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# Unveiling metabolome heterogeneity in three species from *Coccoloba* and *Ruprechtia* through multiple approaches of UPLC/HRMS and chemometric analysis in relation to antidiabetic, antioxidant and antiglycation activities

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## Abstract

**Background** Diabetes mellitus (DM) is a major intricate metabolic disorder, being one of the chief causes of mortality worldwide. *Coccoloba* and *Ruprechtia* are two of the most intriguing polyphenol-rich genera within the Polygonaceae family. The potential of *Coccoloba uvifera*, *Coccoloba peltata* and *Ruprechtia salicifolia* total extracts and fractions as antioxidant, antidiabetic and anti-glycating agents was evaluated and correlated with their chemical composition via multiple approaches of metabolic profiling.

**Results** All the total ethanolic extracts of plant leaves revealed remarkable antioxidant activities in terms of scavenging DPPH and ABTS radicals, as well as ferric reducing antioxidant power (FRAP). Despite having more or less comparable total phenolic and flavonoid contents, *C. uvifera* extract showed the highest inhibitory activity against  $\alpha$ -glucosidase enzyme ( $IC_{50}$   $7.985 \pm 1.08$   $\mu$ g/mL), being more potent than acarbose (20-fold). All total extracts demonstrated moderately high anti-AGEs (> 63% inhibition) in BSA-fructose model. Among all examined fractions, *C. uvifera* 50% MeOH fraction exhibited the most potent antioxidant activity in DPPH, ABTS and FRAP assays ( $5697.33 \pm 360.7$ ,  $3078.9 \pm 249$ ,  $1664.02 \pm 220$   $\mu$ M ascorbic acid equivalent/mg extract, respectively) and the highest  $\alpha$ -glucosidase inhibitory activity ( $IC_{50}$   $3.36 \pm 1.04$   $\mu$ g/mL). A total of 140 compounds, belonging to different classes, were annotated in the three species via UPLC-HRMS, where flavonoids and phenolic acids represented the major classes. Multivariate and correlation analyses revealed the key phytochemicals contributing to  $\alpha$ -glucosidase inhibition as 1-O-vanilloyl-hexoside, 1,3-O-diferuloylglycerol, drovomifoliol-O-glucopyranoside, protocatechuic acid glucoside, digalloyl glucose and coumaric acid sulphate.

**Conclusion** *C. uvifera* leaves extract and its 50% MeOH fraction had a superb potential to alleviate DM and its complications through their antioxidant, antiglycation and  $\alpha$ -glucosidase inhibitory activities mediated by their versatile polyphenolic phytochemicals.

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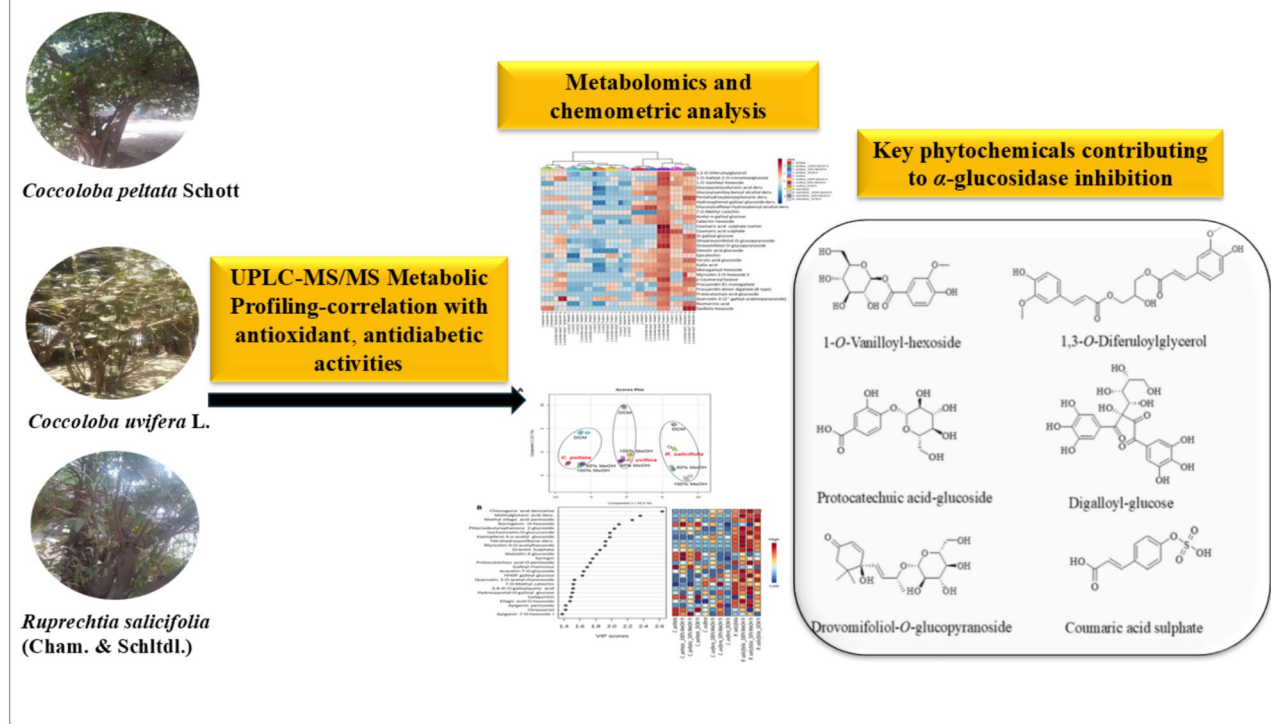
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**Keywords** *Coccoloba uvifera*, *Coccoloba peltata*, *Ruprechtia salicifolia*, Antidiabetic activity, Metabolomics, Advanced glycation end-products (AGEs)

## Graphical abstract



## Background

Diabetes mellitus (DM) is a major and intricate metabolic disorder that significantly affects health and patients' life expectancy. Diabetes is one of the chief reasons for mortality worldwide, with 6.7 million deaths in 2021, as stated by the International Diabetes Federation. Globally, 537 million person suffer from diabetes, expected to reach 643 million in 2030 [1]. Prevention of diabetes is a continuous challenge to save people all over the world and minimize healthcare costs burden.

Among the strategies to control DM is controlling the digestive enzymes activity, alpha-amylase and alpha-glucosidase, thereby delaying carbohydrates' digestion, glucose absorption and in consequence preventing postprandial hyperglycaemia (increase in serum glucose levels after a meal) [2]. Advanced glycation end-products (AGEs) are various compounds produced via non-enzymatic reaction of reducing sugar's carbonyl groups and protein's amino groups, forming unstable Schiff base, which further rearranges to Amadori product, yielding AGEs upon glycoxidation [3]. AGEs interact with their receptors (RAGE), activating a cascade of

intracellular signalling that disrupts cellular function by inducing oxidative stress and inflammatory response. Thus far, diabetes consequences such as nephropathy, neuropathy, retinopathy and cardiovascular disease, and erectile dysfunction have all been linked to the advancement of AGEs [3]. Uncontrolled hyperglycaemia has been reported to induce AGEs; being produced by combined oxidation and glycation reactions, AGEs are also reported to be a consequences of enhanced oxidative stress [3, 4]. AGEs and oxidative stress are thus considered of the main culprits of DM complications. Hence, the search for natural inhibitors of oxidative stress and AGEs has attracted much attention. Several investigations documented the role of phenolic compounds as antioxidants as well as inhibitors of digestive enzymes and AGEs [5, 6].

*Coccoloba* and *Ruprechtia* are two of the most interesting polyphenol-rich genera in family Polygonaceae considering their biological activities and secondary metabolites. The genus *Coccoloba* has been reported to have a broad range of compounds such as flavonoids, phenolic acids, anthraquinones, diterpenes, triterpenes and sterols [7, 8]. *C. uvifera* has demonstrated

different activities such as antioxidant, antidiabetic, antimutagenic, anti-inflammatory and antimicrobial activities [8, 9]. Also, *C. peltata* has showed cytotoxic, antioxidant, antihyperglycaemic activities [10, 11]. Comparatively, *Ruprechtia salicifolia* has been reported for its immunomodulatory, anti-inflammatory, antibacterial and anticancer activities in addition to possessing a variety of polyphenolic compounds [12].

Plant metabolomics is a fundamental approach for drug discovery. The recent advancements in plant metabolomics techniques for plants have made it possible to consistently figure out hundreds of metabolites and contrast between samples for differences and similarities in an untargeted, semi-automated manner [13]. This was only possible through hyphenated techniques utilizing mass spectrometry (MS) coupled to chromatographic system. The huge amount of data produced by these techniques are to be analysed through advanced chemometric tools in order to get meaningful results and tangible insights [14, 15].

The comparative chemical profiling and detailed biological studies for *C. uvifera*, *C. peltata* and *R. salicifolia* have never been deeply investigated. Thus, this study aims to evaluate and compare the three ethanolic leaves extracts of *C. uvifera*, *C. peltata* and *R. salicifolia* along with their fractions (dichloromethane, 100% and 50% MeOH) for antioxidant,  $\alpha$ -glucosidase, pancreatic lipase and advanced glycation end-products inhibition potentials. In addition, a complete phytochemical profiling of the three total extracts and their fractions using UPLC-ESI-MS/MS analysis and correlating the phytochemical compounds with these activities via a non-targeted metabolomics approach will be performed.

## Methods

### Chemicals and reagents

Analytical grade reagents and solvents were obtained from El-Nasr for chemicals (Cairo, Egypt). LC-MS grade solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) or local vendors. Deionized water was Milli-Q. In this experiment  $\alpha$ -glucosidase was used from *Saccharomyces cerevisiae* (Sigma-Aldrich, St. Louis, MO, USA). *p*-nitrophenyl dodecanoate (*p*-NPD), pancreatic lipase (porcine pancreatic lipase type II, PPL), *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (*p*-NPG), all were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Plant material

The leaves of the three plants *C. peltata*, *C. uvifera*, and *R. salicifolia* were collected in September 2020 from

Al-Orman botanical garden, Giza. The samples were verified by Mrs. Therese Labib Youssef, Former Manager and Taxonomist of Al-Orman Botanical Garden, Giza, Egypt. Voucher samples were deposited at Pharmacognosy Department Herbarium, Faculty of Pharmacy, Cairo University with the following numbers (25.7.23), (26.7.23) and (27.7.23) for *C. peltata*, *C. uvifera*, and *R. salicifolia*, respectively.

### Plant extraction and liquid-liquid partitioning

Fresh leaves were cleaned from dust and air-dried in shade and then grinded into coarse powder. The dried powdered leaves of the three species (700 g/sample) were macerated separately in absolute ethanol (4 × 1.5 L), each time for 3 days. The extract was filtered through Whatman No.1 papers and then evaporated to dryness under vacuum using rotary evaporator (at 55 °C) (Buchi, G. Flawil, Switzerland). The dried extracts were then stored at -3 °C. The extracts were subjected to fractionation as follows. Aliquots of the three ethanolic extracts of *C. peltata*, *C. uvifera* and *R. salicifolia* were dissolved in methanol/water solution (1:1 v/v) and partitioned with dichloromethane (DCM) (4 × 250 mL) to yield methylene chloride fractions. Afterwards, the remaining water was run over a diaion HP-20 column (300 g, 5 × 50 cm) and fractionated into three fractions of 100% H<sub>2</sub>O (2L), H<sub>2</sub>O: MeOH (1:1) (2 L) and 100% MeOH (2 L) where three fractions were obtained from each extract. 100% water fraction was not investigated further in the study because of its content of sugars and pigments. Methylene chloride, 50% MeOH: H<sub>2</sub>O, 100% MeOH fractions together with total ethanolic leaves extracts of the three species were subjected to the biological assays. The yield of the extracts and fractions is represented in Table S1.

### Phytochemical screening of ethanolic leaves extracts

#### Total phenolic content

Colorimetric method was employed to estimate the total phenolics in the three total ethanolic extracts using Folin-Ciocalteu reagent [16]. The tested extracts (100  $\mu$ L) were mixed with Folin-Ciocalteu reagent (10%, 200  $\mu$ L) and vortexed; then, saturated Na<sub>2</sub>CO<sub>3</sub> solution (7%, 800  $\mu$ L) was mixed. In a dark area, the mixture was settled for 1 h at room temperature. MeOH (100  $\mu$ L) or gallic acid (100  $\mu$ L) replaced the tested extracts in blank and standard assays, respectively. Concentrations of the standard gallic acid were (250–15.6  $\mu$ g/mL). Absorbance was measured at 630 nm [17] using microplate reader (TECAN, Männedorf, Switzerland) after transferring 200  $\mu$ L of the extracts, standard and control to a clear 96-well plate. The TPC was expressed in milligrams of gallic acid equivalents (GAE) for every gram of dry sample.

$$\text{Gallic acid equation : } y = 0.0039x + 0.1009$$

#### Total flavonoids content

A colorimetric method was conducted to measure the total content of flavonoids in the extracts, based on the formation of aluminium–flavonoid complex [18].  $\text{AlCl}_3$  aqueous solution (5%, 500  $\mu\text{L}$ ) was mixed with the 500  $\mu\text{L}$  of the tested extracts, methanol or quercetin (100–10)  $\mu\text{g/mL}$ ; then, the mixtures were vortexed. After room temperature incubation for 10 min, aliquots (250  $\mu\text{L}$ ) were transmitted to a clear 96-well plate. The amount of  $\text{AlCl}_3$  solution was replaced by same amount of water in blank. Measurements were performed at 403 nm [18]. The amount of total flavonoids per gram of dried sample was quantified in milligrams of quercetin equivalents.

$$\text{Quercetin equation : } y = 0.008x + 0.0609$$

#### Antioxidant activity assessment of total extracts and fractions

##### 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

The capability of free radicle scavenging of the samples was determined in vitro using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [19]. Methanolic DPPH solution (180  $\mu\text{L}$ ) is mixed with 20  $\mu\text{L}$  of tested extracts, methanol or ascorbic acid (500–15.6  $\mu\text{M}$ ). The mixtures were incubated for 20 min in dark at room temperature with absorbance determined at 540 nm [19]. The DPPH scavenging activity was determined from ascorbic acid standard curve and expressed as ascorbic acid equivalent in  $\mu\text{M}$  per milligram of extract.

$$\text{DPPH inhibition \%} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

##### 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

Samples were tested to the free radical scavenging activity of ABTS following the previously reported methodology [20]. To prepare the ABTS radical cation ( $\text{ABTS}^{+\cdot}$ ), potassium persulphate (140 mM, 88 mL) was combined with ABTS aqueous solution (7 mM, 5 mL). The generated  $\text{ABTS}^{+\cdot}$  solution was diluted using methanol (1:50) to get absorbance (0.7) at 690 nm. A 96-well plate containing 10  $\mu\text{L}$  of the test extract, MeOH or ascorbic acid was mixed with of  $\text{ABTS}^{+\cdot}$  solution (190  $\mu\text{L}$ ) and kept in dark area for 30 min. Absorbances were performed at 690 nm using the microplate reader [21]. By substituting the recorded absorbance of the samples into the ascorbic acid linear regression equation, the antioxidant power was calculated as  $\mu\text{M}$  ascorbic acid equivalents.

$$\text{ABTS inhibition \%} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

#### Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay method relies on the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ , which is subsequently chelated to form the  $\text{Fe}^{2+}$ -TPTZ complex by 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) [22]. Briefly, the FRAP reagent (190  $\mu\text{L}$ ) (sodium acetate buffer 300 mM pH 3.6, ferric chloride 20 mM, and TPTZ 10 mM (10:1:1)) left for 30 min in a dark place at 37 °C with 10  $\mu\text{L}$  of the samples, using absorbance 620 nm for measurement [23]. Using the linear regression equation, the antioxidant effect of the extract was calculated as  $\mu\text{M}$  ascorbic acid equivalents.

$$\text{Ferric reducing power} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

#### Enzymatic assays for extracts and fractions

##### $\alpha$ -glucosidase inhibition assay

$\alpha$ -glucosidase inhibition activity was investigated using the method reported by Gutiérrez-Grijalva [24]. At a concentration of 1 mM, acarbose was prepared in methanol as a positive control. For colorimetric assessment, *p*-nitrophenyl- $\alpha$ -D-glucopyranoside was used as a substrate. In 96-microwell plates, 50  $\mu\text{L}$  of (0.6 U/mL)  $\alpha$ -glucosidase in phosphate buffer (pH 7, 0.1 M) was added to samples/blank (25  $\mu\text{L}$ ) at 37 °C for 10 min. Afterwards, 25  $\mu\text{L}$  of 3 mM *p*-NPG in phosphate buffer (pH 7) was then added and left at 37 °C for 5 min. Measurement was performed at 405 nm. Then  $\alpha$ -glucosidase inhibition percentage was determined.

Regarding the fractions, they were prepared at 25  $\mu\text{g/mL}$  as a final concentration in the assay buffer. At this concentration, samples which exceeded 50% inhibition were prepared to measure their  $\text{IC}_{50}$  values.

$$\alpha - \text{glucosidase inhibition \%} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

##### Pancreatic lipase inhibition assay

*P*-nitrophenyl dodecanoate *p*-NPD was used as the substrate in the determination of the inhibition activity on pancreatic lipase; the assay was applied as previously described with a minor modifications [25]. Solutions of the total extracts were prepared 100  $\mu\text{g/mL}$  as a final concentration. Orlistat prepared in assay buffer was used as a positive control. 50  $\mu\text{L}$  of (1 mg/mL) PPL was mixed with samples /blank 25  $\mu\text{L}$  in a Tris-HCl buffer (100 mM, pH 8) at 37 °C for 10 min. Then, 10  $\mu\text{L}$  of the *p*-NPD solution (10 mM, in isopropanol) was added, and the total volume was then diluted using Tris-HCl buffer to 200  $\mu\text{L}$ . Furthermore, the mixture was left at 37 °C for 20 min; the

absorbance was carried out at 405 nm. Then, the percentage of pancreatic lipase inhibition was assessed.

$$\text{Lipase inhibition \%} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

#### Advanced glycation end-products (AGEs) formation inhibition

Three different assays were used to assess the inhibitory activity of the samples against AGEs. The total extracts and their fractions were prepared at final concentration 1 mg/mL in the assays buffer. At this concentration, samples which exceeded 50% inhibition were prepared to measure their IC<sub>50</sub> values using quercetin as a positive control [26].

