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Phytochemical assay, and *in vitro* assessment of the antioxidant, antibacterial, and anti-inflammatory properties of *Beta vulgaris* L. (Amaranthaceae), and *Corchorus olitorius* L. (Malvaceae) crude leaf extracts

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Abstract

Background and objective: The traditional herbs *Beta vulgaris* and *Corchorus olitorius* are cultivated in Iraq and recorded in the Kurdish ethnobotany for various health problems including expectorant, laxative, diuretic, and anti-inflammatory and to relieve fever and pain. This study aimed to assess and compare the different biological properties of methanolic leaf extracts of *Beta vulgaris* and *Corchorus olitorius* cultivated in Iraq and recognize various classes of phytoconstituents present in each one.

Methods: The bioactive entities in the crude methanolic leaves extracts of *Beta vulgaris* and *Corchorus olitorius* were subjected to phytochemical evaluations. The antioxidant and anti-inflammatory activities were evaluated with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and egg albumin denaturation assays, respectively. The leaf extracts were tested for antibacterial properties using the agar well diffusion and broth microdilution methods.

Results: The crude methanolic extracts of *Beta vulgaris* and *Corchorus olitorius* showed the presence of carbohydrates, alkaloids, cardioactive glycosides, coumarins, flavonoids, phenols, tannins, terpenoids, and steroids. The *Beta vulgaris* displayed higher gallic acid content 417.90±0.52 mg/gm of the extract compared to 79.85±0.99 mg/gm for *Corchorus olitorius* with (P <0.001).The antioxidant activity of *Beta vulgaris* leaves extract was significantly higher with (P = 0.035) in reference to ascorbic acid. Among the selected bacterial species, *Klebsiella pneumoniae* revealed pronounced sensitivity towards the methanolic extracts of both plants. The *Corchorus olitorius* leaves extract showed higher antibacterial activity than *Beta vulgaris*. *Beta vulgaris* presented greater anti-inflammatory activity at 1000 µg/mL with inhibition of 93.0% compared to 83.2% using acetylsalicylic acid as positive control.

Conclusion: The methanolic leaf extracts of *Beta vulgaris* and *Corchorus olitorius* possess antibacterial, antioxidant, and anti-inflammatory potentials.

Keywords: *Beta vulgaris*; *Corchorus olitorius*; Antioxidant; Egg albumin denaturation; Agar well diffusion assay.

Introduction

The elevated reactive oxygen species (ROS) levels are a result of an imbalance in the favor of oxidative stress and results in an increase in the levels of several free radicals like hydroxyl, peroxide, and superoxide in the physiologic system which could be very destructive to macromolecules including DNA, lipids, and proteins. The impairment of macromolecules may lead to carcinogenesis, immunosuppression, Parkinson's disease, Alzheimer's disease, atherosclerosis, inflammation, infectious disease, chronic obstructive pulmonary disease, cataract formation, diabetes, hypertension, aging, and hair loss.[1]

Previous works on animal models demonstrated that synthetic antioxidants like butylated hydroxyl anisole, butylated hydroxyl toluene, and propyl gallate, were found to create internal and external

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bleeding at high doses.[2]

Since antiquity, herbs and their parts like fruit, vegetables, and spices were used for several illnesses. The rapid development of multiple-drug resistance against antimicrobials and anticancer agents in addition to the toxicities raised from impurities of synthetic agents suggests the discovery of new antibacterial, antioxidant, and anti-inflammatory drugs from alternative sources.[3] Inflammation is the response progression of living tissues to stimuli induced by inflammatory mediators like harmful chemical irritations, physical damages, microbial infections, and heat. As a consequence of cellular response to inflammation, certain pathological changes like redness, swelling, heat, and pain will be associated with impaired physiological functions. Inflammation is involved in the pathogenesis of many diseases including cancer, arthritis, and stroke. Arthritis is an inflammatory disease that occurs as a result of protein denaturation and incidence of the inflammatory response to various stimuli.[4]

Beta vulgaris, commonly known as Swiss Chard, Al-Salq in both Arabic and Kurdish, belongs to the Amaranthaceae family. It is originated from subtropical and tropical countries in Africa and Asia, and cultivated in Iraq.[5] *Beta vulgaris* has been reported to possess medicinal and nutritional properties and used in food preparations. The *Beta vulgaris* roots have been traditionally used as an expectorant, diuretic, laxative, and in the treatment of liver and mental disorders. While the plant leaves were applied for inflammatory conditions, liver disease, spleen problem, paralysis, act as a tonic, and diuretic.[6] According to the previous studies, *Beta vulgaris* considered as a good source for omega-3, phenolic acid, proteins, fibers, flavonoids, carotenoids (ß-carotene, lutein, and zeaxanthin), minerals (magnesium, copper, calcium, sodium, etc.), and vitamins (A, C, K, and B-complex group).[7]

Corchorus olitorius commonly known as Jute Mallow and Mulukhiyah in Arabic and Kurdish belongs to the Malvaceae family, is an annual herb, and is widely distributed in Nigeria, Egypt, Sudan, Malaysia, South America, the Philippines, India, Caribbean, and cultivated in Iraq.[8] Jute Mallow is a medicinal herb, used as a vegetable. This plant is widely used in traditional medicines for fever, tumor, pain, appetite, diuretic, demulcent, and chroniccystitis.[9] Phytochemical investigations of *Corchorius olitorius* have resulted in the identification of phenolic acids, flavonoids, minerals (iron, phosphorus, calcium, and potassium), carotene, vitamins (B1, B2, C, and E), and aminoacids.[10]

A small number of studies were concerned with the biological properties of *Beta vulgaris* and *Corchorus olitorius* leaves. Therefore, the main objective of the current study was to determine the plant metabolites in both species and assess the antioxidant, antibacterial, and anti-inflammatory activities of methanolic crude leaves extracts of *Beta vulgaris* and *Corchorus olitorius* which are cultivated in Iraq.

