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EXPERIMENTAL PAPER

LC-ESI-MS/MS profiling and antioxidant activity of okra (*Abelmoschus esculentus* (L.) Moench) fruit pericarp from different accessions cultivated in Biskra, South Algeria

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Summary

Introduction: Okra (*Abelmoschus esculentus* (L.) Moench) is a widely consumed vegetable valued for its rich nutritional content and diverse bioactive compounds.

Objective: The objective of this study was to evaluate the extraction yield, phenolic composition and antioxidant capacity of hydro-ethanolic extract of fresh fruit pericarp of late-maturity okra from six accessions grown in Biskra (South Algeria) where the pericarp is considered as an agricultural by-product.

Methods: Total phenolic and flavonoids contents were analysed by Folin Ciocalteu and aluminum chloride assays, respectively. Antioxidant capacity was studied *in vitro* by using DPPH radical scavenging activity, ABTS and phenanthroline assays. Phenolic compounds were analysed by LC-ESI-MS/MS.

Results: The hydroethanolic extract from the BA accession collected from Beni Abbes region had the highest extraction rate at 17.91±0.37%. The GH accession collected from Ghardaia region exhibited the highest values of total phenolic contents (38.77±2.6 μg GA/mgDE). This accession showed the highest activity in the DPPH assay (IC_{50}=372.99±2.84 μg/ml). The extract from BI1 accession (collected from Biskra) showed a higher capacity to scavenge the $ABTS^{\bullet+}$ radical (IC_{50}=187.02±7.00 μg/ml). The extracts from the AD accessions collected from Adrar region demonstrated strong antioxidant capacity in reducing metallic ions ($A_{0.5}$ values=159.75±17.32 μg/ml). Ten distinct phenolic compounds were identified, with most of these compounds being reported for the first time. Notably, *p*-coumaric, caffeic, *o*-coumaric, and salicylic acids were found in the extracts of all accessions.

Conclusion: Okra fruit pericarp at late-stage maturity is considered an important and diverse source of medically phenolic compounds.

Key words: ***Abelmoschus esculentus (L.) Moench), antioxidant activity, fruit pericarp, LC-ESI-MS/MS, phenolic compounds, plant accessions***

Słowa kluczowe: ***Abelmoschus esculentus (L.) Moench), aktywność przeciwutleniająca, owocnia, LC-ESI--MS/MS, związki fenolowe, pochodzenia roślin***

INTRODUCTION

The diversity of inputs in crop species is crucial to improve productivity and thus enable the development of new and more valuable agricultural products [1]. However, the intraspecific diversity in crops is due to environmental factors and genetic variability [2]. Okra is extensively cultivated in tropical and subtropical regions [3], and its optimal temperature for growth is approximately 30°C [4]. It is an annual vegetable crop with a strong, erect, branched stem ranging in height from 50 to 400 cm [5]. Additionally, cultivation methods, pedoclimatic variables, and genotype all affect fruit size and pod characteristics. According to [6], the length of dry mature fruit is 15 cm in the long green fresh fruit accession and 11.69 cm in the short red fresh fruit accession. Okra is a multipurpose crop that is widely recognized for its genetic diversity and its diverse nutritional value and bioactive components. These components include dietary fibre, vitamins, sugars, polyphenols, proteins, and fatty substances, which are present in the fresh leaves, buds, flowers, fruits, stems, and seeds of the plant [6–8]. In addition, okra is a native crop of the south of Algeria, where it is grown to make young okra fruits that are usually used in soups. To our knowledge, studies conducted on Algerian okra accessions focused on morphological, fibre and mineral characteristics of different parts of okra (stem, fruit, pericarp and seed) [6]. Unexpectedly, no studies have examined the phytochemical constituents of Algerian okra accessions in the literature review.

On the other hand, in south Algeria okra fresh pod production is usually available from March to December, with its peak production season occurring during the summer months (personal observation and communication with local growers). However, summer temperatures in these regions can sometimes exceed 47°C in July and August [9] resulting in soil water deficiency, especially when irrigation doses and frequency are inadequate. Water shortages also increase the crude fibre content of okra fruits, making them woody and inedible [10], which is considered a late-stage maturity ripening. The elimination of these fruits is necessary to maintain a good production of fresh fruits and therefore, it can be considered an agricultural by-product. According to [11] some such by-products have been found to be effective sources of phenolic antioxidants. In order to clarify the matter and to increase the value of secondary agricultural products derived from okra fruits, the investigation has been restricted to stems [12].

The objective of this investigation was to assess the antioxidant capacity of hydroethanolic extracts from the pericarp of six okra accessions at the final stage of maturity, which were harvested from Biskra region in Algeria. Subsequently, the identification and quantification of phenolic compounds were conducted using LC-ESI-MS/MS. To our knowledge, there are no published reports on the evaluation of the phenolic compounds of the okra pericarp using LC-ESI-MS/MS.

MATERIAL AND METHODS

Plant material

This study was conducted in three phases: collection, seeding, and sample preparation. In the first phase, local okra seeds were gathered from various locations across Algeria's arid regions, including four major districts (Table 1).

Seeds collected were kept at 4°C in the Plant Physiology and Bioactive Molecules Laboratory at the Scientific and Technical Research Center for Arid Areas (CRSTRA) in Biskra, Algeria.

In the second stage, the collected biological material was sown in field plots at the CRSTRA Bio-Resources experimental station on April 19, 2022, following a completely randomized design. The soil is a loam-clay soil with less than 3% organic matter. No organic fertilizer was used.

In the third stage, okra fruits were harvested from the plants on July 21, 2022. A voucher specimen (fruit) of this harvest was disposed in

Table 1.