##### BSA-fructose assay

The bovine serum albumin BSA-fructose model was used to evaluate the inhibition of AGEs production, as mentioned by the prior study [27]. Briefly, fructose solution (1.25 M) in phosphate buffer (200 mM, pH 7.4) was mixed with sodium azide (0.02%) and with BSA (50 mg/mL) (1:1:1) by volume. The incubations were settled in dark area at 37 °C for 72 h with samples/ blank. Fluorescent AGEs were assessed on microplate reader (Fluostar Omega BMG Labtech, Ortenberg, Germany) using wavelengths for excitation 355 nm and for emission 460 nm. Then the inhibition percentage of AGEs was determined.

$$\% \text{ Inhibition} = [(F_{\text{blank}} - F_{\text{sample}}) / F_{\text{blank}}] \times 100$$

##### BSA-methylglyoxal assay

The activity of the samples for AGEs formation inhibition was assessed following the reported method [27]. Briefly, samples were incubated in black 96-microwell plates with BSA (50 mg/mL, 200 mM phosphate buffer, pH 7.4, with 0.02% sodium azide) together with 53.3 mM methylglyoxal in distilled water), (1:1:1 v/v/v) for 72 h in a dark area at 37 °C. Fluorescent AGEs were determined using wavelengths for excitation 355 nm and for emission 460 nm. Assays were all run in triplicate.

$$\% \text{ Inhibition} = [(F_{\text{blank}} - F_{\text{sample}}) / F_{\text{blank}}] \times 100$$

##### Arginine-methylglyoxal assay

Wang's previously described method was used to determine the inhibitory potential of the tested sample on the development of AGEs in the arginine-methylglyoxal experiment [27]. Simply, the samples were incubated in black 96-microwell plates with 53.3 mM methylglyoxal in distilled water and arginine 106.6 mM (in 200 mM phosphate buffer, pH 7.4, with 0.02% sodium azide), (1:1:1 v/v/v), for 72 h at 37 °C in the dark. Fluorescent AGEs were investigated on a microplate reader (Fluostar Omega/

BMG Labtech/Germany) using wavelengths for excitation 355 nm and for emission 460 nm. Assays were run in triplicate.

$$\% \text{ Inhibition} = [(F_{\text{blank}} - F_{\text{sample}}) / F_{\text{blank}}] \times 100$$

#### Ultrahigh-performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS analysis)

The dried residues of the total leaves extract of the three species and their fractions were dissolved separately in methanol: water (1:1, v/v) to a concentration of 1 mg/mL. Samples were filtered using 0.2-μm PTFE syringe filter and transferred into LC glass vials. Blank and quality control (QC) (a pool that were collected by aliquoting the same volume from all the extracted tubes) samples were included for background removal and quality assessment, respectively. To confirm the stability and reproducibility of the analysis, QC samples were analysed prior to sample acquisition and after every five samples. Samples were randomized before the analysis to exclude run order effect. The UPLC was equipped with High-Strength Silica C18 column (HSS) reversed-phase (RP) T3 (100×2.1 mm and 1.7 μm particles diameter, Waters). The extracts were individually subjected to chromatography. The injection volume was 3 μL, and the flow rate was adjusted to 0.4 mL/min. The chromatographic conditions were performed as using water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The separation gradient was as follows: 0–1 min at 99% A; 1–11 min from 99 to 60%A; 11–13 min from 60 to 30%A; 13–15 min 60 to 1%A; 15–16 min, system was maintained at 1%A; 16.0–17.0 min, mobile phase A was linearly increased from 1 to 99%; 17.0–20.0 min, mobile phase A was maintained at 99%. The column temperature was set to 40 °C [28].

Mass spectra were acquired using heated electrospray ionization (HESI) source coupled with high-resolution Orbitrap mass analyser (QExactive, Thermo-Fisher, Bremen, Germany) [29]. Mass spectra were acquired using data-dependent acquisition (DDA) in negative ionization mode in the range of m/z 100–1500. Following MS<sup>1</sup> analysis, the five most intense precursor ions were selected for subsequent dissociation. The following parameters were applied: ion voltage, 3000 V; sheath gas, 40 psi; auxiliary heating gas, 15 psi; ion source heating temperature, 350 °C; collision energy, 20–40–60 V cycle; MS<sup>1</sup> and MS<sup>2</sup> resolution, 70,000 and 17,500, respectively [28].

### Data processing and multivariate analysis

The chromatograms were visually inspected by Xcalibur 2.1 software package (Thermo-Fisher Scientific Inc., USA). In addition, the obtained MS/MS data were converted from raw files (.RAW) to the mzML file format using the MSConvert software [30]. MZmine software (Version 3.6) was used to attain data matrices [31]. We used the following MZmine parameters (A) Mass detection: MS<sup>1</sup> noise level, 1E5; MS<sup>2</sup> noise level, 1E4. (B) ADAP chromatogram builder: MS-level 1; min. group size in no. of scans, level 2; group intensity threshold, 2E5; m/z tolerance, 0.01 m/z. (C) Chromatogram deconvolution: Local minimum search algorithm. (D) Isotopic peaks grouper: m/z tolerance, 0.01 m/z; RT tolerance, 0.1 min; monotonic shape, yes; maximum charge, 2; representative isotope, lowest m/z. (E) peak alignment: m/z tolerance, 0.02 m/z; weight for m/z, 75; RT tolerance, 0.25 min; weight for RT, 25. (F) Peak list rows filter: Only features with accompanying MS<sup>2</sup> data and their retention time between 0 and 20 min were kept. (G) Duplicate peak filter: filter mode, old average; m/z tolerance, 0.02 m/z; RT tolerance, 0.5 min. High-resolution mass as well as MS<sup>2</sup> analysis fragmentation spectra were used to identify the chemical components in each sample, matching with the authentic standards, public MS/MS libraries and literature-based data. The resulting data were converted into a CSV file and directly exported to multivariate analysis. Data analysis and correlation were carried out using Metaboanalyst 5.0 (<https://www.metaboanalyst.ca/>) [32]. The following settings were used Metaboanalyst parameters: sample normalization, none; data scaling, Pareto-scaled (mean-centred values/square root of the standard deviation); and data transformation, log transformation. Subsequently, the data were subjected to different statistical analysis methods, including unsupervised methods, i.e. principal component analysis (PCA) and hierarchical cluster analysis (HCA) and the supervised partial least squares discriminant analysis (PLS-DA). Heatmap clustering analysis was performed on the transformed and scaled data using the Euclidian distance method utilizing ward clustering algorithm.

### Statistical analysis

The significance of the differences was calculated using one-way ANOVA followed by Tukey's HSD test ( $p \leq 0.05$ ) as post-tests for multiple comparisons. The graphics were done using Microsoft excel 365 while GraphPad Prism version 6.0 was used to perform all statistical analysis. The expression of the data was illustrated as mean  $\pm$  standard deviation of the mean, and all analyses were done in triplicate.  $p$  values less than 0.05 were deemed statistically significant.

**Table 1** Total phenolic and flavonoid contents of the tested extracts

| Extract               | Total phenolic content*           | Total flavonoid content**       |
|-----------------------|-----------------------------------|---------------------------------|
| <i>C. peltata</i>     | 209.77 $\pm$ 3.811 <sup>a</sup>   | 26.475 $\pm$ 1.635 <sup>b</sup> |
| <i>C. uvifera</i>     | 204.26 $\pm$ 0.361 <sup>a</sup>   | 31.162 $\pm$ 0.574 <sup>b</sup> |
| <i>R. salicifolia</i> | 190.632 $\pm$ 10.636 <sup>b</sup> | 33.933 $\pm$ 4.596 <sup>a</sup> |

\* mg/g gallic acid equivalent  $\pm$  SD

\*\*mg/g quercetin equivalent  $\pm$  SD

<sup>a, b</sup> Means with different letters superscript indicate significant difference between them, while means with the same letter superscript have no significant difference between them (Tuckey's—HSD multiple range post hoc test,  $P < 0.05$ )

## Results

### Total phenolic and flavonoid contents

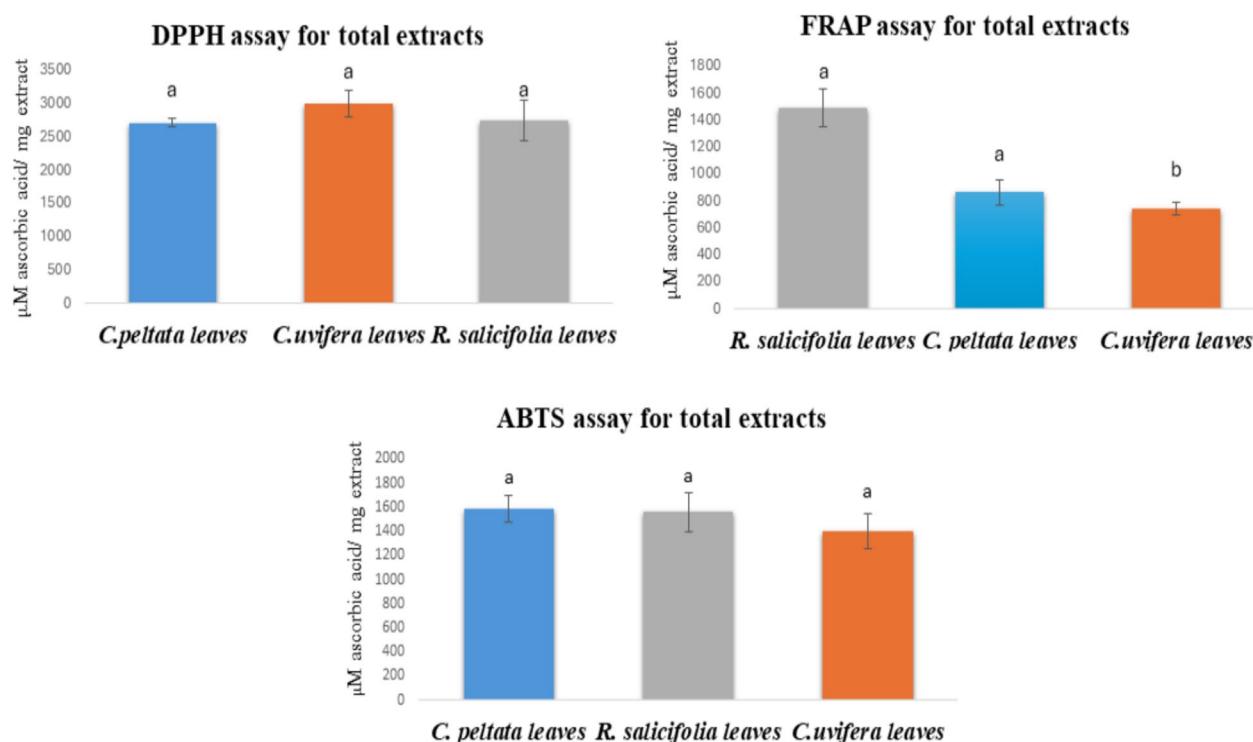
Total phenolic and flavonoid contents of the three species total ethanolic extracts were quantified. *C. peltata* and *C. uvifera* possessed the highest total phenolic content (209.77 and 204.26 mg/g GAE, respectively), while *R. salicifolia* had the highest total flavonoid content (33.933 mg/g QE) (Table 1).

### Antioxidant capacity

Strong association exists between oxidative stress and many chronic diseases such as cardiovascular diseases, atherosclerosis, diabetes neurological disorders and others. At this point, herbs are considered vital sources of plethora of variable antioxidants [33]. The antioxidant activities of the total extracts and their fractions were investigated in terms of scavenging DPPH and ABTS radicals, as well as ferric reducing antioxidant power (FRAP) to assess different antioxidant mechanisms.

The highest DPPH scavenging activity was exhibited by *C. uvifera* extract (2996.556  $\pm$  205.7  $\mu$ M ascorbic acid equivalent (AAE)/mg extract), followed by *R. salicifolia* then *C. peltata* (Fig. 1). However, no significant difference existed between the three extracts as revealed by ANOVA testing. Similarly, in ABTS assay, the extracts revealed remarkable activities, with no significant differences between them. Regarding the FRAP assay, *R. salicifolia* showed higher reducing power (1492.44  $\pm$  141.42  $\mu$ M AAE/ mg extract), compared to the other two extracts (Fig. 1).

As for the antioxidant activity of fractions, 50% and 100% MeOH fractions of *C. uvifera* were the most potent DPPH scavengers (5697.33  $\pm$  360.7 and 5548.67  $\pm$  308.36  $\mu$ M AAE/ mg extract, respectively), with no significant difference between them, followed by *C. peltata* 100% MeOH fraction (3363.64  $\pm$  91.07  $\mu$ M AAE/ mg extract), then both the 50% and 100% MeOH fractions of *R. salicifolia* (2622.89  $\pm$  360.2 and



**Fig. 1** Antioxidant activities of the three total extracts using DPPH, ABTS and FRAP assays, <sup>a, b</sup>Means with different letters superscript indicate significant difference between them, while means with the same letter superscript have no significant difference between them (Tuckey's—HSD multiple range post hoc test,  $P < 0.05$ )

2389.77 ± 80.57 μM AAE/ mg extract), respectively, with no significant difference between them. *C. peltata* 50% MeOH fraction was the least effective fraction as DPPH scavenger (Fig. 2). The 50% MeOH fraction of *C. uvifera* also demonstrated the highest ABTS scavenging activity (3078.9 ± 249 μM AAE/mg extract), followed by *C. uvifera* 100% MeOH fraction and *R. salicifolia* 50% MeOH fraction (2621.6 ± 151.56 and 2279.4 ± 195.2 μM AAE/mg extract, respectively, with no significant difference), then the 100% MeOH fraction of *R. salicifolia* (1784.7 ± 215.9 μM AAE/ mg extract). *C. peltata* 100% and 50% MeOH fractions were the least active, with no significant difference between them (Fig. 2). In the FRAP assay, again *C. uvifera* 50% and 100% MeOH fractions revealed the highest ferric reducing power (1664.02 ± 220 and 1434.5 ± 60.5 μM AAE/ mg extract, respectively), with no significant difference among them. *R. salicifolia* 50% and 100% MeOH fractions owed the second highest activity (1312.36 ± 56.8 and 1287.6 ± 103.16 μM AAE/mg extract, respectively), followed by *C. peltata* 50% and 100% MeOH fractions (441.94 ± 61.2 and 237.9 ± 31.5 μM AAE/mg extract, respectively) (Fig. 2).

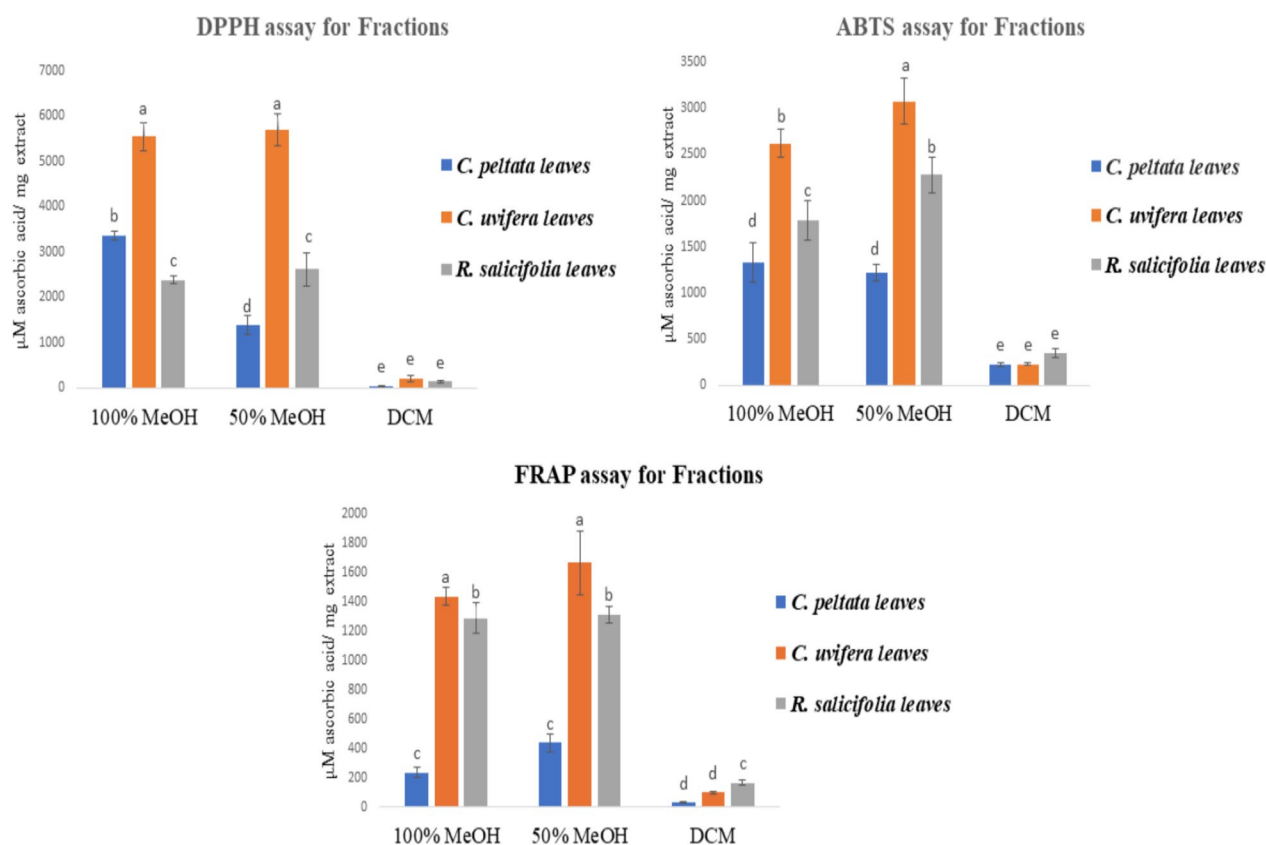
Accordingly, *C. uvifera* 50% and 100% MeOH fractions displayed the highest free radical scavenging activity,

followed by 50% and 100% MeOH fractions of *R. salicifolia* in ABTS and FRAP assays. Conversely, DCM fractions of the three species demonstrated the least antioxidant activities in the three utilized assays.

A correlation analysis between different antioxidant assays was further performed, revealing a positive correlation (>0.7) between the three antioxidant assays: ABTS, DPPH, FRAP, as demonstrated in Table S2. The highest correlation (0.92) was evidenced between ABTS and DPPH, with a similar mechanism of action. The positive correlations between ABTS, DPPH scavenging activities and FRAP are also illustrated in Table S2; Fig. S1.

