Italian Journal of Food Science, 2024; 36 (4): 193–207

Phytochemical profile and biological activities of *Brassica oleracea* var. *elongata* leaf and seed extracts: An *in vitro* study

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Received: 6 July 2024; Accepted: 19 September 2024; Published: 1 October 2024

ORIGINAL ARTICLE

Abstract

This study investigated the phytochemical composition and *in vitro* biological activities of leaf and seed extracts of *Brassica oleracea* var. *elongata*. Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and reducing sugars in both methanolic and aqueous extracts. Quantitative analysis showed that methanolic extracts contained higher levels of polyphenols and flavonoids than aqueous extracts, with methanolic seed extract exhibiting the highest polyphenol content (4.399±0.14 mg GAEq/g). High-performance liquid chromatography analysis identified significant concentrations of gallic acid (1180.20 µg/100 mg), chlorogenic acid (8323.831 µg/g), and quercetin (5512.58±0.02 µg/100 mg) in extracts. Antioxidant activity assays demonstrated potent effects, with methanolic leaf extract showing the strongest 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (IC_{50}: 0.058±0.001 mg/mL) and aqueous leaf extract exhibiting the highest activity in the β-carotene bleaching method (EC_{50}: 0.049±0.001 mg/mL). Ultraviolet protection potential was assessed, revealing promising sun protection factor (SPF) values of all extracts, with methanolic seed extract having the highest SPF of 45.58±10. Evaluation of anti-inflammatory activity revealed the aqueous leaf extract's remarkable inhibition of inflammation (94.8%). Antimicrobial assays showed notable antibacterial effects, particularly against Gram-positive bacteria. These findings underscore the therapeutic potential of *Brassica oleracea* extracts and warrant further exploration into their applications in both pharmaceutical and cosmetic industries.

Keywords: *Brassica oleracea* var. *elongata*; *in vitro* biological activities; HPLC analysis; phytochemical compounds

Introduction

Recent developments in functional foods and phytochemical products derived from different parts of medicinal and food plants, such as leaves, seeds, and fruits, have profoundly and positively impacted various aspects of human health and its nutritional life (Adeleke & Babalola, 2020; Banwo *et al.*, 2021; Teklić *et al.*, 2021). The mustard family, also known as *Brassicaceae* or *Cruciferae*, is a distinct group of flowering plants of the order Brassicales. Brassicaceae is a rich source of primary and secondary metabolites and (Franzke *et al.*, 2011; Jalal *et al.*, 2023).

Phytochemical screening is essential in analyzing plant-derived compounds for their therapeutic benefits. It involves identifying and characterizing bioactive compounds found in plants, such as alkaloids, flavonoids, tannins, terpenoids, and phenols (Ahmed *et al.*, 2018; Kumar *et al.*, 2015). This screening helps scientists to understand the chemical composition of plants and discover specific compounds that may have medicinal properties. Understanding the phytochemical profile helps uncover the potential pharmacological activities of plants and their applications in drug development, nutrition, and disease prevention (Kabera *et al.*, 2014).

In recent years, significant advancements are observed in functional foods and phytochemical products derived from medicinal and food plants. These developments have profoundly and positively impacted various aspects of human health and nutritional life (Adeleke & Babalola, 2020; Banwo *et al.*, 2021; Teklić *et al.*, 2021). One notable group of plants that has garnered attention is the mustard family, also known as Brassicaceae or *Cruciferae*. This distinct group of flowering plants, belonging to the order Brassicalesi, is known for its rich composition of primary and secondary metabolites, such as amino acids, sugars, indoles, phenolics, and glucosinolates. Many species within the Brassicaceae family have been identified as potential sources of health-promoting compounds (Franzke *et al.*, 2011).

This knowledge of phytochemical profiles enables researchers to explore the potential pharmacological activities of these plants, paving the way for their applications in drug development, nutrition, and prevention of diseases (Kabera *et al.*, 2014). The importance of phytochemical screening in analyzing plant-derived compounds cannot be overstated, as it plays a crucial role in unraveling the therapeutic potential and chemical diversity of natural plant sources.

Brassicaceae is a rich source of phytochemicals, including glucosinolates and phenolic compounds, as well as important nutrients such as vitamins, minerals, and fiber. As a result, a diet rich in Brassicaceae offers several health advantages (Jaiswal, 2020). Numerous scholarly investigations demonstrated a connection between broccoli's anticancer, antioxidant, antibacterial, anti-inflammatory, and antihypertensive characteristics (Elhouda-Mekhadmi *et al.*, 2024) and its abundance of bioactive compounds. Owing to its advantageous makeup, it may also be utilized to treat various medical conditions, such as diabetes, hypercholesterolemia, heart disease, and photosensitivity disorders (Jang *et al.*, 2015).

This study conducted the first-ever examination of the phytochemical composition and *in vitro* biological properties of *Brassica oleracea* var. *elongata*, a plant commonly utilized in traditional cuisine in the El Oued province of Algeria. Four distinct extracts of *Brassica oleracea* were prepared and analyzed, encompassing aqueous extracts of leaves and seeds, along with methanolic extracts of leaves and seeds. Phytochemical analysis was undertaken to identify and quantify various bioactive compounds within the extracts, such as alkaloids, flavonoids, terpenoids, phenols, tannins, and saponins. Quantitative analysis aimed to ascertain total phenolic content (TPC) and total flavonoid content (TFC) of extracts. Additionally, high-performance liquid chromatography (HPLC) analysis was employed to identify and quantify the profile of phenolic compounds of diverse extracts. Subsequently, the extracts underwent assessment for various *in vitro* biological activities, such as antioxidant, anti-hemolytic, sun protection factor (SPF), anti-inflammatory, and antibacterial activities.

Materials and Method

Plant Material

Aerial parts of *Brassica oleracea* (Brassicaceae) were collected in April 2022 from the Hassi Khelifa region (33°33'50.6" N, 7°0'19.3" E) in El-Oued, southeast Algeria. The plant studied in this work grew in a wild area where no human intervention occurred. The botanical identification of the plant material was carried out by Prof. Atef Chouikh, Department of Biology, El Oued University, Algeria. A representative voucher specimen was assigned the accession number LOST.St09/10 and deposited in the herbarium of the Faculty of Life and Natural Sciences at the same university for future reference. To ensure the quality and integrity of the plant material, harvested leaves were carefully air-dried in a shaded area, and protected from direct sunlight exposure. Once dried, the leaves were stored in airtight containers at ambient temperature until required for experimental procedures. In contrast, the seeds were partially crunched just before their use in the experiments to maintain their freshness and prevent any potential deterioration of their bioactive compounds.