Geographic location of okra seeds collected

City	Code	Longitude	Latitude	Collection date	Altitude [m.s.d]
Ghardaia	GH	31°58'42.25"N	3°44'51.04"E	2021	426
Beni Abbes	BA	30° 7'45.76"N	2°10'34.38"W	2022	407
Biskra	BI1	34°42'27.71"N	5°53'13.54"E	2021	25
Biskra	BI2	34°47'11.14"N	5°49'30.37"E	2021	59
Biskra	BI3	34°49'43.84"N	5°54'56.67"E	2021	118
Adrar	AD	27°51'44.78"N	0°18'16.48"W	2022	328

the herbarium of the Laboratory of Biosystematic, Scientific and Technical Research Center for Arid Areas (CRSTRA), Biskra, Algeria under number (020.CRSTRA.0001). After being dried in the shade at room temperature, the seeds were removed. For this study, only the pericarps (fruits without seeds) were used. These pericarps were then cleaned and ground using a mechanical grinder.

Ultrasound-assisted extraction

Ultrasound-assisted extraction was carried out using a Bandelin Sonorex Digetec model (Type: DT 514, Germany), operating at 250 W power and a frequency of 35 kHz. Separate 3 g samples were placed in beakers and extracted for 40 min with 100 ml of a 70:30 *v/v* ethanol–water mixture at a constant temperature of 60°C. The extract solution was filtered through filter paper, and the solvent was removed from the extracts by vacuum evaporation at 40°C using a rotary evaporator (R215, BÜCHI Labortechnik AG, Flawil, Switzerland). The resulting dry extracts were then collected and stored in the dark at 4°C.

Extraction yield of total phenols content (TPC) calculation

The extraction yield of the samples was calculated as dividing the weight of the extract by the weight of the sample, then multiplying the result by 100.

Total phenols content (TPC)

A 96-well microplate reader (Thermo Scientific TMMultiskan Sky, Singapore, ref: 5111700DP) was used to analyse the total phenolic content of the extracts according to the method of [13]. The protocol was based on mixing 20 μl sample with 100 μl Folin Ciocalteu reagent and 75 μl (7.5%) sodium carbonate. Absorbance was measured at 740 nm after 2 h incubation in darkness at a room temperature. Results were expressed in micrograms of gallic acid equivalents per milligrams of dry extract (μg GAE/mg DE). The calibration curve for the determination of total phenols was as follows: $y = 0.002\ x + 0.010$ ($R^2 = 0.989$).

Total flavonoids content (TFC)

Total flavonoid content was assessed according to the method of [14] with slight modifications to enhance accuracy. 20 μl of each diluted extract solution was mixed with 10 μl of 10% aluminium nitrate, 10 μl of potassium acetate (1 M) and 130 μl of methanol. After 40 min incubation at room temperature, the absorbance was measured at 415 nm. The flavonoid content was expressed as micrograms per milligram of quercetin equivalents of dry extract (μg QE/mg DE). The calculation was based on a calibration curve with the equation $y = 4.63x - 0.0516$ ($R^2 = 0.9988$).

LC-ESI-MS/MS analysis

Bioactive compounds in six hydro-ethanolic extracts were identified and quantified using LC-ESI-MS/MS analysis, conducted on an Agilent 1260 Infinity II LC System connected to a tandem mass spectrometer. The method had a flow rate of 0.4 ml/min, with a total run time of 30 min, and the oven temperature was maintained at 25°C.

Chromatographic separation was performed using a reversed-phase Agilent Poroshell120 EC-C18 analytical column (100 mm × 3.0 mm, 2.7 μm). Eluent A, comprising water with 5 mM ammonium formate, and eluent B, consisting of acetonitrile with 0.1% formic acid, were used as mobile phases under isocratic conditions with a composition of 75% A and 25% B. Mass spectrometry was performed using an Agilent 6460 Triple Quadrupole Mass Spectrometer System with electrospray ionization (LC–ESI–MS/MS) to detect the compounds. The acquisition was carried out in both positive and negative ionization modes. Agilent Mass Hunter Software was used to perform data analysis. A multiple reaction monitoring (MRM) method was employed to precisely identify and quantify the phytochemical compounds. Collision energies were optimized to ensure optimal fragmentation and transmission of the target ions. The mass spectrometer was operated with a nitrogen (N2) drying gas flow of 15 ml/min, a nitrogen nebulizing gas flow of 11 ml/min, a capillary voltage of 4 kV, and a gas temperature set at 350°C. As stated by [15], the validation parameters for the method, such as the limit of detection (LOD), limit of quantification (LOQ), and linearity range, were examined and determined. The samples were prepared using the method of [16] with slight modifications. A 50 mg portion of hydroethanolic extracts was dissolved in a mixture of 1 ml ethanol and 1 ml hexane in a 2 ml Eppendorf tube. The solution was vortexed for 2 min at 4°C using a Bioprep-24 homogenizer and then centrifuged at 9.000 rpm for 10 min at 4°C using a Hettich Universal 320R centrifuge (Germany). A 100 μl sample was collected from the separated methanol phases and diluted with distilled water at 900 μl. The final samples were then filtered through a Captiva premium syringe filter, featuring a polypropylene shield, a 25 mm diameter nylon membrane with a pore size of 0.45 μm, and an injection volume of 5.12 μl. The gradient elution was optimized with the following steps: 0–5 min, 10–20% A; 5–10 min, 20–30% A; 10–15 min, 30–50% A; 15–20 min, 50-70% A; 20–25 min, 70–90% A; 25–30 min, 90–50% A; and 30–35 min, returning to the initial conditions. The bioactive compounds in each extract were identified based on their retention times, mass-to-charge ratios (m/z), and characteristic fragmentation patterns obtained by LC-ESI-MS/MS analysis. The acquired MS/MS spectra were compared with data from the literature and, when available, with reference spectral databases.

Antioxidant activity evaluation

DPPH free radical-scavenging capacity

The microplate reader analysis of DPPH$^{\bullet}$ scavenging activity of hydro-ethanolic extracts was evaluated using the method of [17]. About 40 μl of each methanolic extract concentrations (18.75, 37.5, 75, 150, 300, 600 and 1200 μg/ml) was added to 160 µl of methanolic DPPH solution (6 mg DPPH dissolved in 100 mg methanol). The mixture was shaken vigorously and left at a room temperature in the dark for 30 min. The experiment was performed in triplicate and the absorbance was determined at 517 nm by using a 96-well microplate reader. The ability to scavenge the DPPH radical of each extract concentrations was calculated using the following equation [1]:

DPPH scavenging effect (%) = [(A0 – A1/A0)] × 100 [1],

where A0 was the absorbance of the negative control and A1 was the absorbance of the sample at 30 min. The obtained inhibitions were plotted with the concentrations of the samples. These resulting plots were then utilized to determine the IC_{50} values, which represent the concentration of the samples necessary to reduce DPPH by 50%.

