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Antidiabetic potential of *Abelmoschus esculentus* leaves and fruits: a comparative study assisted by chemical profiling, *in vitro* and *in silico* studies

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Abelmoschus esculentus (L.) is an edible plant from the Malvaceae family known for its nutritional value. The phytochemical content and medicinal usefulness of the fruits of this plant have been extensively discussed, but the leaves have not been adequately investigated. The present research provides a comparative study of leaves and fruits in terms of phytochemical content and biological potential. Interestingly, the current findings highlight the higher contents of phenolics and flavonoids in the leaf extracts than in the fruit extracts. The results of GC-MS and LC-HRMS/MS analyses indicated the rich and diverse content of both organs. LC-HRMS/MS analysis allowed the annotation of seventy-four metabolites, with the leaf extract being richer (60 annotated metabolites) than the fruit extract (32 metabolites). Flavonoids, phenolics, and fatty acids were the most predominant classes of the detected metabolites. Remarkably, fatty acid derivatives, coumarins, iridoids, and lignans were reported for the first time in the genus *Abelmoschus*. The investigation of the potential activities of the two organs concluded that the antioxidant activity of leaves (9.9 ± 0.71 mg AAE per g) is better than that of fruits (7.32 ± 0.91 mg AAE per g). Similarly, the IC_{50} values for the anti-enzymatic activity of the leaf extract (4.47 ± 0.1 mg mL⁻¹, 3.54 ± 0.08 mg mL⁻¹, 0.385 ± 0.019 μg mL⁻¹, and 1.044 ± 0.05 mg mL⁻¹ against α-glucosidase, α-amylase, DPP-4, and lipase enzymes, respectively) were lower than those for the anti-enzymatic activity of the fruit extract (10.4 ± 0.2 mg mL⁻¹, 6.53 ± 0.15 mg mL⁻¹, 2.669 ± 0.132 μg mL⁻¹, and 14.66 ± 0.67 mg mL⁻¹, respectively). Additionally, the molecular docking simulation study concluded the distinct role of flavonoids in the observed bioactivities. In conclusion, *A. esculentus* leaves, which are considered agriculture waste, show richer metabolic content and more potent activities than the fruits. Therefore, okra leaves should be valued for these results.

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1 Introduction

Human well-being is directly related to nutrition and food. At the health level, dietary habits play a valuable and certain role. In recent years, there has been an increase in evidence-based research focused on functional foods and its various effects on human health, preventing numerous health disorders and blocking degenerative diseases.^{1–3} Growing research has proven the protective effect of a plant-rich diet against various health disorders, while a diet poor in plants might lead to human disorders.² A huge number of publications and research have emphasized that vegetables and fruits act as a bioactive barrier

against cancer and are identified as “chemo-preventers” owing to presence of plant phytochemicals.³ Therefore, numerous edible plants have been explored for their nutritional values and biological properties.

Diabetes mellitus (DM) is a metabolic disease commonly associated with alterations in carbohydrate, protein and fat metabolism.⁴ DM has been categorized as one of the five directing causes of death worldwide, with statistics indicating that more than 450 million people between the ages of 20 and 79 have been affected by diabetes.⁵ It is a progressive disease in which inflammation and oxidative stress have been recognized as its most common causes.⁶ Diabetes occurrence has been closely related to disorders in functional enzymes such as α-glucosidase and α-amylase. These enzymes are closely related to type 2 diabetes as they are involved in the digestion process of carbohydrates.⁷ Moreover, a high lipid profile and obesity have been accompanied by type 2 diabetes.⁸

Abelmoschus esculentus (L.) is a member of the Malvaceae family and it is commonly known as lady finger or okra. Okra is an edible plant that can be eaten fresh, cooked and used in salads, stews and soups. Okra is native to Africa, and is now

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widely cultivated throughout the world: Southern Europe, Middle East, Asia, and America.⁹ Okra has been used traditionally as a diuretic and in the management of acute inflammation, diarrhea, stomach irritation, dysuria, and gonorrhea infection, bronchitis and pneumonia.¹⁰ Also, it was reported that okra can be used as an antihyperlipidemic as it decreases lipid and cholesterol absorption. Moreover, it was found that the polysaccharide contents of okra fruits can lower the glucose levels in the body and improve the glucose tolerance,¹¹ while the polysaccharide content of okra leaves showed potent antioxidant potential.¹² The seeds and the fleshy part of okra are rich in polyphenolic constituents such as isoquercetin, quercetin, quercetin-3-*O*-gentibioside, rutin, and catechin derivatives, hydroxycinnamic derivatives which are known to be main bioactive metabolites in the plant.^{13–15} Therefore, okra extract may be used to develop novel products with different applications in the nutraceutical section, which could include functional foods with significant antioxidant, antidiabetic, and other health-promoting bioactive properties.^{16,17} Additionally, the ingestion of okra extract reduces peroxidation of lipids, boosts the levels of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase and reduces glutathione levels in diabetes, which is a chronic disease characterized by organ damage induced by oxidative stress.¹⁸

Based on the previous data, the aim of this work is based on a comparative study between the leaves and fruits of okra (*A. esculentus*). The study includes phytochemical analysis, involving the determination of total phenolic and total flavonoids as well as GC-MS and LC-HRMS/MS. Biological investigations include *in vitro* determination of the antioxidant capacity and the inhibitory potential of the extracts against α -glucosidase, α -amylase, DPP-4 (dipeptidyl-peptidase-4) and lipase enzymes. In addition, *in silico* studies were performed to elucidate the mode of interactions between the identified metabolites in the leaves and fruit extracts against the tested enzymes.

2 Materials and methods

2.1. Plant materials

The leaves and fruits of *A. esculentus* were collected from the cultivated plants in Beni-Suef governate, South Egypt. The plant parts were air dried and separately ground into a fine powder to yield 500 g of the powdered leaves and fruits, then kept separately in an airtight container until extraction.

2.2. Solvents and chemicals

The solvents used during this work (methanol, *n*-hexane and ethanol) were purchased from El-Nasr Company for chemicals; solvents used in LC/MS/MS analysis were obtained from Sigma-Aldrich. Chemicals used for total phenolic (TPC) and flavonoid (TFC), and antioxidants were Folin-Ciocalteu (F-C), sodium carbonate, gallic acid, aluminum chloride, sodium hydroxide, sodium nitrite, quercetin, ascorbic acid and DPPH, which were purchased from Sigma-Aldrich.

2.3. Preparation of the plant extracts

For LC/MS analysis and biology, 100 g of the leaves and fruits was extracted with methanol (300 mL \times 3) till complete extraction and then filtered and dried to obtain 5 g and 1 g of dry extract, respectively. One hundred mg of the dried extract was used for LC-MS/MS analysis and biological evaluation. For GC-MS analysis, 10 g of the dried plant powders were extracted with 50 mL *n*-hexane, then filtered and dried. The *n*-hexane extract was used for GC-MS analysis. For TPC, TFC and antioxidant, one gram of the dried powder of leaves and fruits was extracted with 50 mL methanol twice using an orbital shaker for 2 h, filtered and the collected filtrates were adjusted to 100 mL in a volumetric flask.

2.4. Total phenolic content (TPC)

The TPC of the leaves and fruits was determined using F-C reagent as follows: 100 μ L of the extract was mixed with 0.75 mL of F-C reagent (diluted tenfold with distilled water); after 5 min, 0.75 mL of 6% sodium carbonate was added, the mixture was kept for 90 min in the dark at room temperature, and the developed color was measured at 725 nm using a spectrophotometer (Jenway 6300). Different concentrations of gallic acid (25–200 μ g mL⁻¹) were used to plot the standard curve and the results were calculated as mg gallic acid equivalents/g dry plant powder.^{19,20}

2.5. Total flavonoid content (TFC)

The TFC of the leaves and fruits was measured using 10% AlCl₃ as follows: in a test tube, 0.5 mL of the extract was diluted with 2.25 mL of distilled water and 0.15 mL of 5% NaNO₂ solution was added. After 6 min, 0.3 mL of 10% AlCl₃·6H₂O solution was added to the mixture and allowed to stand for another 5 min. Finally, 1.0 mL of 1 M NaOH was added. The mixture was mixed well with a vortex. The absorbance was immediately measured at 510 nm using a spectrophotometer. The results were expressed as mg quercetin equivalents in 1 g of dried sample.^{19,20}

2.6. GC-MS profiling of the *n*-hexane extract of the leaves and fruits of *A. esculentus*

GC-MS analysis of the *n*-hexane extracts was performed using a TRACE GC Ultra gas chromatograph (Thermo Scientific Corp., USA) coupled with a Thermo Scientific mass spectrometer detector (ISQ single quadrupole mass spectrometer). The GC-MS apparatus was outfitted with a TR-5 MS column (30 m \times 0.32 mm i.d., 0.25 μ m film thicknesses). Helium was used as the carrier gas with flow rate of 1.0 mL min⁻¹ and a split ratio of 1 : 10 using the following temperature program: 60 $^{\circ}$ C for 1 min, rising at 4.0 $^{\circ}$ C min⁻¹ to 240 $^{\circ}$ C and held for 1 min. The injector and detector were held at 210 $^{\circ}$ C. Mass spectra were obtained by electron ionization (EI) at 70 eV using a spectral range of 40–450 *m/z*. The chemical constituents were identified bases on Wiley and NIST libraries as well as comparison of the retention indices. The compounds were identified after comparison with the available data in the computer library (NIST and Wiley) attached to the GC-MS instrument.



2.7. Metabolites profiling of the alcoholic extract of the leaves and fruits of *A. esculentus* using LC-MS/MS

Separation was performed using a Thermo Scientific C18 column (Acclaim™ Polar Advantage II, 3 × 150 mm, 3 μm particle size) on an UltiMate 3000 UHPLC system (Dionex). Gradient elution was performed at a flow rate of 0.4 mL min⁻¹ and a column temperature of 40 °C using H₂O + 0.1% formic acid (A) and 100% acetonitrile (B) with a total run time of 22 minutes. The injection volume of the sample was 3 μL. The gradient started at 5% B to 80% B in 15 min, followed by a reverse gradient back to 5% B at 22 min. High resolution mass spectrometry was carried out using a MicroTOF QIII Bruker Daltonic with ESI positive ionization and the following settings: capillary voltage: 4500 V; nebulizer pressure: 2.0 bar; drying gas: 8 L min⁻¹ at 300 °C. The mass range was 50–1000 *m/z*. The accurate mass data of the molecular ions provided by the TOF analyzer were processed by Compass Data Analysis software (Bruker Daltonik GmbH).