Yield

Using methanol and water, the study extracted the plant's two parts (leaves and seeds). For methanolic extract, 5 g of each part was soaked in 50 mL of methanol for 24 h and concentrated with Buchi Rotavapor R-200. The aqueous extract was obtained by soaking 5 g of dried leaves and seeds in 50 mL of water for 24 h, followed by evaporation at 55°C; the extraction process was repeated for three times. Extract yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{P_1}{P_2} \times 100, \quad (1)$$

where P_1: weight of the extract dried (in grams), and P_2: weight of the plant starting material (in grams).

Phytochemical Study

Chemical screening

A comprehensive screening process was performed using standard precipitation and coloration reactions to evaluate the qualitative phytochemical composition of crude extracts. These tests aimed to identify the primary natural chemical groups and secondary metabolites present in the plant material. A combination of well-established methods was employed to characterize thoroughly the phytochemical profile of medicinal plants. The screening tests were conducted on both methanolic and aqueous extracts of *Brassica oleracea* leaves and seeds by following conventional protocols and utilizing appropriate reagents as outlined by Sofowara (Sofowara, 1993). The dry matter of *Brassica oleracea* leaves and seeds was subjected to a series of phytochemical tests to ascertain the presence of biologically active compounds, such as alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and reducing sugars, as described by Evans (Evans, 2009). This systematic approach ensured a comprehensive analysis of the plant's phytochemical constituents.

Determination of total polyphenols

The total polyphenol content (TPC) of *Brassica oleracea* extracts was quantified using the Folin–Ciocalteu (FC) reagent method, following the protocol described by Singleton and Rossi with minor modifications (Singleton & Rossi, 1965). In brief, 0.2 mL of extract solution (prepared by dissolving 0.2 mg of dry extract in 1 mL of methanol) was combined with 1.8 mL of deionized water in a test tube. Subsequently, 10 mL of 10% FC reagent and 8 mL of 7.5% sodium carbonate solution were added to the mixture. The reaction was allowed to proceed for 30 min, during which development of a characteristic blue color was observed. The absorbance of the resulting solution was measured at 7,600 nm using a spectrophotometer. A calibration curve was constructed using gallic acid as a standard reference compound, with concentrations ranging from 0 mg/L to 10 mg/L, plotting concentration versus respective absorbance. The linearity of the calibration curve was confirmed by a coefficient of determination (R^2) of 0.983. The TPC of *Brassica oleracea* extracts was calculated based on the calibration curve and expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight (DW) of extract. This standardized method enables the accurate determination of TPC in plant extracts, facilitating comparison with other studies.

Determination of total flavonoids

The TFC of four *Brassica oleracea* extracts was determined using the aluminum chloride colorimetric method, as described by (Zahnit *et al.*, 2022), with slight modifications. Quercetin was employed as a standard reference compound for the quantification of flavonoids. A 0.5-mL aliquot of each extract solution (prepared by dissolving 0.2 mg of dry extract in 1 mL of methanol) was mixed with an equal volume of 2% aluminum chloride ($AlCl_3$) solution. The mixture was incubated at room temperature for 50 min to allow the formation of a flavonoid–aluminum complex. The absorbance of the resulting solution was measured at 506 nm using a spectrophotometer. A calibration curve was constructed using quercetin standards at concentrations ranging from 0 mg/L to 10 mg/L, with R^2 = 0.988. The TFC of *Brassica oleracea* extracts was calculated based on the quercetin calibration curve and expressed as milligrams of quercetin equivalent per gram (mg QE/g) of DW of extract.

Reverse-phase HPLC fractionation and analysis

Being a widely used technique, this study used reverse-phase high-performance liquid chromatography (RP-HPLC) to separate, identify, and quantify phenolic compounds in *Brassica oleracea* extracts (Bart, 2005). The separation principle of HPLC is based on the differential partitioning of analytes between a stationary phase and a mobile phase. The RP-HPLC method was optimized according to Hemmami *et al.* (Hemmami *et al.*, 2020), utilizing a column with non-polar aliphatic residues as a stationary phase and a gradient system of acetonitrile (eluent A) and water–acetic acid (eluent B, 98.8:0.2, v/v) as a mobile phase. The column temperature was maintained at room temperature, and the flow rate was set at 1 mL/min. The column was equilibrated with a mobile phase for 300 min before sample injection. A sample volume of 20 µL (1 mg/mL plant extract solution) was injected, and the elution profile was monitored using an ultraviolet/visible light (UV/Vis) detector set at 268 nm. The gradient elution program was as follows: initial conditioning of 900% B, decreasing to 860% B at 60 min, 830% B at 160 min, 810% B at 230 min, and 77% B at 280 min. The composition was held at 77% B from 28–35 min, then decreased to 60% B at 38 min, and finally

returned to 90% B at 50 min, with a total run time of 50 min. The separation of compounds in the plant extract was achieved based on their molecular weight and polarity. The separated peaks were quantified by calibrating with standard compounds, including ascorbic acid, gallic acid, chlorogenic acid, caffeic acid, vanillin, rutin, and quercetin. Standard curves were constructed for each reference compound to determine the phenolic composition of extracts (Cheng *et al.*, 2006).

In vitro biological activity

Antioxidant activity

The ability of different extracts (leaves methanolic [LM] and leaves aqueous [LA] extracts as well as seeds methanolic [SM] and seeds aqueous [SA] extracts) of *Brassica oleracea* to inhibit 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was investigated by the procedure described by Wang *et al.* (Wang *et al.*, 2022): 40 µL of plant extracts at different concentrations were combined with a freshly prepared 0.1-M DPPH solution in methanol for reaction. Utilizing a microplate reader (Perkin Elmer EnSpire, Singapore), the mixture was incubated in the dark for 30 min before the absorbances were measured at 517 nm. The synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as standards. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$\%\text{Inhibition} = \frac{\left(\text{Abs}_{517}\text{blank} - \text{Abs}_{517}\text{sample}\right)}{\text{Abs}_{517}\text{blank}} \times 100, \quad (2)$$

where Abs_{517} blank: absorbance of control reaction, and Abs_{517} sample: absorbance of test sample. The percentage inhibition curve at various concentrations was used to calculate IC_{50} value, which corresponds to the concentration of extract and expressed as µg/mL.