#### ***α-glucosidase and pancreatic lipase inhibition assays***

Postprandial hyperglycaemia represents a continuous risk factor with a linear link to cardiovascular disorders, atherosclerosis and strokes [34]. *α*-Glucosidase inhibitors can slow down the release of glucose from oligo- or polysaccharides, thereby reducing the rate of glucose absorption and the subsequent postprandial plasma glucose levels [35, 36]. Thereof, *α*-glucosidase inhibitors represent potential therapeutic approach to restrain postprandial hyperglycaemia, which can be a helpful mechanism in the development of antidiabetic medications. In this



**Fig. 2** Antioxidant activities of the three total extracts fractions (100%MeOH, 50%MeOH and DCM) using DPPH, ABTS and FRAP assays, <sup>a–e</sup>Means with different letters superscript indicate significant difference between them, while means with the same letter superscript have no significant difference between them (Tuckey's—HSD multiple range post hoc test,  $P < 0.05$ )

**Table 2**  $IC_{50}$  values of the three total leaves extracts in  $\alpha$ -glucosidase inhibition assay

| Extract               | $IC_{50}$ $\mu\text{g/mL} \pm \text{SE}$ |
|-----------------------|------------------------------------------|
| <i>C. uvifera</i>     | $7.985 \pm 1.08^a$                       |
| <i>C. peltata</i>     | $13.74 \pm 1.03^b$                       |
| <i>R. salicifolia</i> | $> 500 \mu\text{g/mL}^d$                 |
| Acarbose              | $161.4 \pm 1.05^c$                       |

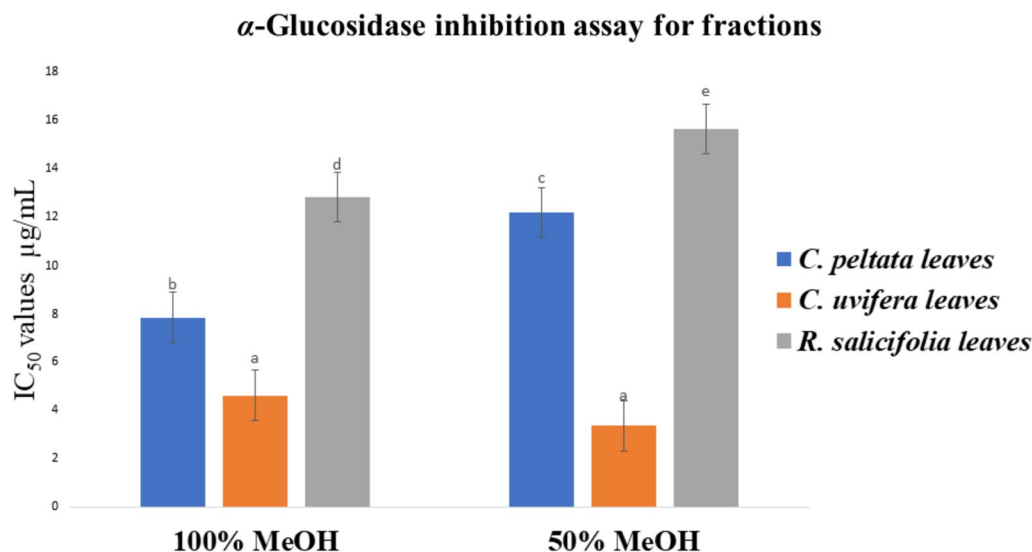
<sup>a–d</sup>Means with different letters superscript indicate significant difference between them, while means with the same letter superscript have no significant difference between them (Tuckey's—HSD multiple range post hoc test,  $P < 0.05$ )

aspect, polyphenolic compounds display a significant role via blocking the action of digestive enzymes [5]. In a similar scenario, inhibition of lipases lowers fatty acids absorption by interfering with fat hydrolysis from ingested lipids. As a result, pancreatic lipase inhibitors are regarded as a beneficial treatment medication for treating people with diet-related obesity [37]. Consequently,  $\alpha$ -glucosidase and pancreatic lipase inhibition assays were conducted to investigate the antidiabetic

and antihyperlipidemic activities of the tested samples, respectively.

The results of  $\alpha$ -glucosidase inhibition assays of the total ethanolic extracts are demonstrated in Table 2, while  $IC_{50}$  values of the fractions exhibiting more than 50% enzyme inhibition at 25  $\mu\text{g/mL}$  are demonstrated in Fig. 3 ( $IC_{50}$  values curve for total extracts and fractions Fig. S2). *C. uvifera* leaves extract exhibited the highest inhibitory activity against  $\alpha$ -glucosidase enzyme, followed by *C. peltata* extract with  $IC_{50}$  values of  $7.985 \pm 1.08$  and  $13.74 \pm 1.03$   $\mu\text{g/mL}$ , respectively; it was worth noting that these extracts were significantly 12–20 times more potent than acarbose (Table 2). In addition, both the 50% and 100% MeOH fractions of *C. uvifera* were the most potent  $\alpha$ -glucosidase inhibitors ( $IC_{50}$  values  $3.36 \pm 1.04$  and  $4.61 \pm 1.03$   $\mu\text{g/mL}$ , respectively), presenting no significant difference, followed by 100% MeOH fraction of *C. peltata* ( $IC_{50}$   $7.86 \pm 1.03$   $\mu\text{g/mL}$ ). On the contrary, all DCM fractions showed less than 50% enzyme inhibition at 25  $\mu\text{g/mL}$ .

Conclusively, *C. uvifera* extract and its fractions (50% and 100%MeOH) showed more significant inhibition of



**Fig. 3** IC<sub>50</sub> values µg/mL  $\pm$  SE for extracts fractions (100%MeOH and 50%MeOH) in  $\alpha$ -glucosidase inhibition assay, <sup>a–e</sup>Means with different letters superscript indicate significant difference between them, while means with the same letter superscript have no significant difference between them (Tuckey's—HSD multiple range post hoc test,  $P < 0.05$ )

$\alpha$ -glucosidase than the other two tested species. On the other hand, all extracts revealed weak inhibitory activity against pancreatic lipase, less than 30% inhibition at a concentration of 100 µg/mL.

Pearson's correlation coefficients ( $r$ ) were employed to unveil the relation between the antioxidant and antidiabetic activities (Table S2; Fig. S3). A strong positive correlation existed between the results of DPPH and ABTS assays on one side and the IC<sub>50</sub> values of the  $\alpha$ -glucosidase inhibition on the other side ( $r \geq 0.8$ ,  $P < 0.001$ ). However, the FRAP assay results were poorly correlated with  $\alpha$ -glucosidase inhibition ( $r = 0.54$ ,  $P < 0.001$ , Table S2); it can thus be concluded that metal reduction or chelation is not correlated with  $\alpha$ -glucosidase inhibition.

#### Advanced glycation end-products (AGEs) inhibition assays

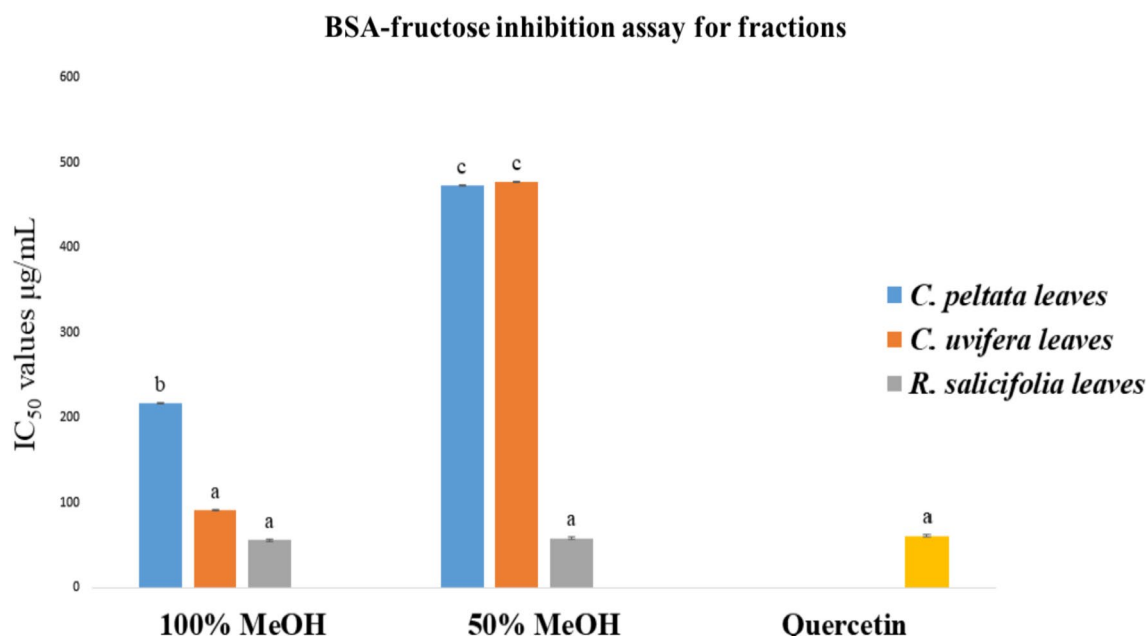
Glycation is a long term non-enzymatic process, involving three phases: first, unstable Schiff bases are formed through the reaction of reducing sugars' carbonyl groups with protein's amino moieties which further rearrange into the more stable ketoamines (Amadori products);

second, Schiff bases or Amadori products break down into different dicarbonyl compounds as glyoxal and methylglyoxal; finally advanced glycation end-products (AGEs) are formed via a series of reactions. Thereafter, AGEs bind to their specific receptors (RAGE) and stimulate intracellular signalling pathways which induce oxidative stress, inflammation and tissue damage, resulting in cardiovascular diseases, nephropathy, retinopathy and neuropathy [3, 6]. Persistent hyperglycaemia and oxidative stress induce accelerated generation of AGEs, which play a key role in the pathogenesis of diabetic complications. At this point, we evaluated the in vitro antiglycation activity of the three species using three models viz: BSA-fructose, arginine-methylglyoxal, and BSA-methylglyoxal assays. BSA was used as the model protein, being 90% homologous with human serum albumin so used as its analogue. Fructose was used as glycating agent, as it mediates protein glycation ~ ten times faster than glucose [27]. Additionally, in diabetic patients, fructose accumulates in the peripheral nerves, ocular lens and blood vessels after increased glucose supply, thus contributing

**Table 3** BSA-fructose, BSA-methylglyoxal and arginine-methylglyoxal inhibition assays for total extracts at 1000 µg/mL

| Extract               | BSA-fructose inhibition %     | BSA-methylglyoxal inhibition % | Arginine-methylglyoxal inhibition % |
|-----------------------|-------------------------------|--------------------------------|-------------------------------------|
| <i>C. peltata</i>     | 63.83 $\pm$ 1.16 <sup>a</sup> | 21.26 $\pm$ 2.8 <sup>a</sup>   | 34.6 $\pm$ 0.66 <sup>a</sup>        |
| <i>C. uvifera</i>     | 63.1 $\pm$ 1.59 <sup>a</sup>  | 8.1 $\pm$ 5 <sup>b</sup>       | 16.9 $\pm$ 0.9 <sup>b</sup>         |
| <i>R. salicifolia</i> | 67.27 $\pm$ 2.7 <sup>a</sup>  | 23 $\pm$ 1.6 <sup>a</sup>      | 17.3 $\pm$ 0.3 <sup>b</sup>         |

<sup>a, b</sup> Means in the same column with different letters superscript indicate significant difference between them (Tuckey's—HSD multiple range post hoc test,  $P < 0.05$ )



**Fig. 4**  $IC_{50}$  values of the extract's fractions (100%MeOH and 50%MeOH) in BSA-fructose inhibition assay, <sup>a-c</sup>Means with different letters superscript indicate significant difference between them, while means with the same letter superscript have no significant difference between them. (Tuckey's—HSD multiple range post hoc test,  $P < 0.05$ )

to vascular complications [38]. Hence, inhibition of fructose is considered of paramount importance. The three total ethanolic leaves extracts (1000 µg/mL) inhibited AGEs formation in BSA-fructose model by >63% (Table 3). However, in the arginine-methylglyoxal and BSA-methylglyoxal models, the inhibition of AGEs by the total extracts was less than 50% at 1000 µg/mL. In consequence, BSA-fructose inhibition assay was selected for assessing the fractions' inhibitory activities.

$IC_{50}$  values of BSA-fructose inhibition assay for fractions are demonstrated in Fig. 4 ( $IC_{50}$  values curve Fig S4). 100% MeOH and 50% MeOH fractions of *R. salicifolia* showed the highest AGEs inhibitory potential ( $IC_{50}$  values  $56.09 \pm 1.08$  and  $58.32 \pm 1.5$  µg/mL, respectively), followed by *C. uvifera* 100% MeOH fraction ( $91.22 \pm 1.16$  µg/mL), presenting no significant differences between their  $IC_{50}$  values, and being nonsignificant from the positive control quercetin ( $IC_{50}$   $61.27 \pm 1.04$  µg/mL) ( $P < 0.05$ ). *C. peltata* 100% MeOH fraction was secondly ranked, with  $IC_{50}$  value of  $217.2 \pm 1.07$  µg/mL, whereas 50% MeOH fractions of *C. peltata* and *C. uvifera* extracts came in the third rank, compared to other fractions.

It was obvious that the activities of the same extracts varied based on the used glycation model. In a similar way, the same phenolic compound exhibited varied degrees of inhibition according to the used AGEs model [39]. In the BSA-fructose model, fructose and its

oxidation products mediate protein glycation yielding AGEs; thus, this model can evaluate all phases of protein glycation [39]. Nonetheless, BSA-MGO model assesses only the middle phase of protein glycation, where MGO is directly used for glycation, omitting the initial conversion steps from sugars to carbonyls [27, 39]. It is worth noting that methylglyoxal is detected at high concentrations in diabetic patients, which results in protein glycation a way more faster than sugars. On the other hand, the arginine-methyl glyoxal model specifically gauges reactions of methylglyoxal with the arginine guanidine group [27].

Overall, the three tested extracts as well as their polar fractions, in particular the 100% MeOH fractions, inhibited protein glycation in BSA-fructose model, whereas nonpolar fractions (DCM) were much less effective, which could be partly attributed to their low polyphenolic contents. Considering the favoured generation of AGEs by oxidative reactions, together with the antioxidant efficacies of the three tested species proven herein, antioxidants in the investigated samples may have inhibited the early stage of protein glycation.

#### Identification of metabolites by ultrahigh-performance liquid chromatography electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS)

Natural products have shown a fascinating improvement in human health for a long time. With the advent of using

nutraceuticals as dietary supplements for protection and healing, there has been a rise in the importance of medicinal plants as a source of biologically active compounds. Besides, plant-derived bioactive compounds possess a variety of chemical characteristics in addition to varying biological effects both in vitro and in vivo [40].

Owing to the robust antioxidant potential and promising inhibitory effects of the total extracts of *C. uvifera*, *C. peltata* and *R. salicifolia* against  $\alpha$ -glucosidase and AGEs, their chemical profiles were analysed for first time via ultrahigh-performance liquid chromatography electrospray ionization mass spectrometry (UHPLC-ESI-MS/MS) to distinguish the phytochemical constituents correlated with the biological activities. The results of this analysis are summarized in Table 4; Fig. S5. Based on MS/MS data in comparison with published literature, a total of 140 compounds were putatively identified. About 69 compounds were newly identified in the genus *Coccoloba*, whereas about 57 compounds were newly identified in the genus *Ruprechtia*.

#### Phenolic acids and derivatives

LC-MS analysis of the ethanolic extract of the three species showed 31 hydroxybenzoic acid derivatives. Generally, there was a discernible fragmentation that involved the cleavage of the intact sugar and then the ion that matched the fragment of phenolic acid was released. These compounds were **8, 10–16, 18, 20, 22, 25, 29–31, 38, 39, 44, 45, 60, 65, 72, 77, 78, 88, 93, 96, 101, 108, 113, 115**. They were identified according to their MS and MS/MS pattern as gallic acid and its derivatives, protocatechuic acid and its glycosides, ellagic and vanillic acids glycosides. Compound **101** was putatively identified as digalloyl quinic acid with  $m/z$  495.0786 which generated the fragments of  $MS^2$  at  $m/z$  343 ( $[M-H-152]^-$ ) in addition to  $m/z$  191 ( $[M-H-304]^-$ ) (i.e. quinic acid ion) resulting from the elimination of one and two galloyl moieties, respectively, then  $m/z$  173 ( $[M-H-18]^-$ ) due to water loss, while  $m/z$  169 fragment referred to gallic acid anion. Thus, the compound was identified as digalloyl quinic acid (Fig. S6) [41].

In addition, compound **38** was putatively assigned as vanillic acid pentoside hexoside with  $m/z$  461.1307 which provided the fragments of  $MS^2$  at  $m/z$  329.05 ( $[M-H-132]^-$ ) corresponding to a pentose loss, followed by hexose loss  $m/z$  167.03 ( $[M-H-294]^-$ ) (i.e. vanillic acid ion), in addition to methyl  $m/z$  153.01 moieties [42].

For cinnamic acid derivatives,  $CO_2$  losses were observed as well as  $[M-CH_3]^-$  formation in case of methoxylated compounds. Twelve compounds were putatively identified as cinnamic acid derivatives, compounds **17, 26, 37, 40, 46, 48, 54, 68, 104, 116, 136 and 139**. The compounds were rosmarinic acid, ferulic

acid glucoside, *p*-coumaroyl hexose, coumaric acid sulphate, coumaric acid isomer sulphate, sinapoyl-hexoside, chlorogenic acid derivative, 1-*O*-galloyl-2-*O*-cinnamoyl-glucose, feruloyl tyramine, 3,4-di-*O*-caffeoyl-5-*O*-sinapoylquinic acid and caftaric acid respectively. Compound **136**  $m/z$  721.1761 yielded fragment ions at  $m/z$  397.13 (sinapoylquinic acid),  $m/z$  223 corresponding to sinapic acid ion and  $m/z$  179 corresponding to sinapic acid—COO (Fig. S7) [43, 44].

#### Flavonoids

The analysis of the three ethanolic extracts showed 65 flavonoids based on the  $MS^2$  fragmentation pattern, in comparison with available literature and mass spectral libraries. Glucose and  $H_2O$  were the characteristic neutral losses of flavonoids, and many of these flavonoids showed a distinctive fragment at  $m/z$  151, an outcome of the retro Diels–Alder (RDA) reaction. Compounds **24, 35, 36, 42, 43, 49, 50, 52, 53, 59, 69 and 90** were flavanols, identified as galocatechin, catechin hexoside, epicatechin, EGCG dimer (prodelphinidin dimer), catechin-3-*O*-gallate, catechin, epigallocatechin gallate, catechin-7-*O*- $\alpha$ -L-rhamnopyranoside, epicatechin dimethyl gallate, procyanidin B1 monogallate, procyanidin dimer digallate (B-type), 7-*O*-methyl catechin, respectively.

Compound **69** was putatively identified as proanthocyanidin dimer digallate (B-type) with  $m/z$  881.1591 according to its  $MS^2$  fragmentation pattern. The formation of the conspicuous product ion peak at  $m/z$  729.1476 ( $[M-H-152]^-$ ) was obtained from the neutral loss of either the flavanol B ring or a galloyl group combined with a portion of the C ring from RDA reaction. Additionally, subsequent expulsions of one or two more 152 Da fragments due to collision-induced dissociation produced ions with  $m/z$  values of 577 and 407 (425— $H_2O$ ) as well. Furthermore, an interflavan bond cleavage took place, resulting in (epi)catechin and its gallate ester ions at 289 and 441 Da, respectively (Fig. S8) [45].

Thirteen flavones were tentatively identified (compounds **34, 47, 56, 57, 82, 99, 114, 122, 123, 125, 135, 137 and 138**) based on their  $MS^2$  fragmentation pattern. The compounds were tentatively identified as apigenin-*O*-hexoside I, apigenin pentoside, isorhamnetin-3-*O*-glucoside, isorhamnetin-*O*-glucuronide, orientin, apigenin-*O*-hexoside II, acacetin-*O*-glucoside, chrysoeriol hexoside, tricin, isoliquiritin, chrysoeriol, apigenin and chrysin derivative, respectively. Compound **57** with  $m/z$  491.0812 was putatively identified as isorhamnetin glucuronide according to its  $MS^2$  spectrum; the base peak ion at  $m/z$  315 ( $[M-H-176]^-$ ) demonstrated a loss of glucuronyl unit, resulting in the isorhamnetin aglycone at  $m/z$  315 [46].