Methods

Plant collection and extraction

The fresh leaves of *Beta vulgaris* and *Corchorus olitorius* were collected from the Erbil, Kurdistan Region, Iraq. The plant was authenticated by Assist. Prof. Dr. Alaadin Naqishbandi and a voucher specimen (code: A-16, 17) was deposited at the Pharmacognosy Department, Pharmacy College, Hawler Medical University. The fresh leaves were rinsed from debris and dried under shade at 40-45°C. About 400 gm of the powdered dried leaves from each plant were subjected for sonication (Greatsonic, China) in 500 mL methanol (Chem-Lab, Belgium) for one hour at 40 °C. The supernatant was then evaporated to dryness under vacuum and reduced temperature (40-50 °C) with a rotary evaporator (Buchi Rotavator®, Switzerland).[11,12]

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Phytochemical analysis

Qualitative screening tests

The crude methanolic extracts of both *Beta vulgaris* and *Corchorus olitorius* were exposed for qualitative chemical assays to identify the target metabolites. The involved chemical tests included Molisch's test for carbohydrates, Fehling's test for reducing sugars, Dragendorff's and Hager's test for alkaloids, foam test for saponins, Borntrager's test for anthraquinones, Keller -killani test for cardioactive glycosides, sodium hydroxide test for coumarins, aluminum chloride, and potassium hydroxide test for flavonoids, ninhydrin test for proteins and amino acids, ferric chloride test for phenols and tannins, hydrochloric acid for phlobatannins, Salkowski test for terpenoids and steroids, sodium hydroxide and hydrochloric acid for volatile oils according to standard procedures.[13,14]

Quantitative screening tests

Total phenolic contents

The total amount of phenolic compounds was assessed using the Folin-Ciocalteu method against gallic acid (GA) as a standard.[15,16] A stock solution from the crude leaves methanolic extracts, and gallic acid solutions were prepared. To find the total phenolic contents, a serial dilution from each stock solution was prepared in the following sequence, 12.5, 25, 50, 100, and 200µg/mL and 100 µL, then were added into separate test tubes containing 1.5mL of Folin-Ciocalteu reagent (Merck, Germany). Following 5 min, 4mL of 20% sodium carbonate solution (Thomas Baker PVT., India) was added to the tube and continued the volume to 10mL with distilled water. The sample was incubated for an additional 30 min in the dark and absorbance was read against the blank at 738 nm using a spectrophotometer (UV-Vis Shimadzu). Each test was achieved in triplicate. The results were expressed as µg gallic acid per gram of the dried extract, using the equation ($y=0.0043x+0.0203$, $R^2 = 0.996$) obtained from a calibration curve of gallic acid, Figure 1.

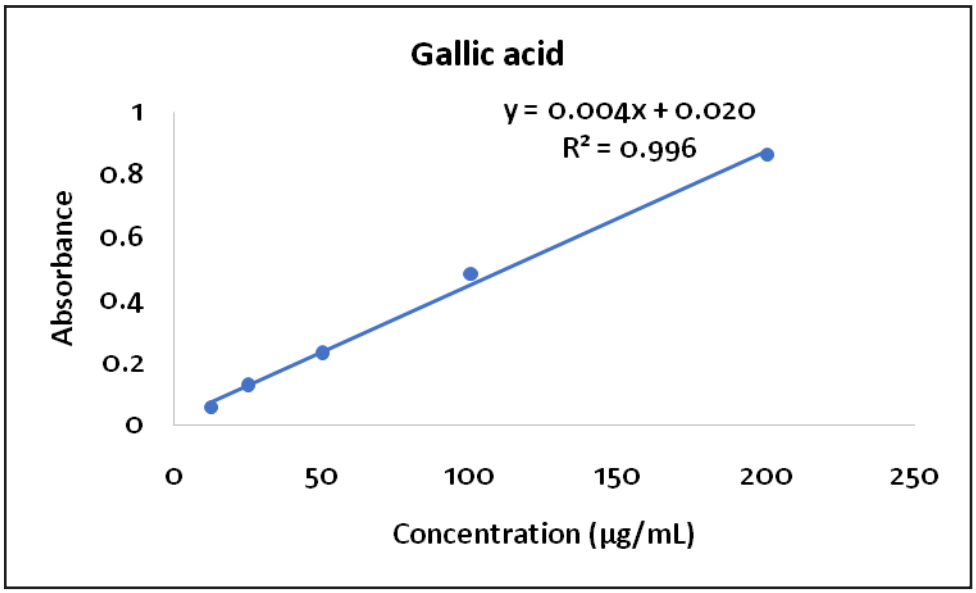

Figure 1 Linear regression plots and correlation coefficients of total phenolic contents of *Beta vulgaris* and *Corchorus olitorius* leave extracts