The ferric-reducing antioxidant power (FRAP) assay was employed to evaluate the total antioxidant capacity (TAC) of aqueous and methanolic extracts derived from the leaves and seeds of *Brassica oleracea*. The assay was performed according to the method described by Miraliakbari and Shahidi (Miraliakbari & Shahidi, 2008), with slight modifications. Briefly, 75 µL of each sample of various concentrations (0, 125, 250, 500, and 1,000 µM) was added to 1.5-mL micro-centrifuge tubes and 1.5 mL of freshly prepared working FRAP solution. The mixtures were then incubated in the dark for 30 min at 37°C for the reduction reaction to take place. Following incubation, the absorbance of the resulting solutions was measured at 593 nm (A593) using a spectrophotometer with 1-cm path length. To ensure accuracy and reproducibility, 200 µL aliquots of each reaction mixture were transferred in quadruplicate to a microplate reader (Perkin Elmer EnSpire, Singapore) for absorbance measurements at 593 nm. All experiments were conducted in triplicate to account for any variability in results.

The β-carotene–linoleic acid bleaching method was employed to assess the capacity of different extracts of *Brassica oleracea* (methanolic and aqueous extracts of leaves and seeds) to inhibit β-carotene oxidation in a model system, as described by Miraliakbari and Shahidi (Miraliakbari & Shahidi, 2008). The assay involved the preparation of β-carotene–linoleic acid emulsion by dissolving 0.5 mg of β-carotene in 1 mL of chloroform, followed by the addition of 20 µL of linoleic acid and 200 µL of tween 40 in a flask. Then chloroform was removed using a vacuum evaporator, and the mixture was vigorously agitated with 50 µL of hydrogen peroxide (H_2O_2). The absorbance of emulsion was adjusted to 0.8–0.9 at 470 nm. Aliquots of 160 µL of emulsion were mixed with 40 µL of plant extracts or synthetic antioxidants (BHT) at various concentrations in each well of a 96-well microplate. The microplate was incubated at 50°C, and the absorbance was measured at 470 nm at regular intervals (t = 0, 30, 60, 90, and 120 min). The percentage of β-carotene bleaching inhibition was calculated using the following equation:

$$\text{Inhibition, I (\%)} = 1 - \frac{(\text{AH}_0 - \text{AH}_t)}{(\text{AC}_0 - \text{AC}_t)} \times 100, \quad (3)$$

where I (%) is the percentage of inhibition, AH_0 and AH_t are the absorbance values of extracts at t = 0 and t = 120 min, respectively, and AC_0 and AC_t are the absorbance values of negative control at t = 0 and t = 120 min, respectively.

The total antioxidant activity of different extracts of *Brassica oleracea* was determined by TAC as reported by (Prieto *et al.*, 1999).

The anti-hemolytic activity of *Brassica oleracea* extracts was evaluated *in vitro* following the method described by Afsar *et al.* (Afsar *et al.*, 2016), with minor modifications. Briefly, 2 mL of each extract was incubated with 40 µL of 10% human red blood cells (RBC) suspension for 5 min at 37°C. After incubation, the mixture was exposed to oxidative stress by adding 40 µL of 30-mM H_2O_2, 40 µL of 80-mM $FeCl_3$, and 40 µL of 50-mM ascorbic acid, followed by an additional incubation period of 1 h at 37°C. The samples were then centrifuged at 700 rpm for 5 min, and absorbance of the supernatant was measured at 540 nm. The percentage of hemolysis was calculated using the following equation:

$$\text{Hemolysis (\%)} = \frac{\text{Absorbance}_{\text{control}}}{\text{Absorbance}_{\text{sample}}} \times 100, \quad (4)$$

where $Absorbance_{control}$ represents the absorbance of untreated RBC suspension, and $Absorbance_{sample}$ represents the absorbance of the extract-treated sample. Ascorbic acid was used as a standard reference compound in this assay. The results were expressed in terms of Hly_{50} value, the extract concentration required to achieve 50% inhibition of RBC hemolysis.

In order to evaluate the photoprotective properties of *Brassica oleracea* extracts, SPF was determined using a spectrophotometric method adapted from Mansur *et al.* (Mansur *et al.*, 1986). The extracts were dissolved in ethanol at 1 mg/mL, and the absorbance was measured at seven different wavelengths ranging from 290 nm to 320 nm. The SPF value was then calculated using the following equation:

$$SPF_{Spectrophotometric} = CF \times \sum 320 - 290 \times EE(\lambda) \times I(\lambda) \times DO(\lambda), \quad (5)$$

where CF is the correction factor equal to 10, EE(λ) represents the arrhythmogenic effect of radiation at wavelength λ, I(λ) denotes solar intensity spectrum at wavelength λ, DO(λ) corresponds to spectrophotometric absorbance values of the sample at wavelength λ. EE(λ) and I(λ) are constants obtained from literature. A commercially available sunscreen, Avene®, was used as a positive control to validate the method and compare the SPF values of *Brassica oleracea* extracts.

According to the European recommendations (Miksa *et al.*, 2016), sunscreen products should possess an SPF value of at least 6 and do not exceed 50+ (equivalent to an SPF value of 60 or higher). Furthermore, the SPF value should be accompanied by a qualitative description denoting the level of protection, categorized as low, medium, high, or very high protection (Table 1).

Anti-inflammatory activity

In vitro anti-inflammatory activity of *Brassica oleracea* var. *elongata* extracts was evaluated using the method described by Messaoudi *et al.* (2022), which assessed the ability of extracts to inhibit the denaturation of egg albumin. Varying concentrations of crude extracts, ranging from 0.1 mg/mL to 1 mg/mL, were prepared in a reaction mixture containing 0.2 mL of fresh hen's egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH 6.8), and 0.2–2 mL of corresponding extract concentrations. Double-distilled water and dimethyl sulfoxide (DMSO) were used as negative controls, with volumes equivalent to those of test samples. The reaction mixtures were incubated at 37±2°C for 30 min, followed by heating at 75±2°C for 10 min to induce protein denaturation. After allowing the samples to cool, their absorbance was measured at 660 nm using vehicle as a blank.

Table 1. Category of sunscreen products' sun protection factor (SPF).

Protection level	SPF value
Low protection	6, 10
Medium protection	15, 20, 25
High protection	30, 40
Very high protection	50+

Diclofenac, at a final concentration of 10 mg/mL, was employed as a positive control and subjected to the same experimental conditions as test samples. Its absorbance was measured accordingly. To ensure reliability of the results, the experiment was conducted in triplicate. The percentage inhibition of protein denaturation was calculated using the following equation:

$$\text{Inhibition, I}\,(\%) = \frac{(\text{Abs C} - \text{Abs T})}{\text{Abs C}} \times 100, \quad (6)$$

where Abs T represents absorbance of the test sample, and Abs C represents absorbance of the control.