2.8. Biological activities

2.8.1 *In vitro* antioxidant evaluation. The antioxidant evaluation of the extracts was evaluated using DPPH as follows:^{19,20} 100 μL of the extracts were mixed with 1.0 mL of 500 μM (DPPH) in absolute ethanol. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. The developed yellow color was measured at 517 nm using a spectrophotometer (Jenway 6300). A control sample was prepared by mixing 100 μL of methanol with 1.0 mL of DPPH. The free radical scavenging activity was calculated from the following equation:

$$\text{Scavenging effect (\%)} = \left[\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \right] \times 100$$

The calibration curve was plotted using different concentrations of ascorbic acid. The result was calculated as mg ascorbic acid equivalent antioxidant capacity in 1 g of sample (mg AEAC per g).

2.8.2 Evaluation of inhibitory effects of the leaves and fruits extracts of *A. esculentus* on digestive enzymes. The leaves and fruits extracts were separately subjected to *in vitro* evaluation of the antidiabetic potential by measuring the inhibitory activity of the extracts against the digestive enzymes, such as α-glucosidase, α-amylase, dipeptidyl peptidase (DDP-4) and lipase. Solutions of the extracts and reference drugs: Acarbose for the α-glucosidase and α-amylase assay, Sitagliptin for the DPP-4 assay and Orlistat for the lipase assay were prepared in dimethyl sulfoxide (DMSO) at different concentrations: 10 mg mL⁻¹, 1 mg mL⁻¹, 100 μg mL⁻¹, 10 μg mL⁻¹, 1 μg mL⁻¹, and 0.1 μg mL⁻¹.

2.8.2.1. *In vitro* α-glucosidase inhibitory assay. The assay was performed according to the instructions in the kit protocol (Bio-vision, catalog # K938) as follows: in a 96 well clear plate, 10 μL of the sample (plant extract, Acarbose) was mixed with 10 μL of the assay buffer and 10 μL of the enzyme (dilute 2 μL of α-glucosidase with 38 μL of α-glucosidase assay buffer), adjust the volume to 80 μL with the buffer, mix well and incubate at room

temperature for 15–20 min. Twenty μL of the enzyme substrate (*p*-nitrophenyl-α-D-glucopyranoside (PNPG)) was mixed and the absorbance was measured at 410 nm. The IC₅₀ for the extracts and Acarbose were calculated.^{19,21}

2.8.2.2. *In vitro* α-amylase inhibitory assay. The inhibitory potential of the extracts and the reference drug Acarbose was evaluated *in vitro* according to the instructions in the kit protocol (Bio-vision, catalog # K482): in a 96 well microplate, 50 μL of the extract or the standard was mixed with 50 μL of the enzyme solution, mixed well and incubated at room temperature for 10 min, followed by the addition of 50 μL of the starch solution and incubated for 3 min. Then, 50 μL of DNS reagent (3,5-dinitrosalicylic acid) was added to stop the reaction the reaction and boiled for 10 min at 85–90 °C in a water bath. The mixture was cooled to room temperature and the absorbance was measured at 405 nm.²²

2.8.2.3. *In vitro* DPP-4 inhibitory assay. The activities of the extracts as inhibitors of DPP-4 were measured using a DPP-4 inhibitor screening assay kit (RayBio Quantichrom DPP-4 inhibitor screening kit) and compared with Sitagliptin (reference drug) according to the manufacturer's protocol. In a 96 well plate, 50 μL of the diluted DPP-4 enzyme solution was mixed with 25 μL of the sample (extract or control), mixed well, and incubated for 10 minutes at 37 °C. Twenty-five μL of the substrate was added to each well and incubated for 30 minutes at 37 °C. After incubation, the fluorescence was measured at Ex/Em = 360/460 nm.^{23,24}

2.8.2.4. *In vitro* lipase inhibitory assay. Lipase inhibition activity of the leaves, fruit extracts and the reference drug (orlistat) was evaluated according to.²⁵ In this method, the activity was assayed using *p*-nitrophenyl butyrate (PNB) as a substrate. Fifty μL of the sample (extract or standard) was added to 20 μL of the enzyme (pancreatic lipase, type II, ≥125 units per mg protein from Sigma-Aldrich), diluted with 120 μL of Tris-base buffer solution, and incubated for 25 minutes at 37 °C. Then, 20 μL of the substrate was added, and the amount of *p*-nitrophenol released in the reaction was measured using a Robonik p2000 ELISA reader at 450 nm.

2.9. Molecular docking study

AutoDock Vina software was used in all molecular docking experiments.²⁶ All dereplicated compounds were docked against the active sites of human α-amylase, α-glucosidase, and dipeptidyl peptidase-4 (DPP4) (with PDB codes 4W9, 3L4W, and 2ONC, respectively).^{27,28} The docking sites were determined according to the enzyme's co-crystallized ligands. The coordinates of the grid boxes were *x* = -9.682; *y* = 4.274; *z* = -23.145; and *x* = 45.424; *y* = 92.375; *z* = 34.811; and *x* = -10.456; *y* = 17.557; *z* = -76.278, respectively. The size of the grid box was set to 20 Å³. The exhaustion was set to 24. Ten poses were generated for each docking experiment. Docking poses were analyzed and visualized using Pymol software.²⁶

2.10. Statistical analysis

All the data was expressed as means ± standard deviation (SD) from three experiments. The data of TPC, TFC and antioxidant



was calculated from the linear calibration curve plotted by Excel software using different concentrations of the standards. In addition, the difference for α -glucosidase, α -amylase, DDP-4 and lipase was considered significant at a P value < 0.05 using one-way analysis of variance (ANOVA) for comparison of the group's differences followed by Tukey's test for multiple comparisons using GraphPad Prism 8 (La Jolla, CA, USA).

3 Results and discussion

A. esculentus, known as okra, is a species in the Malvaceae family whose fruit is widely consumed in the human diet. The nutritional value of different parts of okra have been thoroughly investigated and found to be rich in proteins, fibers, polysaccharides, vitamins, and minerals.²⁹ Its medicinal value has also been discussed in several studies that reported its antioxidant, antimicrobial and cytotoxic effects.^{12,30–32} The current research aims to value okra leaves that are considered as agriculture waste and to compare their phytochemical content and biological efficacy with those of fruits (the edible part of the plant). The metabolic content of the two organs was explored using GC-MS and LC-MS/MS analyses, then the DPPH scavenging effect and the antidiabetic activity of the two organs extracts were compared. It is noteworthy that this study is the first report of the metabolic profiling and antidiabetic potential of an alcoholic extract of *A. esculentus* (okra) leaves. Moreover, an *in silico* study was conducted to figure out which of the dereplicated metabolites is effective in inhibiting the tested enzymes.

3.1. Total phenolics and total flavonoids

The TPC of the leaves and fruits were evaluated using Folin-Ciocalteu (FC) reagent. The phenolic compounds reduce the FC reagent, forming a blue complex measured at 725 nm, revealing that the leaves showed higher content of phenolics (6.84 ± 0.19 mg GAE per g dry weight (DW)) than fruits, which showed 3.89 ± 0.034 mg GAE per g DW. A previous study of the phenolic content of the leaves, fruits and seeds of okra indicated higher content of phenolics in leaves;³³ these results support the current findings. TFC was also evaluated using 10% aluminum chloride and the results were expressed as quercetin equivalent (QE) per g dry weight. The flavonoid content in leaves was quantified as 2.04 ± 0.2 mg QE per g DW while the fruits exhibited 1.4 ± 0.08 mg QE per g DW. Similar results were previously reported by Wu *et al.* 2020 who investigated the flavonoid content of the fruits in different okra cultivars and concluded variable contents of flavonoids ranging from 1.75 to 3.39 mg RE per g DW.³⁴

3.2. GC-MS profiling of the *n*-hexane extract of leaves and fruits of *A. esculentus*

The constituents in the *n*-hexane extracts from leaves and fruits were analyzed using GC-MS and the identified compounds were recorded in Table 1. The results noted that the linoleic acid, palmitic acid and oleic acid fatty acids with peak areas 14.52%, 10.73% and 8.83%, respectively are the major components in

the fruit extract. Oleic acid, 2-methylhexacosane, and hexadecanoic acid ethyl ester with peak areas of 12.16%, 10.58% and 7.13%, respectively were the major identified compounds in the leaf extract. Reviewing the relevant literature, several studies were found discussing GC-MS analysis of okra.^{35,36} Osman *et al.*, analyzed okra fruit extracts using GC-MS and concluded that hexadecanoic acid methyl ester and 9,12-octadecadienoic acid methyl ester were among the major detected components.³⁶ Interestingly, the current findings stated the identification of hexadecanoic acid methyl ester (7.13%) and 9,12-octadecadienoic acid ethyl ester (4.98%) in the leaf extract, while 9,12-octadecadienoic acid (14.52%) was detected in the fruit extract.

3.3. Metabolomic profiling of the alcoholic extract of leaves and fruits of *A. esculentus*

Metabolic profiling using LC-HRMS/MS of *A. esculentus* led to the annotation of 74 metabolites with variable chemical structures: 60 metabolites were identified in the leaf extract while 32 metabolites were identified in the fruit extract. The annotated metabolites (Table 2 and Fig. 1–3) could be classified according to their chemical identity as follows.

3.3.1 Flavonoids. Twenty six flavonoids were annotated from the mass ion peaks at m/z : 275.0945, 259.0922, 303.1318, 287.1299, 507.2122, 599.2448, 523.1986, 579.1621, 627.2373, 597.1400, 465.1011, 300.1219, 291.0967, 627.2658, 625.2477, 449.3742, 433.2750, 335.2147, 479.2969, 595.3724, 317.1164, 611.4198, 463.2982, 319.2203, 307.2546, and 551.3482 as: luteoliflavan (**10**), davidigenin (**11**), quercetin (**16**), kaempferol (**17a**) or luteolin (**17b**), quercetin 3-*O*-(6-*O*-acetyl- β -D-glucopyranoside) (**22**), delphinidin 3-*O*-sambubioside (**24**), floramanoside F (**26**), apigenin 7-*O*-neohesperidoside (**27**), quercetin 3,7 diglucoside (**28a**), quercetin 3-*O*-sophoroside (**28b**), quercetin 3-*O*-[β -D-xylosyl-(1 \rightarrow 2)- β -D-glucoside] (**30**), quercetin-3-*O*-glucoside (**33**), hispidulin (**38**), epicatechin (**39**), myricetin 3-*O*-rutinose (**41**), floramanoside D (**42**), kaempferol-3-*O*-glucoside (**48**), apigenin-7-glucoside (**49**), hibiscetin (**53**), quercetin 4'-*O*-methyl-3-*O*- β -D-glucopyranoside (**59**), tiliroside (**61**), isorhamnetin (**63**), isorhamnetin 3-*O*-glucoside-7-*O*-xyloside (**64**), scutellarin (**65**), myricetin (**69a**) or gossypetin (**69b**), gallo catechin (**72**), and quercetin-3-*O*-(malonyl) glucoside (**74**), respectively. Among the detected metabolites flavonoids, the most predominant were flavonoid numbers (**16**)^{13,52} (**24**),⁶² (**28a**),³⁷ (**28b**),³⁷ (**39**),⁷³ (**59**),⁸³ (**61**),^{42,49} (**64**),⁶² (**72**)⁷³ and (**74**),¹³ which were previously reported in *A. esculentus*. Interestingly, a signal with a molecular ion mass at m/z 286.6171 was compatible with the molecular formula $C_{15}H_9O_6$ and could be tentatively identified as kaempferol (**17a**) that was earlier identified in *A. esculentus* seeds and skins *via* HPLC coupled with DAD and an ESI/MS/MS detector¹³ or luteolin (**17b**), which has been identified in the methanolic extract of the flowers of *H. sabdariffa*⁵³ and also isolated from *Malva parviflora* leaf extract.⁵⁴ Similarly, a signal at m/z 318.2130 agreed with the molecular formula $C_{15}H_{10}O_8$ and could be identified as myricetin (**69a**), which has been isolated from *A. manihot* (L.) Medik. flowers⁵² or gossypetin (**69b**) that has been identified in the petals of *A.*