ABTS radical scavenging assay

The microplate reader analysis of $ABTS^{\bullet+}$ scavenging activity was assessed using the method described by [18]. The $ABTS^{\bullet+}$ was obtained by mixing 19.2 mg of 7 mM ABTS solution and 3.3 mg of 2.45 mM potassium persulphate solution, this emulsion was stored for 16 h at room temperature in the dark. Subsequently, the $ABTS^{\bullet+}$ was diluted with ethanol to the absorbance of 0.750 (±0.020) at 734 nm. Then, 160 μl of $ABTS^{\bullet+}$ solution was added to 40 μl of sample solution in methanol at different concentrations (18.75, 37.5, 75, 150, 300, 600 and 1200 μg/ml) and these mixtures were homogenized. The experiment was done in triplicate and the absorbances were recorded at 734 nm by using a 96-well microplate reader. After 10 min, the inhibition percentage of each concentration was calculated relative to a blank absorbance (methanol), following the equation [1].

Phenanthroline activity

Fe^{2+}-phenanthroline reduction was conducted following the method of [19]. In a 96-well microplate, 10 μl of the tested extract at various concentrations (37.5, 75, 150, and 300 μg/ml) was combined with 50 μl of $FeCl_3$ (0.2%), 30 μl of 1,10-phenanthroline

(0.5%), and 110 μl of methanol. The mixture was incubated at 30°C for 20 min, after which the absorbance was measured at 510 nm. The results are expressed in absorbance and in $A_{0.5}$ (μg/ml) corresponding to the concentration indicating absorbance 0.50.

Statistical analysis

Data were analysed using Statistix 6 for ANOVA, regression and co-relation analyses. Probability levels (p<0.05). were considered to analyse the difference among treatment means. Analysis of one-way variance (ANOVA) was performed and significant differences among means were determined using Newman-Keuls Multiple Range test (p<0.05).

RESULTS AND DISCUSSION

The extraction was carried out using an ultrasound-assisted extraction method with ethanol/water solvent. Due to its efficiency, this method has become increasingly popular for extracting natural compounds, necessitating less solvent and energy. Additionally, it is regarded as a sustainable "green" technology [20]. However, the choice of solvent plays a crucial role in determining the types of molecules extracted [21, 22]. Several studies have employed a combination of different solvents in different ratios to enhance the extraction of phenolic compounds [23, 24]. Additionally, a number of studies have emphasized the use of hydroethanolic solvents for phenolic compound extraction [25, 26], since this solvent is thought to be safe for human consumption and to be useful in natural medicinal applications [27].

Extraction yields, TPC and TFC contents of okra fruit pericarp accessions

The extract yield TPC and TFC contents of six accessions of okra fruit pericarp obtained by the ultrasound-assisted extraction techniques using ethanol and water at *v/v* ratios of 70:30 is illustrated in Table 2. BA accession extract yield gave the highest extraction yield by 17.91±0.37% followed by accession from GH (14.53±0.55%) in the second group, and AD with BI3 accessions in the third group (12.94±1.15% and 12.49±0.45%, respectively). Contrarily, the lowest yields were observed in BI1 and BI2 accessions (12.50±0.40% and 11.18±1.67%, respectively). Although the accessions in this study were cultivated under identical conditions, the okra fruits were harvested on the same day and at the same stage of maturity with the extraction process being standardized. However, significant differences in extract yield were observed across the various accessions. Similarly, [28] observed variability in extraction yields from the rhizomes of ten *Curcuma aeruginosa* accessions, which were grown under the same conditions and extracted using the same method. This suggests that genetic factors are likely responsible for the variation in extract yields.

Table 2.

Extraction yields, total phenol and total flavonoid contents in okra fresh fruit pericarp at late-stage maturity

Okra Accessions	Extraction yields [%]	TPC [μg GAE/mg DE]	TFC [μg QE/mg DE]
AD	12.94±1.15^{bc}	14.07±1.21^{d}	–
BA	17.91±0.37^{a}	21.29±1.83^{b}	–
BI1	12.50± 0.40^{c}	17.85±1.42^{d}	–
BI2	11.18±1.67^{c}	16.00±2.03^{cd}	–
BI3	12.49±0.45^{bc}	13.52 ± 0.84^{e}	–
GH	14.53±0.55^{b}	38.77±2.6^{a}	–

The values are presented as mean ±SD (n=3). AD refers to okra accessions from the Adrar region, BA represents okra accessions from the Beni Abbes region, BI1, BI2, and BI3 accessions from different sites in the Biskra region, and GH refers to okra accessions from the Ghardaia region. TPC stands for total phenolic content, TFC for total flavonoid content, μg GAE/mg DE: microgram gallic acid equivalent per milligram of dry plant extract. μg QE/mg DE: microgram quercetin equivalent per milligram of dry plant extract. Letters indicate statistical differences among means (p<0.05).

The amounts of total polyphenols varied significantly between the fruit pericarps of okra accessions. GH accession showed maximum quantity of TPC (38.77±2.6 μg GAE/ mg DE) followed by the BA accession (21.29±1.83 μg GAE/ mg DE). While, BI2 and AD accessions (13.52±0.84, 14.07±1.21 μg GAE/mg DE, respectively) had the lowest TPC. A similar finding was reported by [29], who observed variation in the phenolic content of five okra fruit skin cultivars collected in China. This variation was significantly affected by genotype factors [30]. The total flavonoid content was not detected in all extracts. This absence of detectable total flavonoid content in the hydro-ethanolic extracts may be attributed to the low absorbance recorded during spectrophotometric analysis, as previously reported [31]. Another plausible explanation is the limited affinity between flavonoid compounds and the extraction solvent used. As is well known, the efficiency of compound extraction is strongly influenced by solvent polarity [32].

