Hindawi
Journal of Chemistry
Volume 2021, Article ID 5583001, 10 pages
https://doi.org/10.1155/2021/5583001

Research Article

Chemical Profile, *In Vitro* Antioxidant, Pancreatic Lipase, and Alpha-Amylase Inhibition Assays of the Aqueous Extract of *Elettaria cardamomum L.* Fruits

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Received 24 February 2021; Revised 14 May 2021; Accepted 20 May 2021; Published 29 May 2021

Academic Editor: Jean-Marie Nedelec

Phytochemical and pharmacological investigations of *Elettaria cardamomum* L. were mostly focused on its essential oil and organic crude extracts with little attention on its aqueous extracts. Therefore, the current study aimed to investigate the phytochemical profile, *in vitro* antiobesity, and antidiabetic activities of the aqueous extract of *Elettaria cardamomum* L. family Zingiberaceae. UPLC-ESI-MS/MS analysis is used for the aqueous extract characterization in both ionization modes. The analysis revealed the tentative identification of forty-seven compounds based on their MS/MS fragmentation pattern and comparison with the reported data. The identified compounds include eight flavones, thirteen phenolic and nonphenolic acids, one coumarin, and nineteen anthocyanins. Moreover, the *in vitro* antiobesity and antidiabetic activities were also studied. The results showed that *E. cardamomum* L. aqueous extract inhibits pancreatic lipase and α-amylase enzyme in a concentration-dependent manner, as 1 mg/mL extract was able to inhibit pancreatic lipase and α-amylase by 62.25% ± 0.58 (IC_{50} = 288.75 ± 1.3 μg/mL) and 70.42% ± 1.5 (IC_{50} = 220.5 ± 1.3 μg/mL), respectively. Conclusively, the current study indicated that the investigated biological activities of *Elettaria cardamomum* aqueous extract were attributed to the existence of biologically active metabolites such as flavones, phenolic and nonphenolic acids, coumarins, and anthocyanins. Moreover, it proposed that the aqueous cardamom extract can be used as a natural potential source in different pharmaceutical preparations to protect against variable chronic disorders, especially obesity and diabetes. Deeper *in vivo* investigations, isolation, purification, and structural elucidation of the major active metabolites from cardamom are recommended.

1. Introduction

Plants and their secondary metabolites have played a crucial and important role in the human's life particularly those which are used in traditional medicine or as food due to their healing and nutritional properties[1]. Spices are often used as food additives and considered a promising source for finding newer digestive enzyme inhibitors that do not have the same side effects as synthetic ones [2, 3].

Spices are well known to improve gastric function by increasing salivary flow and gastric juice secretions and help in digestion [3]. *Elettaria cardamomum* L. is a member of the family Zingiberaceae. It is known as "Queen of Spices" and considered as the second essential "national spice" of India [4]. Traditionally, cardamom is used to treat different disorders, such as gum infections, asthma, cataracts, and cardiac, digestive, and kidney diseases. It also has antidiabetic, anti-inflammatory, antioxidant, and anticarcinogenic effects [5, 6].

Most of the previous biological investigations focused on cardamom volatile oil [7] which has valuable constituents such as terpene, esters, and flavonoids. The major constituents of cardamom volatile oil are 1,8-cineole (36.3%) and α-terpinyl acetate (31.3%). It was also reported that organic

fractions of cardamom crude extracts contain many flavonoid constituents such as luteolin, quercetin, kaempferol, and pelargonidin [5, 8]. However, nothing was reported about the nonvolatile constituents of the aqueous extract of cardamom or its biological activities. Therefore, the aim of the present study is to investigate the aqueous extract of cardamom fruits using UPLC-ESI-MS/MS and to evaluate its *in vitro* antiobesity and antidiabetic activities.

2. Materials and Methods

2.1. Preparation of Cardamom Aqueous Extracts. Green cardamom (obtained from Spices Board, Cochin, Kerala, India) was used for this study. The plant was verified by Prof Dr. Husain Abdel Basset, professor of Taxonomy, Faculty of Science, Zagazig University, Egypt. A voucher specimen (#EC-519) was prepared and deposited at the herbarium in the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University. Eighty grams of the fruits and seeds of *E. cardamomum* were ground, and the powder was mixed with 800 mL of distilled water and agitated at 150 rpm at room temperature for 24 h. The solution was then filtered using muslin cloth. The filtrate was paper filtered, then poured into a bottle, and placed in a freezer at −70°C. The frozen filtrate was subjected to lyophilization (α 1-4 LDplus freeze dryer, Christ Co.) for 48 h. A 1.350 g sample of the powder was collected for further analysis by UPLC-ESI-MS/MS.

2.2. Chemicals. All chemicals and reagents used in the study were of analytical grade. α-Amylase inhibitor, dinitro salicylic acid (DNS, a colour reagent), porcine pancreatic lipase, and *p*-nitrophenyl butyrate (NPB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents used for extractions and quantification were purchased from Sigma-Aldrich (Al-Khobar, KSA), and HPLC-grade methanol for ESI-MS analyses was sourced from Gulf Scientific Corporation, Ltd. (Dubai, UAE). Formic acid and acetic acid were purchased from Sigma-Aldrich.