Table 1 GC-MS analysis results of the *n*-hexane extract of the leaves and fruits of *Abelmoschus esculentus*

Compound name	R_t	Fruit	Leaves	Molecular formula	
1	Tridecanol	4.28	2.19	—	C ₁₃ H ₂₈ O
2	2,7-Dimethyl-1-octanol	4.86	3.94	—	C ₁₀ H ₂₂ O
3	1-Octadecanethiol	5.26	1.56	—	C ₁₈ H ₃₈ S
4	Propane, 1-(dodecyloxy)-2,3-epoxy-	5.49	1.40	—	C ₁₅ H ₃₀ O ₂
5	<i>Trans</i> -3-decen-1-ol	5.86	3.60	—	C ₁₀ H ₂₀ O
6	4-Tridecene, (<i>Z</i>)-	6.76	4.89	—	C ₁₃ H ₂₆
7	1-Octadecyne	7.12	1.08	—	C ₁₈ H ₃₄
8	1-Hexadecyne	7.38	1.41	—	C ₁₆ H ₃₀
9	1-Chlorooctadecane	8.01	3.64	—	C ₁₈ H ₃₇ Cl
10	1-Octene	8.95	2.22	—	C ₈ H ₁₆
11	1-Octanol, 2-butyl-	9.13	2.42	—	C ₁₂ H ₂₆ O
12	<i>n</i> -Heptadecylcyclohexane	9.53	2.27	—	C ₂₃ H ₄₆
13	1-Chlorohexadecane	9.95	1.21	—	C ₁₆ H ₃₃ Cl
14	Benzene, (2-decyldodecyl)	10.06	1.76	—	C ₂₈ H ₅₀
15	10-Heneicosene, 11-phenyl-	10.57	3.74	—	C ₂₇ H ₄₆
16	Oxirane, dodecyl	10.97	1.49	—	C ₁₄ H ₂₈ O
17	Neophytadiene	19.98	—	3.58	C ₂₀ H ₃₈
18	Hexahydrofarnesyl acetone	20.06	—	1.71	C ₁₈ H ₃₆ O
19	1-Heptatriacotanol	21.19	—	2.46	C ₃₇ H ₇₆ O
20	Hexadecanoic acid, methyl ester	21.29	—	2.16	C ₁₇ H ₃₄ O ₂
21	Hexadecanoic acid, ethyl ester	22.31	—	7.13	C ₁₈ H ₃₆ O ₂
22	Methyl octadecanoate	21.36	1.24	—	C ₁₉ H ₃₈ O ₂
23	Tetradecanoic acid	23.11	3.94	—	C ₁₄ H ₂₈ O ₂
24	Phytol	24.05	1.16	2.59	C ₂₀ H ₄₀ O
25	Dodecanoic acid	24.15	1.46	—	C ₁₂ H ₂₄ O ₂
26	9,12-Octadecadienoic acid (9 <i>Z</i> ,12 <i>Z</i>), ethyl ester	24.68	—	4.98	C ₂₀ H ₃₆ O ₂
27	Hexadecanoic acid	24.85	10.37	—	C ₁₆ H ₃₂ O ₂
28	Heptadecanoic acid, ethyl ester	25.12	—	1.68	C ₁₉ H ₃₈ O ₂
29	Oleic acid	25.51	8.83	12.16	C ₁₈ H ₃₄ O ₂
30	9,12-Octadecadienoic acid (<i>Z,Z</i>)-	27.41	14.52	—	C ₁₈ H ₃₂ O ₂
31	Stigmasterol	29.36	—	3.74	C ₂₉ H ₄₈ O
32	Di- <i>n</i> -octyl phthalate	30.19	1.87	6.16	C ₂₄ H ₃₈ O ₄
33	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	31.74	—	6.45	C ₃₃ H ₅₄ O ₃
34	2,6,10-Trimethyltetradecane	31.83	—	1.89	C ₁₇ H ₃₆
35	2-Methylhexacosane	32.00	1.68	10.58	C ₂₇ H ₅₆
36	Squalene	32.71	—	4.70	C ₃₀ H ₅₀
37	(9 <i>E</i>)-8-Methyl-9-tetradecenyl acetate	32.82	—	1.05	C ₁₇ H ₃₂ O ₂
38	Methyl 4,4-difluororetinoate	33.01	—	3.29	C ₂₁ H ₂₈ F ₂ O ₂
39	Methoprene	33.24	—	3.59	C ₁₉ H ₃₄ O ₃
40	Octadecanoic acid	33.47	—	1.14	C ₁₈ H ₃₆ O ₂
41	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	34.76	—	1.10	C ₃₃ H ₅₄ O ₃
42	Cholest-4-en-3-one	35.15	—	1.58	C ₂₇ H ₄₄ O
43	Cedryl propyl ether	35.23	—	2.34	C ₁₈ H ₃₂ O
44	Cholesta-4,6-dien-3-ol	35.48	—	1.91	C ₂₇ H ₄₄ O

esculentus.⁸⁹ Both myricetin and gossypetin have been identified in the corolla of *A. manihot* by UPLC-triple TOF-MS/MS.^{59,90} Flavonoids number 22,⁵⁹ 26,⁵² 30,⁵⁹ 33,^{52,68} 41,⁵⁹ 42,^{59,75} 63,^{85,86} 69a,⁵² and 69b,^{59,90} were previously reported from *A. manihot* while flavonoids number 27,⁶⁴ 38,⁴² 48,^{42,93} 49,^{42,54} 53,^{42,53,78} 61,^{42,49} and 65,⁸⁷ were previously reported in other Malvaceae plants: *H. sabdariffa*,⁵³ *Malva parviflora*,⁵⁴ *Kitaibelia vitifolia*,⁶⁴ *Alcea setosa*,⁴² and *Adansonia digitata*.⁸⁷ Notably luteoliflavan (10) davidigenin (11) flavonoids are reported in the Malvaceae family for the first time; however, (10) was isolated from the methanol extract of *Pandanus tonkinensis* fruits (Pandanaceae),⁴⁷ and (11) has been isolated from the methylene chloride fraction of *Mascarenhasia arborescens* A. DC. (Apocynaceae).⁴⁸

3.3.2 Phenolics. Fourteen phenolics were dereplicated from the recorded masses at *m/z*: 211.0719, 461.2013, 138.0548, 167.0891, 328.1380, 337.1060, 369.1162, 197.1164, 171.0994, 195.1375, 181.121, 199.1336, 361.2195, and 355.281 as 5-hydroxyferulic acid (3), paeonolide (4), salicylic acid (5), methyl 2-(2-hydroxyphenyl) acetate (12), *p*-coumaroyl-hexose (13), 5-caffeoylshikimic acid (21), feruloylquinic acid (32), acetosyringone (35), gallic acid (36), ferulic acid (43), caffeic acid (44), syringic acid (47), rosmarinic acid (57), and chlorogenic acid (73), respectively. The second abundant class in okra was the phenolics, where compounds 12,⁴⁹ 13,¹³ 36,^{70,71} 43,^{70,71} 44,³⁷ 47,³⁷ 57,⁷⁰ and 73,⁷¹ were previously isolated from different parts of *A. esculentus*. In addition, four metabolites were reported in other



Table 2 LC-HRMS-MS dereplication results of the alcoholic extract of the leaves and fruits of *Abelmoschus esculentus*