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Total flavonoid contents

The total amount of flavonoids was assessed by the colorimetric method against aluminum chloride using quercetin as a standard.[17] A stock solution from the crude leaves methanolic extracts and quercetin (QU) was prepared. To find the total flavonoid content, a serial dilution from each stock solution was prepared using the sequence, 12.5, 25, 50, 100 and 200 µg/mL, and 100 µL. Aliquots of 1mL from each sample was mixed with 4 mL distilled water, 3mL (10%) sodium nitrate solution (Thomas Baker PVT., India), 3mLof (10%) aluminum chloride solution (Sigma-Aldrich, Germany), and 2.0mL of (5%) sodium hydroxide solution (Thomas Baker PVT., India). This mixture was maintained in darkness at room temperature for 30 min, and the absorbance was measured at 510 nm. Each test was achieved in triplicate. The results were expressed as µg quercetinper gram of the dry extract, using the equation ($y = 0.007x + 0.036$, $R^2 = 0.983$) obtained from a calibration curve of quercetin, Figure 2.

Biological activity assessment

Antioxidant activity assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was conducted for evaluation of the antioxidant potential of crude leaf extracts, be consistent according to the previously established method by González-Palma et al.[18] Briefly, stock solutions had been prepared by dissolving 10 mg of the leaf extract and ascorbic acid (Positive control) (Sigma-Aldrich, Germany) in 10 mL methanol. Aliquots from the serial dilution samples of the stock solutions were added to 1.5 mL (0.1 mM) DPPH solution (Merck, Germany) to produce a final concentration (31.25-500 µg/mL). The negative control was displaced by methanol instead of the extract solution. The tested solutions were maintained in the dark for 30 min, and the absorbance was measured at 517 nm. Three parallel measurements were achieved for each sample.

The free radical scavenging capacity is expressed as a percentage of inhibition, and calculated using the following equation:

Inhibition% =(Acontrol – Asample)/Acontrol*100

Where (Acontrol) is the absorbance of the negative control, and (Asample) is the absorbance of the DPPH solution following the addition of the sample. The EC_{50} was calculated graphically from the regression plots as the effective concentration of the extract utilized for the scavenging of 50% of the free radicals and expressed as mean (µg/mL) ± standard deviation (SD).

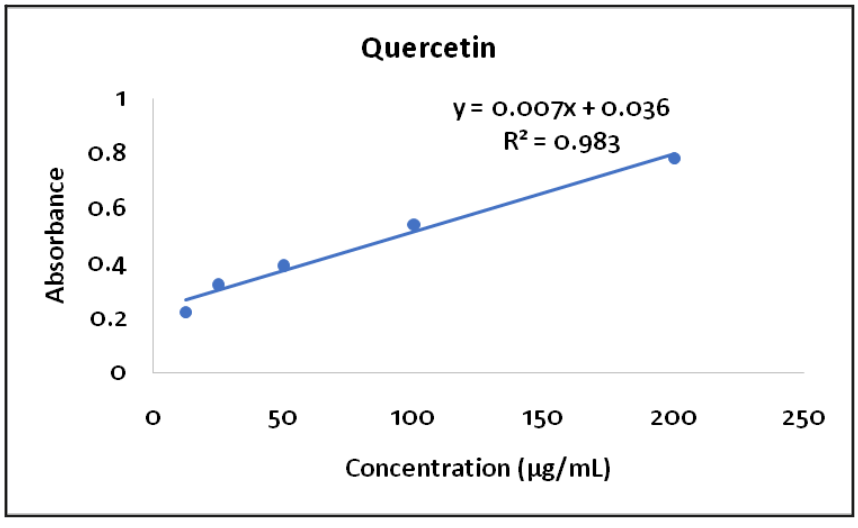

Figure 2 Linear regression plots and correlation coefficients of total flavonoid contents of *Beta vulgaris* and *Corchorus olitorius* leave extracts

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Antibacterial activity assay

Preparation of Inoculum

The antimicrobial activities of crude extracts were tested against Gram-positive *Staphylococcus aureus, Staphylococcus epidermidis,* and Gram-negative *Escherichia coli* and *Klebsiella pneumoniae* isolated pathogenic bacteria from human urine. The isolated bacteria strains were identified and confirmed by morphological studies and biochemical tests in the Microbiology Laboratory of the Biology Department, College of Education, Salahaddin University.

The selected microorganisms were pre-cultured in Mueller Hinton agar (Rashmi diagnostic PVT, Ltd, India) overnight at 37° C, then deposited at 4°C until used.