2.3. UPLC-ESI-MS/MS Instrument and Separation Technique. The UPLC- ESI-MS/MS (ultra-performance liquid chromatography with electrospray ionization quadrupole linear ion trap tandem mass spectrometry) analysis performed on ESI-MS positive and negative ionization acquisition modes was carried out on a XEVO TQD triple quadruple instrument mass spectrometer. For the quantitative determination of the phytochemicals, a multiple-reaction monitoring (MRM) mode method was employed. *E. cardamomum* L. fruits aqueous extract was analyzed by UPLC to get the chromatographic profiles of the highly polar portions of the extracts, which contain polar compounds such as phenolic and flavonoid compounds. HPLC-grade methanol was used to dissolve the sample and filtered through 0.2 μm membrane disc filter and resulting solution concentrations were in 0.2 to 0.5 mg/mL range, depending on each fraction. The UPLC system was a Waters Corporation, Milford, MA01757 U.S.A, mass spectrometer. The reverse-phase separations were performed (ACQUITY UPLC BEH C18 1.7 μm, 2.1 × 50 mm Column) (50 mm × 1.2 mm (inner diameter) and 1.7 μm particle size) at 0.2 mL\min flow rate. The gradient program previously reported in [9] was used for the analysis. The mobile phase consists of acidified water containing 0.1% formic acid (A) and acidified methanol containing 0.1% formic acid (B). The elution conditions were 0–2 min 10% B isocratic; 2–5 min, linear gradient B 10 to 30%; 5–15 min, linear gradient from 30% to 70% B; 15–22 min, linear gradient from 70% to 90% B; 22–25 min, 90% B isocratic and lastly washing and reconditioning of column was carried out. To obtain more data, electrospray ionization (ESI) was performed in both negative and positive ion modes. The analysis parameters were carried out using negative ion mode as follows: source temperature 150°C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440°C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h. Mass spectra were detected in the ESI between *m/z* 100–1000 atomic mass units. The identification of the phytochemical constituents was done by their fragmentation patterns and ESI-QqQLIT–MS/MS spectra. Peaks and spectra were processed using the MassLynx 4.1 software and tentatively identified by comparing its retention time (R_t), mass spectrum with reported data, and library search (such as FooDB (http://www.Foodb.ca)).

2.4. In Vitro Antidiabetic Assay Using α-Amylase Inhibition Method. In this method, the solution of the enzyme was prepared by dissolving α-amylase in 20 mM phosphate buffer (6.9) at the concentration of 0.5 mg/mL. 1 mL of the extract of various concentrations (7.81–1000 μg/mL) and 1 mL of enzyme solution were mixed and incubated at 25°C for 10 min. After incubation time, 1 mL of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10 min. The reaction was stopped by the addition of 2 mL of dinitro salicylic acid (DNS, colour reagent), heating in a boiling water bath (5 min). After cooling, the absorbance was measured using a calorimeter at 565 nm (Spectrumlab S23A, Globe Medical, England). The percentage of inhibition was calculated using the given formula,

$$\%\text{inhibition} = \left(1 - \frac{As}{Ac}\right) \times 100, \quad (1)$$

where *As* is the absorbance of tested extracts and *Ac* is the absorbance of control. Acarbose was used as a control [10]. The IC_{50} value is the concentration of α-amylase inhibitor to inhibit 50% of its activity under the assay conditions. Nonlinear regression analysis of GraphPad Prism 5 software (GraphPad software, San Diego, California) was used to calculate IC_{50} from graphic plots of the dose response curve for each applied concentration. Each experiment was performed in triplicates, and all values are represented as means ± SD.

2.5. In Vitro Antiobesity Using Pancreatic Lipase Inhibitory Assay. The lipase inhibition activity of *E. cardamomum* aqueous extract was determined by a method in [11]. In this method, the porcine pancreatic lipase activity was measured

using *p*-nitrophenyl butyrate (NPB) as a substrate. Lipase solution (100 μg/mL) was prepared in a 0.1 mM potassium phosphate buffer (pH 6.0). Samples with different concentrations (7.81–1000 μg/mL) were preincubated with 100 μg/mL of lipase for 10 min at 37°C. The reaction was then started by adding 0.1 mL NPB substrate. After incubation at 37°C for 15 min, *p*-nitrophenol amount released in the reaction was measured using Multiplate Reader (BioTek, Synergy HT, VT, USA). Orlistat was used with the same concentrations as a control. The results were expressed as percentage inhibition, which was calculated using the formula,

$$\text{inhibitory activity}\,(\%) = \left(1 - \frac{As}{Ac}\right) \times 100, \quad (2)$$

where (Ac) is the absorbance of control and (As) is the absorbance in the presence of test substance. The concentration of pancreatic lipase inhibitor to inhibit 50% of its activity under the assay conditions is the IC_{50} value. Its IC_{50} was done from dose response curve graphic plots for each concentration by using nonlinear regression analysis of GraphPad Prism 5 software. Each experiment was performed in triplicates, and all values are represented as means ± SD of triplicates.

3. Results and Discussion

3.1. Characterization of the Phytoconstituents of the Aqueous Extract of E. cardamomum L. Fruits. The polyphenolic constituents of *E. cardamomum* L. fruit aqueous extract was investigated for the first time using UPLC-ESI-MS/MS to identify its bioactive constituents related to their *in vitro* antiobesity and antidiabetic activities. Some of the identified compounds were previously reported in *E. cardamomum.*

Forty-seven compounds were tentatively identified by UPLC-ESI-MS/MS (negative and positive ionization modes) from the aqueous fraction of *E. cardamomum* (Figure 1). Table 1 shows the identified compounds with their retention time (R_t), detected mass (M ± H), and MS/MS fragment ions. Chromatograms of some identified compounds are shown in Figure 2. The identified compounds include thirteen phenolic and nonphenolic acids (2–7, 9, 11, 16, 18, 25, 37, and 38), eight flavones (1, 14, 15, 17, 20, 22, 24, and 31), one coumarin (8), and nineteen anthocyanins (10, 12, 13, 19, 21, 23, 24, 26, 27, 30, 32, 33, 34, 35, 36, 42, 44, and 45) and two unknown compounds were also detected.