Antimicrobial activity

In this study, the widely used method for evaluating the antimicrobial activity of plant or microbial extracts is the Well-Agar Diffusion method (Balouiri *et al.*, 2016). The antibacterial activity of compound was assessed using the well diffusion method (WD) against the following four bacterial strains: *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922. The compound was prepared as 5 mg/mL and 10 mg/mL in DMSO. Mueller–Hinton agar was used as a growth medium. Bacterial strains were cultured on agar plates and incubated at 37°C for 24 h. To ensure a consistent inoculum size, the bacterial cultures were mixed with 0.9% NaCl solution and adjusted to a turbidity equivalent of McFarland 0.5 standard (10^8 CFU/mL). Subsequently, the agar was poured into Petri dishes, and the bacterial suspension was swabbed evenly onto the agar surface. Wells with a diameter of 6 mm were created in the agar, and 50 µL of the compound at respective concentrations were added to each well. The plates were then incubated at 37°C for 24 h. The experiment was performed in triplicate to ensure accuracy and reproducibility. The zone of inhibition observed around the wells indicated the antibacterial activity of different extracts of *Brassica oleracea* against tested bacterial strains.

Statistical analysis

All assays were performed in triplicate. The data were presented as mean values ± standard deviation (SD), with

a sample size, n = 3. Statistical analysis was carried out using the GraphPad Prism 8.0 software. One-way analysis of variance (ANOVA) was employed to determine significance of differences among various treatment groups. Tukey's *post hoc* test was subsequently applied to identify specific pair-wise differences between treatment groups. Significance levels were set at $p < 0.05$, $p < 0.01$, and $p < 0.001$, providing a comprehensive assessment of the statistical significance of observed differences.

Results and Discussion

Phytochemical Study

Chemical screening

Table 2 summarizes the findings of initial phytochemical analysis conducted on methanolic and aqueous extracts derived from the leaves and seeds of *Brassica oleracea*. The screening process aimed to detect the presence of various secondary metabolites, such as alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and reducing sugars, in these extracts.

The results of this study matched with the findings of Ayadi *et al.* (2022), who investigated the phytochemical composition of four *Brassica* species: *Brassica rapa*, *Brassica oleracea*, *Brassica napus*, and *Brassica juncea*. Employing advanced analytical methods, such as gas chromatography-mass spectrometry (GC-MS) and HPLC, the researchers successfully identified and quantified an extensive array of bioactive compounds in the aqueous and organic extracts of Brassica seeds. These compounds included volatile oils, glycosides, reducing sugars, polyphenols, phenolic acids, flavonoids, alkaloids, saponins, terpenoids, tannins, and glucosinolates, all of which were discovered in significant quantities.

Similarly, Parikh and Khanna (Parikh & Khanna, 2014) undertook a detailed phytochemical screening of six *Brassica* species: *Brassica oleracea* var. *capitata*, *Brassica juncea*, *Brassica napus*, *Brassica rapa*, *Brassica carinata*, and *Brassica nigra*. Employing qualitative and quantitative methods, they characterized the occurrence and relative abundance of key bioactive compounds, such as alkaloids, flavonoids, phenols, tannins, saponins, and glucosinolates. Their findings underscored the diverse phytochemical profiles of these *Brassica* species, highlighting their potential as valuable sources of naturally occurring bioactive compounds.

Total polyphenol and total flavonoid contents

The study investigated TPC and TFC in leaf and seed extracts of *Brassica oleracea* (Table 3). Methanolic extracts generally exhibited higher levels of phenols and flavonoids, compared to aqueous extracts. Methanolic seed extract had the highest phenols content (4.399±0.14 mg GAEq/g), while the aqueous seed extract showed slightly lower phenols content (2.160±0.13 mg GAEq/g). Similar trends were observed for flavonoids, with the methanolic seed extract (0.432±0.15 mg QEq/g) surpassing the aqueous seed extract (1.241±0.17 mg QEq/g). Methanolic leaf extract exhibited higher phenols content (3.462±0.17 mg GAEq/g), compared to the aqueous leaf

Table 2. Preliminary phytochemical screening of *Brassica oleracea* leaf and seed extracts.

Secondary metabolites	Sample	Leaf extract		Seed extract	
		Methanolic	Aqueous	Methanolic	Aqueous
Alkaloids	Observation	White precipitate			
	Results	+	+	+	+
Flavonoïds	Observation	Intense red	Red-pink	Intense red	Pink
	Results	+++	++	+++	+
Terpenoids	Observation	Purple ring			
	Results	+	+	+	+
Phenols	Observation	Dark blue-green	Green	Dark blue-green	Pale green
	Results	+++	++	+++	+
Tannins	Observation	Black color	Bluish-green color	Black color	Bluish-green color
	Results	+	–	+	–
Saponins	Observation	–	Forms foam in all tubes	–	Forms foam in all tubes
	Results	–	++	–	++
Reducing sugars	Observation	Brown	Brick-red	Brick-red	Brick-red
	Results	++	+	+	+

+ denotes presence (+++high concentration, ++moderate concentration, +low concentration), **–** denotes absence or not detected.

Table 3. Yield of total polyphenols and total flavonoids in the leaf and seed extracts of *Brassica oleracea*.

	Leaf extract		Seed extract	
	Methanolic	Aqueous	Methanolic	Aqueous
Yield (%)	9.52±0.12[b]	15.45±0.20[a]	6.18±0.09[c]	7.75±0.11[c]
Polyphenols (mg of GAEq/g)	3.46±0.17[b]	2.02±0.2[c]	4.40±0.14[a]	2.16±0.13[c]
Flavonoïds (mg QEq/g)	0.67±0.05[c]	1.40±0.17[a]	0.43±0.15[c]	1.24±0.17[b]

The values are given as mean ± SD (n = 3).
[a–c]Mean values with distinct letters in each row differ substantially ($p < 0.05$).

extract (2.015±0.2 mg GAEq/g), but lower flavonoids content (0.671 ± 0.05 mg QEq/g), compared to the aqueous leaf extract (1.399 ± 0.17 mg QEq/g). These results emphasized the significant influence of solvent type on concentrations of phenols and flavonoids in different extracts.