Furthermore, flavonoid distribution within okra is known to vary significantly depending on the plant organ, developmental stage, and environmental conditions. In the present study, the analysis focused on the pericarp of okra fruits harvested at the final maturity stage, which may contain lower flavonoid levels compared to other tissues such as seeds, leaves, or flowers reported in earlier studies. In addition, thermal stress and growth conditions may further influence flavonoid biosynthesis and accumulation.

To the best of our knowledge, comprehensive studies specifically investigating flavonoid content in okra fruits using hydro-ethanolic or semi-polar solvent extracts remain limited. This lack of available data makes direct comparison difficult and highlights the originality of the present work, while also suggesting that future studies employing a wider range of solvents and analytical approaches may be necessary to fully elucidate the flavonoid profile of okra fruit tissues.

LC-ESI-MS/MS results

Using chromatographic methods, earlier research has documented the phenolic content of various okra tissues. In a separate study, the identification and quantification of isoquercitrin, protocatechuic acid, quercetin-3-*O*-gentiobioside, quercetin, and rutin were achieved through the use of HPLC-DAD/ESI-MS to analyze a methanol extract of fresh okra fruit [33]. Additionally, quercetin derivatives and kaempferol derivatives were identified and quantified in hydroethanolic extracts of fresh okra fruits [8]. Similarly, the phenolic compounds in methanolic extracts of okra fruits were analysed by HPLC-DAD. The study identified and quantified quercetin-3-*O*-gentiobioside, quercetin-3-*O*-glucoside (isoquercitrin), rutin, a quercetin derivative, protocatechuic acid, and a catechin derivative [34]. In a separate study, [35] determined the phenolic compounds in the ethanolic extract of fresh okra skins and seeds using HPLC/DAD. The results indicated that the seeds are primarily composed of oligomeric catechins and flavonol derivatives, while the skins are primarily composed of hydroxycinnamic acids and quercetin derivatives. Another study also demonstrated that flavonoid glycosides, including quercetin-3-*O*-gentiobiopyranoside, quercetin-3-*O*-[β-D-xyl-(1 → 2)]-*β*-D-glucopyranoside and quercetin-4"-*O*-methyl-3-*O*-β-D-glucopyranoside are common compounds in okra peel, seed, leave, and flower [7]. Also, research has indicated that quercetin 3-m-diglucoside, quercetin 3-m-glucoside, and quercetin-3-m-gentiobiose are especially abundant in okra seeds [36, 37]. This study is the first to use LC-ESI-MS/MS to report on the phenolic compound profiles in the pericarp of fresh okra fruits, expanding on the body of existing knowledge. The hydroethanolic extract from the pericarp of fresh okra fruits harvested at the final maturity stage was analyzed using LC-ESI-MS/MS analysis, as illustrated in Table 3. A total of seven phenolic acids were identified, namely protocatechuic, caffeic, *p*-coumaric, salicylic, *trans*-ferulic, *o*-coumaric, and vanillic acids. Also, two phenolic aldehydes, vanillin and hydroxybenzaldehyde, as well as a flavonoid, isoquercitrin, were detected. In general, the presence or absence of these phenolic compounds varied across the different okra accessions. Specifically, three phenolic acids; caffeic, salicylic, and *o*-coumaric acids, were detected in all six okra accessions. Hydroxybenzaldehyde was identified in all accessions except for BA accession. Vanillic acid was found in all accessions except for the BI3 accession, while *trans*-ferulic acid was present in all accessions except for the AD accession. Protocatechuic acid was only detected in the BA and GH accessions and was absent in the other accessions. *P*-coumaric acid was present only in AD and BI1 accessions and was absent in all accessions. Vanillin was not detected in the BA and GH accessions. Isoquercitrin was only absent in the BI3 and BI2 accessions. According to the studies mentioned above, in the present work, protocatechuic acid and isoquercetin were also identified in some okra accessions. In the same vein, the okra pericarp was the site of the first report

Table 3.

Phenolic compounds identified and quantified by LC-ESI-MS/MS analysis in the fruit pericarp of six okra accessions at late-stage maturity

Compound		Concentrations [mg/g extract]												
		Accessions												
		Rt	AD	BA	BI1	BI2	BI3	GH	Ion Transitions	Ion Mod	R^2	LOQ [μg/l]	LOD [μg/l]	Linearity Range [μg/l]
1	Protocatechuic acid	6.96	ND	0.015	ND	ND	ND	0.017	**153.0≥109.0**	**Negative**	0.99	13.17	3.15	15.625–250
2	Hydroxybenzaldeyde	8.64	0.010	ND	0.012	0.012	0.013	0.007	121.0≥92.0	**Negative**	0.99	12.86	4.97	15.625–250
3	Vanillic acid	7.68	0.223	0.281	0.794	0.241	ND	0.349	167.0≥151.8	**Negative**	0.99	1424.21	219.04	1250–20000
4	Caffeic acid	7.81	0.020	0.013	0.028	0.008	0.030	0.008	178.9≥135.1	**Negative**	0.99	24.16	6.92	31.25–500
5	Vanillin	8.59	0.006	ND	0.015	0.009	0.012	ND	153.0≥125.0	**Negative**	0.99	40.54	14.58	62.5–1000
6	*p*-coumaric acid	8.84	0.006	ND	0.007	ND	ND	ND	163.0≥119.0	**Negative**	0.99	17.54	3.53	31.25–500
7	Salicylic acid	9.02	0.185	0.171	0.182	0.266	0.300	0.229	137.0≥93.1	**Negative**	0.99	82.96	47.66	112.5–1800
8	*trans*-ferulic acid	9.50	ND	0.079	0.061	0.061	0.066	0.066	193.1≥133.9	**Negative**	0.99	11.52	6.11	31.25–1000
9	*o*-coumaric acid	8.84	0.024	0.027	0.039	0.046	0.040	0.028	163.0≥119.1	**Negative**	0.99	7.99	4.01	15.625–500
10	Isoquercitrin	8.65	0.006	0.005	0.004	ND	ND	0.004	464.9≥302.8	**Negative**	0.99	11.26	9.93	18.75–300
	Total quantification		0.4	0.591	1.142	0.643	0.461	0.708						

Rt: Retention time, AD: okra accessions from the Adrar region, BA: okra accessions from the Beni Abbes region, BI1, BI2, and BI3: accessions from different sites in the Biskra region, GH: okra accessions from the Ghardaia region, R2: Linearity coefficient of correlation, LOQ [μg/l]: limit of quantification, LOD [μg/l]: limit of detection, ND: Compound not detected above the LOD.