3.1.1. Phenolic and Nonphenolic Acids. In the aqueous extract, phenolic and nonphenolic acids were identified based on mass measurement, MS^2 fragmentation patterns, and previous studies. Chromatograms in Figure 1 show the presence of thirteen acidic compounds. Compounds 6, 7, 9, 11, and 18 (R_t 2.53, 4.90, 5.74, 8.69, and 9.56) showed a common pseudomolecular ion peak at *m/z* 353 [M-H] and MS^2 fragments at *m/z* 191, 179, 173, and 135 from a chlorogenic acid isomer, neochlorogenic acid, cryptochlorogenic acid, and chlorogenic acid, respectively [19]. Compounds 2 to 5, with MS^1 at 297 [M – H], 191 [M – H], 171 [M + H], and 155 [M + H], and MS^2 base peak fragment ions at *m/z* 161, 111, 125, and 109, respectively, were identified as protocatechuic hexoside [14, 15], citric acid [16], gallic acid [17], and protocatechuic acid [18], respectively. Compound 16 with MS^1 at *m/z* 137 [M-H] and MS^2 base peak fragment ions at 107 [M-H-CHO] were tentatively identified as protocatechualdehyde. It is noteworthy that protocatechualdehyde has been previously identified in *E. cardamomum* [18]. Compounds 25, 37, 38, and 43 showed pseudomolecular ions at *m/z* 187 $[M - H]^-$, 313 $[M + H]^+$, 225 $[M + H]^+$, and 153$[M + H]^+$. They were tentatively identified as azelaic, caftaric, sinapic, and methyl salicylic acids, respectively, based on the MS^2 data reported in Table 1 and [27, 34, 35, 38].

3.1.2. Flavone Compounds. Compound 1 showed a pseudomolecular ion peak at *m/z* 641 $[M + H]^+$ and a base peak fragment ion at *m/z* 305, representing a loss of 336 amu (quinoyl hexoside moiety) [35], leaving dihydroquercetin as an aglycone [12]. It was thus concluded to be dihydroquercetin (taxifolin) quinoyl hexoside. Compounds 14 and 20 showed a common pseudomolecular ion peak at *m/z* 447 [M – H], and mass data showed the same fragment ion at *m/z* 285 [M – H-162], corresponding to the loss of hexose moiety. Based on these results, compounds 14 and 20 were concluded to be kaempferol-3-*O*-hexoside or luteolin-7-*O*-hexoside [13]. Compound 15 showed a pseudomolecular ion peak at *m/z* 463 [M – H] and a base peak fragment ion at *m/z* 301, a loss of 162 amu, which was identified as quercetin-3-*O*-hexoside. Compounds 17 and 22 were identified as isorhamnetin-7-*O*-dihexoside and naringenin-7-*O*-hexoside from MS^1 and MS^2 data presented in Table 1 [26]. Compounds 29 and 31 showed pseudomolecular ion peaks at *m/z* 489 $[M - H]^-$ and 475 $[M + H]^+$ and base peak fragment ions at *m/z* 285 and 271, respectively, with neutral loss of 204 amu (acetyl hexoside) moiety so that these compounds were tentatively identified as kaempferol-3-*O*-acetyl hexoside [23] and apigenin-7-*O*-acetyl hexoside [30, 31], respectively.

3.1.3. Anthocyanins. Nine compounds were identified as cyanidin derivatives based on their MS fragmentation, leading to cyanidin aglycone (*m/z* 287) in the positive mode. Compound 10 was identified as cyanidin-3-*O*-acetyl rhamnoside with *m/z* 475 and an MS^2 fragment at 287, a loss of 188 amu from acetyl rhamnoside [12]. Compounds 13 and 44 had a common protonated pseudomolecular ion peak at *m/z* 595, with fragment ions at *m/z* 449 and 287 and neutral losses of 146 and 162 amu from coumaroyl and hexose moieties, respectively; they were identified as cyanidin-3-*O*-coumaroyl hexoside [24]. Compound 24 had a $[M + H]^+$ ion at *m/z* 449 and MS^2 fragment ion at *m/z* 287, which is consistent with cyanidin-3-*O*-hexoside [22].

Compounds 26 and 28 had a common [M + H] ion at *m/z* 535, which is consistent with cyanidin-3-*O*-malonyl hexoside [25]. Compound 34 had a $[M + H]^-$ ion at *m/z* 595 and an MS^2 fragment at *m/z* 287 (a loss of 308 amu), which is consistent with cyanidin-3-*O*-rutinoside [28]. Peak 35 had $[M + H]^+$ at *m/z* 593, with main fragment ion at *m/z* 287 (loss of 306 amu from cinnamoyl glucuronide) and was