The quantification of flavonoids and polyphenols in various *Brassica* species has been a topic of interest in multiple research studies. Cartea *et al.* (Cartea *et al.*, 2010) comprehensively analyzed polyphenol content in different cultivars of *Brassica oleracea*, such as kale, cabbage, and broccoli. Their findings revealed a wide range of TPC, varying from 6.93 mg GAE/g DW to 32.86 mg GAE/g DW, with kale displaying the highest concentrations among the studied cultivars. Similarly, Jaiswal *et al.* (Jaiswal *et al.*, 2012), focused on TFC in the subspecies *of Brassica rapa*, reporting values between 0.52 mg QE/g DW and 3.17 mg QE/g DW. Among the analyzed samples, the highest flavonoid levels were exhibited in Chinese cabbage leaves.

Matching the previous findings, the leaves of *Brassica oleracea* demonstrated higher TFC than its seeds, especially in the aqueous extract (1.399 ± 0.17 mg QEq/g). However, our investigation also emphasized the crucial role of extraction solvent in quantifying these bioactive compounds. The methanolic extracts consistently yielded higher quantities of phenols and flavonoids than the aqueous extracts, indicating that selecting an appropriate extraction method was critical in accurately determining the levels of these valuable phytochemicals in the *Brassica* species.

HPLC fractionation and analysis

High-performance liquid chromatography analysis (Table 4) revealed distinct patterns of phenolic compounds in various extracts of *Brassica oleracea*. Gallic acid was present in high concentrations in methanolic leaf and seed extracts, with 811.59±0.02 µg/100 mg and 1180.20 µg/100 mg, respectively. Chlorogenic acid exhibited high concentrations in methanolic seed and aqueous seed extracts, with respective values of 626.26±0.01 µg/100 mg and 790.12±0.02 µg/100 mg. Vanillin was detected in both LM and SM extracts, with respective values of 485.67±0.03 µg/100 mg and 461.61±0.01 µg/100 mg. *P*-coumaric acid was found in methanolic seed and aqueous seed extracts, with values of 345.37±0.02 µg/100 mg and 32.90±0.02 µg/100 mg. Vanillic acid was observed solely in the LM extract, with a value of 4.72±0.03 µg/100 mg. Naringin, a bitter flavonoid, was detected in both LA and SA extracts, with respective values of 2776.25±0.03 µg/100 mg and 48.56±0.03 µg/100 mg. Quercetin was present exclusively in LA extract, with a value of 5512.58±0.02 µg/100 mg.

Gallic acid exhibited notable concentrations in methanolic leaf extract (LM; 404.550 µg/g) (Table 5) and methanolic seed extract (SM) (557.831 µg/g) but was absent in aqueous leaf extract (LA), with a comparatively lower presence in aqueous seed extract (SA) (14.603 µg/g). Chlorogenic acid was present prominently in SM (8323.831 µg/g) and SA (790.122 µg/g) extracts but was indiscernible in LM or LA extract. Vanilin was detected in both LM (465.786 µg/g) and SM (252.329 µg/g) extracts but not in LA or SA. Likewise, *p*-coumaric acid was absent in LM and LA extracts but present in SM (151.344 µg/g) and SA (32.908 µg/g) extracts. Vanillic acid was present in LM extract (263.309 µg/g) but absent in SM, LA, and SM extracts. Naringin was present in LA (308.106 µg/g) and SA extracts (48.568 µg/g) but was not found in LM or SM extract. Quercetin, however, was solely observed in LA extract (2192.635 µg/g).

These findings matched the established scientific comprehension of *Brassica*'s phenolic compounds and their antioxidative attributes. Phenolic compounds, such as gallic acid and chlorogenic acid, contribute to antioxidant potential and cellular defense against oxidative stress (Cartea *et al.*, 2010). Presence or absence of specific phenolic compounds in distinct extracts could be attributed to variations in extract composition, growth conditions, and genetic factors. This nuanced distribution indicated diverse phenolic profiles within *Brassica* species, potentially linked to their health advantages and unique defense mechanisms against oxidative harm (Al-Blesh, 2017).

Table 4. Results of RP-HPLC-UV analysis of phenolic compounds in *Brassica oleracea* extracts.

Compounds		Retention time (min)	Leaf extract		Seed extract	
			Methanolic	Aqueous	Methanolic	Aqueous
Phenolic acid (µg/100 mg ED)	Gallic acid	5.29	811.59±0.02^{b}	265.71±0.01^{c}	1180.20±0.01^{a}	14.60±0.01^{d}
	Chlorogenic acid	13.39	3330.89±0.01^{a}	690.84±0.02^{c}	626.26±0.01^{d}	790.12±0.02^{b}
	Vanillic acid	15.53	4.72±0.03^{d}	434.02±0.03^{b}	2769.18±0.01^{a}	9.09±0.03^{c}
	Caffeic acid	16.27	–	62.63±0.03^{b}	–	231.84±0.03^{a}
	Vanillin	21.46	485.67±0.03^{a}	95.34±0.01^{c}	461.61±0.01^{a}	247.93±0.01^{b}
	p-coumaric acid	23.81	–	–	345.37±0.02^{a}	32.90±0.02^{b}
Flavonoid (µg/100 mg ED)	Rutin	28.37	745.48±0.01^{b}	27.74±0.02^{d}	6751.99±0.01^{a}	36.96±0.01^{c}
	Naringin	34.78	2776.25±0.03^{a}	–	–	48.56±0.03^{b}
	Quercetin	45.04	5512.58±0.02^{a}	–	3717.39±0.01^{b}	–

ED: dry extract. The values are given as mean ± SD (n = 3).
$^{a–d}$Mean values with distinct letters in each row differ substantially ($p < 0.05$).

Table 5. *In vitro* antioxidant activity of *Brassica oleracea* extracts.