**Table 4** Characterization of compounds in three species *C. peltata*, *C. uvifera* and *R. salicifolia* ethanolic leaves extracts and their fractions using UPLC-ESI-MS/MS in negative ion mode

| Number | Compound name                                             | Molecular formula                               | Monoisotopic mass | Retention time | Experimental m/z | Mass error (ppm) | MS/MS fragmentation                    | References |
|--------|-----------------------------------------------------------|-------------------------------------------------|-------------------|----------------|------------------|------------------|----------------------------------------|------------|
| 1      | Glucose                                                   | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>   | 180.06339         | 0.63           | 179.0552         | -2.04            | 164.8355, 162.8384, 160.8414           | [74]       |
| 2      | Quinic acid                                               | C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>   | 192.06339         | 0.69           | 191.0557         | 0.71             | 173.0449, 127.0391                     | [47]       |
| 3      | Dihexose                                                  | C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> | 342.116215        | 0.7            | 341.1093         | 2.67             | 179.0555                               | [75]       |
| 4      | 4-Methoxy phenylethan-ol-O-coumarate                      | C <sub>18</sub> H <sub>18</sub> O <sub>4</sub>  | 298.120509        | 0.71           | 297.1139         | 4.09             | 163.0607, 145.0500, 135.0654, 117.0549 | [76]       |
| 5      | Shikimic acid                                             | C <sub>7</sub> H <sub>10</sub> O <sub>5</sub>   | 174.052824        | 0.91           | 173.0448         | -1.16            | 155.0345, 129.0377                     | [77]       |
| 6      | Succinic acid                                             | C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>    | 118.02661         | 1.69           | 117.0183         | -4.14            | 73                                     | [78]       |
| 7      | 1-Methyl emodin                                           | C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>  | 284.068475        | 1.77           | 283.0637         | 8.30             | 241.0027, 225.0782, 182.9544           | [79]       |
| 8      | Gallic acid                                               | C <sub>7</sub> H <sub>6</sub> O <sub>5</sub>    | 170.021525        | 2.54           | 169.0134         | -0.67            | 108.0209, 124.0254, 125.0236, 151.0026 | [80]       |
| 9      | Taxifolin-hexoside                                        | C <sub>21</sub> H <sub>22</sub> O <sub>12</sub> | 466.11113         | 3.19           | 465.1026         | -1.52            | 303.1029, 285.0629, 151.0029           | [81]       |
| 10     | Protocatechuic acid                                       | C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>    | 154.02661         | 3.36           | 153.0185         | -1.86            | 109.0286                               | [82]       |
| 11     | Galloyl-rhamnose                                          | C <sub>13</sub> H <sub>16</sub> O <sub>9</sub>  | 316.079435        | 3.48           | 315.0726         | 3.14             | 169.0137                               | [83]       |
| 12     | Monogalloyl-hexoside                                      | C <sub>13</sub> H <sub>16</sub> O <sub>10</sub> | 332.07435         | 3.68           | 331.0638         | -8.23            | 271.8394, 211.0609, 169.0137, 125.0234 | [84]       |
| 13     | 1-O-Vanilloyl-hexoside1                                   | C <sub>14</sub> H <sub>18</sub> O <sub>9</sub>  | 330.095085        | 3.71           | 329.0881         | 2.55             | 167.0343, 152.0108, 123.0442           | [84]       |
| 14     | Methyl gallate-hexoside                                   | C <sub>14</sub> H <sub>18</sub> O <sub>10</sub> | 346.09            | 3.88           | 345.0824         | 0.65             | 183.0294, 168.0058, 124.0157           | [85]       |
| 15     | Protocatechuic acid glucoside                             | C <sub>13</sub> H <sub>16</sub> O <sub>9</sub>  | 316.079435        | 3.94           | 315.0725         | 2.82             | 153.0186                               | [86]       |
| 16     | Acetyl-O-galloyl glucose                                  | C <sub>15</sub> H <sub>18</sub> O <sub>11</sub> | 374.084915        | 4              | 373.0745         | -6.94            | 313.0958, 271.0988, 169.0137, 151.0392 | [83]       |
| 17     | Rosmarinic acid                                           | C <sub>18</sub> H <sub>16</sub> O <sub>8</sub>  | 360.08452         | 4.1            | 359.0789         | 6.14             | 197.0453, 179.0708, 161.0450, 135.0442 | [87]       |
| 18     | Protocatechuic acid pentoside hexoside                    | C <sub>18</sub> H <sub>24</sub> O <sub>13</sub> | 448.121695        | 4.11           | 447.1151         | 2.75             | 315.0746, 271.1011, 152.0118           | [88]       |
| 19     | 3,4-Dihydroxy-7-O-(4'-O-glucosylvanilloyl) benzyl alcohol | C <sub>21</sub> H <sub>24</sub> O <sub>11</sub> | 452.131865        | 4.22           | 451.1252         | 2.66             | 167.0344, 123.0443, 152.0110, 329.0887 | [89]       |
| 20     | Elenolic acid glucoside                                   | C <sub>18</sub> H <sub>28</sub> O <sub>10</sub> | 404.16825         | 4.3            | 403.1622         | 4.40             | 223.0611, 161.0448                     | [90]       |
| 21     | 4-O-(6'-O-Glucosylcaffeoyl)-4-hydroxybenzyl alcohol       | C <sub>22</sub> H <sub>24</sub> O <sub>10</sub> | 448.13695         | 4.38           | 447.1261         | -6.77            | 323.1353, 179.0557, 138.0553           | [91]       |
| 22     | Hydroxyacetyl-O-galloyl glucose                           | C <sub>15</sub> H <sub>18</sub> O <sub>12</sub> | 390.07983         | 4.44           | 389.0725         | 1.10             | 313.0569, 271.0456, 169.0137, 151.0394 | [83]       |
| 23     | Protocatechuic aldehyde                                   | C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>    | 138.031695        | 4.54           | 137.0247         | 6.06             | 108.0207                               | [92]       |
| 24     | Gallocatechin                                             | C <sub>15</sub> H <sub>14</sub> O <sub>7</sub>  | 306.073955        | 4.61           | 305.067          | 2.85             | 179.0556, 169.0137, 163.0394, 125.0236 | [93]       |
| 25     | 3,4-di-O-Galloylquinic acid                               | C <sub>21</sub> H <sub>20</sub> O <sub>14</sub> | 496.08531         | 4.67           | 495.0786         | 2.25             | 191.0556, 169.0136, 137.0235, 109.0287 | [94]       |
| 26     | Ferulic acid glucoside                                    | C <sub>16</sub> H <sub>20</sub> O <sub>9</sub>  | 356.110735        | 4.68           | 355.1053         | 6.73             | 193.0503, 134.0365, 119.0340           | [95]       |
| 27     | Torachrysone-8-O-glucopyranoside                          | C <sub>20</sub> H <sub>24</sub> O <sub>9</sub>  | 408.142035        | 4.69           | 407.1319         | -5.67            | 245.0458, 229.9163                     | [50]       |
| 28     | 1,3-O-Diferuloylglycerol                                  | C <sub>23</sub> H <sub>24</sub> O <sub>9</sub>  | 444.142035        | 4.71           | 443.1328         | -3.18            | 235.0608, 193.0502, 134.0365           | [96]       |
| 29     | Coumaroyl-O-galloyl-glucose                               | C <sub>22</sub> H <sub>22</sub> O <sub>12</sub> | 478.11113         | 4.75           | 477.1021         | -2.53            | 331.0833, 313.0578, 169.0138, 125.0236 | [97]       |

**Table 4** (continued)

| Number | Compound name                           | Molecular formula                               | Monoisotopic mass | Retention time | Experimental m/z | Mass error (ppm) | MS/MS fragmentation                              | References |
|--------|-----------------------------------------|-------------------------------------------------|-------------------|----------------|------------------|------------------|--------------------------------------------------|------------|
| 30     | Protocatechiuc acid-O-pentoside         | C <sub>12</sub> H <sub>14</sub> O <sub>8</sub>  | 286.06887         | 4.76           | 285.0618         | 1.77             | 153.0186, 109.0285                               | [98]       |
| 31     | Di-galloyl-glucose                      | C <sub>20</sub> H <sub>20</sub> O <sub>14</sub> | 484.08531         | 4.84           | 483.0786         | 2.31             | 125.0235, 151.0394, 169.0137, 313.0572, 331.0686 | [99]       |
| 32     | 3-Glucosyl- pentahydroxybenzophenone    | C <sub>19</sub> H <sub>20</sub> O <sub>11</sub> | 424.100564        | 4.85           | 423.0944         | 3.92             | 314.0605, 169.0137, 109.0286                     | [75]       |
| 33     | Quercetin-3-O-glucuronide               | C <sub>21</sub> H <sub>18</sub> O <sub>13</sub> | 478.074745        | 4.86           | 477.0629         | -8.42            | 301.0361, 299.0564, 271.1046                     | [53]       |
| 34     | Apigenin-O-hexoside                     | C <sub>21</sub> H <sub>20</sub> O <sub>10</sub> | 432.105649        | 4.88           | 431.0968         | -2.38            | 269.1035                                         | [100]      |
| 35     | Catechin hexoside                       | C <sub>21</sub> H <sub>24</sub> O <sub>11</sub> | 452.131865        | 4.92           | 451.1203         | -8.29            | 289.0722, 245.0858, 179.0556                     | [96]       |
| 36     | Epicatechin                             | C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>  | 290.07904         | 4.96           | 289.0728         | 5.48             | 125.0236                                         | [53]       |
| 37     | <i>P</i> -coumaroyl hexose              | C <sub>15</sub> H <sub>18</sub> O <sub>8</sub>  | 326.10017         | 5.01           | 325.0931         | 1.77             | 163.0394, 147.0452, 187.0259, 146.0367, 119.0493 | [76]       |
| 38     | Vanillic acid pentoside hexoside        | C <sub>19</sub> H <sub>26</sub> O <sub>13</sub> | 462.137345        | 5.03           | 461.1307         | 2.56             | 329.0534, 299.0532, 167.0343, 152.0109           | [42]       |
| 39     | Vanillic acid hexoside                  | C <sub>14</sub> H <sub>18</sub> O <sub>9</sub>  | 330.095085        | 5.08           | 329.0881         | 2.55             | 167.0344, 152.0067, 123.0442                     | [42]       |
| 40     | Coumaric acid sulphate                  | C <sub>9</sub> H <sub>8</sub> O <sub>6</sub> S  | 244.004162        | 5.13           | 242.9967         | 1.49             | 163.0394, 119.0493                               | [100]      |
| 41     | Tuberonic acid glucoside                | C <sub>18</sub> H <sub>28</sub> O <sub>9</sub>  | 388.173335        | 5.24           | 387.1630         | -6.48            | 207.0661, 163.0395, 101.0233                     | [78]       |
| 42     | EGCG dimer (prodelphinidin dimer)       | C <sub>44</sub> H <sub>34</sub> O <sub>22</sub> | 914.15418         | 5.33           | 913.1489         | 2.79             | 743, 573, 555, 429, 169, 125                     | [101]      |
| 43     | Catechin-3-O-gallate                    | C <sub>22</sub> H <sub>18</sub> O <sub>10</sub> | 442.0900          | 5.37           | 441.0839         | 3.91             | 289.0720, 245.0459, 169.0137                     | [99]       |
| 44     | Gentisic acid                           | C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>    | 154.02661         | 5.39           | 153.0185         | -0.44            | 109.0287                                         | [102]      |
| 45     | HHDP galloyl glucose                    | C <sub>27</sub> H <sub>22</sub> O <sub>18</sub> | 634.08062         | 5.42           | 633.0735         | 1.11             | 463.0916, 301.1079, 248.9607, 169.0137, 125.0236 | [99]       |
| 46     | Coumaric acid isomer sulphate           | C <sub>9</sub> H <sub>8</sub> O <sub>6</sub> S  | 244.004162        | 5.47           | 242.9967         | 1.49             | 163.0394, 119.0493                               | [100]      |
| 47     | Apigenin pentoside                      | C <sub>20</sub> H <sub>18</sub> O <sub>9</sub>  | 402.095085        | 5.51           | 401.0854         | -4.64            | 269.1035, 161.0448, 112.9846                     | [103]      |
| 48     | Sinapoyl-hexoside                       | C <sub>17</sub> H <sub>22</sub> O <sub>10</sub> | 386.1213          | 5.56           | 385.1145         | 2.66             | 225.0775, 223.0612                               | [104]      |
| 49     | Catechin                                | C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>  | 290.07904         | 5.68           | 289.0693         | -6.62            | 245.0821/137.0235/125.0235                       | [53]       |
| 50     | Epigallocatechin gallate                | C <sub>22</sub> H <sub>18</sub> O <sub>11</sub> | 458.084915        | 5.71           | 457.0784         | 2.87             | 305.0672, 261.0407, 219.0658, 169.0136           | [105]      |
| 51     | Drovomifoliol-O-β-D-glucopyranoside     | C <sub>19</sub> H <sub>30</sub> O <sub>8</sub>  | 386.1940          | 5.76           | 385.1875         | 3.26             | 223.1339, 205.1231, 153.0914                     | [106]      |
| 52     | Catechin 7-O-α-L-rhamnopyranoside       | C <sub>21</sub> H <sub>24</sub> O <sub>10</sub> | 436.13695         | 5.78           | 435.1284         | -1.67            | 289.0724, 169.0136, 125.0235                     | [75]       |
| 53     | Epicatechin dimethyl gallate            | C <sub>24</sub> H <sub>22</sub> O <sub>10</sub> | 470.1213          | 5.83           | 469.1169         | 7.30             | 271.0636, 319.0485, 169.0137, 125.0235           | [107]      |
| 54     | Chlorogenic acid derivative             |                                                 |                   | 5.83           | 405.1405         |                  | 383.2081, 191.0346, 161.0449                     | [108]      |
| 55     | Dihydrovomifoliol-O-β-D-glucopyranoside | C <sub>19</sub> H <sub>32</sub> O <sub>8</sub>  | 434.2152          | 5.85           | 387.2035         | 4.15             | 225.1495, 161.0448                               | [106]      |
| 56     | Isorhamnetin-3-O-glucoside              | C <sub>22</sub> H <sub>22</sub> O <sub>12</sub> | 478.11113         | 6.05           | 477.1045         | 2.66             | 314.0439, 315.0482, 271.0598                     | [109]      |
| 57     | Isorhamnetin-O-glucuronide              | C <sub>22</sub> H <sub>20</sub> O <sub>13</sub> | 492.090395        | 6.05           | 491.0812         | -2.79            | 315.1430, 271.0463, 255.0272                     | [46]       |
| 58     | Phloridzin (phloretin-2-O-glucoside)    | C <sub>21</sub> H <sub>24</sub> O <sub>10</sub> | 436.13695         | 6.06           | 435.1293         | 0.40             | 273.0789                                         | [81]       |

**Table 4** (continued)

| Number | Compound name                                            | Molecular formula                                 | Monoisotopic mass | Retention time | Experimental m/z | Mass error (ppm) | MS/MS fragmentation                                        | References |
|--------|----------------------------------------------------------|---------------------------------------------------|-------------------|----------------|------------------|------------------|------------------------------------------------------------|------------|
| 59     | Procyanidin B1 monogallate                               | C <sub>37</sub> H <sub>30</sub> O <sub>16</sub>   | 730.15339         | 6.08           | 729.1411         | −9.75            | 577.1219, 451.1033, 441.0815, 407.0768, 289.0728, 287.0542 | [53]       |
| 60     | Galloylquinic acid-O-hexoside                            | C <sub>20</sub> H <sub>26</sub> O <sub>15</sub>   | 506.127175        | 6.09           | 505.1172         | −4.26            | 343.0461, 313.0350, 169.0137                               | [110]      |
| 61     | 3-(benzoyloxy)–2-hydroxypropyl glucopyranosiduronic acid | C <sub>16</sub> H <sub>20</sub> O <sub>10</sub>   | 372.105649        | 6.13           | 371.0985         | 1.82             | 249.0618, 121.0285                                         | [111]      |
| 62     | Myricetin 3-O-glucoside                                  | C <sub>21</sub> H <sub>20</sub> O <sub>13</sub>   | 480.090395        | 6.23           | 479.0834         | 1.73             | 317.0283, 316.0229, 287.0204, 271.0250, 151.0029           | [97]       |
| 63     | Myricetin-O-glucuronide                                  | C <sub>21</sub> H <sub>18</sub> O <sub>14</sub>   | 494.06966         | 6.29           | 493.063          | 2.60             | 317.0305, 315.0153, 287.0206, 178.990                      | [53]       |
| 64     | Myricetin-3-O-galactoside                                | C <sub>21</sub> H <sub>20</sub> O <sub>13</sub>   | 480.090395        | 6.33           | 479.0839         | 3.10             | 317.0290, 316.0228, 287.0201, 271.0250                     | [53]       |
| 65     | Syringic acid                                            | C <sub>9</sub> H <sub>10</sub> O <sub>5</sub>     | 198.052824        | 6.47           | 197.045          | 0.00             | 179.0553, 135.0441, 123.0078                               | [78]       |
| 66     | Phlorisobutyrophene-2-glucoside                          | C <sub>16</sub> H <sub>22</sub> O <sub>9</sub>    | 358.126385        | 6.5            | 357.1182         | −1.01            | 195.0658                                                   | HMDB       |
| 67     | Chrysophanol-8'-O-(6'-O-galloyl)-glucose                 | C <sub>28</sub> H <sub>24</sub> O <sub>13</sub>   | 568.121695        | 6.58           | 567.1151         | 2.17             | 331.0809, 313.0576, 271.0616, 211.0598, 169.0137           | [110]      |
| 68     | 1-O-Galloyl-2-O-cinnamoylglucose                         | C <sub>22</sub> H <sub>22</sub> O <sub>11</sub>   | 462.116215        | 6.6            | 461.1051         | −7.13            | 169.0137, 125.0236                                         | [50]       |
| 69     | Procyanidin dimer digallate (B-type)                     | C <sub>44</sub> H <sub>34</sub> O <sub>20</sub>   | 882.16435         | 6.62           | 881.1591         | 2.92             | 729.1479, 559.1294, 407.0781, 711.1370                     | [45]       |
| 70     | Dihydrokaempferol hexoside                               | C <sub>21</sub> H <sub>22</sub> O <sub>11</sub>   | 450.116215        | 6.62           | 449.1096         | 2.88             | 287.0529, 259.0616, 125.0236                               | [112]      |
| 71     | Quercetin-3-O-(O-galloyl)-hexoside                       | C <sub>28</sub> H <sub>24</sub> O <sub>16</sub>   | 616.10644         | 6.68           | 615.0998         | 1.93             | 463.0895, 301.0352, 271.0453, 169.0136                     | [47]       |
| 72     | 3-Methoxy-4-hydroxyphenol-1-O-(6'-O-galloyl)-glucoside   | C <sub>20</sub> H <sub>22</sub> O <sub>12</sub>   | 454.11113         | 6.74           | 453.1045         | 2.60             | 313.1278, 169.0137, 151.0394, 125.0236                     | [99]       |
| 73     | Myricetin-3-O-pentoside                                  | C <sub>20</sub> H <sub>18</sub> O <sub>12</sub>   | 450.07983         | 6.79           | 449.0735         | 3.33             | 316.0230, 287.0207, 178.9981                               | [110]      |
| 74     | Myricetin 3-O-α-rhamnoside                               | C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>   | 464.09548         | 6.86           | 463.0887         | 2.26             | 317.0284, 316.0227, 287.0199, 271.0250, 178.9979, 151.0028 | [97]       |
| 75     | Quercetin-3-O-glucoside                                  | C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>   | 464.09548         | 6.97           | 463.0889         | 2.69             | 301.0357, 300.0280, 271.0253                               | [97]       |
| 76     | Phloretin 3',5'-dihexoside                               | C <sub>27</sub> H <sub>34</sub> O <sub>15</sub>   | 598.189775        | 6.98           | 597.1835         | 3.54             | 357.0986, 209.0798, 167.0343, 123.0439                     | [113]      |
| 77     | Ellagic acid-O-hexoside                                  | C <sub>20</sub> H <sub>16</sub> O <sub>13</sub>   | 464.059095        | 7.01           | 463.0518         | 1.14             | 300.0280, 301.0348, 283.0254, 257.0465, 169.0136           | [114]      |
| 78     | Ellagic acid pentoside                                   | C <sub>19</sub> H <sub>14</sub> O <sub>12</sub>   | 434.04853         | 7.28           | 433.0401         | −1.40            | 300.0281, 179.0575                                         | [99]       |
| 79     | Quercetin 3-O-pentoside I                                | C <sub>20</sub> H <sub>18</sub> O <sub>11</sub>   | 434.084915        | 7.36           | 433.0779         | 1.87             | 301.0699, 300.0280, 151.0393                               | [97]       |
| 80     | Kaempferol-3-O-rhamnoside                                | C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>   | 432.105649        | 7.41           | 431.0967         | −2.61            | 285.0397, 284.0331, 255.0301                               | [110]      |
| 81     | Quercetin 3-(2"-galloyl)rhamnoside                       | C <sub>28</sub> H <sub>24</sub> O <sub>15</sub>   | 600.111525        | 7.42           | 599.1059         | 3.67             | 300.0280, 301.0343, 447.0941, 169.0137                     | [115]      |
| 82     | Orientin (Luteolin 8-C-glucoside)-Sulphate               | C <sub>21</sub> H <sub>20</sub> O <sub>14</sub> S | 528.057382        | 7.5            | 527.0505         | 1.79             | 447.0946, 327.0724                                         | [105]      |
| 83     | Myricetin-3-O-acetylhexoside                             | C <sub>23</sub> H <sub>22</sub> O <sub>14</sub>   | 522.10096         | 7.61           | 521.0949         | 3.39             | 317.0268, 316.0229, 271.0248, 151.0394                     | [116]      |