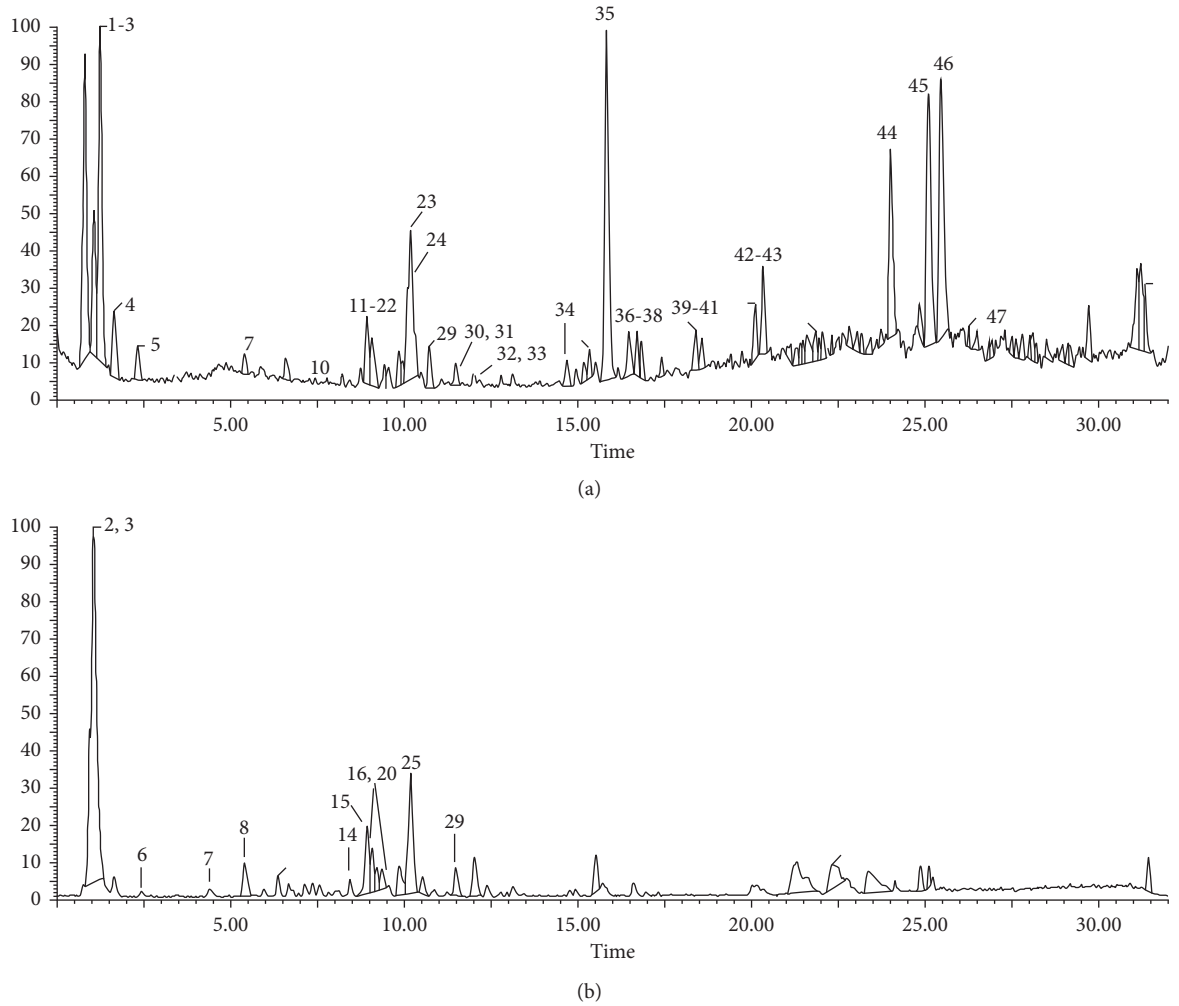

Figure 1: UPLS-ESI-MS chromatograms of *E. cardamomum* L. aqueous extract: (a) positive ion mode; (b) negative ion mode.

Table 1: Tentatively identified metabolites in the aqueous extract of *E. cardamomum* L. fruits (Card A) using UPLC-ESI-MS in negative and positive ionization modes.

No.	Tentative assignment	R_t (min)	$[M+H]^+$ (m/z)	$[M-H]^-$ (m/z)	MS^2 fragments (m/z)	Reference
1	Taxifolin quinoyl hexoside	0.96	641		305 (100%)	[12, 13]
2	Protocatechoyl glucoside	0.95	299	297	161 (100%)	[14, 15]
3	Citric acid	1.29	193	191	111 (100%)	[16]
4	Gallic acid	1.64	171		125	[17]
5	Protocatechuic acid	2.32	155		109	[18]
6	Chlorogenic acid isomer	2.53		353	135 (100%)	[19]
7	Neochlorogenic acid	4.90		353	191 (100%)	[19]
8	Umbelliferone	5.39	163		135 (100%), 107	[20]
9	Cryptochlorogenic acid	5.74		353	191, 179, 173 (100%), 135	[19]
10	Cyanidin-3-*O*-acetyl rhamnoside	8.19	475		287 (100%)	[13]
11	Chlorogenic acid isomer	8.69	355	353	191 (100%)	[19]
12	Pelargonidin-3-*O*-feruloyl glucoside	8.77	609		271 (100%)	[21]
13	Cyanidin-3-*O*-coumaroyl glucoside	8.87	595		449, 287 (100%)	[22]
14	Kaempferol or luteolin-3-*O*-glucoside	8.93	449	447	285 (100%)	[23]
15	Quercetin-3-*O*-glucoside	9.20	465	463	301	[23]
16	Protocatechualdehyde	9.34	139	137	107 (100%)	[18]
17	Isorhamnetin-3-*O*-diglucoside	9.38	641		317 (100%), 163	[24]
18	Chlorogenic acid isomer	9.56	355		193	[19]
19	Pelargonidin-3-*O*-glucoside	9.83	433		271(100%)	[25]

Table 1: Continued.