Agar well diffusion assay

The agar well diffusion method was used to screen the antibacterial activity of the crude leaves extracts of the selected plants and streptomycin was used as a positive control (Panpharma SA, France) against various pathogenic bacteria.[19] One hundred µL of the newly activated bacterial strain was prepared at turbidity equivalent to 0.5 McFarland, was spread with sterile cotton swabs over a Muller Hinton agar Petri dish. 5.0 mm wells were made using a sterile cork borer into the agar plates followed by the addition of 100 µL of pre-prepared extract in 10% DMSO (Sigma-Aldrich, Taufkirchen, Germany) at 500 and 250 mg concentrations, a 10 µM of streptomycin was added to each well. The plates were placed in acold area for 15-30 min to allow extract distribution throughout the agar medium, followed by incubation at 37°C for 18-24 h, latter the plates were examined for zone inhibition appearance.[20]

Determination of minimum inhibitory concentrations (MIC) using broth microdilution method

Stock solutions of 1000 mg/mL of each crude leave extracts were prepared by re suspending extracts in 10 % DMSO. From each stock solution, twofold serial dilutions of the crude leaves' extracts were prepared directly in a 96-flat sterile well plates containing nutrient broth (Rashmi diagnostic PVT, Ltd, India) to achieve the desired concentrations. The bacterial suspensions' concentrations were adjusted to 0.5 McFarland turbidity using 10 µL samples from the test microorganisms and added to the adjusted microplate wells. The plates were covered and incubated for 24 h at 37°C and a solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (Taizhou Xianju, Pharma. Co., China) at a concentration 2% was added in each well of the 96-well plate and was incubated at 37°C for a further 30 min. The wells holding the bacterial growth changed to violet color whereas the well without bacterial growth remained yellow. The MIC was measured as the lowest concentration of the leave extract that prevented bacterial growth.[21]

Anti-inflammatory activity assay

The anti-inflammatory activity of the methanolic crude leaf extracts of both plants was evaluated using the egg albumin denaturation assay.[22] Briefly, 1 mL of the leaf extracts or acetylsalicylic acid (Positive standard) (Bayer, Germany) at a concentration (250, 500, and 1000 µg/mL) and 3 mL of phosphate-buffered saline (pH 6.5) was mixed with 2 mL of egg albumin and incubated at 25°C for 15 min. The mixture was denatured in a 65°C water bath for 12 min. Distilled water was used as a negative control. After cooling, the absorbance was measured at 660 nm by using double distilled water as the blank. Each test was achieved in triplicate. The protein denaturation inhibition percentage was assessed with the following formula:

Inhibition (%) =As-Ac /Ac*100

Where As= absorbance of sample;
Ac= absorbance of control.

Statistical analysis

In vitro, biological activity data were expressed as mean ± standard deviation (SD) of the three replicates using Microsoft Excel 2016. The Student's t-test (for two independent samples) was used for statistical analysis with the SPSS software

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program (version 18.0). The differences were considered statistically significant when $P < 0.05$.

Results

Phytochemical study of the *Beta vulgaris* and *Corchorus olitorius* crude leaves extracts

The yield, color, and consistency of both plants' crude methanolic extracts are shown in Table 1. The results demonstrated obvious differences between the yields of *Beta vulgaris and Corchorus olitorius*. The higher quantity of *Beta vulgaris* methanolic crude extract (8.85%) was yielded by sonication. The preliminary phytochemical investigation was achieved on methanolic extracts of *Beta vulgaris* and *Corchorus olitorius*, the outcomes showed the presence of carbohydrates, alkaloids, cardio active glycosides, coumarins, flavonoids, phenols, tannins, phlobatannins, terpenoids, and steroids, in both plants. Reducing sugars and proteins were only present in *Beta vulgaris*. Anthraquinone and saponin glycosides were absent in both plants, Table 2. The color intensity results were expressed as (+Ve) for faint colors and (++Ve) for more intense colorsoutcomes.[13,14]

Table 1 Extraction yield percentage by ultrasonic-assisted extractor method.

Plants	Yields (%)	Color	Consistency
Beta vulgaris	8.85	Green	Solid
Corchorus olitorius	6.55	Green	Solid

Table 2 Preliminary phytochemical screening of the *Beta vulgaris* and *Corchorus olitorius* leaves

Phytochemical compounds	Test performed	*Beta vulgaris*	*Corchorus olitorius*
Carbohydrates	Molisch's test	+	+
	Fehling test	+	-
Alkaloids	Dragendorff's test	+	++
	Hager's test	+	++
Glycosides	Foam test	-	-
	Bontrager's test	-	-
	Keller-Killani test	+	++
	Coumarin test	+	++
	Aluminum chloride test	+	+
	Potassium hydroxide test	+	+
Proteins and amino acids	Ninhydrin test	++	-
Phenols and tannins	Ferric chloride test	+	+
Phlobatannins	1% Hydrochloric acid	+	++
Terpenoids and Steroids	Salkowski test	+	++
Volatile oils	NaOH &HCl	++	+

*(-) Referred to the absence of phytochemical constituents, (+) referred to the presence of mild amounts of phytochemicals (Light color), (++) referred to the presence of moderate amounts of phytochemicals (Intense color). Foam test (-) referred to lack of foam formation in plant extracts.