Table 4.

Antioxidant capacity of the methanolic extracts in okra fresh fruit pericarp at late-stage maturity by the DPPH assay.

Concentration [μg/ml]	Inhibition [%]					
	AD	BA	BI1	BI2	BI3	GH
18.75	–	–	3.53±2.99	–	–	–
37.5	1.31±1.09	–	7.01±6.63	–	–	10.91±4.34
75	4.02±2.31	3.76±2.77	8.30±0.85	–	–	13.18±3.55
150	13.11±2.01	11.91±3.34	19.07±1.98	9.40±4.22	1.42±3.78	22.25±2.33
300	32.20±1.71	30.16±4.50	35.41±2.47	31.80±5.39	16.02±2.73	50.34±6.48
600	53.01±2.10	52.17±5.81	46.20±1.04	45.08±9.50	25.76±13.55	65.55±6.81
1200	62.76±2.60	59.16±3.43	52.10±1.31	55.54±5.62	42.85±2.55	–
IC_{50} (µg/mL)	**565.41±1.94^{b}**	**539.52±34.14^{b}**	**1078±8.67^{c}**	**1063.57±53.57^{c}**	**>1200^{d}**	372.99±2.01^{a}

Values expressed as mean ±SD (n=3). In the last line followed by a different letter (a-c) are significantly different (p<0.05). AD: okra accessions from the Adrar region, BA: okra accessions from the Beni Abbes region, BI1, BI2, and BI3: accessions from different sites in the Biskra region, GH: okra accessions from the Ghardaia region

of all other compounds. Moreover, most of these compounds were detected also in the ethanolic extract of *Physalis acutifolia* areal parts, which are protocatechuic acid, hydroxybenzaldehyde, caffeic acid, vanillin, *o*-coumaric acid, salicylic acid and isoquercitrin [38].

In terms of quantity, the concentration of these compounds ranged between accessions from 0.004 mg/g to 0.794 mg/g. The vanillic acid content ranged from 0.223 mg/g to 0.794 mg/g, while salicylic acid levels varied between 0.171 mg/g and 0.300 mg/g, with these two compounds being the most abundant. In contrast, isoquercitrin and *p*-coumaric acid were found in minimal quantities across all accessions containing these compounds, with levels ranging from 0.004 mg/g to 0.006 mg/g. Vanillin was present in low amounts in BI2 and AD accessions (0.009 mg/g and 0.006 mg/g, respectively). Caffeic acid was detected in small quantities in BI2 and GH accessions (0.008 mg/g) and hydroxybenzaldehyde present in small amounts in GH accession (0.007 mg/g). Similarly, under identical agricultural conditions, several studies have reported that variation in the quantity and quality of phenolic compounds is influenced by cultivars and accessions [39–41]. Therefore, this variation is likely driven by genetic factors [42]. A closer examination of the identified compounds reveals several bioactive molecules of notable pharmacological interest. Caffeic acid, *o*-coumaric acid and salicylic acid are important phenolic compounds in fruits and grains and are known for their therapeutic effects [43, 44]. Then, salicylic acid (aspirin's precursor) plays also an important role in mitigating abiotic stresses, such as drought resistance [45]. This could suggest that the accessions were exposed to water stress, possibly due to the excessive heat in July [46]. Vanillic acid is known for its potential as a nutritional supplement to help prevent Alzheimer's disease [47]. Additionally, *trans*-ferulic acid is of particular interest due to its potential uses as an antioxidant in edible products, a food preservative, and a health supplement. It is also recognized for its therapeutic applications, including in the treatment of diabetes and cancer [48]. Hydroxybenzaldehyde exhibits its antioxidant and antibacterial properties [49]. Moreover, vanillin,

Table 5.

Antioxidant capacity of the methanolic extracts in okra fresh fruit pericarp at late-stage maturity by the ABTS assay

Concentration [μg/ml]	Inhibition [%]					
	AD	BA	BI1	BI2	BI3	GH
18.75	17.65±1.01	9.93±0.37	13.36±1.14	14.95±0.20	8.74±1.00	11.81±16.15
37.5	20.36±1.21	18.03±2.29	23.01±1.73	22.10±1.73	13.68±1.38	16.75±15.5
75	27.22±1.95	24.74±2.63	32.74±1.92	30.75±1.24	28.56±2.16	29.19±22.73
150	41.23±0.58	39.16±2.71	39.91±10.88	47.16±0.86	40.17±2.66	–
300	58.95±2.07	56.52±0.29	66.00±1.24	65.64±2.95	56.42±2.14	65.50±2.99
600	86.69±1.63	87.87±2.20	–	80.60±7.58	81.91±5.98	–
1200	87.84±3.54	–	–	83.31±2.75	–	–
IC_{50} [μg/ml]	**230.52±11.12^{b}**	**242.64±12.51^{b}**	**187.02±4.95^{a}**	**192.51±12.03^{a}**	**236.88±10.68^{b}**	**202.31±25.07^{a}**

Values expressed as mean ± SD (n=3). In the last line followed by a different letter (a-b) are significantly different (p<0.05). AD: okra accessions from the Adrar region, BA: okra accessions from the Beni Abbes region, BI1, BI2, and BI3: accessions from different sites in the Biskra region, GH: okra accessions from the Ghardaia region.

a safe-for-use food flavouring agent, has anti-metastatic potential and decreases the invasiveness of breast cancer cells, with great potential for use as cancer treatment [50]. Additionally, the small amount of *p*-coumaric acid detected should not be ignored because the synergistic effects of different compounds can affect the overall biological activity of plant organs [51]. From a broader phytochemical perspective, the presence of isoquercetin in okra pericarp aligns with findings reported in other species of the same genus [52]. At the family level, the detection of p-coumaric acid is consistent with reports in *Malva parviflora* L. leaves [53]. further supporting the relevance of these phenolic compounds within the *Malvaceae* family. Other, the isolation and purification of these bioactive molecules and the assessment of their therapeutic potential open new scientific perspectives for future studies.