No.	Tentative assignment	R_t (min)	$[M+H]^+$ (m/z)	$[M-H]^-$ (m/z)	MS^2 fragments (m/z)	Reference
20	Kaempferol or luteolin-3-*O*-hexoside	9.94	449	447	285 (100%)	[23]
21	Peonidin-3-*O*-hexoside	9.96	463		301 (100%)	[25]
22	Naringenin-7-*O*-hexoside	9.98	435		273 (100%)	[26]
23	Peonidin-3-*O*-coumaroyl glucoside	10.16	609		301 (100%)	[25]
24	Cyanidin-3-*O*-hexoside	10.18	449		287 (100%)	[25]
25	Azelaic acid	10.52		187	125 (100%)	[27]
26	Cyanidin-3-*O*-malonyl hexoside	10.57	535		287 (100%)	[28]
27	Delphinidin-3-*O*-feruloyl hexoside	11.01	641		303	[21, 29]
28	Cyanidin-3-*O*-malonyl hexoside	11.48	535		449. 287 (100%)	[28]
29	Kaempferol or luteolin-3-*O*-acetyl hexoside	11.48		489	285	[30]
30	Cyanidin-3-*O*-acetyl hexoside	12.10	491		287(100%)	[25]
31	Apigenin-7-*O*-acetyl hexoside	12.20	475		271 (100%), 151	[31]
32	Peonidin-3-*O*-coumaroyl hexoside	12.81	609		301(100%)	[25]
33	Peonidin-3-*O*-feruloyl hexoside	13.38	639		301 (100%)	[21, 29]
34	Cyanidin-3-*O*-rutinoside	15.60	595		287 (100%)	[32]
35	Cyanidin-3-*O*-cinnamoyl glucuronide	15.82	593		287 (100%)	—
36	Pelargonidin-3-*O*-diacetyl hexoside	16.60	517		271 (100%)	[33]
37	Caftaric acid	16.80	313		179	[23, 34]
38	Sinapic acid	16.95	225 (100%)		209, 179, 151	[35]
39	Maslinic acid	18.39[M + H-H_2O]	457		439, 411, 357, 339, 248, 235, 205, 179	[34]
40	Maslinic acid isomer	18.56 [M + H-H_2O]	457		357, 339, 248, 235, 205, 179	[23]
41	Vanillin	18.86	153		138, 125, 121 (100%), 107, 89, 81, 77	[36]
42	Malvidin-3-*O*-feruloyl glucoronide	21.51	683		331 (100%)	[37]
43	Methyl salicylic acid	21.60	153		121, 91	[38]
44	Cyanidin-3-*O*-coumaroyl hexoside	24.11	595		449, 287 (100%),	[22]
45	Delphinidin-3-*O*-acetyl rhamnoside	27.30	491		303 (100%)	[39]
45	Unknown	25.46	381[M+H-H_2O]		335, 267	—
46	Catechin derivative	25.50	595		291 (100%)	—
47	Delphinidin-3-*O*-acetyl rhamnoside	27.30	491		303 (100%)	[39]

tentatively identified as cyanidin-3-*O*-cinnamoyl glucuronide. Compound 30 had $[M+H]^+$ at m/z 491 with main fragment ion at m/z 287 (loss of 204 amu from acetyl hexose). It was tentatively identified as cyanidin-3-*O*-acetyl hexoside [22]. Four compounds were identified as peonidin derivatives based on their MS fragmentation leading to peonidin aglycone (m/z 301) in the positive mode. Compounds 21, 23, 32, and 33 were identified as peonidin-3-*O*-hexoside, peonidin-3-*O*-coumaroyl hexoside, peonidin-3-*O*-coumaroyl hexoside, and peonidin-3-*O*-feruloyl hexoside, respectively [29, 32].

Compounds 12, 19, and 36 were also identified as pelargonidin-3-*O*-feruloyl-hexoside [29], pelargonidin-3-*O*-hexoside [22], and pelargonidin-3-*O*-diacetyl hexoside [21], respectively, according to their mass spectra, with a distinctive fragment ion at m/z 271. Peaks 27 and 45 were identified as delphinidin-3-*O*-feruloyl-hexoside [29, 32] and delphinidin-3-*O*-acetyl rhamnoside, with MS^1 at m/z 641 and 491, respectively, and a common MS^2 fragment at m/z 303, a loss of 338 amu from feruloyl hexoside moiety and of 188 amu from acetyl rhamnoside moiety, respectively [33]. Compound 42 was tentatively identified as malvidin derivative. It produced an $[M+H]^+$ ion at m/z 683 and MS^2 fragment ion at m/z 331, neutral loss of 352 amu (feruloyl glucuronide) moiety. It was tentatively identified as malvidin-3-*O*-feruloyl glucuronide [39].

3.1.4. Coumarins. One coumarin was identified in the aqueous extract of *E. cardamomum*fruits. Compound 8 was identified as umbelliferon, with m/z 163 $[M+H]^+$ and an MS^2 base peak fragment ion at m/z 135 [37].

