine and pharmaceuticals.

Material and Methods

Sampling sites

Despite being situated within the arid Samawa desert, the three sampling sites in the Salman area exhibit notable ecological variation due to differences in topography, soil composition, moisture availability, and exposure to wind and solar radiation. The northern region (31.5°N, 45.5°E) is slightly elevated, characterized by compact sandy-loam soil with moderate drainage and occasional rainfall runoff, which enhances soil moisture retention and supports more continuous vegetation across partially stabilized sand dunes. In contrast, the southern region (30.5°N, 45.5°E) features looser, sandier soils with low water-holding capacity, heightened solar radiation, and pronounced temperature fluctuations, all of which contribute to increased wind erosion and drought stress, thereby selecting for xerophytic plant adaptations. Meanwhile, the eastern region (31.48°N, 44.9°E), located near sedimentary flatlands, contains fine silt deposits that produce compacted and less permeable soils, often accompanied by saline patches resulting from shallow groundwater. These conditions affect surface temperatures and ion uptake, fostering salt tolerance in local flora. Collectively, these microhabitat differences impose varied environmental pressures that drive morphological, physiological, and genetic adaptations in plant species, contributing to the ecological and evolutionary diversity observed within this desert ecosystem

Plant sample collection

Plants of three Brassicaceae species *Diplotaxis harra*, *Eruca vesicaria*, and *Lepidium coronopus* were collected during their pre-flowering stage (March–April) from three distinct microhabitats within the Salman region of the Samawa desert, southern Iraq. All three species were sampled across the following coordinates: Northern region: 31.5°N, 45.5°E, Southern region: 30.5°N, 45.5°E, and Eastern region: 31.48°N, 44.9°E.

The observed variations in plant traits likely reflect habitat adaptations. Plant samples were washed, and rotten leaves were discarded. Identification was made using the Flora of Iraq and the herbarium of the University of Baghdad. Leaves were air-dried (25-30 °C), powdered, and kept in labeled containers with species name, part used, and date. Samples were kept in the herbarium as well.

Moisture content was measured by weighing 10 grams of fresh leaves, air-drying, and re-weighing, applying the formula: Moisture (%) = [(Initial weight − Dry weight) / Initial weight] × 100

For pH analysis, a mixture of 10 g powder and 50 mL distilled water was prepared, shaken for ten minutes, filtered, and the pH was measured (Agafonova et al., 2019). Crude extracts and alkaloids' MIC against pathogenic bacteria were tested. ANOVA and LSD at 0.05 levels of significance compared MIC variations (Sokal and Rohlf, 1987).

Plant extract preparation

The hot alcoholic extraction technique in Azwanida (2015) was employed. Through this procedure, 30 g of each powder of a plant species was individually weighed and loaded on top of the Soxhlet equipment loaded with 250 mL of 80% methanol. It was extracted for 7 hours at 60 °C. After filtration with Whatman No. 1 filter paper and evaporation in rotary-evaporator to yield a sticky solution. The solution was left to dry to a solid at room temperature to yield a dry powder, which was used as the crude extract.

Microorganisms

Bacterial reference strains were acquired from Baghdad University of Botany. Five pathogenic strains of clinical isolates (*E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. aureus*, and *P. aeruginosa*) were employed. They were preserved at 4 °C and then subcultured on agar plates (Mueller-Hinton agar) at 37 °C for 24 h prior to any experiments were performed.

Test for of alkaloids

Alkaloids were detected following the standard protocol by Das et al.(Das et al., 2020). Ten grams of dried leaves from each plant species were boiled in 50 mL of 4% hydrochloric acid (HCl) prepared in distilled water. The mixture was filtered, cooled, and then assayed using the following reagents: white precipitate formation with Mayer's reagent indicating alkaloid presence (Lifongo et al., 2014).

Test for glycosides

The detection of glycosides was carried out using the Evans (2009) protocol. During the first test, equal quantities of plant extract were used and mixed with Fehling's solution, and a red precipitate formed after 10 minutes of boiling, proving the presence of glycosidic compounds. For confirmatory test, 5 mL of the extract was mixed with a few drops of Kedde's reagent. Purple coloration shows the presence of glycosides.

Test for essential oil

The essential oils were assayed as per the method described in the Indian Ayurveda Pharmacopoeia (Joshi et al., 2017). Ten (10) ml of each filtered leaf extract was spilled over filter paper and subjected to ultraviolet radiation at 365 nm wavelength. The development of a pale pink color was taken as an indicator of the essential oil presence.