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The methanolic crude leaves extracts of both *Beta vulgaris* and *Corchorus olitorius* showed the highest phenolic compounds contents compared to flavonoids, Table 3. The total phenolic contents of *Beta vulgaris* was 417.90±0.52 mg GA/gm extract, which is higher than that of *Corchorus olitorius* 79.85±0.99 mg GA/ gm extract with (*P* <0.001). Additionally, the flavonoid content of *Corchorus olitorius* 12.85±0.89 mg QU/gm extract was higher compared to *Beta vulgaris* 10.93±1.51 mg QU/gm extract with (*P = 0.042*).

DPPH radical scavenging analysis

The antioxidant activities of both plants extract and ascorbic acid were assessed at concentrations 31.25-500 µg/mL.

The DPPH free radical scavenging action was parallel to the used plants crude extracts and ascorbic acid samples, Figure 3, especially at 500 µg/mL. In general, the higher ratio of radical scavenging activity and lower EC_{50} values denote a greater antioxidant activity.[18]

Beta vulgaris methanolic extract revealed betterfree radical neutralization 93.28% (EC_{50}:195.49±1.63 µg/mL), compared to ascorbic acid 82% (EC_{50}: 197.93±1.22 µg/mL), (*P* = 0.035), Table 3 and Figure 3.

On the other hand, *Corchorus olitorius* extract presented weak antioxidant activity when compared to ascorbic acid with an EC_{50} value of 463.77±0.45 µg/mL (*P* = 0.028).

Table 3 The EC_{50} values of DPPH scavenging effect, TPC, and TFC of *Beta vulgaris* and *Corchorus olitorius* leaf extracts (Mean±SD)

Samples	DPPH (EC_{50})	[1]TPC (mg GA/gm extract)	*P*-value*	[2]TFC (mg QU/gm extract)	*P*-value*
Beta vulgaris	195.49±1.63	417.90±0.52	<0.001	10.93±1.51	0.042
Corchorus olitorius	463.77±0.45	79.85±0.99		12.85±0.89	
Ascorbic acid	197.93±1.22				

[1]TPC, Total phenolic content; [2]TFC, Total flavonoid content; GA, Gallic acid; QU, Quercetin; DPPH, 2,2-diphenyl-1-picrylhydrazyl.**P*-value represent differences between *Beta vulgaris* and *Corchorus olitorius.*

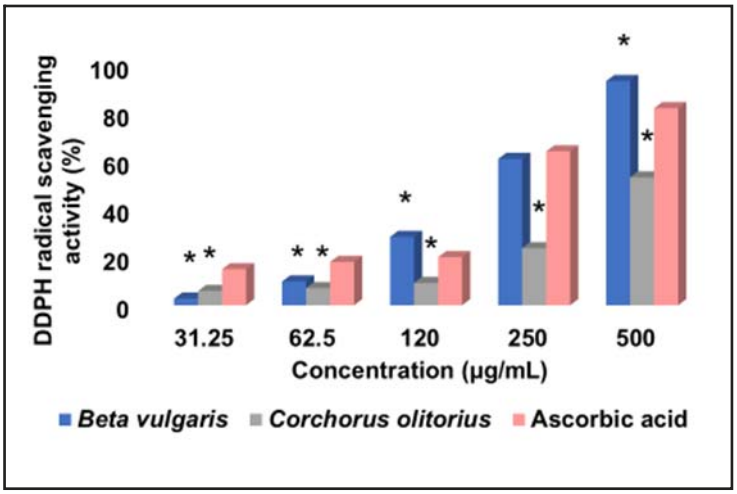

Figure 3 The DPPH radical scavenging capacity of ascorbic acid, *Beta vulgaris,* and *Corchorus olitorius* leaf extracts at concentration range (31.25-500 µg/mL) expressed as a percentage (n=3). *P*-values (**P <0.05*)

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Antibacterial activity

The *in vitro* antibacterial potentials of *Beta vulgaris* and *Corchorus olitorius* were estimated against four isolated bacterial strains. The consequences of screening of leave extracts, Table 4 revealed that *Beta vulgaris* and *Corchorus olitorius* were exerted more pronounced activity against the selected Gram-negative strains than Gram-positive bacteria. Among the tested microorganisms, *Klebsiella pneumoniae* were more sensitive against tested samples with inhibition zones diameters 12±0.62 mm for *Beta vulgaris* and 14±2.62 mm for *Corchorus olitorius* at 500 mg/mL, along with MIC values ranging 62.5-125 mg/mL, Table 5.

By contrast, *Staphylococcus epidermidis* displayed resistance toward extracts. The results of Table 5 exhibited statistically significant differences between MIC of *Beta vulgaris* and *Corchorus olitorius* against *Staphylococcus aureus* and *Klebsiella pneumoniae* with (P <0.001) and (P = 0.021) respectively.