Antioxidant capacities evaluation

The experimental data of DPPH free radical scavenging capacity of hydroethanolic extracts shown in Table 4. DPPH$^{\bullet}$ scavenging ability increased with an increase in concentrations between 18.75 and 1200 μg/ml and the inhibition percentage of DPPH$^{\bullet}$ radical in GH accession was the highest with a low IC_{50} value (372.99±2.84 μg/ml) which indicated a high potency of this extract followed by BA and AD accessions (IC_{50}=539.52±48.28 and 565.41±2.74 μg/ml, respectively). Conversely, the BI3 accession exhibited the lowest scavenging percentages (>1200 μg/ml). The ABTS$^{\bullet+}$ scavenging effect percentages of okra pericarp extracts increased with increasing concentrations, similar to the DPPH$^{\bullet}$ test results (Table 5). All okra skin accessions had a stronger capacity to quench ABTS$^{\bullet+}$ at concentrations ≥ 300 μg/ml. Therefore, BI1 and BI2 extracts exhibited higher capacity to scavenging the ABTS$^{\bullet+}$ radical than the author accessions ($A_{0.5}$= 187.02±7.00 and 192.51±12.03 μg/ml). Variation in DPPH radical scavenging activity and ABTS assay, among fresh okra fruit cultivars grown in China has also been reported by [29].

In the phenanthroline assay (Table 6), an increase in absorbance indicated a greater capacity

Table 6.

Antioxidant capacity of the methanolic extracts in okra fresh fruit pericarp at late-stage maturity by the phenanthroline activity.

Concentration [μg/ml]	Absorbance					
	A	BA	BI1	BI2	BI3	GH
37.5	0.25±0.01	–	–	–	–	0.24±0.002
75	0.43±0.03	0.38±0.01	0.37±0.01	0.32±0.01	0.34±0.03	–
150	0.47±0.05	0.43±0.02	0.47±0.03	0.42±0.03	0.39±0.02	0.44±0.060
300	0.52±0.12	0.49±0.05	0.39±0.03	0.46±0.06	0.46±0.00	0.55±0.022
IC_{50} [μg/ml]	**159.75±17.32^{a}**	**>300^{c}**	**>300^{c}**	**>300^{c}**	**>300^{c}**	**255±2.83^{b}**

Values expressed as mean ±SD (n=3). In the last line followed by a different letter (a-d) are significantly different (p<0.05)

for reducing ability and a stronger antioxidant potential. The AD extract was found to exhibit the highest reducing capacity ($A_{0.5}$= 159.75±17.32). No studies were found in the literature that investigated the phenanthroline activity of okra fruit.

CONCLUSION

This study demonstrated that the hydroethanolic extract of fresh okra fruit pericarp, harvested at the late stage of maturity from six different accessions, exhibited significant extraction yields total phenol and flavonoid contents, as well as antioxidant capacity. The LC-ESI-MS/MS analysis revealed considerable variability among the accessions at a quantitative and qualitative level. A total of ten phenolic compounds ware detected including; protocatechuic, caffeic, *p*-coumaric, salicylic, *trans*-ferulic, *o*-coumaric, and vanillic acids, vanillin, hydroxybenzaldehyde and isoquercitrin. This work offers important insights into food chemistry, waste valorization, and variety selection, providing a foundation for further studies on the agropharmaceutical properties of okra. Our research strongly supports the preservation and development of local food crops in arid regions.

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Ethical approval

The research conducted is not related to either human or animal use.

Conflict of interest

Authors declare no conflict of interest.

REFERENCES

1. Blanco-Díaz MT, Font R, Martínez-Valdivieso D, Del Río-Celestino M. Diversity of natural pigments and phytochemical compounds from exocarp and mesocarp of 27 *Cucurbita pepo* accessions. Sci Hortic 2015; 197:357–365. https://doi.org/10.1016/j.scienta.2015.09.064

2. Ezin V, Kpanougo CMI, Ahanchede, A. Genetic diversity and environmental influence on morphological and yield parameters of maize in Benin. Heliyon 2022; 8:e09670. https://doi.org/10.1016/j.heliyon.2022.e09670

3. Mkhabela SS, Shimelis H, Gerrano AS, Mashilo J. Phenotypic response of okra (*Abelmoschus esculentus* [L.] Moench) genotypes under drought-stressed and non-stressed conditions. South Afri J Bot 2022; 145:293–302. https://doi.org/10.1016/j.sajb.2021.11.008

4. Ayenan MAT, Vihou F, Ambali M, Opoku JA, Ibiotye DO, Schafleitner R. Tapping into the

potential of okra (*Abelmoschus* spp.) in Africa: integrating value-added traits into breeding. Front Plant Sci 2025; 16:1631221. https://doi.org/10.3389/fpls.2025.1631221

5. Alam MS, Khan GMA. Chemical analysis of okra bast fiber (*Abelmoschus esculentus*) and its physico-chemical properties. J Text Appar Tech Manag 2007; 5:1–9.