Test for tannins

The tannin was detected according to Das et al. (Das et al., 2020) protocol. Five grams of leaf extract from every plant was pounded and boiled afterwards with 50 mL of distilled water. The extract was cooled and filtered. The resulting

filtrate was equally divided into two equal portions:

• To the first half, 1% lead acetate solution was poured.

• To the second half, 1% ferric chloride solution was added.

Flavonoid determination

Flavonoid identification was performed according to Evans (Evans, 2009). During the process:

• Solution A: 10 g of leaf powder was dissolved in 5 mL of 95% ethanol

• Solution B: A mixture of 10 mL of 50% ethanol and 10 mL of 50% KOH solution was prepared.

To detect flavonoids, solutions A and B were mixed in equal proportions. The presence of yellow color showed the presence of flavonoid compounds.

Test for coumarin

Coumarin identification was performed as described by Zhang et al. (2024). A small amount each of the plant extracts was pipetted into test tubes and filter paper was wetted with diluted NaOH solution. The test tubes were heated in a water bath for a few minutes. The filter paper, while being heated, was exposed to ultraviolet light. Yellow-green fluorescence in ultraviolet light indicated the presence of coumarins.

Test for resin

Resins were identified according to the process described by Zhang et al. (2024). A 10 mL aliquot of each of the extracts was blended with 20 mL of acidified distilled water (4% HCl). Solution turbidity is evidence of the presence of resins.

Test for saponin

Detection of saponin was done according to Cheok et al. (2014). For this test, the plant extract was shaken thoroughly in a test tube. The presence of stable foam indicates the presence of saponins. The addition of 0.5 mL $HgCl_2$ to 1.5 mL extract resulted in a white precipitation, confirming saponin presence.

Media preparation

Nutrient agar (23 g, Sigma-Aldrich, Germany) was dissolved in 1 L distilled water by boiling. The solution was autoclaved (121 °C, 15 min), left to cool down to room temperature, then poured into Petri dishes at ~45 °C. Plates were left to solidify for 30-40 min on a flat surface.

Antibacterial assay

The antimicrobial activity of plant extracts and alkaloid fractions was determined by the disc diffusion method. Bacterial strains' overnight culture was diluted to an equivalent turbidity of 0.1 at 600 nm (A600) or approximately 3.2×10^8 CFU/100 µL. Suspension of 20 µL was uniformly spread over sterile plates of nutrient agar (20 mL/plate) with sterile cotton swabs. Plates were dried by incubation for 3 minutes before use.

Sterile 5 mm diameter discs of filter paper were loaded with 100 µL of plant extracts (40 mg/disc) and placed on inoculated agar plates. Reference antibiotic discs (kanamycin, 30 µg/disc) were used. plates were maintained at 37 °C (standard microbiological temperature) for a 24-hour incubation period. Inhibition zone diameters were determined in millimeters by using a ruler after incubation. The experiments were conducted in triplicate, and the results were presented as means ± standard deviation.

Minimum inhibitory concentration (MIC)

Quantitative antibacterial activity was assessed by standardized broth dilution methods. Methanolic extracts of *D. harra*, L. coronopus, and *E. vesicaria* were made in serial dilutions between 1.3 and 32 mg/ml and were added to the nutrient broth with test bacterial strains. The tubes were incubated for 24 hours at 37 °C, after which turbidity check showed bacterial growth.

Anatomical methods

During the 2021-2022 growing season, fresh leaves and young stem segments of Brassicaceae species were collected from the southern region

Table 1
Antimicrobial effects of methanol extracts (5-30 mg/mL) on pathogenic bacteria (mm)

Plant extract	Zone of inhibition		
	Microorganism	ME	Average
Diplotaxis harra	*S. aureus*	16.5	17.0
	E. coli	11.3	13.9
	P. vulgaris	12.0	14.5
	P. aeruginosa	10.0	10.2
	K. pneumoniae	6.2	6.6
Eruca vesicaria	*S. aureus*	15.9	16.4
	E. coli	11.5	12.2
	P. vulgaris	11.4	11.7
	P. aeruginosa	9.5	10.1
	K. pneumoniae	7.3	7.4
Lepidium coronopus	*S. aureus*	14.8	15.3
	E. coli	11.6	12.4
	P. vulgaris	10.0	11.5
	P. aeruginosa	9.0	10.3
	K. pneumoniae	6.2	6.5