		Leaf extract		Seed extract		Ascorbic acid	BHT
		Methanolic	Aqueous	Methanolic	Aqueous		
DPPH	IC_{50}	0.058±0.001^{b}	2.097±0.087^{e}	0.200±0.001^{c}	0.337±0.008^{d}	0.006±0.001^{a}	–
β-carotene	EC_{50}	0.076±0.001^{c}	0.049±0.001^{b}	0.134±0.009^{d}	0.047±0.001^{b}	–	0.023±0.001^{a}
Anti-hemolysis	Hly_{50}	0.081±0.033^{c}	0.008±0.015^{a}	0.095 ±0.054^{d}	0.08 ±0.005^{c}	0.075 ±0.001^{b}	–
FRAP	EC_{50}	0.814±0.052^{b}	3.134±0.096^{d}	0.873±0.030^{b}	1.515±0.014^{c}	0.021±0.003^{a}	–
TAC	α-TEAC	55.274±0.007^{d}	100.685±0.08^{b}	74.714±0.004^{c}	70.889±0.001^{c}	210±0.001^{a}	–

The values are given as mean ± SD (n = 3).
$^{a–e}$Mean values with distinct letters in each row differ substantially ($p < 0.05$).
α-TEAC: α-tocopherol equivalent antioxidant capacity (nmol α-tocopherol/g); DPPH: 2,2-diphenyl-1-picrylhydrazyl; BHT: butylated hydroxytoluene; TAC: total antioxidant capacity; FRAP: ferric-reducing antioxidant power assay.

The compound-specific disparities across extracts underlined the intricate interaction of phytochemicals in *Brassica* varieties, further emphasizing the necessity for comprehensive analysis to comprehend fully their health-enhancing effects.

Multiple research studies have focused on identifying and characterizing the phenolic compounds present in various plant species within the genus *Brassica*. Cartea *et al.* (Cartea *et al.*, 2010) conducted a comprehensive analysis of the phenolic profiles of several *Brassica* vegetables, including *Brassica oleracea* var. *acephala* (kale) and *Brassica oleracea* var. *italica* (broccoli). Their findings revealed the presence of similar phenolic compounds, such as gallic acid, chlorogenic acid, caffeic acid, and p-coumaric acid, in both varieties, indicating a shared phenolic composition among these closely related plants.

In a separate study, Olsen *et al.* (Olsen *et al.*, 2009) investigated the phenolic composition of *Brassica oleracea* var. *capitata* and discovered the presence of gallic acid, caffeic acid, and p-coumaric acid, among other phenolic compounds. This further supported the notion that certain phenolic compounds were consistently found across different varieties of *Brassica oleracea*.

Moreover, Taveira *et al.* (Taveira *et al.*, 2009) explored the phenolic profile of *Brassica rapa* var. *rapa* (turnip) and identified the presence of vanillic acid and p-coumaric acid. The detection of these specific phenolic compounds in a different *Brassica* species suggested that they could have common constituents within the genus. The findings of the present study, which identified gallic acid, chlorogenic acid, vanillic acid, and p-coumaric acid in the methanolic and aqueous extracts of *Brassica oleracea* seeds, matched the results of previous investigations. This consistency in phenolic compounds identified across various *Brassica* species and varieties highlights the potential of these plants as a valuable source of bioactive phenolic compounds.

The flavonoid composition of the four samples analyzed in this study was found to be distinct from previous reports (Hertog *et al.*, 1992; Miean & Mohamed, 2001; Trejo-Téllez *et al.*, 2019). Interestingly, kaempferol, a flavonoid commonly associated with *Brassica* species, was not discovered in any of the samples. Instead, predominant flavonoids identified were myricetin and quercetin. In broccoli sample, three different flavonoids were quantified: myricetin (62.5 mg/kg), quercetin (60.0 mg/kg), and luteolin (74.5 mg/kg). The Chinese cabbage sample, on the other hand, contained only two flavonoids, namely myricetin (31 mg/kg) and apigenin (187.0 mg/kg). It is noteworthy that the concentrations of these flavonoids in the current study were generally higher than those reported previously in literature. This variation in TFC could be attributed to differences in cultivar, growing conditions, or analytical methods employed. These findings contributed to our understanding of diverse flavonoid profiles present in *Brassica* species and highlighted the importance of comprehensive analyses to characterize fully their phytochemical composition.

In vitro biological activity

Antioxidant power assay

The antioxidant activity of various *Brassica oleracea* extracts was assessed using DPPH, β-carotene bleaching method, anti-hemolysis, and FRAP and TAC assays (Table 6). The LM extract exhibited the strongest antioxidant activity in DPPH assay ($IC_{50:}$ 0.058±0.001 mg/mL), while the LA extract showed the highest activity in β-carotene bleaching method (EC_{50}: 0.049±0.001 mg/mL). The LA extract demonstrated the strongest anti-hemolysis activity (Hly_{50}: 0.008±0.015 mg/mL), while the LM extract had the highest reducing power in FRAP assay (EC_{50}: 0.814±0.052 mg/mL). In TAC assay, the LM extract exhibited the strongest TAC (IC_{50}: 55.274±0.007 mg/mL).

β-carotene bleaching method: Both LA and SA extracts showed the highest antioxidant activity with EC_{50} values of 0.049±0.001 mg/mL and 0.047±0.001 mg/mL, respectively. The LM extract had an EC_{50} value of 0.076±0.001 mg/mL, while the SM extract had an EC_{50} value of 0.134±0.009 mg/mL. BHT, used as a reference, had an EC_{50} value of 0.023±0.001 mg/mL.

The antioxidant activity of *Brassica oleracea* extracts was investigated using five different assays, each with its unique mechanism of action. The DPPH assay measured the ability of antioxidants to scavenge stable-free DPPH radical by donating hydrogen atoms or electrons (Cheng *et al*., 2006). The LM extract exhibited the strongest antioxidant activity in this assay, which was attributed to the high contents of phenolic compounds and flavonoids. These phytochemicals possess hydroxyl groups that readily donate hydrogen atoms to neutralize DPPH radicals, thereby reducing their stability and purple color (Gawlik-Dziki *et al.*, 2013).

The β-carotene bleaching method evaluated the ability of antioxidants to prevent the oxidation of β-carotene in the presence of linoleic acid and oxygen (Miller, 1971). Both LA and SA extracts showed the highest antioxidant activity in this assay, probably because of the presence of water-soluble antioxidants, such as ascorbic acid and glucosinolates. These compounds neutralized free radicals generated during linoleic acid oxidation, thereby preventing the degradation of β-carotene (Podsędek, 2007).

Phenolic compounds and flavonoids also contributed to this activity by chelating metal ions involved in the initiation of lipid peroxidation (Evans, 2009). The anti-hemolysis activity assessed the ability of antioxidants to protect RBCs from oxidative damage induced by free radicals, such as peroxyl radicals (Cherrada *et al.*, 2023). The LA extract exhibited the strongest anti-hemolysis activity, followed by SA and LM extracts. Phenolic compounds and flavonoids prevent hemolysis by scavenging free radicals, donating hydrogen atoms to neutralize peroxyl radicals, and stabilizing cell membranes (Carvalho *et al.*, 2010). The FRAP assay measures the ability of antioxidants to reduce ferric ions (Fe^{+3}) to ferrous ions (Fe^{+2}) ions under acidic conditions (Benzie & Strain, 1996).