No.	Tentative identification	R_t	m/z^d	Molecular formula	MS/MS-fragments	Chemical class	Plant organ		Reference
							Leaves	Fruits	
1	Shikimic acid	1.3	175.1186	C ₇ H ₁₀ O ₅	116.0699, 130.0970, 175.1181	Hydroxy carboxylic acid	–	+	37
2	Azelaic acid	1.6	189.1118	C ₉ H ₁₆ O ₄	130.0871, 189.1247	Fatty acid	–	+	38 and 39
3	5-Hydroxyferulic acid	1.7	211.0719	C ₁₀ H ₁₆ O ₅	211.0719	Phenolic acid	+	–	40
4	Paeonolide	1.9	461.2013	C ₂₀ H ₂₈ O ₁₂	461.2013	Phenolic compound	+	–	41
5	Salicylic acid (organic acid)	1.9	138.0548	C ₇ H ₆ O ₃	120.0825, 138.0545	Phenolic acid	+	+	42
6	Agnuside	2.0	467.1908	C ₂₂ H ₂₆ O ₁₁	467.1908	Iridoid glycoside	+	–	43 and 44
7	Limifolin A	2.2	305.1330	C ₁₇ H ₂₀ O ₅	215.1339, 227.1079, 304.1513, 305.1378	Sesquiterpene lactones	+	+	45
8	Vernolic acid	2.3	297.0977	C ₁₈ H ₃₂ O ₃	297.0977	Fatty acid	+	+	46
9	Iridotrial glucoside	2.8	345.1357	C ₁₆ H ₂₄ O ₈	165.0549, 280.1175, 308.1125, 326.1238	Iridoid glycoside	+	–	44
10	Luteolinflavan	2.9	275.0945	C ₁₅ H ₁₄ O ₅	130.0497, 192.0652, 226.0715, 238.0708	Flavonoid	+	–	47
11	Davidigenin	3.0	259.0922	C ₁₅ H ₁₄ O ₄	130.0495, 131.0523, 171.0776, 223.0984	Flavonoid	+	+	48
12	Methyl 2-(2-hydroxyphenyl) acetate	3.6	167.0891	C ₉ H ₁₀ O ₃	120.0809, 131.0485, 166.0831	Phenolic compound	+	–	49
13	<i>p</i> -Courmaryl-hexose	4.1	328.1380	C ₁₅ H ₁₈ O ₈	166.0859, 264.1225, 292.1171, 310.1277	Phenolic compound	+	+	13
14	37(β)-9,18-Dihydroxyolean-12-en-3-yl acetate	4.7	485.2498	C ₃₂ H ₅₂ O ₃	485.2498	Triterpene	+	–	50
15	7-Hydroxydodecanoate	5.0	217.1725	C ₁₂ H ₂₄ O ₃	114.103, 132.1133, 216.1704	Fatty acid ester	+	–	51
16	Quercetin	7.0	303.1318	C ₁₅ H ₁₀ O ₇	195.0906, 285.1224, 303.1329	Flavonoid	+	–	13 and 52
17	17a-Kaempferol 17b-Luteolin	7.1	287.1299	C ₁₅ H ₁₀ O ₆	210.0777, 215.5759, 286.1290	Flavonoid	+	–	13
18	Traumatic acid	7.1	229.1551	C ₁₂ H ₂₀ O ₄	229.1551	Fatty acid	–	+	53 and 54
19	(3β,21β)-19,21-Epoxyilup-20(29)-en-3-yl acetate	7.4	483.1977	C ₃₂ H ₅₂ O	483.1977	Triterpene	+	–	50
20	Isobergapten	7.7	217.1725	C ₁₂ H ₈ O ₄	144.0805, 217.0959	Furanocoumarin	+	+	56
21	5-Caffeoylshikimic acid	7.8	337.1060	C ₁₆ H ₁₆ O ₈	337.1060	Phenolic acid	+	–	57 and 58
22	Quercetin 3-O-(6-O-acetyl-β-D-glucopyranoside)	7.9	507.2122	C ₂₃ H ₂₂ O ₁₃	507.2122	Flavonoid glycoside	+	–	59
23	Cycloart-23-ene-3β,25-diol or cycloart-25-en-3,24-diol	8.0	443.2240	C ₃₀ H ₅₀ O ₂	132.1125, 177.0542, 443.2242	Triterpene	+	–	60 and 61
24	Delphinidin 3-O-sambubioside	8.2	599.2448	C ₂₆ H ₃₀ O ₁₆	599.2448	Flavonoid glycoside	+	–	62
25	Abscisic acid	8.3	265.0961	C ₁₅ H ₂₀ O ₄	206.0830, 247.0867, 265.0961	Sesquiterpene	+	–	63
26	Floranoside F	8.4	523.1986	C ₂₃ H ₂₁ O ₁₄	523.1986	Flavonoid glycoside	+	–	52
27	Apigenin 7-O-neohesperidoside	8.6	579.1621	C ₂₇ H ₃₀ O ₁₄	313.0691, 397.0888, 415.0998, 433.1100	Flavonoid glycoside	+	–	64
28	28a-Quercetin 3,7 diglucoside, 28b-Quercetin 3-O-sophoroside	8.7	627.2373	C ₂₇ H ₃₀ O ₁₇	127.0379, 145.0492, 303.0496	Flavonoid glycoside	–	+	37
29	Calanolide A	8.8	371.2023	C ₂₂ H ₂₆ O ₅	133.1005, 137.0946, 209.1531	Coumarin	+	–	65 and 66





Table 2 (Contd.)

No.	Tentative identification	R_t	m/z^d	Molecular formula	MS/MS-fragments	Chemical class	Plant organ			Reference
							Leaves	Fruits	Leaves	
30	Quercetin	8.8	597.1400	$C_{26}H_{28}O_{16}$	127.0384, 303.0486	Flavonoid glycoside	–	+	59	
31	3-O- $[\beta$ -D-xylosyl-(1 \rightarrow 2)- β -D-glucoside]	9.1	521.2143	$C_{26}H_{32}O_{11}$	177.0543, 191.0691	Lignan	+	–	67	
32	(–)Pinoresinol glucoside Feruloylquinic acid	9.1	369.1162	$C_{17}H_{21}O_9$	145.0276, 177.0541, 178.0567	Phenolic acid	+	–	57 and 58	
33	Quercetin-3-O-glucoside	9.2	465.1011	$C_{21}H_{20}O_{12}$	127.0374, 303.0494	Flavonoid glycoside	–	+	52 and 68	
34	Herniarin	9.2	177.0537	$C_{10}H_8O_3$	117.0339, 145.0269	Coumarin	+	–	69	
35	Acetosyringone	9.4	197.1164	$C_{10}H_{12}O_4$	145.0277, 149.0583, 177.0541	Phenolic compound	+	+	42	
36	Galic acid	9.6	171.0994	$C_7H_6O_5$	171.1492	Phenolic acid	+	+	70 and 71	
37	Comiferyl ferulate	9.6	357.1456	$C_{20}H_{20}O_6$	357.1456	Lignan	+	–	72	
38	Hispidulin	9.7	300.1219	$C_{16}H_{12}O_6$	121.0645, 163.0385, 300.1204	Flavonoid	+	–	42	
39	(+)-Epicatechin	9.7	291.0967	$C_{15}H_{14}O_6$	159.0911, 188.0695, 227.0794	Flavonoid	+	–	73	
40	<i>N-E</i> -Feruloyltyramine (moupinamide)	9.9	314.0853	$C_{18}H_{19}NO_4$	121.0638, 177.0540, 255.2301	Nitrogenous metabolite	+	+	49 and 74	
41	Myricetin 3-O-rutinoside	10.1	627.2658	$C_{27}H_{30}O_{17}$	177.0539, 314.1378	Flavonoid glycoside	+	+	59	
42	Floramanoside D	10.2	625.2477	$C_{28}H_{32}O_{16}$	201.0537, 460.1715, 488.1653	Flavonoid glycoside	+	+	59 and 75	
43	Ferulic acid	10.7	195.1375	$C_{10}H_{10}O_4$	135.1174, 194.1164	Phenolic acid	+	–	70 and 71	
44	Caffeic acid	10.9	181.121	$C_9H_8O_4$	135.1151, 163.1107, 181.1210	Phenolic acid	+	–	37	
45	Allamandin	11.1	309.0819	$C_{13}H_{16}O_7$	263.0803, 281.0908, 309.0852	Iridoid	+	–	76	
46	9,12,13-Trihydroxy-octadecenoic acid	11.2	331.2463	$C_{18}H_{34}O_5$	277.2149, 295.2245	Fatty acid	+	+	77	
47	Syringic acid	11.3	199.1336	$C_9H_{10}O_5$	199.1336	Phenolic acid	+	–	37	
48	Kaempferol-3-O-glucoside (astragalol)	11.7	449.3742	$C_{21}H_{19}O_{11}$	186.1231, 225.1974, 449.2699	Flavonoid glycoside	+	+	42	
49	Apigenin-7-glucoside	11.9	433.2750	$C_{21}H_{20}O_{10}$	137.0446, 415.2666	Flavonoid glycoside	–	+	54 and 42	
50	β -Sitosterol	12.0	415.2082	$C_{29}H_{50}O$	119.0851, 120.0878	Sterol	+	–	60 and 61	
51	Pinoresinol	12.1	359.2367	$C_{20}H_{22}O_6$	359.2367	Lignan	+	–	67	
52	Scopoletin	12.3	193.1471	$C_{10}H_8O_4$	135.0791, 191.1415	Coumarin	+	–	42	
53	Hibiscetin	12.5	335.2147	$C_{15}H_{10}O_9$	333.2022, 335.2168	Flavonoid	–	+	42, 53 and 78	
54	(12Z)-9,10-Dihydroxyoctadec-12-enoic acid	12.6	315.2493	$C_{18}H_{34}O_4$	133.0993, 147.1153, 279.2298	Fatty acid	–	+	79	
55	Stigmast-4,22-dien-3,6-dione	12.9	425.3736	$C_{29}H_{44}O_2$	137.1312, 407.3639	Sterol	+	+	60 and 61	
56	Stearidonic acid (6,9,12,15-octadecatetraenoic acid)	13.0	277.1126	$C_{18}H_{28}O_2$	121.1003, 135.1150, 277.2066	Fatty acid	+	+	80	
57	Rosmarinic acid	13.0	361.2195	$C_{18}H_{16}O_8$	129.0146, 361.2341	Phenolic acid	+	–	70	
58	Ammosesinol	13.1	383.1963	$C_{24}H_{30}O_4$	281.1330, 327.1381, 383.2028	Coumarin	+	–	81 and 82	
59	Quercetin-4'-O-methyl-3-O- β -D-glucopyranoside	13.2	479.2969	$C_{22}H_{22}O_{12}$	337.2713, 479.3372	Flavonoid glycoside	–	+	83	
60	Yamogenin 3-O-neohesperidoside	13.3	723.4957	$C_{39}H_{62}O_{12}$	723.4957	Saponin glycoside	+	–	84	

Table 2 (Contd.)

No.	Tentative identification	R_t	m/z^a	Molecular formula	MS/MS-fragments	Chemical class	Plant organ			Reference
							Leaves	Fruits		
61	Tilioside	13.5	595.3724	$C_{30}H_{26}O_{13}$	578.3660, 595.3750	Flavonoid glycoside	+	+	49 and 42	
62	Linolenic acid	13.7	279.2291	$C_{18}H_{29}O_2$	123.1162, 137.1305, 279.2275	Fatty acid	+	+	77	
63	Isorhamnetin	13.8	317.1164	$C_{16}H_{12}O_7$	317.2073	Flavonoid	+	+	85 and 86	
64	Isorhamnetin 3-O-glucoside-7-O-xyloside	13.9	611.4198	$C_{27}H_{30}O_{16}$	317.2074, 318.2095, 358.2330	Flavonoid glycoside	–	+	62	
65	Scutellarin	13.9	463.2982	$C_{21}H_{18}O_{12}$	231.0635, 341.0973, 463.2981	Flavonoid	+	–	87	
66	Oleic acid	14.1	283.2604	$C_{18}H_{34}O_2$	283.2604	Fatty acid	+	–	77	
67	Esculentoside	14.3	331.2801	$C_{13}H_{18}N_2O_8$	239.2366, 313.2694	Nitrogenous metabolites (pyridine-imidazole derivative)	+	–	49 and 74	
68	(4Z,7Z,10Z,13Z,16Z,19Z)-Docosahexaenoic acid ethyl ester	14.5	357.2955	$C_{24}H_{36}O_2$	135.1162, 247.2425, 265.2481	Fatty acid ester	+	–	88	
69	69a-Myricetin (syn.: Myricetol or cannabiscetin) 69b-Gossypetin (syn.: articulatin and equisporol) (–)-Isoamijiol	14.6	319.2203	$C_{15}H_{10}O_8$	318.3111, 319.2224	Flavonoid	–	+	52	
70		14.6	305.2447	$C_{20}H_{32}O_2$	121.1004, 135.1147, 305.2424	Diterpene	+	–	91	
71	Linoleic acid	14.8	281.2451	$C_{18}H_{32}O_2$	133.0975, 135.1124, 45.2270	Fatty acid	–	+	92	
72	Gallocatechin	15.6	307.2546	$C_{15}H_{14}O_7$	123.1153, 261.2210	Flavonoid	+	+	73	
73	Chlorogenic acid	16.0	355.281	$C_{16}H_{18}O_9$	245.2238, 263.2341, 337.2715	Phenolic acid	+	+	71	
74	Quercetin-3-O-(malonyl) glucoside	16.1	551.3482	$C_{24}H_{32}O_{15}$	534.3579, 549.2513	Flavonoid glycoside	+	+	13	

^a All the compounds were detected as M + H.