Values are mean inhibition zone (mm) ± S.D of three replicates. ME: methanol

of Iraq for anatomical and micromorphological analysis. Immediately after harvest, samples were fixed in FAA solution (formalin–acetic acid–alcohol) for at least 48 hours to preserve tissue structure, using a standard botanical fixative composed of 90 mL of 70% ethanol, 5 mL of glacial acetic acid, and 5 mL of 40% formaldehyde. Following fixation, specimens were transferred to 70% ethanol to prevent dehydration and maintain tissue integrity until further processing. Transverse and longitudinal sections (10-15 µm thick) of the midrib, lamina, and stem were prepared using a Leica RM2125 rotary microtome. These sections underwent a double-staining procedure with Safranin O and Fast Green FCF, following Johansen's (1940) protocol with slight modifications: staining in 1% Safranin O (in 50% ethanol) for 2-3 hours to highlight lignified tissues, followed by rinsing in 50% ethanol and counterstaining with 0.5% Fast Green FCF (in 95% ethanol) for 30 seconds to 1 minute to distinguish non-lignified tissues(Karuppaiyan and Kagita, 2006). The sections were then dehydrated through a graded ethanol series, cleared with xylene, and permanently mounted in DPX on clean slides. Microscopic examinations were conducted using an Olympus CH4 compound light microscope at 100×, 400×, and 1000× magnifications (the latter using oil immersion for epidermal detail), with images captured via a DCE-2 digital camera integrated with image analysis software. Quantitative and qualitative data on stomatal density and distribution across adaxial and abaxial leaf surfaces were recorded from calibrated micrographs, and the stomatal index (SI) was calculated based on the formula proposed by Mansfield (2012). Stomatal types and epidermal cell patterns were classified according to Inamdar et al. (1986). Additionally, trichome types, density, and distribution were documented using light microscopy, with representative images obtained for interspecific morphological comparisons.

Results

In the present study, 80% methanol was used for the extraction of leaf samples of the three Brassicaceae species, *E. vesicaria, D. harra, and L. coronopus*. The percentage yield of Leaf extracts varied between species. The highest percentage yield was in *L. coronopus* (8.7%), and the lowest was in *E. vesicaria* (5.8%) (Table 4 and Fig. I). This yield variation is caused by the characteristic phytochemical composition and tissue nature of the specific plant, which controls the extraction of bioactive substances. Maximum activity against *Staphylococcus aureus* in antibacterial screening

Table 2
Phytochemical analysis of active compounds in the leaves of Brassicaceae Species

Active Ingredients	*Diplotaxis harra* (Leaves)	*Eruca vesicaria* (Leaves)	*Lepidium coronopus* (Leaves)	Positive result	Reagent
Alkaloids	+	+	+	Orange precipitate	Dragendorff, Mayer
Glycosides	+	+	+	Red precipitate	Benedict, Fehling, kedd
Essential Oils	+	+	+	Pink color	UV exposure
Tannins	+	+	+	Gel-like precipitate	Lead acetate, Ferric Chloride
Flavonoids	+	+	-	Yellow color	Ethyl alcohol + KOH
Coumarins	+	+	+	Yellow-green color	UV exposure
Resins	+	+	+	Cloudiness	Add water acidified with HCl
Saponins*	-	-	-	Thick foam	Vigorous shaking, mercury chloride

* Saponins were not detected in any of the tested species.

was demonstrated by *D. harra* with the highest inhibition zone diameter (17.0 mm). MIC test revealed that *L. coronopus* extract exhibited the lowest MIC value against *Staphylococcus aureus* and hence the highest antibacterial activity against *Staphylococcus aureus*. Phytochemical screening of a few plant extracts is given in Table 2. The leaves of all three plants showed the presence of tannins, glycosides, alkaloids, and coumarins. Glycosides and Alkaloids are outstanding metabolic substances characteristic of the mustard family. Saponin test was negative in the leaves. The resins and volatile oils, which were also analyzed, presented varying results from one species to another. The flavonoid test was also positive in both *D. harra* and *E. vesicaria* crude extracts, but negative for *L. coronopus*. Most alkaloids (1.76%), tannin (8%), and flavonoids were found in L. coronopus while the lowest alkaloid (0.96%), tannin (4%), and coumarin (0.03%) contents were in *E. vesicaria*. The highest coumarin content was in *D. harra* leaves at (0.10%). Additionally, glycoside estimation results in Table (2) indicated that *L. coronopus* leaves contained the highest glycoside content at 0.64%.