3.2. Antiobesity Activity. Obesity which is recently known as "new world syndrome" results from the interaction of nutritional, environmental, and genetic factors [1, 40]. It is one of the worldwide health problems as it is associated with various chronic disorders such as hypertension, type 2 diabetes, dyslipidaemia, osteoarthritis, and cardiovascular, diseases. Pancreatic lipase enzyme secreted by the pancreas is responsible for 50–70% digestion of fats into monoglyceride and free fatty acids. Orlistat is an antiobesity drug which inhibits the dietary triglycerides hydrolysis and consequently decreases the subsequent intestinal absorption of monoglycerides and free fatty acids (lipolysis products). Despite of being the only drug for long-term control of

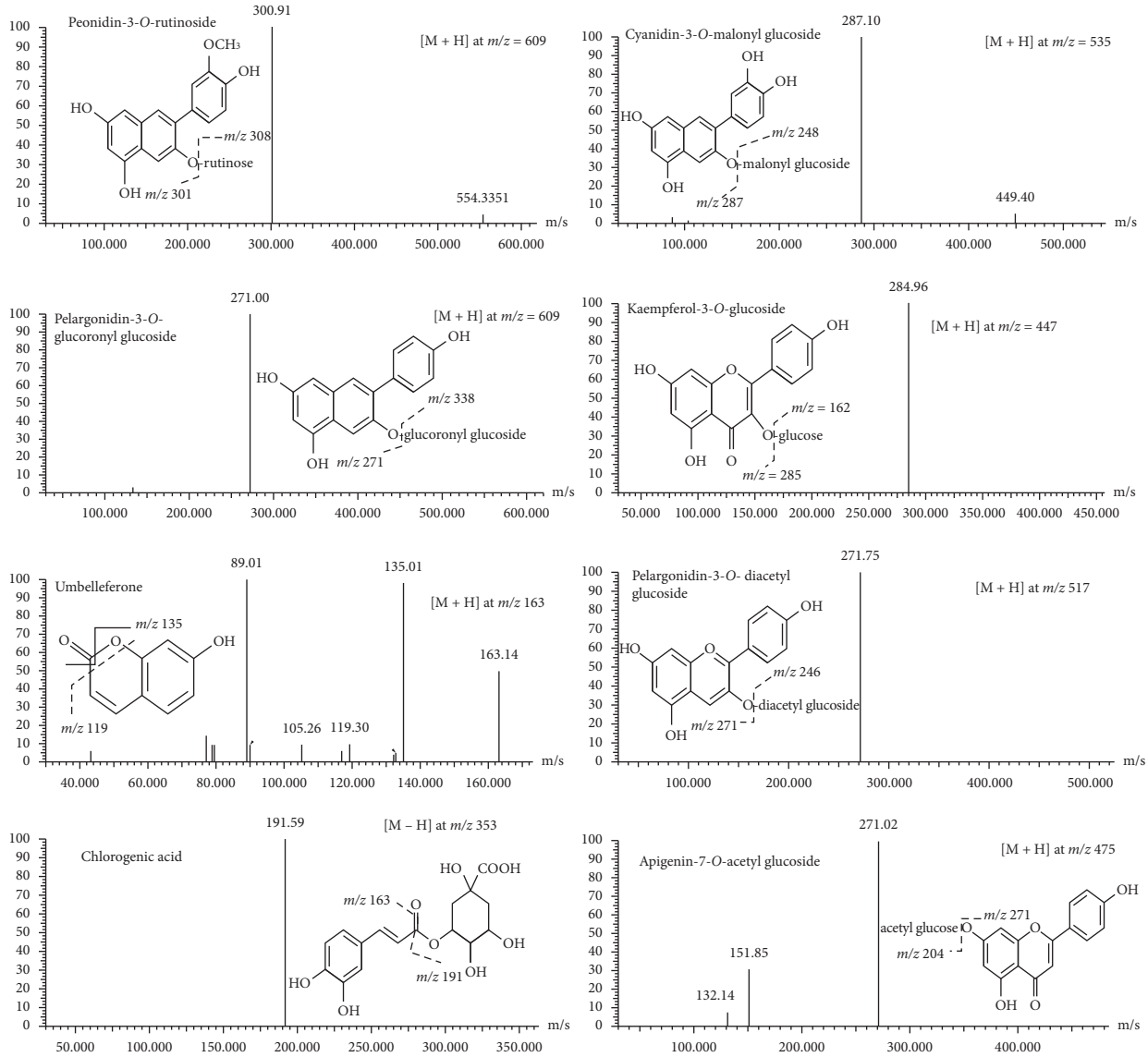

Figure 2: UPLC-ESI-MS/MS fragmentation of some identified compounds in *E. cardamomum* aqueous extract.

obesity, it has gastrointestinal side effects [40–42]. Therefore, natural products and plant-derived compounds have attracted the attention for the obesity management by developing safe and efficient antiobesity drugs [43].

The inhibitory activity of the aqueous extract on pancreatic lipase enzyme is shown in Table 2 and Figures 3(a) and 3(b). The results demonstrated that *E. cardamomum* aqueous extract inhibits pancreatic lipase in a concentration-dependent manner; the inhibition % ranged from 24.13% ± 1.2 to 70.42% ± 1.5 from the lowest to the highest concentration (31.25–1000 μg/ml). The IC_{50} of the extract was (288.75 ± 1.3 μg/ml) while that of the orlistat standard was (IC_{50} = 23.8 ± 0.7 μg/ml). *E. cardamomum* aqueous extract *in vitro* inhibition of pancreatic lipase could be attributed to the presence of major biologically active polyphenolic metabolites such as anthocyanins and flavonoid compounds. Our findings are consistent with those previously published studies. Rahman et al. [36] reported that hepatic damage, dyslipidaemia, and oxidative stress can be prevented by cardamom powder supplementation in high carbohydrate and high fat diet fed rats.