Table 4 Antibacterial activity of *Beta vulgaris* and *Corchorus olitorius* leaf extracts against four microorganisms

Bacteria	*Beta vulgaris*		*Corchorus olitorius*		Streptomycin
	Inhibition zone diameter (mm)[a]				
	250 mg	500 mg	250 mg	500 mg	10µg
Staphylococcus aureus	9±2.61	10 ± 1.41	10±1.33	13±0.71	30±1.94
Staphylococcus epidermidis	R	R	R	R	28±0.41
Escherichia coli	8±0.72	10 ± 0.59	9±0.99	12 ± 1.66	28±0.23
Klebsiella pneumoniae	10±1.44	12 ± 0.62	10±1.88	14±2.62	27±1.84

[a] Values are means of triplicate determination (n = 3) ± standard deviations. R, no zone of inhibition was found. Data presented as Mean ±SD

Table 5 MIC value of *Beta vulgaris* and *Corchorus olitorius* leaf extracts against different microorganisms (Mean±SD)

Bacteria	MIC (mg/mL)		*P*-value	MIC (mg/mL)
	Beta vulgaris	*Corchorus olitorius*		Streptomycin
Staphylococcus aureus	250 ± 1.46	62.5 ± 0.99	<0.001	0.039 ± 1.96
Escherichia coli	250 ± 0.73	250 ± 1.42	0.732	0.009±0.13
Klebsiella pneumoniae	125 ± 0.95	62.5 ± 1.36	0.021	0.039 ± 0.22

MIC; Minimum inhibitory concentration.

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Anti-inflammatory activity analysis

The anti-inflammatory properties of the tested samples demonstrated a concomitant rise in the protein denaturation inhibition as the concentration of methanolic crude leaves extracts and acetylsalicylic acid increased, Table 6. *Beta vulgaris* leaf extract exposed maximum protein denaturation inhibition 93.0%, followed by acetylsalicylic acid of 83.2%, then *Corchorus olitorius* of 70.5% especially at 1000 µg/mL.

Discussion

Natural products are one of the sources of important active constituents that should be investigated for their different biological activity.[23] The *in vitro* antioxidant study results demonstrated noticeable activity of methanolic leaves extracts and ascorbic acid with EC_{50} below 500 µg/mL. *Beta vulgaris* the methanolic crude leaves extract showed higher antioxidant activity 93.28% in comparison to standard ascorbic acid 82%.

The dissimilarity in the antioxidant behavior between the methanolic extracts resulted mainly from the existence of diverse types and concentrations of active constituents in *Beta vulgaris* and *Corchorus olitorius* as presented in Tables 2 and 3. The superior effect may be due to the higher phenolic contents 417.90±0.52 mg GA/gm extract in comparison to *Corchorus olitorius* 79.85±0.99 mg GA/gm extract. Generally, the high plant phenolic contents in the extracts could be a main contributory factor to the strong antioxidant behavior. Diverse investigations have been conducted on *Beta vulgaris* and *Corchorus olitorius* extracts using different diluents were in agreement with the present study results.[24,25]

The antibacterial activities of *Beta vulgaris* and *Corchorus olitorius* against various bacterial strains examined in this study and their effectiveness were qualitatively and quantitatively evaluated by the existence or absence of inhibition zones and the MIC values. The methanolic leaves extracts of both plants exhibited varied antibacterial activity across three studied bacterial strains. These outcomes are in accordance with the previous studies reporting antibacterial activity of *Beta vulgaris* leaves extract against *Escherichia coli, Bacillus cereus, Staphylococcus aureus*, and *Salmonella enteritidis*.[26]

Formerly reported antibacterial activity of crude leaves of *Corchorus olitorius* against *Salmonella enteriditis, Citrobacter fruendii, Enterobacter aerogenes, Klebsiella pneumoniae, Vibrio cholerae, Vibrio parahemolyticus and Salmonella typhi.*[27] In this study, Gram-negative bacteria displayed higher sensitivity toward the

Table 6 Anti-inflammatory effect of *Beta vulgaris* and *Corchorus olitorius* leaf extracts

Samples	Concentrations (µg/mL)	Inhibition of protein denaturation (%)
Acetylsalicylic acid	1000	83.2 ± 1.72
	500	80.0 ± 0.25
	250	75.5 ±1.28
Beta vulgaris	1000	93.0 ± 0.88
	500	59.7 ± 0.41
	250	55.1± 0.85
Corchorus olitorius	1000	70.5 ± 2.62
	500	54.7 ± 1.64
	250	6.3 ± 2.99

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methanolic leaves extracts compared to Gram-positive bacteria, due to the presence of biologically active components in methanolic leaf extracts with various polarities and different concentrations. The amphipathic nature of phytoconstituents plays an important role in the sensitivity, or resistance, of the microorganisms toward plant extracts. The outer membrane of the Gram-negative bacteria is possessed lipopolysaccharide molecules that provide a hydrophobic environment and facilitates the permeability of lipophilic molecules, at the same time small hydrophilic molecules can easily pass via the outer membrane due to the existence of porin proteins in the membrane.[28]

The methanolic extracts exhibited several phytoconstituents with various concentrations in each plant. A previous study stated that tannins, phenolic acid, and flavonoids have strong antibacterial activity and act via different mechanisms such as inactivation of microbial adhesions, transport-proteins, enzymes, and cell envelop, as well as their ability to form a complex with extracellular, soluble protein, and bacterial cell wall.[29]

Corchorus olitorius displayed the strongest antibacterial activity in comparison to *Beta vulgaris,* due to the high quantity of constituents with pronounced antibacterial activity.