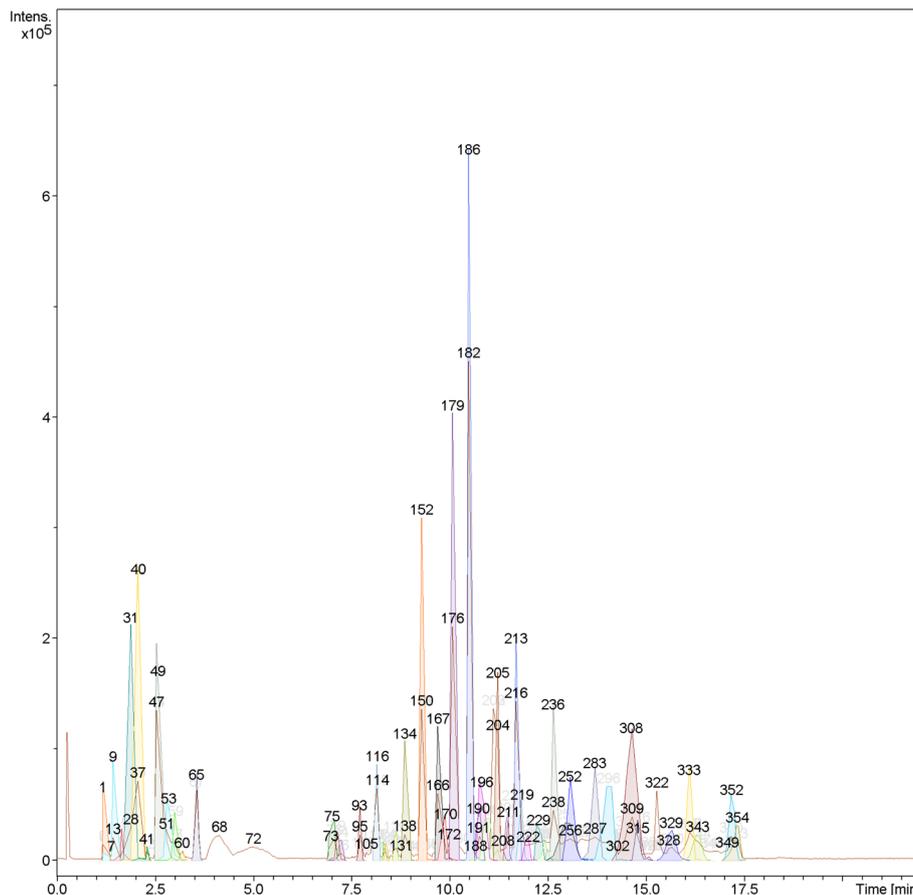


Fig. 1 LC-HRMS chromatogram of dereplicated metabolites from the alcoholic extract of the leaves of *Abelmoschus esculentus* (positive mode).

Malvaceae plants, where metabolites no. **21** and **32** were isolated from the leaves and flowers of *H. sabdariffa*^{57,58} and compounds **5** and **35** from *Alcea setosa* flowers.⁴² Remarkably, the remaining two phenolic compounds (**3** and **4**) were not previously reported from the Malvaceae family; however, **3** was isolated from the leaves of *Wasabia japonica* (Brassicaceae),⁴⁰ and **4** from *Paeonia ostii* roots (Paeoniaceae).⁴¹

3.3.3 Acids. Twelve acids (one hydroxy acid and eleven fatty acids) were dereplicated based on the peaks at m/z 175.1186, 189.1118, 297.0977, 217.1725, 229.1551, 331.2463, 315.2493, 277.126, 279.2291, 283.2604, 357.2955, and 281.2451 as shikimic acid (**1**), azelaic acid (**2**), vernolic acid (**8**), 7-hydroxydodecanoate (**15**), traumatic acid (**18**), 9,12,13-trihydroxyoctadecenoic acid (**46**), (12*Z*)-9,10-dihydroxyoctadec-12-enoic acid (**54**), stearidonic acid (**56**), linolenic acid (**62**), oleic acid (**66**), (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosahexaenoic acid ethyl ester (**68**), and linoleic acid (**71**), respectively. Shikimic acid (**1**) is a hydroxy-acid previously isolated from the Tunisian okra pods (*A. esculentus* L. Moench).³⁷ Fatty acid numbers: **8**,⁴⁶ **46**,⁷⁷ **62**,⁷⁷ **66**,⁷⁷ **68**,⁸⁸ and **71**,⁹² have been reported in several Malvaceae plants: *Malva arboreus*, *Hibiscus cannabinus*, *H. sabdariffa*, *Sida galheirensis*, *M. sylvestris*, *M. sylvestris* L. var. *mauritanica* and *Althaea officinalis* L. Interestingly, compound number **15** is a 7-hydroxylkanoic acids previously detected in eight species of the

genus *Mucor*⁵¹ and **18** was reported as a plant wound hormone.⁵⁵ On the other hand, **2** was previously isolated from *Fagopyrum esculentum* in the Polygonaceae family,^{38,39} **54** was identified as one of the linoleic acid metabolites,⁷⁹ and **56** was identified as the first metabolite of α -linolenic acid.⁸⁰

3.3.4 Terpenes. Six terpenes were annotated as linifolin A (**7**), (3 β)-9,18-dihydroxyolean-12-en-3-yl acetate (**14**), (3 β ,21 β)-19,21-epoxylup-20(29)-en-3-yl acetate (**19**), cycloart-23-ene-3 β ,25-diol or cycloart-25-en-3,24-diol (**23**), abscisic acid (**25**), and (–)-isoamijiol (**70**), in addition to one triterpenoidal saponin annotated as yamogenin 3-*O*-neohesperidoside (**60**) from the molecular masses at m/z 305.1330, 485.2498, 483.1977, 443.2240, 265.0961, 305.2447, and 723.4957, respectively. The identified terpenes included three triterpenes, two sesquiterpenes, one diterpene and one triterpenoidal saponin. Triterpenes **14**,⁵⁰ **19**,⁵⁰ and **23**,^{60,61} as well as the sesquiterpene compound **25**,⁶³ were formerly detected in *A. esculentus* (L.) Moench. The saponin compound **60** and the diterpene **70** were previously identified in the Malvaceae plants: *Abutilon Panosum*⁸⁴ and *Malvaviscus arboreus* Cav.,⁹¹ respectively. Sesquiterpene **7** was earlier isolated from *Helenium aromaticum* (Asteraceae).⁴⁵

3.3.5 Coumarins. The ion peaks at m/z 217.1725, 371.2023, 177.0537, 193.1471, and 383.1963 were characterized as the



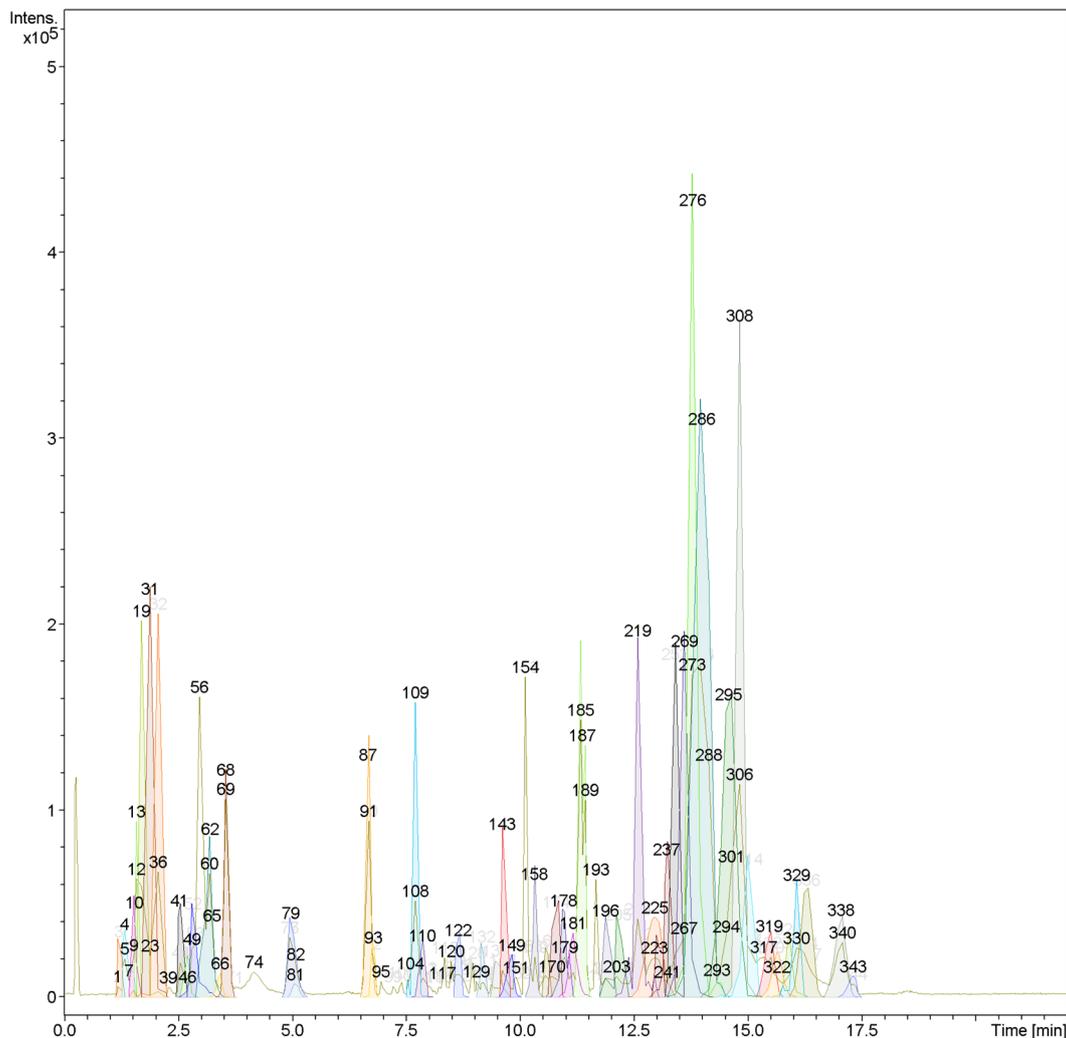


Fig. 2 LC-HRMS chromatogram of dereplicated metabolites from the alcoholic extract of the fruits of *Abelmoschus esculentus* (positive mode).

coumarin derivatives isobergaptin (20), calanolide A (29), herniarin (34), scopoletin (52), and ammosesin (58), respectively. Compounds 20 and 52 were reported in the Malvaceae plants: *Abutilon figarianum* Webb⁵⁶ and *Alcea setosa*,⁴² respectively. Meanwhile, coumarin derivatives 29, 34, and 58 were isolated from *Calophyllum lanigerum* (Calophyllaceae),^{65,66} *Matricaria chamomilla* L. (Asteraceae),⁶⁹ and *D. ammoniacum* (Apiaceae),^{81,82} respectively.