**Table 4** (continued)

| Number | Compound name                                            | Molecular formula                               | Monoisotopic mass | Retention time | Experimental m/z | Mass error (ppm) | MS/MS fragmentation                                        | References |
|--------|----------------------------------------------------------|-------------------------------------------------|-------------------|----------------|------------------|------------------|------------------------------------------------------------|------------|
| 84     | Cyanidin-3-glucoside                                     | C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> | 449.10839         | 7.62           | 447.0938         | 2.37             | 284.0331                                                   | [117]      |
| 85     | Quercetin 3-O-rhamnoside                                 | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 448.100564        | 7.68           | 447.0891         | −8.14            | 301.0351, 300.0280, 255.0300, 179.0557                     | [105]      |
| 86     | Kaempferol-3-O-hexoside I                                | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 448.100564        | 7.74           | 447.0943         | 3.49             | 285.0398, 255.0302, 227.0349                               | [110]      |
| 87     | Dihydroferuloyl-glucosyl-3-hydroxy-3-methylglutaric acid | C <sub>22</sub> H <sub>30</sub> O <sub>13</sub> | 502.16864         | 7.75           | 501.1621         | 2.55             | 399.1293, 357.1230, 195.0660, 151.0030                     | [118]      |
| 88     | Digalloyl shikimic acid                                  | C <sub>21</sub> H <sub>18</sub> O <sub>13</sub> | 478.074745        | 7.78           | 477.0648         | −4.44            | 325.0335, 169.0137, 125.0236                               | [99]       |
| 89     | Quercetin-3-O-acetyl hexosidel                           | C <sub>23</sub> H <sub>22</sub> O <sub>13</sub> | 506.106045        | 7.78           | 505.0995         | 2.88             | 300.0282, 301.0352, 463.0891                               | [119]      |
| 90     | 7-O-Methyl catechin                                      | C <sub>16</sub> H <sub>16</sub> O <sub>6</sub>  | 304.09469         | 7.82           | 303.0854         | −4.83            | 285.0398, 193.0504, 151.0394, 125.0236                     | [96]       |
| 91     | Kaempferol-3-O-pentoside                                 | C <sub>20</sub> H <sub>18</sub> O <sub>10</sub> | 418.09            | 7.85           | 417.0837         | 3.66             | 285.0394, 284.0332, 255.0303, 169.0137                     | [120]      |
| 92     | Myricetin 3-O-(4"-acetyl)-α-l-rhamnopyranosides          | C <sub>23</sub> H <sub>22</sub> O <sub>13</sub> | 506.106045        | 7.88           | 505.0997         | 3.32             | 317.0276/316.0230/271.0232                                 | [121]      |
| 93     | Gallic acid-mono-hydrate                                 | C <sub>7</sub> H <sub>8</sub> O <sub>6</sub>    | 188.03209         | 7.97           | 187.0237         | −3.02            | 169.0137, 125.0236                                         | [96]       |
| 94     | Quercetin-3-O-acetyl hexosidelI                          | C <sub>23</sub> H <sub>22</sub> O <sub>13</sub> | 506.106045        | 8.02           | 505.0994         | 2.34             | 300.0282, 301.0352, 463.0891                               | [119]      |
| 95     | Myricetin                                                | C <sub>15</sub> H <sub>10</sub> O <sub>8</sub>  | 318.03757         | 8.12           | 317.0301         | 1.12             | 191.0347, 178.0502, 151.0030                               | [122]      |
| 96     | Homovanillic acid                                        | C <sub>9</sub> H <sub>10</sub> O <sub>4</sub>   | 182.05791         | 8.21           | 181.05           | −0.47            | 137.0238, 89.0230                                          | [123]      |
| 97     | Syringin                                                 | C <sub>17</sub> H <sub>24</sub> O <sub>9</sub>  | 372.142035        | 8.23           | 371.135          | 2.13             | 209.0816, 148.0842                                         | [114]      |
| 98     | Quercetin-3-O-acetyl hexosidelII                         | C <sub>23</sub> H <sub>22</sub> O <sub>13</sub> | 506.106045        | 8.32           | 505.0994         | 2.34             | 300.0282, 301.0352, 463.0891                               | [119]      |
| 99     | Apigenin-O-hexosidel                                     | C <sub>21</sub> H <sub>20</sub> O <sub>10</sub> | 432.105649        | 8.33           | 431.0988         | 2.26             | 269.0461                                                   | [124]      |
| 100    | Rutin                                                    | C <sub>27</sub> H <sub>30</sub> O <sub>16</sub> | 610.15339         | 8.4            | 609.1442         | −2.24            | 301.0340, 300.0280, 271.0238, 151.0394                     | [110]      |
| 101    | Digalloyl quinic acid                                    | C <sub>21</sub> H <sub>20</sub> O <sub>14</sub> | 496.08531         | 8.49           | 495.0786         | 2.60             | 343.0676, 191.0556, 169.0136                               | [41]       |
| 102    | Tetrahydroxystilbene-2-O-(coumaroyl)-hexoside            | C <sub>29</sub> H <sub>28</sub> O <sub>11</sub> | 552.163164        | 8.49           | 551.151          | −7.87            | 405.1188, 307.0830, 243.0665                               | [54]       |
| 103    | Malvidin-3-glucoside                                     | C <sub>23</sub> H <sub>25</sub> O <sub>12</sub> | 493.134605        | 8.55           | 491.1200         | 2.13             | 329.0630, 328.0595                                         | [117]      |
| 104    | Feruloyl tyramin                                         | C <sub>18</sub> H <sub>19</sub> NO <sub>4</sub> | 313.131409        | 8.56           | 312.1244         | 2.61             | 178.0504, 135.0443                                         | [125]      |
| 105    | Mearnssetin-glucoside                                    | C <sub>22</sub> H <sub>22</sub> O <sub>13</sub> | 494.106045        | 8.58           | 493.0997         | 3.00             | 478.1950, 331.0466, 316.0229, 315.0513, 287.0566           | [126]      |
| 106    | Quercetin 3-(2"-galloyl-α-arabinopyranoside)             | C <sub>27</sub> H <sub>22</sub> O <sub>15</sub> | 586.095875        | 8.63           | 585.0897         | 2.82             | 433.0918, 301.0360, 283.0487, 169.0138                     | [75]       |
| 107    | 4-Methylidaphnetin                                       | C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>   | 192.06339         | 8.64           | 191.0556         | 0.22             | 177.0187                                                   | [127]      |
| 108    | Ellagic acid valeryl rhamnoside                          | C <sub>25</sub> H <sub>24</sub> O <sub>13</sub> | 532.121695        | 8.65           | 531.1154         | 2.88             | 300.0280, 299.0210 489.1031, 471.2553                      | [128]      |
| 109    | Naringenin-O-hexoside                                    | C <sub>21</sub> H <sub>22</sub> O <sub>10</sub> | 434.1213          | 8.68           | 433.1143         | 1.90             | 271.0574, 151.0393, 169.0137                               | [96]       |
| 110    | Trilignol G(8-O-4) G(8-5)G                               | C <sub>30</sub> H <sub>36</sub> O <sub>10</sub> | 556.23085         | 8.71           | 555.2244         | 2.48             | 507.2038, 477.1941, 341.0667, 329.1402, 195.0661, 165.0552 | [53]       |

**Table 4** (continued)

| Number | Compound name                             | Molecular formula                                | Monoisotopic mass | Retention time | Experimental m/z | Mass error (ppm) | MS/MS fragmentation                                                  | References |
|--------|-------------------------------------------|--------------------------------------------------|-------------------|----------------|------------------|------------------|----------------------------------------------------------------------|------------|
| 111    | Kaempferol-3-O-acetyl glucoside           | C <sub>23</sub> H <sub>22</sub> O <sub>12</sub>  | 490.11113         | 8.81           | 489.1046         | 2.65             | 285.0399, 284.0332                                                   | [129]      |
| 112    | Kaempferide -glucuronide                  | C <sub>22</sub> H <sub>20</sub> O <sub>12</sub>  | 476.09548         | 8.84           | 475.0887         | 2.43             | 300.0281, 284.0328                                                   | [130]      |
| 113    | Methyl ellagic acid pentoside             | C <sub>20</sub> H <sub>16</sub> O <sub>12</sub>  | 448.06418         | 8.87           | 447.0595         | 7.03             | 315.1816, 300.0278                                                   | [131]      |
| 114    | Acacetin-O-glucoside                      | C <sub>22</sub> H <sub>22</sub> O <sub>10</sub>  | 446.1213          | 8.95           | 445.1145         | 2.30             | 283.0253                                                             | [132]      |
| 115    | Trigalloylquinic acid                     | C <sub>28</sub> H <sub>24</sub> O <sub>18</sub>  | 648.09627         | 9              | 647.0866         | -2.85            | 495.1948, 343.2147, 191.0551, 169.0137                               | [133]      |
| 116    | Feruloyl tyramin isomer                   | C <sub>18</sub> H <sub>19</sub> NO <sub>4</sub>  | 313.131409        | 9.03           | 312.1244         | 2.61             | 178.0504, 148.0522                                                   | [125]      |
| 117    | Quercetin 3-O-acetyl-rhamnoside           | C <sub>23</sub> H <sub>22</sub> O <sub>12</sub>  | 490.11113         | 9.1            | 489.1047         | 2.85             | 447.0909, 301.0342, 300.028, 271.0659                                | [134]      |
| 118    | Trilignol G(8-O-4) X(8-8)X                | C <sub>31</sub> H <sub>38</sub> O <sub>11</sub>  | 586.241415        | 9.31           | 585.2353         | 2.92             | 537.3228, 507.1869, 371.2435, 359.1522, 195.0659, 165.0553           | [53]       |
| 119    | Quercetin                                 | C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>   | 302.042654        | 9.42           | 301.0356         | 2.56             | 273.0409, 179.0562, 151.0029                                         | [135]      |
| 120    | 3-O-Methylquercetin                       | C <sub>16</sub> H <sub>12</sub> O <sub>7</sub>   | 316.058305        | 9.6            | 315.0514         | 2.92             | 300.0280/271.0640                                                    | [53]       |
| 121    | Emodin hexoside                           | C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>  | 432.105649        | 9.62           | 431.0989         | 2.49             | 269.0461, 241.0507                                                   | [136]      |
| 122    | Chrysoeriol hexoside                      | C <sub>22</sub> H <sub>22</sub> O <sub>11</sub>  | 462.116215        | 9.69           | 461.1047         | -8.00            | 299.0565, 284.0645                                                   | [96]       |
| 123    | Tricin                                    | C <sub>17</sub> H <sub>14</sub> O <sub>7</sub>   | 330.073955        | 9.85           | 329.0657         | -1.31            | 314.0078, 298.9842, 270.9889                                         | [96]       |
| 124    | Kaempferol-3-O-hexoside II                | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>  | 448.100564        | 9.85           | 447.0943         | 3.49             | 285.0398, 255.0302, 227.0349                                         | [110]      |
| 125    | Isoliquiritin                             | C <sub>21</sub> H <sub>22</sub> O <sub>9</sub>   | 418.126385        | 9.92           | 417.1196         | 2.49             | 297.0773, 255.0650, 152.9952                                         | [137]      |
| 126    | Trilignol G(8-O-4) S(8-5)G                | C <sub>31</sub> H <sub>36</sub> O <sub>11</sub>  | 584.225765        | 9.99           | 583.2192         | 2.16             | 368.8896, 357.1333, 195.0661, 165.0552                               | [53]       |
| 127    | Quercetin- O-(O-acetyl)-hexuronide        | C <sub>23</sub> H <sub>20</sub> O <sub>14</sub>  | 520.085309        | 10.52          | 519.0731         | -8.45            | 477.2130, 301.0357                                                   | [138]      |
| 128    | Kaempferol                                | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>   | 286.04774         | 10.66          | 285.041          | 3.81             | 151.0391, 132.8672, 107.0493                                         | [114]      |
| 129    | Kaempferol 3,7,4'-tri-methyl ether        | C <sub>18</sub> H <sub>16</sub> O <sub>6</sub>   | 328.09469         | 10.68          | 327.0881         | 3.78             | 285.0410, 255.2331, 227.0354                                         | [139]      |
| 130    | Oxo-dihydroxy-octadecenoic acid           | C <sub>18</sub> H <sub>32</sub> O <sub>5</sub>   | 328.224975        | 10.79          | 327.218          | 1.99             | 291.1971, 229.1445, 211.1338, 171.1021                               | [140]      |
| 131    | Kaempferol isomer                         | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>   | 286.04774         | 10.92          | 285.041          | 3.81             | 151.0391, 132.8672, 107.0493                                         | [114]      |
| 132    | Methoxyquercetin dimethyl ether           | C <sub>18</sub> H <sub>16</sub> O <sub>8</sub>   | 360.08452         | 10.94          | 359.0755         | -3.33            | 344.0493, 329.2346, 301.0331, 286.0494, 257.9159, 223.0953, 162.0192 | [53]       |
| 133    | Kaempferol-dimethyl ether                 | C <sub>17</sub> H <sub>14</sub> O <sub>6</sub>   | 314.07904         | 11.3           | 313.0698         | -4.52            | 285.0409, 283.0253, 255.2334                                         | [46]       |
| 134    | Undecyl benzene sulphonic acid            | C <sub>17</sub> H <sub>28</sub> O <sub>3</sub> S | 312.175917        | 11.43          | 311.1693         | 3.88             | 248.9605, 183.0117, 170.0169, 119.0493                               | [75]       |
| 135    | Chrysoeriol                               | C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>   | 300.06339         | 12.78          | 299.0565         | 3.13             | 223.0399, 224.0433, 256.0707                                         | [141]      |
| 136    | 3,4-di-O-caffeoyl-5-O-sinapoylquinic acid | C <sub>36</sub> H <sub>34</sub> O <sub>16</sub>  | 722.18469         | 13.44          | 721.1761         | -1.06            | 397.1350, 223.1334, 179.0556                                         | [44]       |
| 137    | Apigenin                                  | C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>   | 270.052825        | 13.48          | 269.0456         | 1.33             | 225.0556, 195.0447, 183.0409, 157.0653                               | [142]      |
| 138    | Chrysin derivative                        |                                                  |                   | 14.02          | 559.313          |                  | 277.2177, 278.2211, 253.0931                                         | [143]      |
| 139    | Caftaric acid                             | C <sub>13</sub> H <sub>12</sub> O <sub>9</sub>   | 312.048135        | 14.8           | 311.0391         | -3.89            | 149.0964, 179.0560, 135.0446                                         | [144]      |
| 140    | Kaempferol -3-O-neohesperidoside          | C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>  | 594.158475        | 15.09          | 593.1499         | -1.26            | 327.2186, 285.0485                                                   | [145]      |

**Table 4** (continued)All compounds were detected as  $[M-H]^-$ . HHDP: Hexahydroxydiphenoyl, EGCG: Epigallocatechin gallate

Thirty-five flavonols were putatively identified as compounds 33, 62–64, 71, 73–75, 79–81, 83, 85, 86, 89, 91, 92, 94, 95, 98, 100, 105, 106, 111, 112, 117, 119, 120, 127–129, 131–133 and 140. The compounds were myricetin and its derivatives, quercetin and its derivatives, kaempferol and its derivatives, kaempferide-glucuronide and mearnssetin-glucoside. Compound 71,  $m/z$  615.0998 was identified as quercetin-3-*O*-(*-O*-galloyl)-hexoside which provided the fragments of  $MS^2$  at  $m/z$  463.08 ( $[M-H-152]^-$ ) corresponding to the loss of galloyl group, hexose  $m/z$  301.03 ( $[M-H-162]^-$ ) (i.e. quercetin aglycone ion), as well as the fragment  $m/z$  169.01 verifies gallic acid anion moiety presence (Fig. S9) [47].

One flavanone was putatively identified as naringenin-*O*-hexoside (**109**). It demonstrated  $[M-H]^-$  ion at  $m/z$  433.1143,  $MS^2$  spectrum gave rise to the fragment at  $m/z$  271 ( $[M-H-162]^-$ ) (i.e. naringenin aglycone ion), corresponding to hexose loss; then, the ions at  $m/z$  151 and 119 could be formed through undergoing a fragmentation mechanism retro Diels–Alder involving cleavage of O1–C2 and C3–C4 bonds in the C ring resulting in loss of water [41].

Two flavanonol were identified in the extracts, compounds **9** and **70** with  $[M-H]^-$  ion at  $m/z$  449.1026 and  $m/z$  465.1096, respectively. The two compounds were putatively identified as taxifolin-glucoside and dihydrokaempferol hexoside. Compound **70** provided fragments at  $m/z$  287 ( $[M-H-162]^-$ ) resulting from hexose moiety loss, followed by  $m/z$  259 ( $[M-H-28]^-$ ) due to CO molecule loss and  $m/z$  125<sup>[1,4]</sup>A. The compound was then identified as dihydrokaempferol hexoside [48].