Non steroidal anti-inflammatory agents are used for inflammation and pain relief attributed to their interference with protein denaturation. However, using these medications is associated with unwanted effects like gastric ulcers and cardiovascular complications.[30] The prevention capacity of natural products for protein denaturation implies potential anti-inflammatory activity.[31] Both plant extracts and acetylsalicylic acids demonstrated pronounced anti-inflammatory activity at tested concentrations (250, 500, and 1000 µg/mL). Interestingly, *Beta vulgaris* exhibited more anti-inflammatory properties compared to acetylsalicylic acids.

Earlier anti-inflammatory investigations for *Beta vulgaris* aqueous crude leaves extract using carrageenan-induced rat paw edema and cotton pellet granuloma methods for acute and chronic inflammations. The results showed dose-dependent anti-inflammatory activity which was comparable to indomethacin at 1000 mg/kg.[32] Another study reported significant anti-inflammatory action of aqueous *Corchorus olitorius* crude leaves' extract when applied on induced-paw edematous skin lesion with histopathological variations and declined TNF-α density in immunoreactive cells.[33]

Conclusion

The methanolic leaf extracts of *Beta vulgaris* and *Corchorus olitorius* were demonstrated various biological activities. The qualitative and quantitative screening revealed the presence of different pharmacologically active compounds like carbohydrates, alkaloids, cardioactive glycosides, coumarins, flavonoids, phenols, tannins, terpenoids, and steroids. The total phenolic content in both plants was greater than that of flavonoid contents. *Beta vulgaris* exhibited higher free radical scavenging activity 93.28%, compared to ascorbic acid 82% with ($P = 0.035$) especially at 500 µg/mL. In the antibacterial activity evaluation, Gram-negative bacteria revealed higher sensitivity against tested methanolic leaf extracts. *Corchorus olitorius* leaves extract presented greater antibacterial activity with inhibition zone diameter 14±2.62 mm than *Beta vulgaris* 12±0.62 mm against *Klebsiella pneumoniae* with MIC value ranging between 62.5±1.36 mg/mL and 125±0.95 mg/mL respectively. *Beta vulgaris* showed better anti-inflammatory activity with inhibition of 93.0% compared to 83.2% using acetylsalicylic acid (ASA) as positive control, specially at 1000 µg/mL.

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Competing interests

The author declares that she has no competing interests.

References

1. Iqbal E, Salim KA, Lim LB. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. J King Saud Univ Sci. 2015; 27(3):224–32. https://doi.org/10.1016/j.jksus.2015.02.003
2. Xu X, Liu A, Hu S, Ares I, Martínez-Larrañaga MR, Wang X, et al. Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action. Food Chem. 2021; 15(353):129488. https://doi.org/10.1016/j.foodchem.2021.129488
3. Gupta PD, Birdi TJ. Development of botanicals to combat antibiotic resistance. J Ayurveda Integr Med. 2017; 8(4):266–75. https://doi.org/10.1016/j.jaim.2017.05.004
4. Macfarlane E, Seibel MJ, Zhou H. Arthritis and the role of endogenous glucocorticoids. Bone Res. 2020; 8(1):1–7. https://doi.org/10.1038/s41413-020-00112-2
5. Maraie NK, Abdul-Jalil TZ, Alhamdany AT, Janabi HA. Phytochemical study of the Iraqi *Beta vulgaris* leaves and its clinical applications for the treatment of different dermatological diseases. World J Pharm Pharm Sci. 2014; 3(8):5–19.
6. Babarykin D, Smirnova G, Pundinsh I, Vasiljeva S, Krumina G, Agejchenko V. Red beet (*Beta vulgaris*) Impact on human health. J Biosci Med. 2019;7(3):61–79. https://doi.org/10.4236/jbm.2019.73007
7. Biondo PBF, Boeing JS, Barizão ÉO, Souza NEd, Matsushita M, Oliveira CCd, et al. Evaluation of beetroot (*Beta vulgaris* L.) leaves during its developmental stages: a chemical composition study. Food Sci Technol. 2014; 34:94–101. https://doi.org/10.1590/S0101-20612014005000007
8. Hasan HT, Kadhim EJ. Phytochemical investigation of *Corchorus olitorius* L. leaves cultivated in Iraq and it's in vitro antiviral activity. Iraqi J Pharm Sci. 2018; 27(2):115–22. https://doi.org/10.31351/vol27iss2pp115-122
9. Boye A, Barku V, Amoateng P, Mbroh L. Anti-nociceptive and antioxidant activities of an aqueous leaf extract of *Corchorus olitorius* L. (Tiliaceae). Int J Biol Chem Sci. 2014; 8(6):2395–406. https://doi.org/10.4314/ijbcs.v8i6.3
10. Choudhary SB, Sharma HK, Karmakar PG, Kumar A, Saha AR, Hazra P, et al. Nutritional profile of cultivated and wild jute ('Corchorus') species. Aust J Crop Sci. 2013; 7(13):1973–82.
11. Adham AN, Naqishbandi AM. HPLC analysis and antidiabetic effect of *Rheum ribes* root in type 2 diabetic patients. Zanco J Med Sci. 2015; 19(2):957–64. https://doi.org/10.15218/zjms.2015.0017
12. Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason Sonochem. 2017; 34:540–60. https://doi.org/10.1016/j.ultsonch.2016.06.035
13. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Int Pharm Sci. 2011; 1(1):98–106.
14. Kumara Swamy M, Sudipta KM, Lokesh P, Rashmi W, Vijay R, Ssn K et al. Phytochemical screening and in vitro antimicrobial activity of *Bougainvillea spectabilis* flower extracts. Int J phytomedicine. 2012; 4(3):375.
15. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants. 2019; 8(4):96. https://doi.org/10.3390/plants8040096
16. Adham AN, Muttalib LY, Naqishbandi AM, Ali SAB. Open-label uncontrolled pilot study on antipsoriatic activity of *Rosa hemisphaerica*. Zanco J Med Sci. 2017; 21(1):1636–44. https://doi.org/10.15218/zjms.2017.014
17. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med. 2012; 12(1):1–2. https://doi.org/10.1186/1472-6882-12-221
18. González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Téllez-Téllez M, Gupta VK, Díaz-Godínez G, et al. Evaluation of the antioxidant activity of aqueous and methanol extracts of *Pleurotus ostreatus* in different growth stages. Front Microbiol. 2016; 7:1099. https://doi.org/10.3389/fmicb.2016.01099
19. Manandhar S, Luitel S, Dahal RK. In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. J Trop Med. 2019; 2019. https://doi.org/10.1155/2019/1895340
20. Adham AN. Comparative antimicrobrial activity of peel and juice extract of citrus fruits growing in Kurdistan/Iraq. Am J Microbiol Res. 2015; 3(5):155–9. https://doi.org/10.12691/ajmr-3-5-1
21. Zarai Z, Kadri A, Chobba IB, Mansour RB, Bekir A, Mejdoub H, et al. The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of *Marrubium vulgare* L. essential oil grown in Tunisia. Lipids Health Dis. 2011; 10(1):1–8. https://doi.org/10.1186/1476-511X-10-161
22. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, da Silva JA, Rokni M, Sharifi-Rad M. Evaluation of biological activity and phenolic compounds of *Cardaria draba* (L.) extracts. J Biol Today's World. 2015; 4(9):180–9.

23. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. Metabolites. 2012; 2(2):303–36. https://doi.org/10.3390/metabo2020303
24. Yakoub ARB, Abdehedi O, Jridi M, Elfalleh W, Nasri M, Ferchichi A. Flavonoids, phenols, antioxidant, and antimicrobial activities in various extracts from Tossa jute leave (*Corchorus olitorus* L.). Ind Crops Prod. 2018; 118:206–13. https://doi.org/10.1016/j.indcrop.2018.03.047
25. Hajihosseini S, Setorki M. The antioxidant activity of Beta vulgaris leaf extract in improving scopolamine-induced spatial memory disorders in rats. Avicenna J Phytomedicine. 2017; 7(5):417.
26. Ahmadi S, Soleimanian-Zad S, Zaeim D. Antibacterial and Antifungal Activity of the Aqueous and Methanolic Extracts and Essential Oils of Red Beets *Beta vulgaris* Leaves. Zahedan J Res Med Sci. 2020; 22(3). https://doi.org/10.5812/zjrms.83725
27. Zakaria ZA, Zaiton H, Henie EF, Jais AM, Kasthuri D, Thenamutha M, et al. The in vitro antibacterial activity of *Corchorus olitorius* and *Muntingia calabura* extracts. J Pharmacol Toxicol. 2010; 5(8):480–6. https://doi.org/10.3923/jpt.2006.108.114
28. Ahmadi S, Soleimanian-Zad S, Zaeim D. Antibacterial and antifungal activity of the aqueous and methanolic extracts and essential oils of red beets *Beta vulgaris* leaves. Zahedan J Res Med Sci. 2020; 22(3):e83725.
29. Sharma A, Patel VK, Rawat S, Ramteke P, Verma R. Identification of the antibacterial component of some Indian medicinal plants against *Klebsiella pneumoniae*. Int J Pharm Pharm. 2010; 2(3):123–7.
30. Sostres C, Gargallo CJ, Arroyo MT, Lanas A. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2010; 24(2):121–32. https://doi.org/10.1016/j.bpg.2009.11.005
31. Bouhlali ET, Hmidani A, Bourkhis B, Khouya T, Ramchoun M, Filali-Zegzouti Y, Alem C. Phenolic profile and anti-inflammatory activity of four Moroccan date (*Phoenix dactylifera* L.) seed varieties. Heliyon. 2020; 6(2):e03436. https://doi.org/10.1016/j.heliyon.2020.e03436
32. Jain S, Garg VK, Sharma PK. Anti-inflammatory activity of aqueous extract of Beta vulgaris L. J Basic Clin Pharm. 2011; 2(2):83.
33. Handoussa H, Hanafi R, Eddiasty I, El-Gendy M, El Khatib A, Linscheid M, Mahran L, Ayoub N. Anti-inflammatory and cytotoxic activities of dietary phenolics isolated from *Corchorus olitorius* and *Vitis vinifera*. J Funct Foods. 2013; 5(3):1204–16. https://doi.org/10.1016/j.jff.2013.04.003