3.3.6 Lignans. Three lignans were generated by investigating the molecular ion peaks at m/z : 521.2143, 357.1456, and 359.2367 as (–)-pinoselin glucoside (31), coniferyl ferulate (37), and pinoselin (51), respectively. The identified lignans are reported for the first time in the Malvaceae family, with 31 and 51 compounds formerly identified in the aerial parts and roots of *Frankenia thymifolia* (Frankeniaceae),⁶⁷ while 37 has been isolated from the radix of *Angelica sinensis* (Apiaceae).⁷²

3.3.7 Iridoids. Three iridoids were dereplicated as agnuside (6), iridotrial glucoside (9), and allamandin (45) from the masses at m/z 467.1908, 345.1357, and 309.0819, respectively. Remarkably, the detected iridoids are reported for the first time from the Malvaceae family. However, 6 has been previously

isolated from the leaves of *Vitex agnus-castus* (Lamiaceae),^{43,44} 9 from *Pedicularis longiflora* Rudolph.var. Tubiformis (Orobanchaceae),⁴⁴ and 45 from *Allamanda cathartica* (Apocynaceae).⁷⁶

3.3.8 Sterols. Ion peak masses at m/z 415.2082 and 425.3736 were dereplicated as β -sitosterol (50) and stigmast-4,22-dien-3,6-dione (55), respectively that were previously isolated from *A. esculentus*.^{60,61}

3.3.9 Nitrogenous metabolites. Two molecular ion peaks at m/z 314.0853 and 331.2801 compatible with the molecular formulae $C_{18}H_{19}NO_4$ and $C_{13}H_{18}N_2O_8$, respectively, were dereplicated as *N*-*E*-feruloyltyramine (syn.: moupinamide) (40), and 3-hydroxy-2,3-dihydroimidazo [1,5- α] pyridin-8(5*H*)-one-5- β -glucopyranoside (67), respectively which were previously isolated from *A. esculentus* (L.) Moench.^{49,74}

In conclusion, compounds 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 15, 18, 17b, 20, 21, 27, 29, 31, 32, 34, 35, 37, 38, 45, 46, 48, 49, 51, 52, 53, 54, 56, 58, 60, 62, 65, 66, 68, 70, and 71 have been identified for the first time in the leaves and fruits extract of the genus *Abelmoschus*. Compounds 12, 13, 14, 16, 17a, 19, 22, 23, 24, 25, 26, 36, 39, 40, 41, 42, 43, 47, 57, 63, 67, 72, and 74 have been discovered for the first time in the leaves extract of *A. esculentus*.



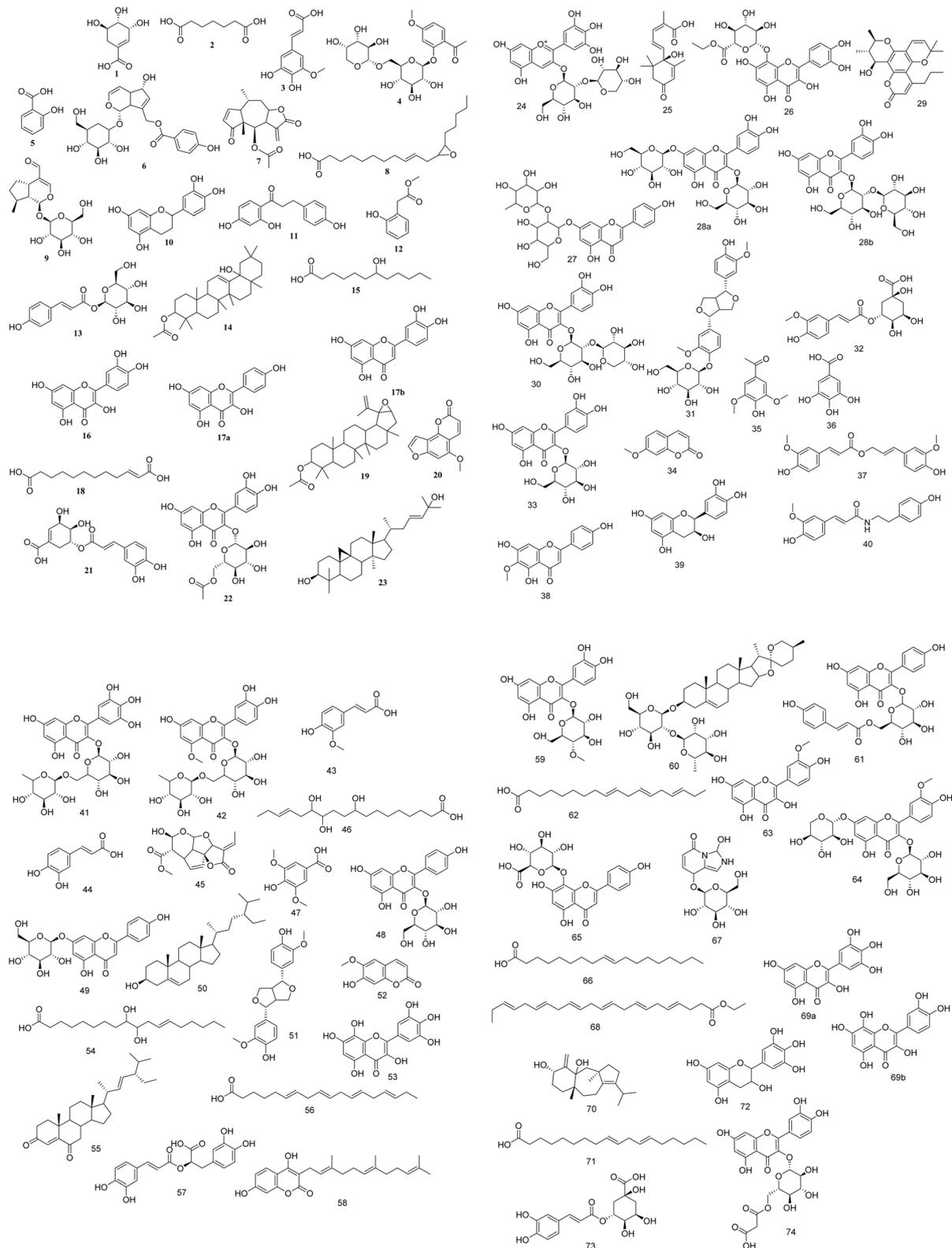


Fig. 3 Structure of the major identified metabolites in the alcoholic extract of the leaves and fruits of *Abelmoschus esculentus*.

On the other hand, compounds 28, 30, 33, 36, 41, 42, 63, 64 and 73 have been detected for the first time in the fruit extract of *A. esculentus*.

3.4. Biological activities

3.4.1 *In vitro* antioxidant assay. The antioxidant activity of the leaves and fruits were measured using DPPH free radical.



The results were expressed as ascorbic acid equivalent (AAE). The leaf extract displayed a higher antioxidant activity of 9.9 ± 0.71 mg AAE per g DW compared with fruit: 7.32 ± 0.91 mg AAE per g DW. A previous report on the antioxidant activity of the fruits of five cultivars of okra was measured by DPPH, ABTS and FRAP assays and the results showed correlation between the phenolic content and the antioxidant activity.³⁴ Another report on the antioxidant activity of leaf and fruit extract by DPPH and ABTS were studied and the results showed that leaves had good activity in the ABTS assay while the fruit was higher in the DPPH assay.⁹⁴ Our study showed a correlation between the phenolic content and the antioxidant activity of the leaves and fruit extracts represented by higher activity of the leaf extract than the fruit extract.

3.4.2. *In vitro* inhibitory effects of the leaves and fruits extracts of *A. esculentus* on digestive enzymes. The leaves and fruits extracts were evaluated for *in vitro* antienzyme activity against α -glucosidase, α -amylase, DPP-4 and lipase enzymes.

3.4.2.1. *In vitro* α -glucosidase inhibitory assay. α -Glucosidase is an enzyme that catalyzes the liberation of glucose from low molecular weight carbohydrates. Inhibition of the enzyme decreases the blood glucose level after a carbohydrate meal.⁹⁵ α -Glucosidase inhibitors represent important candidates in diabetes treatment. The present results indicated a potent activity for the leaf extract with an IC_{50} of 4.47 ± 0.1 mg mL⁻¹ compared with Acarbose standard which exhibits an IC_{50} of 3.2 ± 0.07 mg mL⁻¹, while the fruit extract showed a higher IC_{50} value of 10.4 ± 0.2 mg mL⁻¹ (Table 3). Reviewing the relevant literature, a previous study investigating α -glucosidase inhibition of okra seeds, outer skin and inner skin concluded that there was inhibitory activity for the seeds while the outer and inner skin showed no activity against α -glucosidase.¹⁵

3.4.2.2. *In vitro* α -amylase inhibitory assay. α -Amylase is an enzyme which catalyzes the hydrolysis of starch into low-molecular-weight dextrin and sugars. Inhibition of the enzyme might retard the digestion of carbohydrates and decrease the rate of glucose absorption, which may contribute to glycemic control in type-2 diabetes.⁹⁶ Therefore, the inhibition of α -amylase is considered as a good approach for the development of antidiabetic drugs. Herein, the results revealed a characteristic activity for the leaves extract with an IC_{50} of 3.54 ± 0.08 mg mL⁻¹ compared with Acarbose standard which

reported an IC_{50} of 2.21 ± 0.05 mg mL⁻¹, while the fruit extract showed a higher IC_{50} value expressed as 6.53 ± 0.15 mg mL⁻¹ (Table 3). Remarkably, previous research have stated considerable α -glucosidase and α -amylase inhibitory activities of okra peel and seeds,⁹⁷ while another study also revealed that okra seed protein hydrolysate exhibits potent inhibition of carbohydrate hydrolyzing enzymes together with lipase enzyme.⁹⁸ An additional study exploring the antidiabetic activity of okra fruits unveiled the potential inhibitory activity of the methanolic extract against α -glucosidase and α -amylase with 14.36% to 19.23% inhibition of α -glucosidase and 15.89 to 37.19% inhibition of α -amylase at concentrations of 50–200 mg mL⁻¹.³²