Anthocyanins are flavonoids renowned for having advantageous effects on both humans and animals; they are typically the pigments that give many plants their colours, existing as glycosides in nature. The capacity of the anthocyanin group's structure to alter under various pH settings, resulting in a change in colour, is one of its most noteworthy and distinctive characteristics. In this work, two anthocyanins were putatively identified in the ethanolic extracts of *Coccoloba* leaves. Compounds **84** and **103** with  $m/z$  447.0938 and 491.1200, respectively, showed characteristic fragmentation pattern similar to cyanidin-3-glucoside and malvidin-3-glucoside through the loss of hexose ( $[M-H-162]^-$ ), showing their aglycone fragment ions at  $m/z$  284.03 and 329.06, respectively [49].

### Anthraquinones

Glucosyl or galloyl residues are typically lost by anthraquinones, resulting in a prominent fragment with  $m/z$

equivalent to its corresponding free anthraquinone. This finding may be clearly seen even in their full scan MS spectra. For free anthraquinones with hydroxyl (OH) and carbonyl (CO) groups on the benzene ring, a sequence of losses of 18 and 28 Da was characteristic. Three signals were assigned as anthraquinones compounds **7**, **67** and **121** which were tentatively identified as 1-methyl emodin, chrysophanol-*O*-(*-O*-galloyl)-glucose and emodin hexoside, respectively. Compound **121** was putatively identified as emodin hexoside with  $m/z$  431.0989 which showed loss of hexose then removal of CO to yield  $m/z$  241 ( $[M-H-28]^-$ ), after which one hydroxyl group was lost, resulting in  $m/z$  225 (Fig. S10). Regarding compound **67**: chrysophanol-*O*-(*-O*-galloyl)-glucose, with  $m/z$  567.1151, it gave rise to  $m/z$  253 due to loss of hexose and galloyl moieties. The  $[M-H]^-$  ions of C-3 methylated anthraquinone derivatives firstly lose a neutral molecule of CO and then successively lose a methyl group and a CO molecule, respectively [50, 51].

### Lignin oligomers

It was suggested that lignin was a cross-linked, amorphous macromolecule consisting of lignols, which are phenylpropanoid monomers. A 4-hydroxyphenylring makes up the monolignol H structure. One methoxyl and one hydroxyl group are present in the monolignol guaiacyl G-structure, while one hydroxyl group and two methoxyl groups are present in the monolignol syringyl S-structure. The combinatory process, the most well-known method of biosynthetic polymerization, assumes that dilignols, trilignols and oligolignols are produced by the combination of oxidizing enzymes that form lignol radicals [52]. Three trilignols were identified, compounds **110**, **118** and **126** as trilignol G(8–O–4)G(8–5)G, trilignol G(8–O–4)X(8–8)X (with X being either an S or G unit) and trilignol G(8–O–4)S(8–5)G. The fragment ion of  $m/z$  195 which appeared in all the compounds corresponded to  $A^-$  which is the 8-phenolic end indicating a G unit (guaiacyl, a unit derived from coniferyl alcohol). Compound **126**,  $m/z$  583.2192 gave rise to fragments  $m/z$  368.88 equivalent to ( $[M-H-A^- -H_2O]^-$ ),  $m/z$  357.13 equivalent to ( $[M-H-A^- -CH_2O]^-$ ) and  $m/z$  195 which is the guaiacyl unit (Fig. S11) [53].

### Stilbenes

Compound **102** revealed a molecular ion peak of  $[M-H]^-$  at  $m/z$  551.1510 and characteristic fragments ion at  $m/z$  405 which was generated by the loss of  $C_9H_6O_2$  ( $[M-H-146]^-$ ), followed by elimination of hexose

$C_6H_{10}O_5$  producing fragment at  $m/z$  243 in MS/MS spectra. Additionally, a small ion at  $m/z$  307 was observed. As a result, compound **102** was putatively identified as tetrahydroxystilbene-*O*-(coumaroyl) hexoside [54].

### Naphthalene derivatives

Torachryson-8-*O*-glucoside, compound **27**, was the only naphthalene glycoside in *Coccoloba peltata* ethanolic leaves extract. The  $[M-H]^-$  ion of the compound at  $m/z$  407.1319 produced fragment at  $m/z$  245.04 corresponding to one glucosyl residue loss; then, a  $CH_3$  free radical was eliminated to produce the ion at  $m/z$  229.91 [54].

### Other compounds

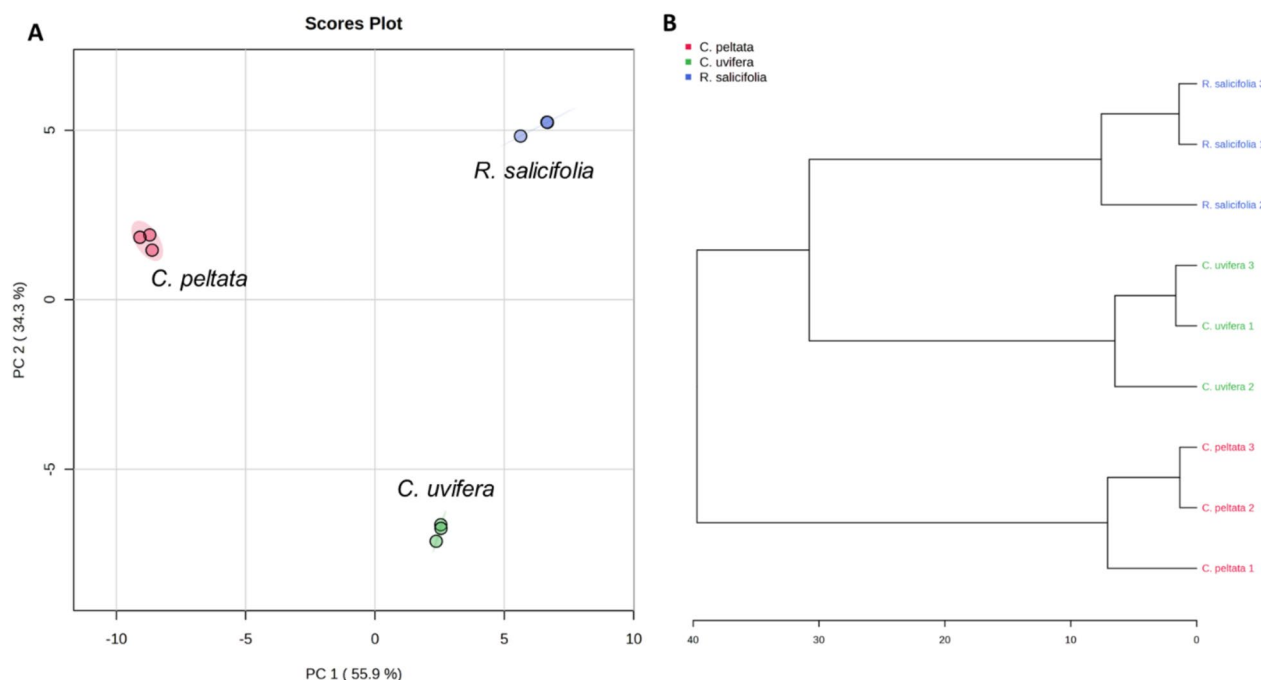
Compounds **58** and **76** were putatively identified as chalcones, compound **130** was a fatty acid derivative, whereas compounds **51** and **55** presented sesquiterpene glycosides. Compounds **1–6**, **19–21**, **23**, **28**, **32**, **41**, **61**, **66**, **87**, **97**, **107** and **134** were also identified in the current study, and their chemical information is listed in Table 4.

### Multivariate and correlation analyses

Multivariate data analysis is an important tool to extract meaningful information from complex data sets. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are the most commonly utilized approaches for untargeted metabolic analysis [55]. Good

discrimination was evident between the three investigated extracts (Fig. 5). PCA score plot (Fig. 5A) revealed three distinct clusters, reflecting different metabolomes. The first two components explained 90.2% of the variation, where PC1 accounted for 55.9% of the total variance, being the major component responsible for discrimination. *C. peltata* located on the plot far-left side (negative PC1 values) was the most distant species. On the right side (positive PC1 values), *R. salicifolia* was top located, while *C. uvifera* was located at the bottom. HCA (Fig. 5B) supported PCA results, showing three clusters, in which *C. peltata* comprised a cluster distantly located from the other two clusters, suggesting variations in its metabolic profile in contrast with other species.

The behaviour of the investigated compounds with regard to each species as a whole was examined applying a heat map to display hierarchical clustering that gathered similar groups in order to uncover cogent patterns among them. A heat map is a visual illustration of data where each value in a matrix is represented by a colour [56]. The heat map was created based on metabolites' abundance in the tested species (Fig. S12). *C. uvifera* extract (the most active antidiabetic/antioxidant extract) showed the accumulation of quercetin and its derivatives mainly, with few phenolic acids and their derivatives alongside a sesquiterpene glycoside. Meanwhile, flavonoids mainly flavones, flavanones, flavonols with few phenolic acid's derivatives and a tetrahydroxy



**Fig. 5** Principal component analysis (PCA) (**A**) and the dendrogram from the hierarchical clustering analysis (HCA) (**B**) of metabolites identified in the total extracts of *C. uvifera*, *C. peltata* and *R. salicifolia*

stilbene derivative dominated *R. salicifolia* metabolome. Additionally, flavonoids, phenolic acids and cinnamic acid derivatives were the discriminating compounds in *C. peltata* extract. Pearson's correlation coefficients (*r*) were used to reveal the strength of relationship between  $\alpha$ -glucosidase inhibition and the top metabolites accumulated in the total extracts of *C. uvifera*, *C. peltata* and *R. salicifolia*, as shown in Fig. S13.

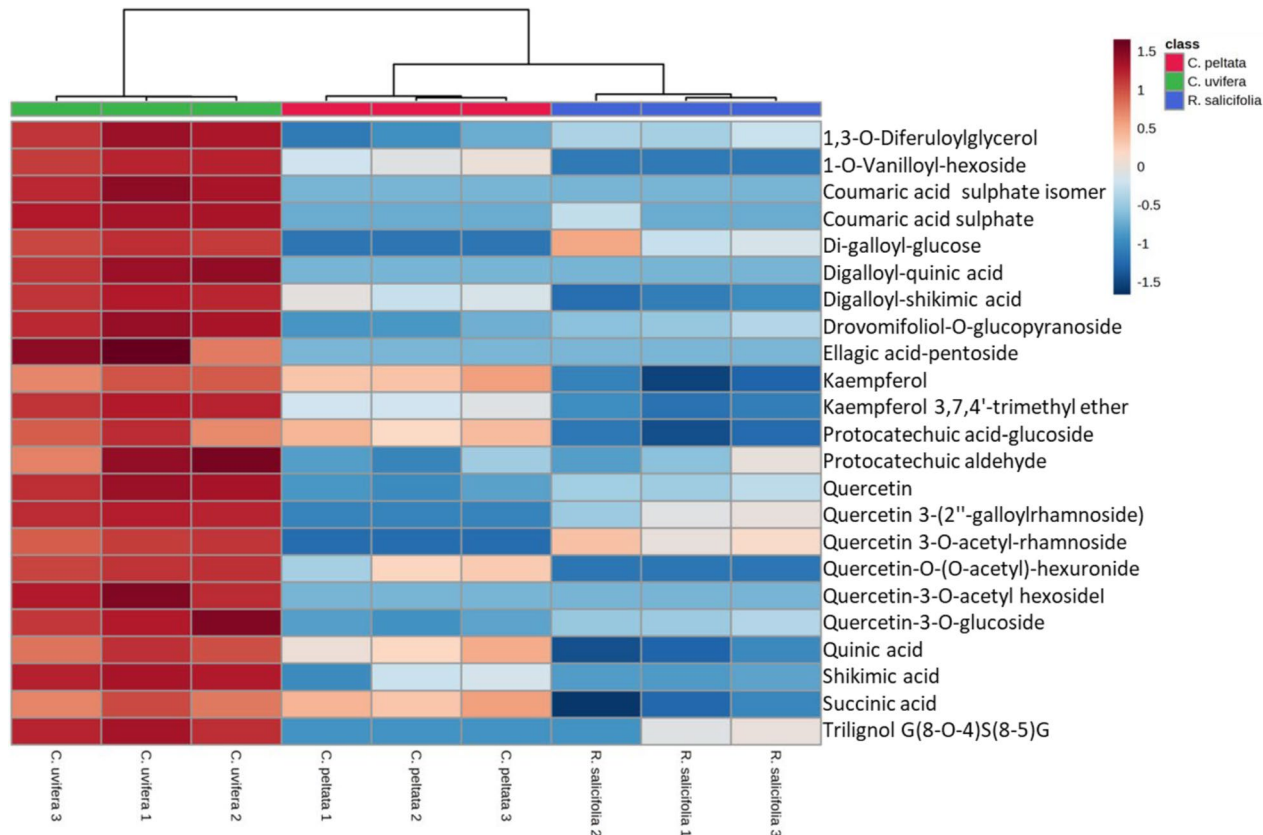
Further analysis focused on *C. uvifera* extract; the most active DPPH antioxidant/antidiabetic extract, in comparison with other species. Subsequently, a heatmap showing the significant metabolites accumulating in the total extract of *C. uvifera* and distinguishing it from *C. peltata* and *R. salicifolia* is illustrated in Fig. 6. In accordance with the former results (Fig. S12), quercetin derivatives, hydroxybenzoic acid derivatives and a sesquiterpene glycoside were the most significantly accumulated metabolites in *C. uvifera* extract. Among these metabolites, eight main compounds positively and significantly correlated with in vitro  $\alpha$ -glucosidase inhibition, as indicated by Pearson's correlation coefficients (Table 5). These metabolites included kaempferol, protocatechuic acid glucoside, quinic acid, quercetin-*O*-(*O*-acetyl)-hexuronide,

**Table 5** Pearson's correlation coefficients (*r*) showing the strength of relationship between  $\alpha$ -glucosidase inhibition and significant metabolites accumulated in the total extract of *C. uvifera* compared to *C. peltata* and *R. salicifolia*

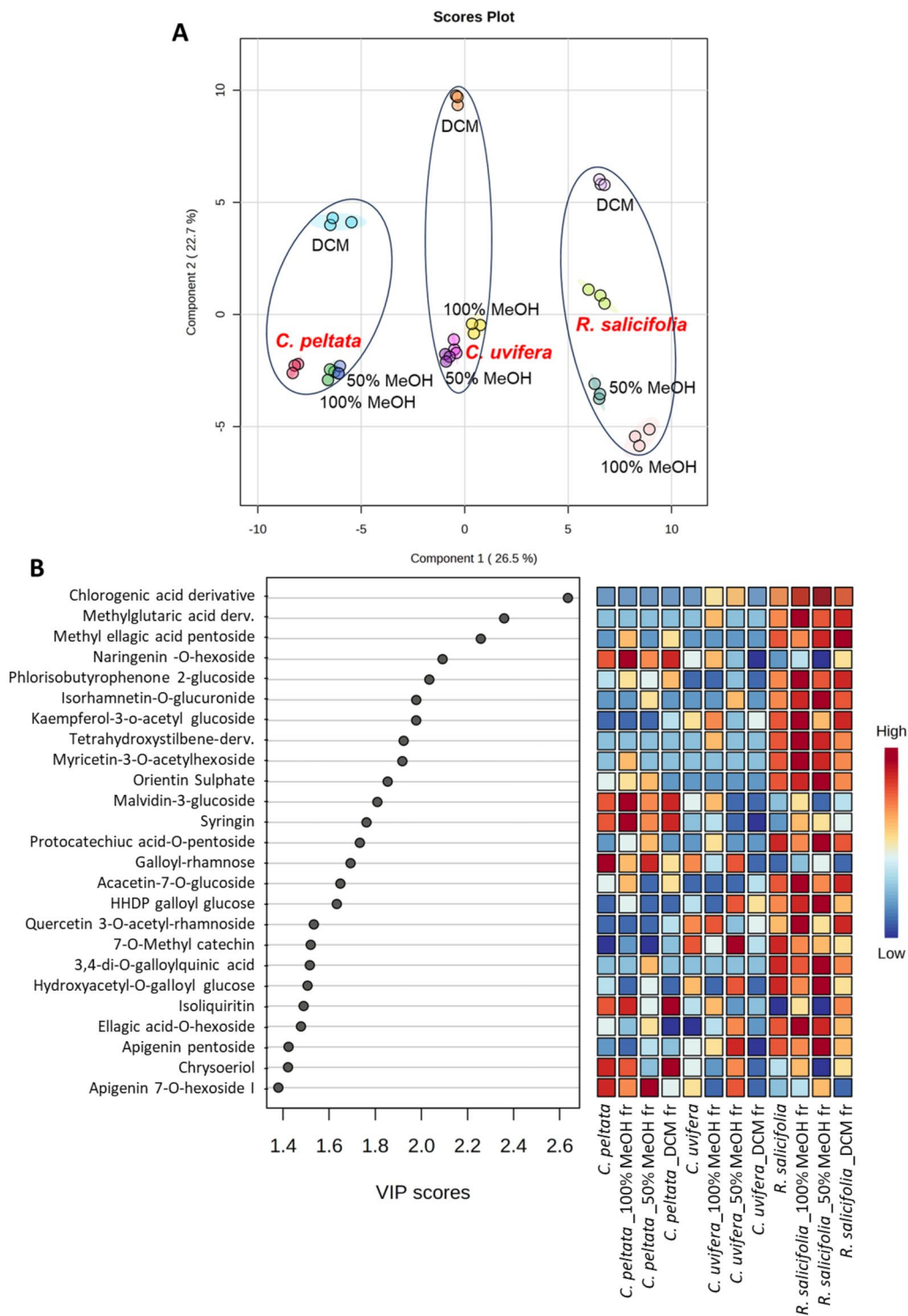
| Metabolites                                         | Correlation | p value  |
|-----------------------------------------------------|-------------|----------|
| $\alpha$ -glucosidase inhibition                    | 1           | 0        |
| Kaempferol                                          | 0.98571     | 1.13E-06 |
| Protocatechuic acid glucoside                       | 0.97974     | 3.82E-06 |
| Quinic acid                                         | 0.96265     | 3.2E-05  |
| Quercetin- <i>O</i> -( <i>O</i> -acetyl)-hexuronide | 0.90676     | 0.000743 |
| 1- <i>O</i> -Vanilloyl-hexoside                     | 0.89755     | 0.001024 |
| Digalloyl shikimic acid                             | 0.88035     | 0.001732 |
| Kaempferol 3,7,4'-trimethyl ether                   | 0.87444     | 0.002039 |
| Shikimic acid                                       | 0.71039     | 0.031975 |

1-*O*-Vanilloyl-hexoside, digalloyl shikimic acid, kaempferol 3,7,4'-trimethyl ether and shikimic acid.

Furthermore, multivariate data analysis including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) was performed to the three extracts and their fractions 50% MeOH, 100% MeOH and DCM (Fig. S14; Fig. 7A). PLS-DA serves to



**Fig. 6** Heatmap showing significant metabolites accumulated in the total extract of *C. uvifera* compared to *C. peltata* and *R. salicifolia*



**Fig. 7** **A** Partial least squares discriminant analysis (PLS-DA) and **B** variable importance in projection (VIP) Top 25 metabolites with VIP score > 1 accumulated in the total extracts and fractions of *C. uvifera*, *C. peltata* and *R. salicifolia*

identify the variables that contain the class separating information and to sharpen the separation among groups of observations by rotating the PCA components with the aim of achieving the largest separation between classes.