6. Labdelli A, Zemour K, Arbaoui F, Lahmadi S, Bengouga K, Tahirine M et al. Morphological, fiber and mineral characteristics of two accessions (genotypes) of okra (*Abelmoschu sesculentus* L. Moench) cultivated in Algeria. Vegetos 2024; 024–01149. https://doi.org/1007/s42535-024-01149-y

7. Lin Y, Lu M, Liao H, Li Y, Han W, Yuan K. Content determination of the flavonoids in the different parts and different species of *Abelmoschus esculentus* L. by reversed phase-high performance liquid chromatography and colorimetric method. Pharmacogn Mag 2014; 10(39):278. https://doi.org/10.4103/0973-1296.137368

8. Haj Romdhane M, Chahdoura H, Barros L, Dias MI, Gomes Corrêa RC, Morales P et al. Chemical composition, nutritional value, and biological evaluation of Tunisian okra pods (*Abelmoschus esculentus* L. Moench). Molecules 2020; 25:4739. https://doi.org/10.3390/molecules25204739

9. Bassoud A, Khelafi H, Mokhtari AM, Bada A. Evaluation of summer thermal comfort in arid desert areas. Case study: Old adobe building in Adrar (South of Algeria). Build Environ 2021; 205:108140. https://doi.org/10.1016/j.buildenv.2021.10814–

10. Kareem I, Faith FA, Kareem SA, Abdulmaliq SY, Adekola OF, Abdulkareem KA et al. NPK partitioning, growth, yield and proximate composition of okra (*Abelmoschus esculentus*) under water deficit stress. Bulgarian Journal of Soil Science Agrochemisty and Ecology 2020; 54(4): 3–24.

11. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem 2006; 99(1):191–203. https://doi.org/10.1016/j.foodchem.2005.07.042

12. Gogoi N, G Minti, Choudhury S. Utilization of agro waste-okra and its potentiality, Asian J Home Sci 2017; 12(1):250–256. https://doi.org/10.15740/HAS/AJHS/12.1/250-256

13. Muller L, Gnoyke S, Popken AM, Bohm V. Antioxidant capacity and related parameters of different fruit formulations. LWT-Food Science and Technology 2010; 43(6):992–999. https://doi.org/10.1016/j.lwt.2010.02.004

14. Da Silva LAL, Pezzini BR, Soares L. Spectrophotometric determination of the total flavonoid content in *Ocimumba silicum* L. (*Lamiaceae*) leaves. Pharmacogn Mag 2015; 11(41):96–101. https://doi.org/10.4103/0973-1296.149721

15. Yilmaz MA, Cakir O, Arica E, Yener İ, Otludil B, Alma MH et al. An LC-MS/MS method validation for the phytochemical quantification of four edible plants. Iğdır Univ J Inst Sci Tech 2018; 8(1):179–187. https://doi.org/10.21597/jist

16. Griffith CM, Thai AC, Larive CK. Metabolite biomarkers of chlorothalonil exposure in earthworms, coelomic fluid, and coelomocytes. Sci Total Environ 2019; 681:435–443. https://doi.org/10.1016/j.scitotenv.2019.04.312

17. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181(18):1119–1200.

18. Re R, Pellegrinis N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free Radical Biol Med 1999; 26(9-10):1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3

19. Szydłowska-Czerniak A, Dianoczki C, Recseg K, Karlovits G, Szłyk E. Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. Talanta 2008; 76(4):899–905. https://doi.org/10.1016/j.talanta.2008.04.055

20. Gadjalova AV, Mihaylova D. Ultrasound-assisted extraction of medicinal plants and evaluation of their biological activity. Food Research 2009; 3(5):530–536.

21. Green RJ. Antioxidant activity of peanut plant tissues. Master's Thesis. North Carolina State University. USA 2004; 82p.

22. Barchan A. Bakkali M, Arakrak A, Pagán R, Laglaoui A. The effects of solvents polarity on the phenolic contents and antioxidant activity of three *Mentha* species extracts. Int J Curr Microbiol App Sci 2014; 3(11):399–412.

23. Trabelsi N, Megdiche W, Ksouri R, Oueslati S, Bourgou S, Hajlaoui H, Abdelly C. Solvent effects on phenolic contents and biological activities of the halophyte *Limoniastrum monopetalum* leaves. LWT-Food Science and Technology 2010; 43 (4):632–639. https://doi.org/10.1016/j.lwt.2009.11.003

24. Falleh H, Ksouri R , Medini F, Guyot S, Abdelly C, Magné C. Antioxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule* L. Ind Crops Prod 2011; 34:1066–1071. https://doi.org/10.1016/j.indcrop.2011.03.018

25. Donkor S, Larbie C, Komlaga G, Emikpe BO. Toxicity of the hydroethanolic leaves extract of *Duranta erecta* L. in rat models. J Pharm Res Int 2020; 32(6):9–18. https://doi.org/10.9734/JPRI/2020/v32i630441

26. Haghighi MS, Arya MM, Mojarrab M, Rahimi Z, Hajialyni M, Hosseinzadeh L et al. Safety assessment of hydroethanolic extract of *Falcaria vulgaris* in Wistar rats: Acute and subchronic toxicities. J Rep Pharma Sci 2022; 11(2):248–256. https://doi.org/10.4103/jrptps.JRPTPS_15_22

27. Ermi Hikmawanti NP, Fatmawati S, Asri AW. The effect of ethanol concentrations as the extraction solvent on antioxidant activity of katuk (*Sauropus androgynus* (L.) Merr.) leaves extracts. IOP Conference Series: Earth and Environmental Science 2021; 755(1):012060. https://doi.org/10.1088/1755-1315/755/1/012060

28. Khumaida N, Syukur M, Bintang M, Nurcholis W. Phenolic and flavonoid content in ethanol extract and agro-morphological diversity of *Curcuma aeruginosa* accessions growing in West Java, Indonesia. Biodiversitas 2019; 20 (3):656–666. https://doi.org/10.13057/biodiv/d200306

29. Fabianová J, Šlosár M, Kopta T,Vargová A,Timoracká M, Mezeyová I et al. 2022. Yield, antioxidant activity and total polyphenol content of olkra fruits grown in Slovak Republic. Horticulturae 2022; 8(10):966. https://doi.org/10.3390/horticulturae8100966

30. Zrcková M, Capouchová I, Paznocht L, Eliášová M, Dvořák P, Konvalina P et al. Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year. Plant Soil Environ 2019; 65:260–266. https://doi.org/10.17221/134/2019-PSE