3.4.2.3. *In vitro* DPP-4 inhibitory assay. Dipeptidyl peptidase (DPP-4) is a serine protease enzyme which acts *via* cleavage and inactivation of peptides as incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Therefore, inhibition of DPP-4 could be a useful approach for treating type 2 diabetes.⁹⁹ Results unveiled a considerable activity for the leaf extract with an IC_{50} of 0.385 ± 0.019 μ g mL⁻¹ compared with Sitagliptin standard (IC_{50} : 0.082 ± 0.004 μ g mL⁻¹) (Table 3). Meanwhile, fruit extract displayed an IC_{50} of 2.669 ± 0.132 μ g mL⁻¹. The antidiabetic activity of okra fruit was previously reported to be attributed to the suppression of DPP-4 signaling.^{100,101}

3.4.2.4. *In vitro* lipase inhibitory assay. Lipase enzyme is one of the important enzymes in the breakdown of triglycerides; therefore, inhibition of pancreatic lipase is a crucial strategy for managing obesity and hyperlipidemia.¹⁰² The current findings highlighted remarkable potent activity for the leaf extract with an IC_{50} of 1.044 ± 0.05 mg mL⁻¹ compared with the Orlistat standard (IC_{50} : 5.05 ± 0.23 mg mL⁻¹), while the fruit extract exhibited an IC_{50} of 14.66 ± 0.67 mg mL⁻¹ (Table 3). Interestingly, the results indicated a better activity of the leaf extract than the Orlistat standard. Previous *in vivo* studies revealed that the dichloromethane and methanol extracts of okra fruits reduced the levels of triglyceride and cholesterol in mice taking tyloxapol to induce hyperlipidemia mice.¹⁰³ Also, okra peel and the seeds extracts showed antihyperlipidemic activity in STZ induced diabetic rats.¹⁰⁴ Another report revealed that okra fruits exhibited remarkable antioxidant potential and potent inhibitory effects on α -glucosidase, α -amylase and lipase digestive enzymes.¹⁰⁵

Table 3 Results of α -glucosidase, α -amylase, DPP-4, and lipase inhibitory activity of the alcoholic extract of leaves and fruits of *Abelmoschus esculentus*^a

Extract	α -Glucosidase	α -Amylase	DPP-4	Lipase
	IC_{50} mg mL ⁻¹	IC_{50} mg mL ⁻¹	IC_{50} , μ g mL ⁻¹	IC_{50} mg mL ⁻¹
Leaves	4.47 ± 0.1 ****	3.54 ± 0.08 ****	0.385 ± 0.019 **	1.044 ± 0.05 ****
Fruits	10.4 ± 0.2 ****	6.53 ± 0.15 ****	2.669 ± 0.132 ****	14.66 ± 0.67 ****
Acarbose®	3.2 ± 0.07	2.21 ± 0.05	—	—
Sitagliptin®	—	—	0.082 ± 0.004	—
Orlistat®	—	—	—	5.05 ± 0.23

^a The data in the table represent means \pm SD, where ** $P < 0.01$ and **** $P < 0.0001$ are considered statistically significant when comparing group differences of Acarbose (for α -glucosidase and α -amylase), Sitagliptin (for DPP-4), and Orlistat (for lipase), using one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons.



3.5. Molecular docking analysis

To gain insights into the potential bioactive metabolites in the alcoholic extracts of leaves and fruits of okra, all the dereplicated compounds were subjected to molecular docking against human α -amylase, α -glucosidase, and dipeptidyl peptidase-4 (DPP-4) with PDB codes 4W9, 3L4W, and 2ONC, respectively. As illustrated in Fig. 4, the docking scores for all the dereplicated compounds ranged from approximately -4 to -12 kcal mol $^{-1}$ with the three enzymes. The majority of compounds scored less than 7 kcal mol $^{-1}$ with the three enzymes: α -amylase, α -glucosidase, and DPP-4. Considering α -amylase, the top-scoring compounds which achieved docking scores below -10 kcal mol $^{-1}$ were quercetin diglucoside (**28**) (detected in fruits), tiliroside (**61**) (detected in leaves-fruits), and isorhamnetin 3-*O*-glucoside-7-*O*-xyloside (**64**) (detected in fruits). They exhibited various binding modes within the enzyme's active site comparable with that of the co-crystallized inhibitor montbretin A (Fig. 5A–D), where they established multiple H-bonds, particularly with ASP-197, GLU-233, and ASP-356. In addition, they established further hydrophobic interactions with TRP-59. Previous reports on tiliroside revealed its effectiveness in inhibiting pancreatic α -amylase *in vitro* and its effective inhibition for plasma glucose levels increase in an oral glucose tolerance test, but not in an intraperitoneal glucose tolerance test. Tiliroside also show inhibitory effects against

glucose uptake which is mediated by both sodium-dependent glucose transporter 1 (SGLT1) and glucose transporter 2 (GLUT2) inhibitors (phlorizin and phloretin, respectively).¹⁰⁶

Regarding α -glucosidase, the best scoring compounds with scores < -10 kcal mol $^{-1}$ were luteoliflavan (**10**) (leaves), quercetin (**16**) (leaves), kaempferol (**17a**) (leaves), (+)-epicatechin (**39**) (leaves), hibiscetin (**53**) (fruits), myricetin (**69a**) (fruits), and gossypetin (**69b**) (fruits) which showed different binding interactions inside the enzyme's active site (Fig. 6). Compounds (**10**) and (**39**) established interactions highly similar to that of the co-crystallized inhibitor miglitol, where H-bonds were the predominant with ASP-203, ASP-327, TRP-406, ASP-443, ASN-449, ARG-526, ASP-542, and HIS-600 (Fig. 6A, C and F). On the other hand, their major hydrophobic interactions were with TRP-406, and PHE-450. The flavonol derivatives (**16**, **53**, **69a** and **69b**) showed fewer H-bonds with only ASP-327 and ASP-203, in addition to three hydrophobic interactions with TYR-299, TRP-406, and PHE-450 (Fig. 6B, D and E).

In 2016, Meng *et al.* investigated the inhibitory activity of myricetin and quercetin previously isolated from *Hovenia dulcis* against α -glucosidase. The two compounds showed a noncompetitive inhibition against the enzyme. The IC₅₀ results of both compounds could be attributed to the presence of a $-OH$ group at the 5'-position of B ring which boosts the inhibitory activity against α -glucosidase.¹⁰⁷

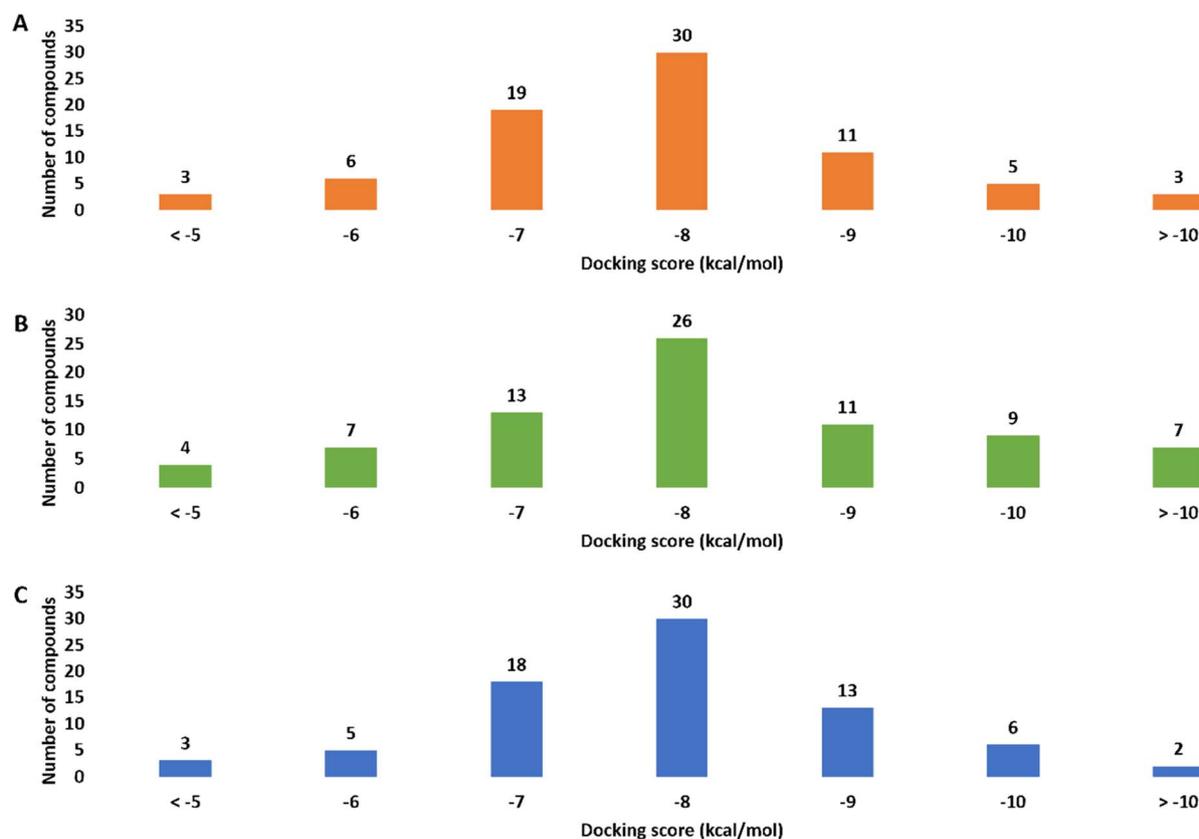


Fig. 4 Docking score distribution of dereplicated metabolites in the leaf and fruit extracts of *A. esculentus* against human α -amylase (A), α -glucosidase (B), and (C) DPP-4 (PDB 4w9, 3l4w and 2onc, respectively).