In the anatomical study, structural differences between the species were evident (Fig. VI). The maximum length of epidermal cells (301 µm) measured on the lower surface of *E. vesicaria* was significantly greater than the minimum length (19 µm) on the upper surface of *L. coronopus*, which could indicate different adaptations to

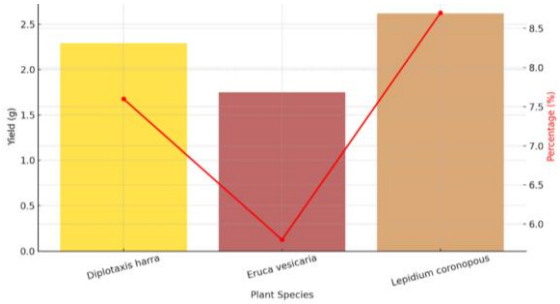

Fig. I. Yield and percentage of different Brassicaceae species

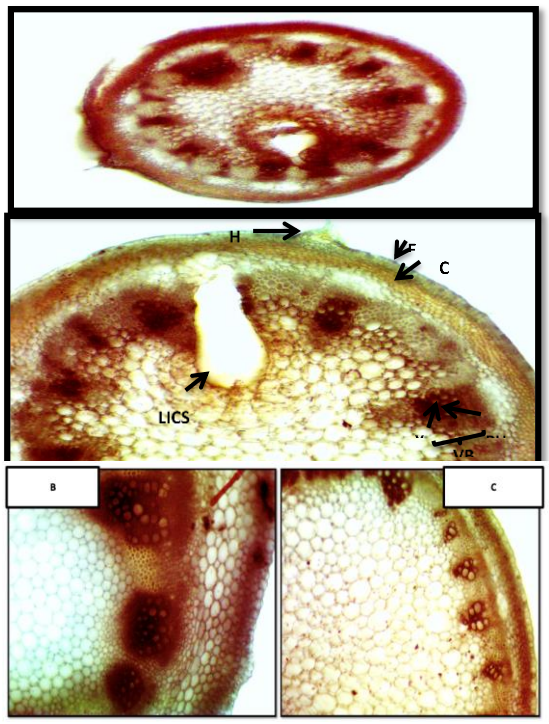

Fig. II. Transverse section of stem (scale 60 µm) *A- D. harra* B- *E. vesicaria* C- *L. coronopus*

Table 3
Minimum inhibitory concentration (MIC, mg/mL) of Brassicaceae leaf extracts against pathogenic bacteria

Bacteria	*Lepidium coronopus*	*Eruca vesicaria*	*Diplotaxis harra*	L.S.D 0.05
S. aureus	1.3	2.0	2.6	1.22
E. coli	2.2	3.3	4.0	1.03
P. vulgaris	2.7	4.0	5.3	1.78
Ps. aeruginosa	4.0	4.0	6.6	8.94
K. pneumoniae	8.0	16.0	16.0	1.79
L.S.D 0.05	1.04	0.94	2.81	----

L.S.D 0.05: Least significant difference at a 0.05 probability level

Table 4
Percentage yield of leaf extracts from the Brassicaceae species.

Plant Species	Initial weight (g)	Yield (%)	Percentage (%)	Morphological characteristics
D. harra	30	2.29	7.6	Yellow powder with a metallic sheen
E. vesicaria	30	1.75	5.8**	Dark brown powder
L. coronopus	30	2.62	8.7*	Yellowish-brown powder

* The highest percentage yield; **The lowest Percentage yield

Table 5
Anatomical characters of leaves in some Brassicaceae species (in micrometers)

No.	Species	Type of mesophyll	Cuticle		Epidermis		Blade				
							Lamina Thickness	Palisade layers		Spongy layers	
			upper	lower	upper	lower		Thickness	No	Thickness	No
1	*Diplotaxis harra*	Isobilateral	1.9-2.5 (2.5)	1.5-3.3 (3.5)	15-25 (20.5)	20 -30 (25.5)	185.3-190.3 (180.2)	77.2-85.3 (80.3)	3	88.5-97.5 (95.5)	5
2	*Lepidium coronopus*	Bifacial	1.5-3.1 (3.3)	2.5-3.4 (3.5)	20.1-25 (23.5)	20.7-35.3 (33.5)	110.5-123.2 (112.4)	60.2-71.5 (66.3)	3	59.2-68.5 (63.4)	3
3	*Eruca vesicaria*	Bifacial	1.6-3.5 (2.5)	2.5-3.1 (2.5)	25.5-35.1 (30.1)	25.3-41.4 (35.5)	260.5-277.2 (274.1)	166.5-176.4 (170.3)	3	99.5-115.8 (100.5)	3