Table 2: Inhibition assay of pancreatic lipase activity of *Elettaria cardamomum* L. (Card A) at different concentrations.

Concentration(μg/ml)	Inhibition %	
	Card A	Standard drug orlistat
7.81	0	29.31 ± 1.4
15.63	0	45.25 ± 3.1
31.25	24.13 ± 1.2	54.36 ± 2.6
62.5	31.85 ± 0.72	60.35 ± 2.1
125	42.87 ± 0.63	65.34 ± 1.5
250	51.46 ± 1.2	80.12 ± 0.58
500	61.98 ± 2.1	86.35 ± 2.1
1000	70.42 ± 1.5	93.25 ± 1.5

Data was represented as mean ± SD.

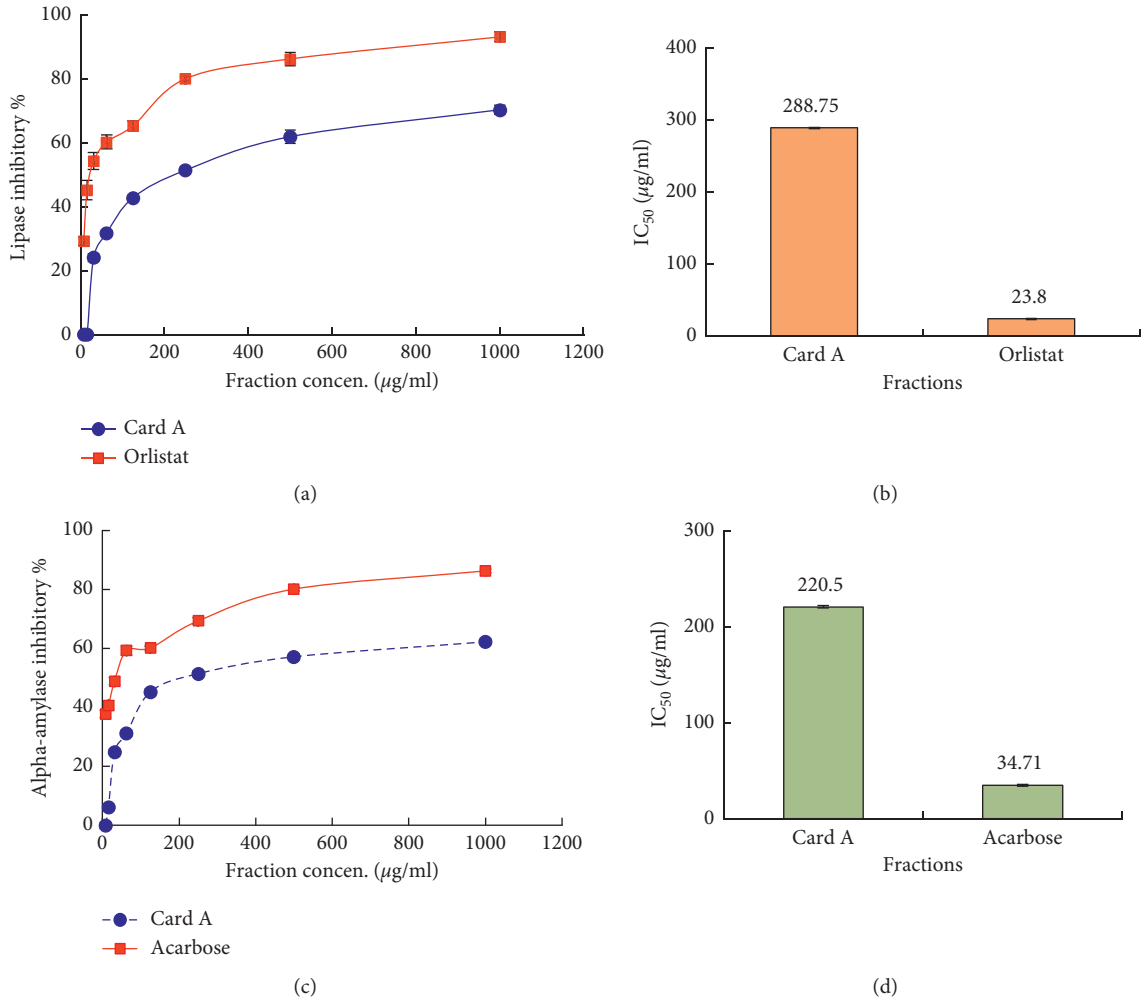

Figure 3: (a) *In vitro* pancreatic lipase inhibitory activity percentage of *E. cardamomum* L. aqueous extract (Card A) compared to orlistat standard. (b) IC_{50} of *E. cardamomum* L. aqueous extract (Card A) and orlistat. (c) *In vitro* α-amylase inhibitory activity of *E. cardamomum* L. aqueous extract (Card A) compared to acarbose standard. (d) IC_{50} of *E. cardamomum* L. aqueous extract (Card A) and acarbose. Data are presented as averages ± standard deviations from three experiments.