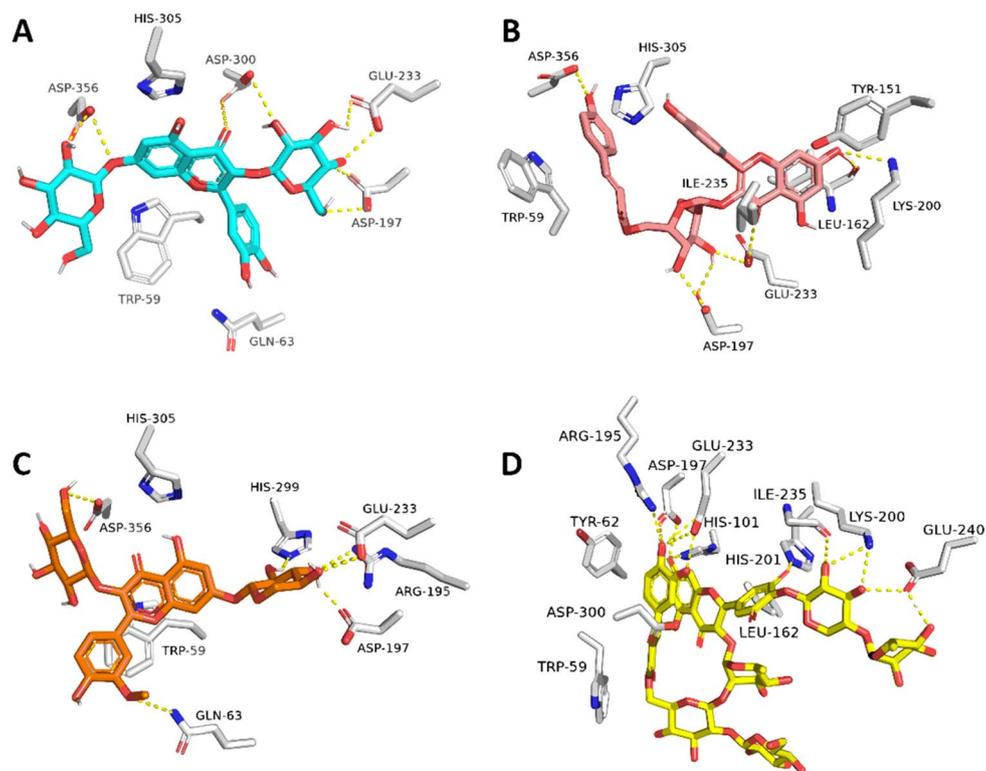


Fig. 5 Docking poses of compounds with docking scores < -10.0 kcal mol $^{-1}$ (i.e., 28, 61, and 64) along with the co-crystallized inhibitor montbretin A inside human α -amylase ((A–D), respectively).

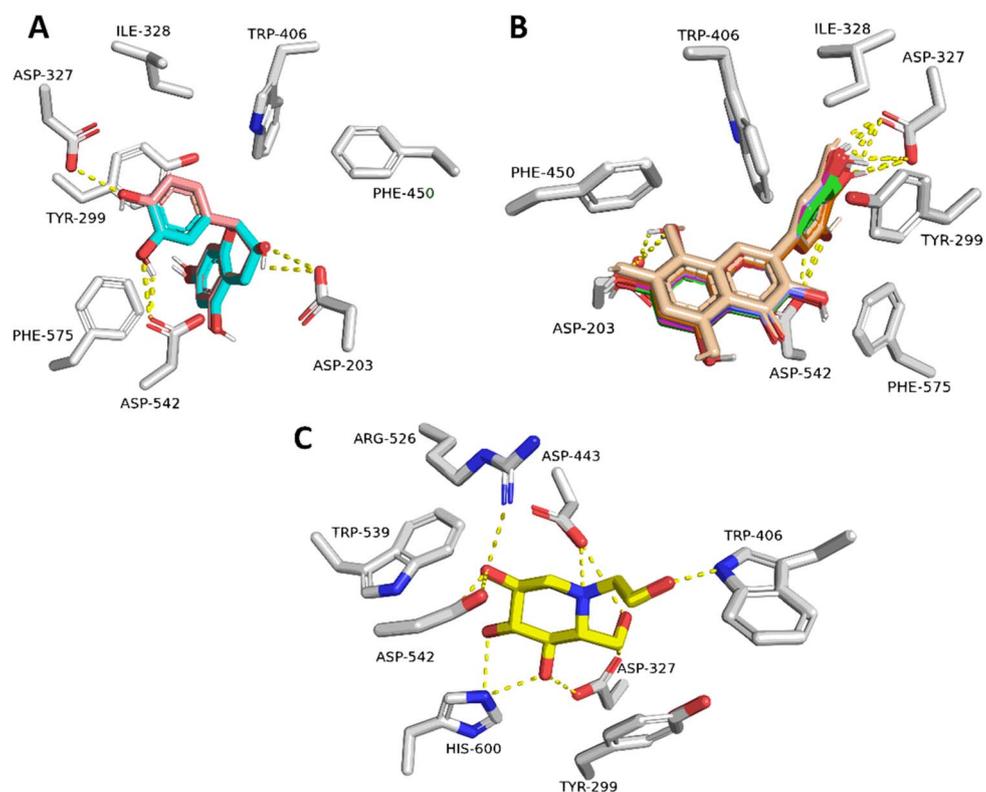


Fig. 6 Docking poses of compounds inside the human α -glucosidase with docking scores < -10.0 kcal mol $^{-1}$. (A) Compounds 10 and 39 (cyan and brick-red structures, respectively) aligned with each other. (B) Compounds 16, 17, 53, 69a, and 69b (green, blue, brown, pink, and orange structures, respectively) aligned with each other. (C) The co-crystallized inhibitor miglitol.



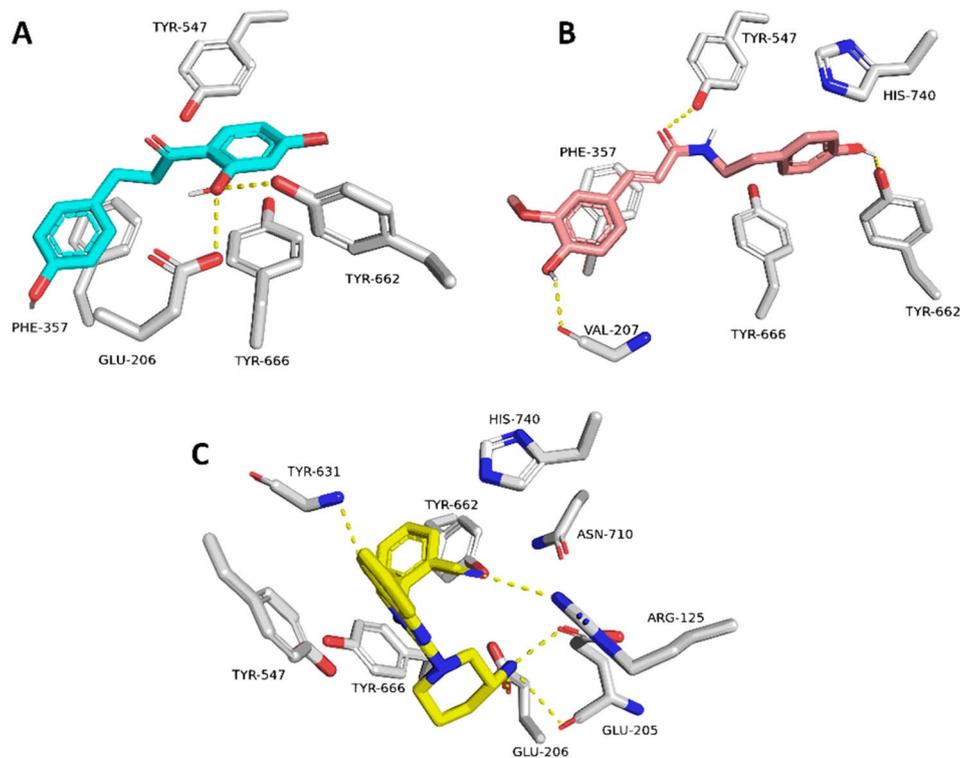


Fig. 7 Docking poses of compounds with docking scores < -10.0 kcal mol $^{-1}$ (i.e., **11** and **40**, along with the co-crystallized inhibitor) inside human DPP-4 (A–C, respectively).

A group of flavonoids previously isolated from *Saccharomyces cerevisiae*, including quercetin, kaempferol, catechin and epicatechin, were chosen for the assessment of their inhibitory activity versus α -glucosidase. The results revealed that the most active compound was quercetin. Analyzing the collected results suggests that the absence of a double bond at C2–C3 in (catechin and epicatechin) may decrease the inhibitory activity. Meanwhile, the occurrence of the catechol group in B ring may improve the inhibitory activity of the flavonoids which was observed in the results of quercetin and kaempferol.¹⁰⁸ Generally, it has been observed that flavonoids are effective in blocking glucose transporters by suppressing the activities of α -amylase and α -glucosidase. A previous study revealed a relationship between the hydroxyl group number on rings A and B in the flavonoid skeleton and the capacity of inhibition.¹⁰⁹ Considering the α -amylase enzyme, the presence of the 4-keto group, the double bond between C2 and C3, and the hydroxyl groups of rings A (at position C6 or C7) and B (at position C4' or C5') potentiate the inhibitory potential of the flavonoid against α -amylase, while the occurrence of an –OH group at C3 and the glycosylation or the methylation of –OH groups in rings A and B may reduce the inhibition activity against α -amylase.¹⁰⁹ Regarding the favorable features of flavonoids for inhibition of α -glucosidase, the existence of the carbonyl group at C4, the double bond at C2 and C3, and the –OH groups at ring C (C3), ring B (C3', 4' and/or 5' positions), and at ring A (5, 6, 7 and/or at 8 positions) enhances the activity of flavonoids against α -glucosidase. The replacement of the hydroxyl groups by the

alkyl or glycosyl moiety in rings A and B may reduce the inhibition activity of the flavonoids.¹⁰⁹

Finally, only two compounds exhibited docking scores below -10 kcal mol $^{-1}$ with human DPP4: davidigenin (**11**) (leaves-fruits) and *N*-E-feruloyltyramine (syn.: moupinamide) (**40**) (leaves-fruits). As depicted in Fig. 7, both compounds shared a common hydrogen bond with TYR-662, along with the co-crystallized inhibitor. Additionally, compound **11** formed a hydrogen bond with GLU-206, similar to the co-crystallized inhibitor.

4 Conclusion

The study of the phytochemical content and biological activities of the leaves and fruits extracts of the edible plant *A. esculentus* revealed higher content of bioactive metabolites in the leaves than in the fruits of the plant. A total of 74 metabolites were dereplicated in the extracts from the two organs, among which flavonoids were the most prevailing chemical class. Investigation of the potential biological activities indicated superior antioxidant and antidiabetic efficacy of the leaves extract compared with the fruit extract. A molecular docking simulation study noted the role of flavonoids in the antidiabetic activity of okra extracts. This study highlighted the medicinal potential of okra leaves. However, due to the limitations of these *in vitro* studies, future research is required to target the *in vivo* validation, bioavailability studies and toxicity assessment to bridge these gaps and provide stronger evidence of the importance of okra leaves that are often treated as waste.



Data availability

All data generated or analyzed during this study are included in this published article.

Author contributions

Conceptualization, A. H. E., and M. H. A. H.; investigation, A. H. E., M. H. A. H., and E. A.; methodology, A. H. E., M. H. A. H., and E. A.; software, A. H. E., M. H. A. H., and A. S.; writing original draft preparation, A. H. E., M. H. A. H., and A. S.; writing, review and editing, A. H. E., M. H. A. H., and E. A.; all authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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