environmental conditions. (Figs. III and IV, Table 7). The maximum length of ECs (301 µm) was measured in the lower surface of *E. vesicaria*, and the minimum (19 µm) in the upper surface of *L. coronopus.* The arrangement of bundles in *E. vesicaria* and *L. coronopus* was a discrete ring type, and in *D. harra* of Irregular ring type, xylem vessels (parallel to the exterior and shaped like a ring), arrangement of phloem fibers (2-4 regular layers around the phloem tissue). *E. vesicaria* was distinguished from the other two species by its hollow stem. The type of mesenchyme in *D. harra* was isobilateral, and in the other two species it is Bifacial. Table 5 and Figure (III) provide a summary of the characteristics and measurements of epidermal cells.

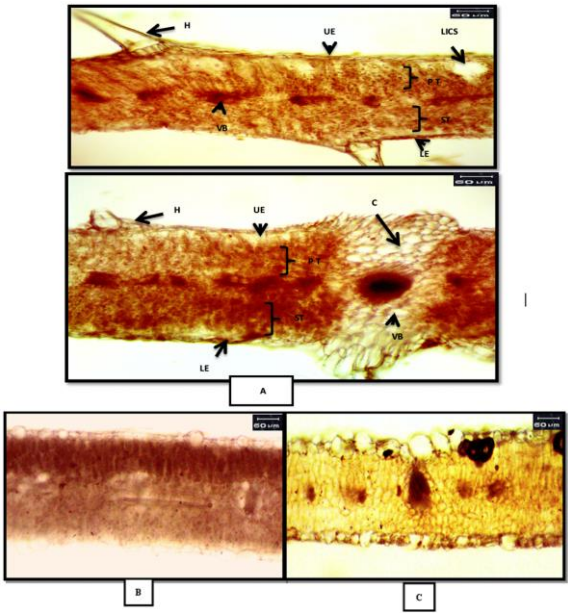

Fig. III. Transverse section of leaf lamina (scale 60 µm) A- *D. harra* B- *E. vesicaria* C- *L. coronopus*

Table 6
Anatomical characters of stems in some Brassicaceae species (in micrometers)

No	Species	Shape of Stem	Stem diameter	Cuticle	Epidermis	Cortex				Vascular Bundle			Pith Diameter
						Collenchyma			Parenchyma				
						Th.	No	Type		dimension	Treachery Column	No of Column Element	
1	*Diplotaxis harra*	Rounded	(2750-1625) 2250.92	3.5-4.5 (4.1)	18.1-25.5 (23.5)	28.5-33.2 (30.5)	2	Angular	20.5-27.1 (25.5)	108.3-112.5 (110.5)	3-6	6-12	107.9-115.5 (113.4)
2	*Eruca vesicaria*	Rectangle	(1300- 850) (1066.66)	2.5-4.1 (3.5)	16.4-20.2 (19. 5)	17.1-22.3 (20.2)	3	Angular	71.6-82.3 (79.5)	41.2-49.5 (47.1)	6-8	6-12	.....
3	*Lepidium coronopus*	Circular sinuate	1985-952 1671.25	2.5-3.6 (2.8)	16.4-22.1 (20.5)	20.1-26.1 (25.5)	3	Angular	23.6-28.9 (27.4)	58.5-66.1 (60.5)	3-4	3-6	339.5-345.1 (340.5)