The results showed three distinguished clusters, each cluster containing the total extract and its 3 fractions discriminated from the two other species. Variable importance projection (VIP) scores are used as a quantitative

evaluation of each particular metabolite discriminatory power to figure out metabolites that significantly contribute to discriminate various samples; metabolites with a VIP score greater than 1 are considered informative metabolite marker. VIP scores discriminated the samples against each other, defining the top accumulating 25 metabolites in the tested samples (Fig. 7B).

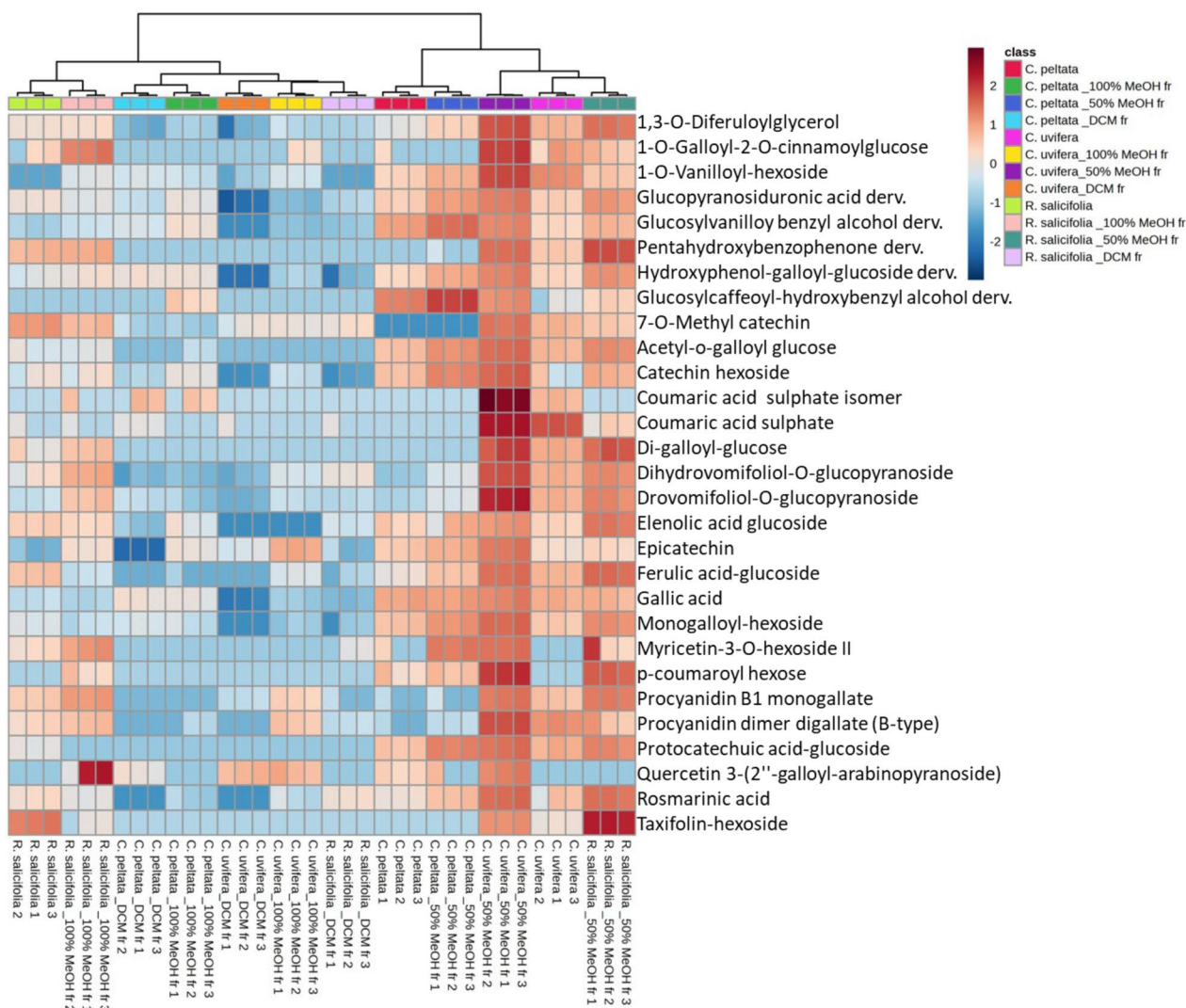
Significant metabolites, responsible for discriminating the most active fraction of *C. uvifera* (50% MeOH fraction) from *C. peltata* and *R. salicifolia*, were further investigated via a heat map (Fig. 8), demonstrating 29 metabolites. Correlation between these metabolites and  $\alpha$ -glucosidase inhibitory activity was next investigated, where Pearson's correlation coefficient ( $r$ ) was  $>0.3$  at  $P<0.05$  (Table S3). This uncovered 25 metabolites including catechins, hydroxybenzoic acids derivatives,

hydroxycinnamic acid derivatives, procyanidins and a sesquiterpene glycoside.

The above results of the most active fraction (50% methanol fraction of *C. uvifera*) were correlated with the significant metabolites accumulated in *C. uvifera* total extract to highlight the most contributing metabolites to  $\alpha$ -glucosidase inhibition and DPPH antioxidant activities. Pearson's correlation coefficient ( $r$ ) revealed the significantly correlated compounds, namely 1-*O*-vanilloyl-hexoside, 1,3-*O*-diferuloylglycerol, drovomifoliol-*O*-glucopyranoside, protocatechuic acid glucoside, digalloyl-glucose and coumaric acid sulphate (Table 6).

## Discussion

This study is providing a quite complete picture for the first time of phytochemical composition of *C. peltata*, *C. uvifera* and *R. salicifolia* leaves ethanolic extract through



**Fig. 8** Heatmap showing significant metabolites accumulated in *C. uvifera* 50% methanol fraction, compared to *C. peltata* and *R. salicifolia*

**Table 6** Pearson's correlation coefficients (*r*) showing the strength of relationship between  $\alpha$ -glucosidase inhibition, DPPH antioxidant activity and significant metabolites accumulated in the total extract and 50% methanol fraction of *C. uvifera* compared to *C. peltata* and *R. salicifolia*

| Metabolites                      | Correlation | p value  |
|----------------------------------|-------------|----------|
| $\alpha$ -glucosidase inhibition | 1           | 0        |
| DPPH                             | 0.88637     | 6.47E-13 |
| 1-O-Vanilloyl-hexoside           | 0.72332     | 6.24E-07 |
| 1,3-O-Diferuloylglycerol         | 0.63727     | 2.92E-05 |
| Drovomifoliol-O-glucopyranoside  | 0.53447     | 0.000785 |
| Protocatechuic acid glucoside    | 0.51258     | 0.001392 |
| Di-galloyl-glucose               | 0.42865     | 0.009096 |
| Coumaric acid sulphate           | 0.3358      | 0.045255 |

UPLC-ESI-MS/MS as well as their potential to possess antidiabetic, antioxidant, antihyperlipidemic and AGEs inhibition activities together with their fractions (DCM, 100% and 50% MeOH). *C. uvifera* extract showed highest antioxidant and antidiabetic activity through inhibition of  $\alpha$ -glucosidase and DPPH free radicle scavenging activity. In addition, 50% MeOH fraction of *C. uvifera* showed the highest  $\alpha$ -glucosidase, DPPH, ABTS and FRAP inhibition activities compared to the other fractions. Pearson's correlation coefficients revealed the significantly correlated compounds in the most active fraction 50% MeOH *C. uvifera* extract with the antioxidant/antidiabetic activity 1-O-vanilloyl-hexoside, 1,3-O-diferuloylglycerol, drovomifoliol-O-glucopyranoside, protocatechuic acid glucoside, digalloyl-glucose, coumaric acid sulphate to be the discriminating compounds.

Vanillic, ferulic, protocatechuic and *p*-coumaric acids were previously revealed to inhibit carbohydrate digesting enzymes:  $\alpha$ -amylase and  $\alpha$ -glucosidase, alongside with their DPPH and ABTS radical scavenging activities as well as  $\text{Fe}^{3+}$  reducing properties [57–60]. The phenolic hydroxyl group and the unsaturated alkyl chain are crucial for the  $\alpha$ -glucosidase inhibitory activity of phenolic acids [61]. Additionally, diferuloyl glycerol, obtained from *Pandanus odoratissimus* flowers, showed promising radical scavenging and reducing properties as well as significant in vitro inhibition of invertase enzyme [62]. Also, the antioxidant activity of vanillic acid  $\beta$ -glucoside was investigated through oxygen radical absorbance capacity ORAC assay [63]. In [64], it is reported that galloyl-based polyphenols with free or unfree galloyl moieties inhibited  $\alpha$ -glucosidase at varying degrees; as the number of free galloyl moieties increased, the inhibitory activity of galloyl-based polyphenols increased. Molecular docking results as well revealed that galloyl-based polyphenols with more galloyl moieties led to more amino acid

residues involved in the interaction; however, too many galloyl moieties and complex structures could not enter the  $\alpha$ -glucosidase active site due to steric hinderance. The monosaccharide core aided the interaction of galloyl moieties with the  $\alpha$ -glucosidase active site [64]. The potent antioxidant activity (ABTS assay) of the sesquiterpene vomifoliol, isolated from *Sesbania grandiflora*, alongside with its promising antidiabetic activity via inhibiting  $\alpha$ -glucosidase enzyme, sevenfold more than acarbose, was reported. Molecular modelling showed two hydrogen bonding interactions between the vomifoliol 6-OH group and the amino acid residues: HIS279 and GLU304, as well as one hydrogen bonding between its enone carbonyl oxygen and ASN241, crucial for binding to  $\alpha$ -glucosidase [65]. Yet, the activity of its glucoside derivative hasn't been investigated; hence, its isolation should now follow to determine its activity as a new antidiabetic agent.

Nevertheless, *R. salicifolia* 100%, and 50% MeOH fractions demonstrated the highest inhibition of AGEs in the BSA-fructose antiglycation model. According to VIP scores (Fig. 7B), fifteen metabolites favourably accumulated in the 100% and 50% MeOH fractions of *R. salicifolia*, designated as chlorogenic acid derivative, methyl glutaric acid derivative, phlorisobutyrophene glucoside, isorhamnetin glucuronide, kaempferol acetylglucoside, tetrahydroxy stilbene derivative, myricetin acetylhexoside, orientin sulphate, protocatechuic acid pentoside, acacetin glucoside, quercetin acetylramnoside, hydroxyl acetyl-O-galloyl glucose, digalloyl quinic acid, ellagic acid hexoside and apigenin pentoside. Polyphenolic compounds as phenolic acids, flavonoids and stilbenes have contributed to the ability of various plant extracts in inhibiting AGEs production [6]. Gallic acid's antiglycation ability had been demonstrated through in vivo and in vitro tests, in addition to reducing AGE-induced inflammatory responses [66]. Also, ellagic acid and its derivatives were investigated for their inhibitory actions on the production of AGEs and rat-lens aldose reductase (AR); ellagic acid exhibited a significantly stronger inhibitory effect on AGEs, compared to aminoguanidine [67]. Protocatechuic acid inhibited AGEs in oleic acid-BSA system more favourably than in glucose-BSA model, by its reducing power [6]. Besides, chlorogenic acid inhibited fluorescent AGEs and prevented BSA-glucose cross-linking substances as detected by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) [68]. Regarding flavonoids, their inhibitory activities were enhanced by increased hydroxylation on the A and B rings; myricetin showed higher inhibition potency against AGEs than quercetin and kaempferol due to the pyrogallol structure of its B ring [69]. The antiglycation

effect of quercetin was reported by many authors, via trapping methylglyoxal and glyoxal [6], in addition to its aldose reductase inhibitory effect [70]. Isorhamnetin was also assessed for its ability to inhibit aldose reductase enzyme and attenuate AGE formation; it could be a promising agent in the management of diabetes and age-related disorders [71]. Glycosylation of the hydroxyl group at C-3 of flavonols increased the AGEs inhibitory activity [69]. For flavones, apigenin reduced the generation of AGEs in a dose-dependent manner in BSA-fructose and BSA-glucose models and inhibited the glycated BSA cross-linking or aggregation, as revealed by the altered molecular weight determined by SDS-PAGE [72]. As reported by Lv, Lishuang, et al. [73], 2,3,5,4'-tetrahydroxystilbene 2-O- $\beta$ -D-glucoside (THSG), a major bioactive constituent in *Polygonum multiflorum* Thunb., efficiently inhibited the formation of AGEs by trapping reactive methylglyoxal under physiological conditions. It is worth noting that chlorogenic acid showed higher binding constant to BSA than ferulic acid, in accordance with molecular docking predictions, where more hydroxyl groups on the benzene ring enhanced the formation of hydrogen bonds. Similarly, molecular docking prediction revealed that BSA-luteolin had a higher binding affinity than apigenin, which may have been as a result of the extra hydroxyl group of luteolin located on the A ring [72]. Nevertheless, the flavones, luteolin and apigenin, were more potent AGEs inhibitors than quercetin and phenolic acids [72], in accordance with [69], reporting higher efficacies of flavones compared to their corresponding flavonols. This may partly rationalize the higher effectiveness of *R. salicifolia* as AGEs inhibitor than the other two investigated species.

## Conclusions

This study reported for the first time the metabolites profile of the extracts and fractions of *C. uvifera*, *C. peltata* and *R. salicifolia* leaves by UHPLC-ESI/MS/MS analysis. *C. uvifera* extract and its 50% MeOH fraction exhibited the most potent antioxidant and  $\alpha$ -glucosidase inhibitory activities. The most correlated metabolites with these activities included 1-O-vanilloyl-hexoside, 1,3-O-diferuloylglycerol, drovomifoliol-O-glucopyranoside, protocatechuic acid glucoside, digalloyl-glucose and coumaric acid sulphate. On the other hand, *R. salicifolia* extract and its polar fractions demonstrated potent antiglycation activities which can be attributable to phenolic acids, flavonols, flavones and stilbene derivative. Further in vivo studies and clinical trials should now follow to validate the use *C. uvifera* and *R. salicifolia* as efficient

complementary therapies in the management of DM and its complications.

## Abbreviations

|           |                                                                          |
|-----------|--------------------------------------------------------------------------|
| UPLC/HRMS | Ultraperformance liquid chromatography high-resolution mass spectrometry |
| DM        | Diabetes mellitus                                                        |
| AGEs      | Advanced glycation end-products                                          |
| RAGE      | Advanced glycation end-products receptors                                |
| DPPH      | 2,2-Diphenyl-1-picrylhydrazyl                                            |
| FRAP      | Ferric ion reducing antioxidant power                                    |
| ABTS      | 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)                   |
| BSA       | Bovine serum albumin                                                     |

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-025-00787-6>.

Supplementary Material 1.

## Acknowledgements

Not applicable.

## Author contributions

Fatma Mohamed involved in methodology, formal analysis, investigation, writing original draft. Mohamed Salem took part in data curation, formal analysis, writing—original draft preparation. Mohammed Khalil involved in conceptualization, investigation, supervision, writing reviewing and editing. Ali El-Halawany involved in conceptualization, supervision, writing, review and editing. Amira El Senousy took part in supervision, validation, investigation, writing—reviewing and editing.

## Funding

No funding.

## Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Received: 29 October 2024 Accepted: 22 February 2025