31. Ladeska V, Elya B, Hanafi M, Kusmardi. Antioxidants, total phenolic and flavonoid content and toxicity assay of ampelas (*Tetracera macrophylla* Wall.Ex Hook.F.& Phcogj.com Thoms) from Kalimantan-Indonesia. Pharmacogn J 2022; 12(5):642– 648. https://doi.org/10.5530/pj.2022.14.147

32. Indarti K, Apriani EF, Wibowo AE, Simanjuntak P. Antioxidant activity of ethanolic extract and various fractions from green tea (*Camellia sinensis* L.) leaves. Pharmacogn J 2019; 11(4):771–776. https://doi.org/10.5530/pj.2019.11.122

33. Wu DT, Nie XR, Shen DD, Li HY, Zhao L, Zhang Q et al. Phenolic compounds, antioxidant activities, and inhibitory effects on digestive enzymes of different cultivars of okra (*Abelmoschus esculentus*). Molecules 2020; 25(6):1276. https://doi.org/10.3390/molecules25061276

34. Shen DD, Li X, Qin YL, Li MT, Han QH, Zhou J et al. Physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of okra (*Abelmoschus esculentus*) fruit at different maturation stages. J Food Sci Technol. 2019; 56(3):1275–1286. https://doi.org/10.1007/s13197-019-03592-1

35. Arapitsas P. Identification and quantification of polyphenolic compounds from okra seeds and skins. Food Chem 2008; 110:1041–1045.

https://doi.org/10.1016/j.foodchem.2008.03.014

36. Hu L, Yu, W, Li Y, Prasad N, Tang Z. Antioxidant activity of extract and its major constituents from okra seed on rat hepatocytes injured by carbon tetrachloride. Biomed Res Int 2014; 1–9. https://doi.org/10.1155/2014/341291

37. Xia F, Zhong Y, Li M, Chang Q, Liao Y, Liu X, Pan R. Antioxidant and anti-fatigue constitu-

ents of okra. Nutrients 2015; 7(10):8846–8858. https://doi.org/10.3390/nu7105435

38. Derbak L, Bendif H, Ayad R, Bensouici C, Yildiz İ, Demirtas I et al. Optimization of polyphenol extraction, phenolic profile by LC-ESI-MS/MS, antioxidant, anti enzymatic, and cytotoxic activities of *Physalis acutifolia*. De Gruyter Brill 2024; 22 (1) 72–80. https://doi.org/10.1515/chem-2024-0040

39. Oszmiański J, Lachowicz S, Gorzelany J, Matłok N. The effect of different maturity stages on phytochemical composition and antioxidant capacity of cranberry cultivars. Eur Food Res Technol 2018; 244:705–719. https://doi.org/10.1007/s00217-017-2994-z

40. Ghimire BK, Yu CY, Kim SH, Chung IM. Assessment of diversity in the accessions of *Setaria italica* L. based on phytochemical and morphological traits and ISSR markers. Molecules 2019; 24(8):1486. https://doi.org/10.3390/molecules24081486

41. Moshrefi Araghi A, Nemati H, Azizi M, Moshtaghi N, Shoor M, Hadian J. Assessment of phytochemical and agro-morphological variability among different wild accessions of *Mentha longifolia* L. cultivated in field condition. Ind Crops Prod 2019; 140:111698. https://doi.org/10.1016/j.indcrop.2019.111698

42. Hadian J, Ebrahimi SN, Salehi P. Variability of morphological and phytochemical characteristics among *Sature jahortensis* L. accessions of Iran. Ind Crops Prod 2010; 32(1):62–69. https://doi.org/10.1016/j.indcrop.2010.03.006

43. Tariq F, Islam F, Atique B, Alawadi AH, Ganesan S, Attique S et al. Bioavailability and bioaccessibility of grain polyphenols: A comprehensive review 2025; 6(3):51–60. https://doi.org/10.1002/efd2.70067

44. Zeng Y, Zhou W, Yu J, Zhao L, Wang K, Hu Z et al. By-products of fruit and vegetables: Antioxidant properties of extractable and non-extractable phenolic compounds 2023; 12(2):418. https://doi.org/10.3390/antiox12020418

45. Bandurska H. Salicylic acid: An update on biosynthesis and action in plant response to water deficit and performance under drought [Salicylic acid]. Plant Growth and Development 2013; 1–14. https://doi.org/10.1007/978-94-007-6428-6_1

46. Faci M, Oubadi M, Matari A, Farhi Y. Analyse des journées caniculaires au Sahara Algérien. Revue des Régions Arides 2018; 44(1):37–42.

47. Sharma N, Tiwari N, Vyas M, Khurana N, Muthuraman A, Utreja P. An overview of therapeutic effects of vanillic acid. Plant Archives 2020; 20(2):3053–3059.

48. Karlsson O. Ferulic acid – A valuable natural compound for sustainable materials. Degree project. Stockholm, Sweden 2020; 21 p.

49. Esmaeili A, Kakavand S. Antioxidant and antibacterial activity evaluation of 3-hydroxybenzaldehyde: the product of thymol oxidation by a new magnetic nanocatalyst. IET Nanobiotechnol. 2017; 11(6):630–636. http://doi.org/10.1049/iet-nbt.2016.0127

50. Lirdprapamongkol K, Sakurai H, Kawasaki N, Choo MK, Saitoh Y, Aozuka Y, et al. Vanillin suppresses *in vitro* invasion and *in vivo* metastasis of mouse breast cancer cells. Eur J Pharm Sci 2005; 25(1):57–65. https://doi.org/10.1016/j.ejps.2005.01.015

51. Djeridane M, Yousfi B, Nadjemi D, Boutassouna P, Stocker N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. J Agric Food Chem 2006; 97:654–660. https://doi.org/10.1016/j.foodchem.2005.04.028

52. Lai X, Liang H, Zhao Y, Wang B. Simultaneous determination of seven active flavonols in the flowers of *Abelmoschus manihot* by HPLC. J Chromatogr Sci 2009; 47:206–210. https://doi.org/10.1093/chromsci/47.3.206

53. Hussein AA, Motar AA. Study of the phenolic compounds of some species of *Malvaceae*. Int J Adv Multidiscip Res Stud 2023; 3(b2):915–918.