Table 7
Measurements of cells, stomata, and stomatal index

Species	Cell dimensions (µm)				No./(mm) of:		Stomata of Adaxial epidermis					Stomata of Abaxial epidermis				
	Upper epidermis		Lower epidermis		Epidermal cells		length	Width	Stomatal index	Anticlinal wall	Type	Length	Width	Stomatal Index	Anticlinal Wall	Type
	Length	Width	Length	Width	Upper	Lower										
Diplotaxis harra	42.5-100 (58.32)	15-30 (24.37)	25-100 (35.75)	12.5-50 (29.37)	600-900 (720)	1440-7780 (980)	15.5-20.9 (17.3)	12.3-16.8 (15.5)	12.5	Wavy	Anomocytic	12.4-14.3 (13.6)	13.2-15.5 (13.6)	13	Wavy	Anomocytic
Lepidium coronopus	19-100 (50.12)	22.5-55 (43.75)	30-50 (39.15)	30-101 (72.22)	600-1260 (984)	840-1200) (1009)	14.1-18.2 (16.4)	10.4-14.5 (11.5)	16.5	Wavy	Anomocytic	15.5-17.1 (16.2)	12.5-15.1 (14.4)	20.5	Wavy	Anomocytic
Eruca vesicaria	37.5-175 (90.42)	17.5-42.5 (26.25)	75-301 (188.22)	20-62.5 (36.07)	550-750 (661)	600-780 (681.42)	15.2-17.2 (16.5)	9.5-11.1 (10.5)	16	Wavy	Anomocytic	11.5-14.4 (13.1)	10.2-13.4 (12.2)	20	Wavy	Anomocytic

Discussion

Recent studies have emphasized the antioxidant and anticancer potential of plants in the Brassicaceae family, but this study provides new insights into the phytochemical diversity and antibacterial potential of three Iraqi species (Khoobchandani, M., et al, 2010). The striking differences in chemical compositions and biological activities of these species highlight their value as potential sources for the development of new therapeutic agents (Wu et al., 2023; Cartea et al., 2008). The results of this study highlight the striking difference in phytochemical compositions and antibacterial potential of leaf-derived extracts from the three Brassicaceae species. Specifically, the varying yields of extracts and active compound contents may reflect the unique biological and chemical characteristics of each plant. This could be attributed to the organic properties of methanol and its strong ability to dissolve a wide range of organic and antibacterial constituents (Cowan, 1999). In particular, *D. harra*, with its remarkable efficacy against Staphylococcus aureus, a multi-antibiotic resistant pathogen, is considered a promising source of novel antimicrobial compounds. This finding is in agreement with prior research on *Diplotaxis*

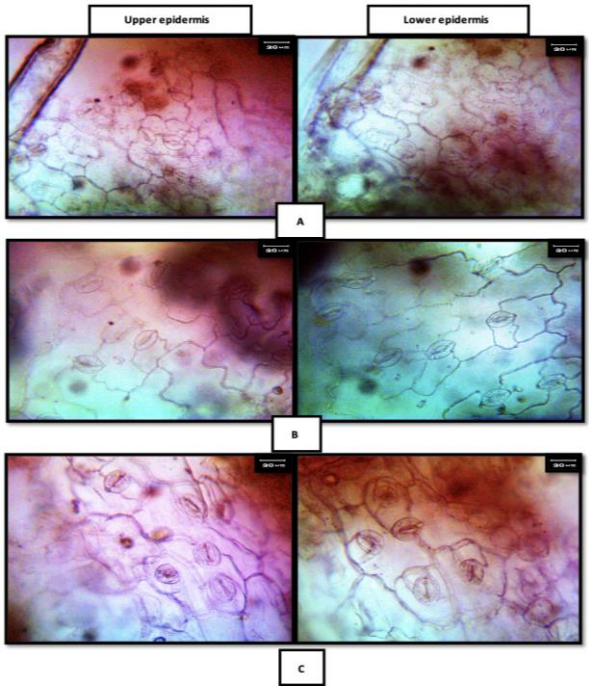

Fig. IV. The upper and lower surface of the leaves (Scale 20 µm); A: *D. harra* B: *E. vesicaria* C*: L. coronopus*

species, which indicates that compounds such as glucosinolates and isothiocyanates (such as sativin) play a key role in inhibiting pathogens (Fechner et al., 2018). The possible mechanism of this activity may include disruption of the bacterial cell membrane, inhibition of essential enzymes (such as DNA gyrase), and induction of oxidative stress (Akiyama et al., 2001; Roy et al., 2022).

The modest antibacterial activity observed in methanol extracts of *L. coronopus* and *D. harra* against *K. pneumoniae* highlights their limited standalone efficacy compared to other plant antimicrobial agents reported. The discrepancy in the obtained results may stem from variations in the chemical composition of essential oils extracted from a specific plant species, which are influenced by factors such as harvest season, plant age, and growth stage. Additionally, blending essential oils derived from different parts of the plant (e.g., leaves, flowers, or roots) can lead to significant differences in the final composition, as each part contains distinct chemical properties (Ashrafi et al., 2017)

The low antibacterial effect of *L. coronopus* and *D. harra* against *K. pneumoniae* can be attributed to several factors underlying the mechanisms of pathogen resistance strategies. *K*. *pneumoniae* exhibits multidrug resistance mainly through the production of β-lactamases, which degrade antibiotics and make them ineffective (Li et al., 2024).