Both LM and SM extracts showed the highest reducing power in FRAP assay, indicating the presence of phenolic compounds and flavonoids that donated electrons to reduce Fe^{+3}. The reducing power of these phytochemicals was related to the number and position of hydroxyl groups in their structure (Pulido *et al.*, 2000). Lastly, the TAC assay evaluated the overall antioxidant potential of a sample by measuring its ability to scavenge stable radical

Table 6. Sun protection factor (SPF) values of different extracts of *Brassica oleracea*.

	Leaf extract		Seed extract		Avene® (control)
	Methanolic	Aqueous	Methanolic	Aqueous	
SPF values	$38±6^{b,c}$	$15±8^{a}$	$46±10^{c}$	$28±5^{b}$	$41±4^{c}$

The data were presented as mean values ± standard deviation (SD), with a sample size, n = 3.
Mean values with distinct superscript letters in each row are statistically different at $p < 0.01$.

Table 7. Evaluation of the percentage of anti-inflammatory inhibition of *Brassica oleracea* extracts.

	Leaf extract		Seed extract		Diclofenac (control)
	Methanolic	Aqueous	Methanolic	Aqueous	
Anti-inflammatory inhibition (%)	17.60±0.04[a]	94.80±0.03	25.70±0.04	55.00±0.02	96.46±0.30[b]

The data were presented as mean values ± standard deviation (SD), with a sample size, n = 3.
[**]Data with different superscript letters are statistically different at $p < 0.01$.

cation (Re *et al.*, 1999). In conclusion, the antioxidant activity of *Brassica oleracea* extracts could be attributed to the presence of phenolic compounds and flavonoids, which had a crucial role in scavenging free radicals, reducing metal ions, preventing lipid peroxidation, and protecting cells from oxidative damage. The varying antioxidant activities observed in different assays could be explained by the specific mechanisms of action and the solubility of antioxidant compounds present in each extract.

Sun protection factor assay

The SPF assay for *Brassica oleracea* yielded valuable SPF values for both aqueous and methanol extracts of leaves and seeds. This study marked the initial investigation into the effectiveness of UV protection within the Brassicaceae family.

Based on the SPF assay, results presented in Table 7 showed that the methanolic and aqueous extracts of *Brassica oleracea* leaves and seeds exhibited promising potential for UV protection. The SM extract demonstrated the highest SPF value of 45.58, followed by the SPF of LM extract as 38.05. Both LA and SA extracts also showed notable SPF values, with SPF of SA being 27.95 and that of LA as 14.77.

These findings suggested that the extracts contained compounds that contributed to their UV protective properties. Polyphenols, particularly flavonoids, play a significant role in protecting the skin from UV radiation. Flavonoids, such as quercetin and rutin, are reported to absorb UV light and act as natural sunscreens (Saewan & Jimtaisong, 2013). They also scavenge free radicals generated by UV exposure, thus reducing oxidative stress and preventing DNA damage (Nichols & Katiyar, 2010).

Terpenes, another class of compounds present in *Brassica oleracea* extracts, have been associated with UV protection. Certain terpenes, such as limonene and geraniol, are able to absorb UV light and protect against UV-induced skin damage (Kaur & Saraf, 2010). Additionally, saponins possess antioxidant properties and contribute to the overall UV protective effect of *Brassica oleracea* extracts (Praveen & Kumud, 2012).

The maxim behind the protective effect of these compounds is in their ability to absorb UV radiation and dissipate energy as heat, thus preventing harmful rays from reaching skin cells. Furthermore, their antioxidant properties help to neutralize free radicals generated by UV exposure, reducing oxidative stress and preventing cellular damage (Pandel *et al.*, 2013).

In vitro anti-inflammatory activity

The anti-inflammatory activity of *Brassica oleracea* extracts, particularly that of the LA extract, was notably high (94.8%), compared to the LM extract (17.6%) (Table 7). This indicated the potential of these extracts in reducing inflammation, with the choice of solvent for extraction influencing the potency of anti-inflammatory compounds present. The LA extract exhibited the highest inhibition percentage (94.8%), closely matching that of the reference compound diclofenac (96.46%) (Figure 1).

The anti-inflammatory activity of *Brassica oleracea* extracts was attributed to the presence of various phytochemical compounds, such as polyphenols, flavonoids, terpenes, and saponins. These compounds had a crucial role in protecting tissues and cells from infections through different mechanisms. (1) *Polyphenolic compounds*, such as phenolic acids and flavonoids, possess

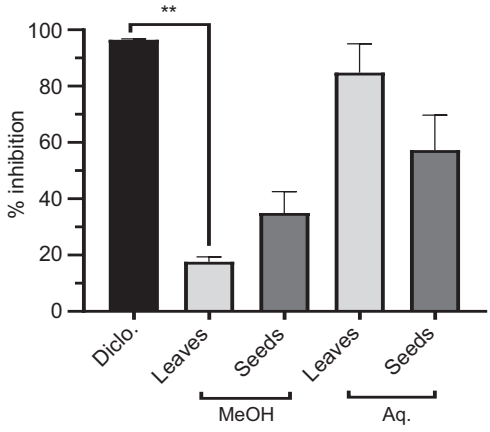

Figure 1. Anti-inflammatory potential of aqueous and methanol extracts of *Brassica oleracea*.

strong antioxidant and anti-inflammatory properties. They scavenge free radicals, reduce oxidative stress, and modulate inflammatory pathways by inhibiting pro-inflammatory enzymes and cytokines (Pandey & Rizvi, 2009). (2) *Flavonoids*, a subclass of polyphenols, are shown to exhibit anti-inflammatory effects by inhibiting enzymes involved in the production of inflammatory mediators, such as cyclooxygenase (COX) and lipoxygenase (LOX). They also suppress the activation of nuclear factor *kappa* B (NF-κB), a key transcription factor in inflammatory response (Serafini *et al.*, 2010). (3) *Terpenes* and *terpenoids* have demonstrated anti-inflammatory properties by inhibiting the release of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interlukin 1β (IL-1β), and reducing the production of nitric oxide (NO) and prostaglandin E2 (PGE2) (Guimarães *et al.*, 2013). (4) *Saponins* possess anti-inflammatory effects by modulating the immune system, inhibiting the production of pro-inflammatory cytokines, and reducing the expression of adhesion molecules involved in the inflammatory process (Sparg *et al.*, 2004). The higher anti-inflammatory activity observed in aqueous extracts, compared to methanolic extracts, suggests that water-soluble phytochemical compounds, such as polyphenols and flavonoids, could be primary contributors to the anti-inflammatory properties of *Brassica oleracea*.