3.3. Antidiabetic Activity. Diabetes mellitus (DM) is a long-term metabolic disease characterized by elevated blood sugar levels. It may be attributed to a lack of insulin secretion, resistance to peripheral insulin's effects, or both. It affects around 10% of the population. Moreover, it is expected that diabetic patients may reach 230 million in 2025. DM is increasing all over the world, due to the prevalence of unhealthy habits, such as obesity, sedentarism, dietary patterns, smoking, or alcohol intake [44, 45].

Diabetes mellitus is usually accompanied by lipid, carbohydrate, and protein metabolic abnormalities. In patients with diabetes mellitus, chronic hyperglycemia, in combination with other metabolic disorders, can damage various organ systems, resulting in potentially fatal health complications, the most common of which are retinopathy, nephropathy, neuropathy, and cardiovascular disease [45]. Despite the availability of other therapeutic options, herbal remedies (medicinal plants or their extracts) are highly recommended for the treatment of diabetes since ancient times [44, 46]. Alpha-amylase and α-glucosidase are digestion enzymes of carbohydrates which can significantly decrease the postprandial elevation of blood glucose after a carbohydrate diet. So, inhibition of these enzymes is considered as essential strategy in blood glucose management [10].

In the present study, the *in vitro* α-amylase inhibitory activity of *E. cardamomum*fruits aqueous extract was investigated using variable doses (7.81–1000 μg/mL). The extract showed a good inhibition of carbohydrate-hydrolyzing enzymes (α-amylase) in a dose-dependent manner, as shown in Table 3 and Figures 3(c) and 3(d). The inhibition % ranged from 6.17 ± 1.7 to 62.25 ± 0.58 from the lowest to the highest concentration (15.63–1,000 μg/ml). However, the highest inhibition % of acarbose standard was 40.75 ± 1.5 to 86.32 ± 0.63 from lowest to highest concentration, as shown in Table 3. The IC_{50} of the extract was (220.5 ± 1.3 μg/ml) while that of the acarbose standard was (IC_{50} = 34.71 ± 0.7 μg/ml). The antidiabetic activity may be attributed to the presence of anthocyanins (cyanidin-3-*O*-cinnamoyl glucuronide, cyanidin-3-*O*-coumaroyl hexoside, cyanidin-3-*O*-hexoside,

Table 3: Inhibition assay of α-amylase activity of *E. cardamomum* L. (Card A) at different concentrations.

Concentration (μg/ml)	Inhibition %	
	Card A	Standard drug acarbose
7.81	0	37.81 ± 1.2
15.63	6.17 ± 1.7	40.75 ± 1.5
31.25	24.93 ± 1.2	48.84 ± 1.2
62.5	31.15 ± 0.72	59.31 ± 1.5
125	45.28 ± 1.5	60.17 ± 0.63
250	51.46 ± 1.2	69.37 ± 1.2
500	57.19 ± 2.1	80.14 ± 0.58
1000	62.25 ± 0.58	86.32 ± 0.63

Data are represented as mean ± SD.

peonidin-3-*O*-coumaroyl glucoside, and malvidin-3-*O*-feruloyl glucuronide) and flavonoids (taxifolin quinoyl hexoside and quercetin-3-*O*-hexoside).

Anthocyanin is considered one of the most important classes of polyphenols which play a key role in the treatment and prevention of type 2 diabetes mellitus [47]. It was reported that cyanidin, delphinidin, malvidin, and cyanidin-3-*O*-glucoside are the most potent anthocyanins for alpha-amylase inhibition. Moreover, cyanidin and its glucosides have been shown to inhibit carbohydrate-digestive enzymes by forming covalent and/or noncovalent bonds between their hydroxyl groups and the polar residues of the enzyme active sites [44, 45].

Recently, flavonoids' activity has been investigated even up to clinical studies for diabetes management in humans. The findings are compiled in several reviews [48–50]. Flavonoids have also been identified as α-amylase inhibitors in other studies [51]. Additionally, flavonoids are considered as natural alternative in diabetes treatment as they can regulate insulin secretions through different mechanisms. For example, taxifolin-3-*O*-rhamnoside works in a mechanism like sulfonylureas by K_{ATP} channel closure and cell surface Ca^{2+} channel signaling pathway opening [52].

4. Conclusion

In the present study, the aqueous extract of *E. cardamomum* fruits was investigated for the first time using UPLC-ESI/MS/MS in both negative and positive ionization modes. The antidiabetic and antiobesity activities of the extract were also evaluated *in vitro*. The results revealed the tentative identification of forty-seven compounds including (anthocyanins, flavones, and phenolic and nonphenolic acids). *E. cardamomum* aqueous extract showed a good antidiabetic and antiobesity activities which may be related to the presence of major biologically active metabolites (cyanidin-3-*O*-coumaroyl hexoside, cyanidin-3-*O*-hexoside, azelaic acid, and taxifolin quinoyl hexoside) with reported antioxidant, antiobesity, and antidiabetic activities. Finally, future bioavailability and pharmacokinetic studies of *E. cardamomum* fruit are recommended to identify and isolate its active metabolites and expand its medical application.

Data Availability

The data used to support the findings of this study are included in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors made considerable contributions to the manuscript. HA, AA, WH, AA, and SA designed the study. WH, HA, and SA performed the experiments. HA, AA, and SA interpreted the results. AA, WH, HA, AA, and SA wrote the manuscript. All authors revised the manuscript and confirmed it for publication.

Acknowledgments

The authors are thankful to the Researchers Supporting Project no. (RSP-2020/132), King Saud University, Riyadh, Saudi Arabia, for supporting the study.

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