The three species harbored alkaloids, glycosides, tannins, and coumarins as phytochemical constituents, and these are generally well known for their medicinal values. Alkaloids and tannins have already been shown in the past to possess antibacterial properties through some work, and this could be the cause of the antibacterial activities observed here, particularly against resistant strains such as Staphylococcus aureus (Akiyama et al., 2001).

Moreover, according to Hossain et al. (2021), tannins are identified by their strong astringent action and their effectiveness against bacteria. The second prominent finding in this study was flavonoids and coumarin in *D. harra* and *E. vesicaria*. Flavonoids have been effective chemotaxonomic indicators in the genus Diplotaxis (Caruso et al., 2018). Additionally, rocket species have been previously reported to be good sources of glucosinolates (GSLs) and flavonoids (Bell and Wagstaff, 2014). Flavonoids and coumarins are anti-inflammatory and antioxidant agents possessing medicinal properties in these plants (Roy et al., 2022). Several studies have validated that coumarins and flavonoids are beneficial to human health since they suppress inflammation and oxidative stress (Das et al., 2020; Roy et al., 2022). Moreover, the greatest MIC of all the extracts was *L. coronopus* against *Staphylococcus aureus*. This offers greater potential for such a plant towards the utilization of antibacterial drugs. With ongoing antibiotic resistance also being one of the most formidable challenges in the management of bacterial infection, the utilization of antibacterial plants as medicine drugs, such as *L. coronopus*, can offer another alternative or ancillary solution to fight resistant forms of bacteria. The conclusions of this study exhibit the huge potential of *D. harra, E. vesicaria*, and *L. coronopus* as pharmaceutical and medicinal products.

Various anatomical studies in Brassicaceae family genera have been conducted to identify their diagnostic significant characters. These studies highlight the presence or absence of chlorenchyma, collenchyma, and pith, as well as epidermal surface features and mesophyll structure, as significant characteristics for species identification within Brassicaceae (Tekin and Martin, 2017).

Two types of mesophylls were observed: Isobilateral mesophyll in *D. harra* and Bifacial mesophyll in *E. vesicaria* and *L. coronopus* species, supporting the findings from earlier studies (Qader, 2018). Moreover, the distribution of vascular bundles (VBs) also differs among these species—forming a discrete ring in *E. vesicaria* and *L. coronopus*, and an irregular ring in *D. harra*. These findings are consistent with the descriptions provided by Alamgir (2017). The epidermal cells (ECs) are polygonal isodiametric or elongated polygonal in shape, featuring non-glandular, unicellular, and unbranched trichomes, as reported by Kadhem and Alnomani (2017). In all

three species, stomata are of anisocytic type, consistent with previous information (Mousavi and Sharifi-Rad, 2014).

These analytical methods are used to better understand the relationship between plant morphology and function in different environments. The striking differences in stomatal type, stem structure, and content of active compounds (such as flavonoids and alkaloids) serve as a suitable tool for identifying species.

The three species, though collected from ecologically similar habitats in southern Iraq, displayed notable variations in their phytochemical composition and antibacterial potential.

Despite these findings, more comprehensive studies and clinical evaluations are required to substantiate the results and explore the clinical relevance of the plant species. Furthermore, more intensive investigations of their chemical composition and bioactivity will further contribute to the creation of new therapeutic agents with specific medicinal effects.

Conclusion

The present study has shown that leaf extracts of Iraqi Brassicaceae species such as *L. coronopus*, *D. harr*, and *E. vesicaria* exhibit excellent antibacterial activity against several pathogenic bacteria such as P. aeruginosa, *S. aureus, P. vulgaris*, *K. pneumoniae*, and *E. coli*. Bioactive compounds such as alkaloids, flavonoids, glycosides, tannins, essential oils, glucosinolates, and isothiocyanates are the main components in their antibacterial activities. These are not only because of the species adaptation to similar ecological conditions in southern Iraq, but also because of their phytochemical diversity. The results are consistent with previous research into the antibacterial activity of compounds of members of this family, and they strengthen their potential for further study against other microorganisms.

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