Antimicrobial activity

Based on the results presented in Table 8, the antibacterial activity of various extracts of *Brassica oleracea* leaves and seeds was evaluated using the well-agar diffusion method. This widely accepted technique allows for the assessment of antimicrobial potential by measuring the zone of inhibition around wells containing tested extracts (Balouiri *et al.*, 2016).

The results demonstrate that both leaf and seed extracts of *Brassica oleracea* exhibited varying degrees of antibacterial activity, with a notable difference in their efficacy against Gram-positive and Gram-negative bacteria. The LM extract showed the highest inhibition against Gram-positive bacteria, with the zone of inhibition diameters being 14 mm and 11 mm for *S. aureus* ATCC 25932 and 12 mm and 16 mm for *E. coli* ATCC 25922 at concentrations of 5 mg/mL and 10 mg/mL, respectively. In contrast, the same extract exhibited lower activity against Gram-negative bacteria, with inhibition zones of 14 mm and 16 mm for *P. aeruginosa* ATCC 27853 and 12 mm and 15 mm for *K. pneumoniae* ATCC 13883 at the same concentrations.

This observed difference in antibacterial activity was attributed to the distinct cell wall structures of both Gram-positive and Gram-negative bacteria. Gram-positive bacteria have a thick peptidoglycan layer in their cell wall, which is more permeable to antimicrobial compounds. On the other hand, Gram-negative bacteria possess an additional outer membrane that acts as a barrier, limiting the entry of antimicrobial agents (Breijyeh *et al.*, 2020).

Both LA and SM extracts demonstrated a similar trend, exhibiting higher antibacterial activity against Gram-positive bacteria, compared to Gram-negative bacteria. This finding further supports the notion that the cell wall structure plays a crucial role in the susceptibility of bacteria to plant-derived antimicrobial compounds (Wang *et al.*, 2022).

The antibacterial activity of *Brassica oleracea* extracts is attributed to the presence of various phytochemical compounds, such as polyphenols, flavonoids, alkaloids, and coumarins. Polyphenols, including flavonoids, are reported to possess antibacterial properties by disrupting bacterial cell membranes, inhibiting bacterial enzymes, and interfering with bacterial DNA synthesis (Cushnie & Lamb, 2005). Alkaloids also exhibit antibacterial activity by inhibiting bacterial cell wall synthesis and altering bacterial cell membrane permeability (Cushnie *et al.*, 2014). Coumarins inhibit bacterial growth by interfering with bacterial DNA replication and transcription (Venugopala *et al.*, 2013).

Previous studies on other *Brassica* species also reported antibacterial activity. For example, a study conducted by Jaiswal *et al.* (Jaiswal *et al.*, 2011) demonstrated the antibacterial activity of methanolic extracts of *Brassica juncea* leaves against various Gram-positive and Gram-negative bacteria. Similarly, *Brassica rapa* var. *rapa* root extract was found to exhibit antibacterial activity against *E. coli* and *S. aureus* (Abdou *et al.*, 1972).

The mechanism of action of plant compounds in killing and inhibiting Gram-negative and Gram-positive bacteria varies. Gram-negative bacteria possess an outer membrane that acts as a barrier to hydrophobic compounds, making them more resistant to certain antibiotics and plant-derived compounds (Delcour, 2009). However, some plant compounds, such as flavonoids, disrupt outer membrane, allowing other compounds to penetrate and exert their antibacterial effects (Tsuchiya, 2015). Gram-positive bacteria, on the other hand, lack an outer membrane, making them more susceptible to antibacterial compounds that directly interact with their cell wall and membrane (Malanovic & Lohner, 2016).

Conclusion

In conclusion, the findings of this study elucidated the phytochemical composition and antioxidant,

Table 8. Antibacterial activity of *Brassica oleracea* extracts.

Sample	Concentration	Zone of inhibition (mm)			
		Gram-negative		Gram-positive	
		P. aeruginosa ATCC 27853	K. pneumoniae ATCC 13883	S. aureus ATCC 25932	E. coli ATCC 25922
Aqueous extract of leaves	5 mg/mL	110.15	90.10	10.000.10	110.10
	10 mg/mL	130.10	110.15	12	130.10
Methanolic extract of leaves	5 mg/mL	140.10	120.10	110.10	120.50
	10 mg/mL	16.000.15	150.10	140.10	160.50
Methanolic extract of seeds	5 mg/mL	80.25	70.15	70.10	90.10
	10 mg/mL	110.15	90.10	100.25	110.15
Aqueous extract of seeds	5 mg/mL	–	–	–	–
	10 mg/mL	90.15	8 0.15	120.15	8 0.25
Ciprofloxacin (CIP-5)	50 µg	22.00 0.4	20 0.15	14 0.2	17.00 0.2

The data are presented as mean values ± standard deviation (SD), with a sample size, n = 3.

anti-inflammatory, and antimicrobial activities of *Brassica oleracea* var *elongata* leaf and seed extracts. The extracts demonstrated significant secondary metabolites, such as alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and reducing sugars. Moreover, the extracts exhibited potent antioxidant properties, UV protection potential, notable anti-inflammatory effects, and significant antibacterial activity against Gram-positive and Gram-negative bacteria. These findings highlighted the biological significance of *Brassica oleracea* extracts and their potential as valuable sources of natural bioactive compounds with diverse pharmacological activities, thus suggesting their promising applications in skincare formulations and pharmaceuticals. Further investigation, optimization of extraction methods, formulation development, and clinical trials are recommended to fully explore and harness the therapeutic potential of *Brassica oleracea* extracts.

Acknowledgments

The authors thanks the technical assistance of the Laboratory of Biology, Environment and Health (LBEH), University of El Oued39000, Algeria for all their helps. Also, The authors acknowledged and extended their appreciation to the Researchers Supporting Project No. RSPD2024R709, King Saud University, Riyadh, Saudi Arabia, for funding this study.

Funding

Tis research was funded by King Saud University, Riyadh, Saudi Arabia, Project Number (RSPD2024R709).

Availability of Data and Materials

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declared no conflict of interest.

Author Contributions

All authors read and agreed to the published version of the manuscript.

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