Published online: 11 March 2025

## References

- Federation IUHWDO (2021) IDF diabetes atlas, 10th edn, Brussels Belgium
- Naveen J, Baskaran V (2018) Antidiabetic plant-derived nutraceuticals: a critical review. *Eur J Nutr* 57:1275–1299. <https://doi.org/10.1007/s00394-017-1552-6>
- Khalid M, Petroianu G, Adem A (2022) Advanced glycation end products and diabetes mellitus: mechanisms and perspectives. *Biomolecules* 12:542. <https://doi.org/10.3390/biom12040542>
- Nowotny K, Jung T, Höhn A, Weber D, Grune T (2015) Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. *Biomolecules* 5:194–222. <https://doi.org/10.3390/biom5010194>
- Ćorković I, Gašo-Sokač D, Pichler A, Šimunović J, Kopjar M (2022) Dietary polyphenols as natural inhibitors of  $\alpha$ -amylase and  $\alpha$ -glucosidase. *Life* 12:1692. <https://doi.org/10.3390/life12111692>
- Khan M, Liu H, Wang J, Sun B (2020) Inhibitory effect of phenolic compounds and plant extracts on the formation of advance glycation end products: a comprehensive review. *Food Res Int* 130:108933. <https://doi.org/10.1016/j.foodres.2019.108933>
- Bailey C, Christian KR, Pradhan S, Nair MG, Christian OE (2011) Anti-inflammatory and antioxidant activities of *Coccoloba uvifera* (Sea-grapes). *Curr Top Phytochem* 10:55–60
- Ramos-Hernández JA, Calderón-Santoyo M, Burgos-Hernández A, García-Romo JS, Navarro-Ocaña A, Burboa-Zazueta MG, Sandoval-Petris E, Ragazzo-Sánchez JA (2021) Antimutagenic, antiproliferative and antioxidant properties of sea grape leaf extract fractions (*Coccoloba uvifera* L.). *Anti-Cancer Agents Med Chem* 21:2250–2257. <https://doi.org/10.2174/1871520621999210104201242>
- Mohamed FA, Sayed RH, Khalil MNA, Salem MA, El SenousyEl-Halawany ASAM (2024) Ameliorative activity of standardized *Coccoloba uvifera* leaves extract against streptozotocin-induced diabetic rats via activation of IRS-1/PI3K/AKT/GLUT2 pathway in liver. *Future J Pharm Sci* 10:132. <https://doi.org/10.1186/s43094-024-00707-0>
- El-Kawe BJCT (2019) A pharmacognostical study of *Coccoloba peltata* Schott family polygonaceae, Master thesis
- Mohamed FA, Khalil MN, Salem MA, El Senousy AS, El-Halawany AMJE-JOC (2024) Pharmacological potentials of the genus *Coccoloba* and its phytochemical constituents: an updated review since 2020. *Egypt J Chem* 67:203. <https://doi.org/10.21608/ejchem.2024.311546.10181>
- Haggag EG, Abdelhady MI, Kamal AM (2013) Phenolic content of *Ruprechtia salicifolia* leaf and its immunomodulatory, anti-inflammatory, anticancer and antibacterial activity. *J Pharm Res* 6:696–703. <https://doi.org/10.1016/j.jopr.2013.07.015>
- Bedair M, Sumner LW (2008) Current and emerging mass-spectrometry technologies for metabolomics. *Trends Anal Chem* 27:238–250. <https://doi.org/10.1016/j.trac.2008.01.006>
- Grata E, Boccari J, Guillaume D, Glauser G, Carrupt P-A, Farmer EE, Wolfender JL, Rudaz SJJOCB (2008) UPLC–TOF–MS for plant metabolomics: a sequential approach for wound marker analysis in *Arabidopsis thaliana*. *J Chromatogr B* 871:261–270. <https://doi.org/10.1016/j.jchro mb.2008.04.021>
- Salem MA, Perez De Souza L, Serag A, Fernie AR, Farag MA, Ezzat SM, Alseekh S (2020) Metabolomics in the context of plant natural products research: from sample preparation to metabolite analysis. *Metabolites* 10:37. <https://doi.org/10.3390/metabo10010037>
- Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat prot* 2:875–877. <https://doi.org/10.1038/nprot.2007.102>
- Albino AM, Gomes DL, Santos AH, Malta GD, Oliveira DH, Souto LF, Teles CB, Fialho SN, Silva MA, Araújo NF, Bay M (2024) Cytotoxicity evaluation and dereplication of flavonoids-guided by antioxidant activity and total phenolics content from ephedrantus amazonicus leaves. *Braz Arch Biol Technol* 67:e24231132
- Pękal A, Pyrzyńska K (2014) Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Anal Method* 7:1776–1782. <https://doi.org/10.1007/s12161-014-9814-x>
- Saeed N, Khan MR, Shabbir M (2012) Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement Altern Med* 12:1–12. <https://doi.org/10.1186/1472-6882-12-221>
- Faitanin RD, Gomes JV, Rodrigues PM, de Menezes LF, Neto AC, Gonçalves RC, Kitagawa RR, Silveira D, Jamal CM (2018) Chemical study and evaluation of antioxidant activity and  $\alpha$ -glucosidase inhibition of *Myrciaria strigipes* O. Berg (Myrtaceae). *J Appl Pharm Sci* 8(3):120–125. <https://doi.org/10.7324/JAPS.2018.8317>
- Sunil D, Isloor AM, Shetty P, Satyamoorthy K, Prasad AB (2010) 6-[3-(4-Fluorophenyl)-1H-pyrazol-4-yl]-3-[(2-naphthylthio) methyl][1, 2, 4] triazolo [3, 4-b][1, 3, 4] thiadiazole as a potent antioxidant and an anticancer agent induces growth inhibition followed by apoptosis in HepG2 cells. *Arab J Chem* 3:211–217. <https://doi.org/10.1016/j.arabjc.2010.06.002>
- Justino AB, Miranda NC, Franco RR, Martins MM, Da Silva NM, Espindola FS (2018) *Annona muricata* Linn. leaf as a source of antioxidant compounds with in vitro antidiabetic and inhibitory potential against  $\alpha$ -amylase,  $\alpha$ -glucosidase, lipase, non-enzymatic glycation and lipid peroxidation. *Biomed Pharmacother* 100:83–92. <https://doi.org/10.1016/j.biopha.2018.01.172>
- Benzie IF, Devaki M, Trends CR, and Applications (2018) The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications. In: Measurement of antioxidant activity capacity, pp 77–106. <https://doi.org/10.1002/9781119135388.ch5>
- Gutiérrez-Grijalva EP, Antunes-Ricardo M, Acosta-Estrada BA, Gutiérrez-Urbe JA, Heredia JB (2019) Cellular antioxidant activity and in vitro inhibition of  $\alpha$ -glucosidase,  $\alpha$ -amylase and pancreatic lipase of oregano polyphenols under simulated gastrointestinal digestion. *Food Res Int* 116:676–686. <https://doi.org/10.1016/j.foodres.2018.08.096>
- Kordel M, Schmid R (1991) Inhibition of the lipase from *Pseudomonas* spec. ATCC 21808 by diethyl p-nitrophenylphosphate. In: Alberghina L, Schmid RD (eds) Hints for one buried active site for lipolytic and esterolytic activity, Verger, pp 385–387
- Alam MM, Ahmad I, Naseem I (2015) Inhibitory effect of quercetin in the formation of advance glycation end products of human serum albumin: an in vitro and molecular interaction study. *Int J Biol Macromol* 79:336–343. <https://doi.org/10.1016/j.ijbiomac.2015.05.004>
- Wang W, Yagiz Y, Buran TJ, Do Nascimento Nunes C, Gu L (2011) Phytochemicals from berries and grapes inhibited the formation of advanced glycation end-products by scavenging reactive carbonyls. *Food Res Int* 44:2666–2673. <https://doi.org/10.1016/j.foodres.2011.05.022>
- Merghany RM, Salem MA, Ezzat SM, Moustafa SF, El-Sawi SA, Meselhy MRJSR (2024) A comparative UPLC–orbitrap–MS-based metabolite profiling of three *Pelargonium* species cultivated in Egypt. *Sci Rep* 14:22765. <https://doi.org/10.1038/s41598-024-72153-0>
- Salem MA, Michel HE, Ezzat MI, Okba MM, El-Desoky AM, Mohamed SO, Ezzat SMJM (2020) Optimization of an extraction solvent for angiotensin-converting enzyme inhibitors from *Hibiscus sabdariffa* L. based on its UPLC–MS/MS metabolic profiling. *Molecules* 25:2307. <https://doi.org/10.3390/molecules25102307>
- Chambers MC, Maclean B, Burke R, Amodei D, Ruderman DL, Neumann S, Gatto L, Fischer B, Pratt B, Egerton JNJB (2012) A cross-platform toolkit for mass spectrometry and proteomics. *Nat Biotechnol* 30:918–920. <https://doi.org/10.1038/nbt.2377>
- Pluskal T, Castillo S, Villar-Briones A, Orešič M (2010) MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinform* 11:1–11. <https://doi.org/10.1186/1471-2105-11-395>
- Pang Z, Chong J, Zhou G, De Lima Morais DA, Chang L, Barrette M, Gauthier C, Jacques P-É, Li S, Xia J (2021) *MetaboAnalyst 5.0*: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res* 49:W388–W396. <https://doi.org/10.1093/nar/gkab382>
- Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M (2023) Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Arch toxicol* 97:2499–2574. <https://doi.org/10.1007/s00204-023-03562-9>
- Leviton EB, Song Y, Ford ES, Liu S (2004) Is nondiabetic hyperglycemia a risk factor for cardiovascular disease?: A meta-analysis of prospective studies. *Arch Intern Med* 164:2147–2155. <https://doi.org/10.1001/archinte.164.19.2147>

35. Hanefeld M, Schaper F (2007) The role of alpha-glucosidase inhibitors (acarbose). *Pharmacotherapy of diabetes: new developments*. Springer, pp 143–152. [https://doi.org/10.1007/978-0-387-69737-6\\_13](https://doi.org/10.1007/978-0-387-69737-6_13)
36. Lawag IL, Aguinaldo AM, Naheed S, Mosihuzzaman M (2012)  $\alpha$ -glucosidase inhibitory activity of selected Philippine plants. *J Ethnopharmacol* 144:217–219. <https://doi.org/10.1016/j.jep.2012.08.019>
37. Valsaraj M, Saseendran N, Koorapally AS, Augustine A (2018) Anti-obesity molecules from plants and their mode of action. *Anti-Obes Drug Discov Dev* 4:197. <https://doi.org/10.2174/9781681085586118040008>
38. Heath H, Hamlett Y (1976) The sorbitol pathway: effect of streptozotocin induced diabetes and the feeding of a sucrose-rich diet on glucose, sorbitol and fructose in the retina, blood and liver of rats. *Diabetologia* 12:43–46. <https://doi.org/10.1007/bf01221963>
39. Park JJ, Lee WY (2021) Anti-glycation effects of brown algae extracts and its phenolic compounds. *Food Biosci* 41:101042. <https://doi.org/10.1016/j.fbio.2021.101042>
40. Fontana A (2023) Recovery, characterization, functionality and applications of bioactive compounds from food-plant products and their by-products. *Plants* 12:3964. <https://doi.org/10.3390/plants12233964>
41. Pacifico S, Piccolella S, Marciano S, Galasso S, Nocera P, Piscopo V, Fiorentino A, Monaco P (2014) LC-MS/MS profiling of a mastic leaf phenol enriched extract and its effects on H<sub>2</sub>O<sub>2</sub> and A $\beta$  (25–35) oxidative injury in SK-B-NE (C)-2 cells. *J Agric Food Chem* 62:11957–11966. <https://doi.org/10.1021/jf504544x>
42. Mekky RH, Abdel-Sattar E, Segura-Carretero A, Contreras MDM (2019) Phenolic compounds from sesame cake and antioxidant activity: a new insight for agri-food residues' significance for sustainable development. *Foods* 8:432. <https://doi.org/10.3390/foods8100432>
43. Du J, Zhong B, Subbiah V, Barrow CJ, Dunshea FR, Suleria HA (2021) LC-esi-qtof-ms/ms profiling and antioxidant activity of phenolics from custard apple fruit and by-products. *Separations* 8:62. <https://doi.org/10.3390/separations8050062>
44. Jaiswal R, Kuhnert N (2010) Hierarchical scheme for liquid chromatography/multi-stage spectrometric identification of 3, 4, 5-triacyl chlorogenic acids in green Robusta coffee beans. *Rapid Commun Mass Sp* 24:2283–2294. <https://doi.org/10.1002/rcm.4639>
45. Rockenbach II, Jungfer E, Ritter C, Santiago-Schübel B, Thiele B, Fett R, Galensia RJFRI (2012) Characterization of flavan-3-ols in seeds of grape pomace by CE, HPLC-DAD-MSn and LC-ESI-FTICR-MS. *Food Res Int* 48:848–855. <https://doi.org/10.1016/j.foodres.2012.07.001>
46. Falcão SI, Vale N, Gomes P, Domingues MR, Freire C, Cardoso SM, Vilas-Boas M (2013) Phenolic profiling of *Portuguese propolis* by LC–MS spectrometry: uncommon propolis rich in flavonoid glycosides. *Phytochem Anal* 24:309–318. <https://doi.org/10.1002/pca.2412>
47. Saldanha LL, Vilegas W, Dokkedal AL (2013) Characterization of flavonoids and phenolic acids in *Myrcia bella* cambess Using FIA-ESI-IT-MSn and HPLC-PAD-ESI-IT-MS combined with NMR. *Molecules* 18:8402–8416. <https://doi.org/10.3390/molecules18078402>
48. Zhang X, Su M, Du J, Zhou H, Li X, Zhang M, Hu Y, Ye ZJFC (2023) Profiling of naturally occurring proanthocyanidins and other phenolic compounds in a diverse peach germplasm by LC-MS/MS. *Food Chem* 403:134471. <https://doi.org/10.1016/j.foodchem.2022.134471>
49. Buzgaia N, Lee SY, Rukayadi Y, Abas F, Shaari KJP (2021) Antioxidant activity,  $\alpha$ -glucosidase inhibition and UHPLC–ESI–MS/MS profile of Shmar (*Arbutus pavarii* Pamp). *Plants* 10:1659. <https://doi.org/10.3390/plants10081659>
50. Jin W, Wang YF, Ge RL, Shi HM, Jia CQ, Tu PF (2007) Simultaneous analysis of multiple bioactive constituents in *Rheum tanguticum* Maxim. ex Balf. by high-performance liquid chromatography coupled to tandem mass spectrometry. *Rapid Commun Mass Sp* 21:2351–2360. <https://doi.org/10.1002/rcm.3086>
51. Wang Q, Lu Z, Zhang L, Zhang Q, Wang M, Zhao H, Liu Y, Fu S, Huang Z, Xie ZJJOSS (2017) Applying characteristic fragment filtering for rapid detection and identification of ingredients in rhubarb by HPLC coupled with linear ion trap–Orbitrap mass spectrometry. *J Sep Sci* 40:2854–2862. <https://doi.org/10.1002/jssc.201700203>
52. Banoub J, Delmas GH Jr, Joly N, Mackenzie G, Cachet N, Benjelloun-Milayab B, Delmas M (2015) A critique on the structural analysis of lignins and application of novel tandem mass spectrometric strategies to determine lignin sequencing. *J Mass Spectrom* 50:5–48. <https://doi.org/10.1002/jms.3477>
53. Méndez D, Escalona-Arranz JC, Pérez EM, Foubert K, Matheeußen A, Tuenter E, Cuyper A, Cos P, Pieters LJP (2021) Antifungal activity of extracts, fractions, and constituents from *Coccoloba cowellii* leaves. *Pharmaceuticals* 14:917. <https://doi.org/10.3390/ph14090917>
54. Yu XA, Ge AH, Zhang L, Li J, An M, Cao J, He J, Gao XM, Chang YX (2017) Influence of different processing times on the quality of *Polygoni Multiflora* Radix by metabolomics based on ultra high performance liquid chromatography with quadrupole time-of-flight mass spectrometry. *J Sep Sci* 40:1928–1941. <https://doi.org/10.1002/jssc.201600913>
55. Castañeda FN, Vidal RBP, Aspromonte J (2024) Untargeted chromatographic methods coupled with chemometric strategies for the analysis of food and related samples. *TrAC Trends Anal Chem* 173:117650. <https://doi.org/10.1016/j.trac.2024.117650>
56. Toddenroth D, Ganslandt T, Castellanos I, Prokosch HU, Bürkle T (2014) Employing heat maps to mine associations in structured routine care data. *Artif Intell Med* 60:79–88. <https://doi.org/10.1016/j.artmed.2013.12.003>
57. Kakkar S, Bais S (2014) A review on protocatechuic acid and its pharmacological potential. *Int Sch Res Not* 2014(1):952943. <https://doi.org/10.1155/2014/952943>
58. Matowane GR, Ramorobi LM, Mashele SS, Bonnet SL, Noreljaleel AE, Swain SS, Makhafola TJ, Chukwuma CIJDM (2022) Complexation potentiated promising anti-diabetic and anti-oxidative synergism between ZN (ii) and ferulic acid: a multimode study. *Diabet Med* 39:e14905. <https://doi.org/10.1111/dme.14905>
59. Oke IM, Ramorobi LM, Mashele SS, Bonnet SL, Makhafola TJ, Eze KC, Noreljaleel AE, Chukwuma CI (2021) Vanillic acid–Zn (II) complex: a novel complex with antihyperglycaemic and anti-oxidative activity. *J Pharm Pharmacol* 73:1703–1714. <https://doi.org/10.1093/jpp/rgab086>
60. Ramorobi LM, Matowane GR, Mashele SS, Bonnet SL, Noreljaleel AE, Swain SS, Makhafola TJ, Chukwuma CIJOFB (2022) Bioactive synergism between zinc mineral and *p*-coumaric acid: a multi-mode glycemic control and antioxidative study. *J Food Biochem* 46:e14360. <https://doi.org/10.1111/jfbc.14360>
61. Ding H, Huang S, Chook CY, Kwek E, Yan C, Ma K, Liu J, Zhu H, Chen Z (2024) Blood glucose-lowering activity of protocatechuic acid is mediated by inhibiting  $\alpha$ -glucosidase. *Food Sci Hum Well* 13:1212–1219. <https://doi.org/10.26599/fshw.2022.9250101>
62. Suvertha SP, Sathishkumar T, Kumaraesan K, Rapheal VS, Muthukumaran V, Thirugnanam N (2022) Purified novel and new diferuloyl glycerate related phenolic acid from *Pandanus odoratissimus* flowers shows antioxidant, invertase inhibition and control against diabetic foot ulcer (DFU) causing bacterial pathogens-an in vitro study to establish an effective regulation over type 2 diabetes mellitus. *Braz J Pharm Sci* 58:e19484. <https://doi.org/10.1590/s2175-97902022e19484>
63. Kayano S-I, Kikuzaki H, Fukutsuka N, Mitani T, Nakatani N (2002) Anti-oxidant activity of prune (*Prunus domestica* L.) constituents and a new synergist. *J Agric Food Chem* 50:3708–3712. <https://doi.org/10.1021/jf0200164>
64. Cao J, Yan S, Xiao Y, Han L, Sun L, Wang M (2022) Number of galloyl moiety and intramolecular bonds in galloyl-based polyphenols affect their interaction with alpha-glucosidase. *Food Chem* 367:129846. <https://doi.org/10.1016/j.foodchem.2021.129846>
65. Thissera B, Visvanathan R, Khanfar MA, Qader MM, Hassan MH, Hassan HM, Bawazeer M, Behery FA, Yaseen M, Liyanage R (2020) *Sesbania grandiflora* L. Poir leaves: a dietary supplement to alleviate type 2 diabetes through metabolic enzymes inhibition. *S Afr J Bot* 130:282–299. <https://doi.org/10.1016/j.sajb.2020.01.011>
66. Umadevi S, Gopi V, Vellaichamy E (2013) Inhibitory effect of gallic acid on advanced glycation end products induced up-regulation of inflammatory cytokines and matrix proteins in H9C2 (2–1) cells. *Cardiovas Toxicol* 13:396–405. <https://doi.org/10.1007/s12012-013-9222-2>
67. Kim JM, Jang DS, Lee YM, Yoo JL, Kim YS, Kim JH, Kim JS (2008) Aldose-reductase and protein-glycation-inhibitory principles from the whole plant of *Duchesnea chrysantha*. *Chem Biodivers* 5:352–356. <https://doi.org/10.1002/cbdv.200890034>
68. Yu L, Wang J, Zhang N, Yang Y, Guo C, Li M (2024) Inhibition of fluorescent advanced glycation end-products by ferulic acid and chlorogenic acid: a fluorescence spectroscopy and molecular docking study. *Food Biosci* 58:103790. <https://doi.org/10.1016/j.fbio.2024.103790>

69. Matsuda H, Wang T, Managi H, Yoshikawa MJB, Chemistry M (2003) Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorg Med Chem* 11:5317–5323. <https://doi.org/10.1016/j.bmc.2003.09.045>
70. Julius A, Hopper W (2017) Inhibition of advanced glycation end-product formation by quercetin and catechin: an alternative therapy for treating diabetic complications. *Asian J Pharm Clin Res* 10(11):173–176. <https://doi.org/10.22159/ajpcr.2017.v10i11.19412>
71. Balaji A, Arthanari A, Parameshwari P (2024) Evaluation of isorhamnetin for its inhibitory activity on amylase and advanced glycation end products. *Int J Appl Eng Technol*. <https://doi.org/10.1021/acs.jafc.8b00650.s001>
72. Qin C, Li Y, Zhang Y, Liu L, Wu Z, Weng P (2021) Insights into oat polyphenols constituent against advanced glycation end products mechanism by spectroscopy and molecular interaction. *Food Biosci* 43:101313. <https://doi.org/10.1016/j.foodchem.2021.101313>
73. Lv L, Shao X, Wang L, Huang D, Ho C-T, Sang S (2010) Stilbene glucoside from *Polygonum multiflorum* Thunb.: a novel natural inhibitor of advanced glycation end product formation by trapping of methylglyoxal. *J Agric Food Chem* 58:2239–2245. <https://doi.org/10.1021/jf904122q>
74. Zhan L, Huang X, Xue J, Liu H, Xiong C, Wang J, Nie ZJFC (2021) MALDI-TOF/TOF tandem mass spectrometry imaging reveals non-uniform distribution of disaccharide isomers in plant tissues. *Food Chem* 338:127984. <https://doi.org/10.1016/j.foodchem.2020.127984>
75. Araujo NMP, Arruda HS, Dos Santos FN, De Moraes DR, Pereira GA, Pastore GM (2020) LC-MS/MS screening and identification of bioactive compounds in leaves, pulp and seed from *Eugenia calycina* Cambess. *Food Res Int* 137:109556. <https://doi.org/10.1016/j.foodres.2020.109556>
76. Hong C, Chang C, Zhang H, Jin Q, Wu G, Wang X (2019) Identification and characterization of polyphenols in different varieties of *Camellia oleifera* seed cakes by UPLC-QTOF-MS. *Food Res Int* 126:108614. <https://doi.org/10.1016/j.foodres.2019.108614>
77. Chen S-D, Lu C-J, Zhao R-ZJM (2014) Qualitative and quantitative analysis of Rhizoma Smilacis glabrae by ultra high performance liquid chromatography coupled with LTQ OrbitrapXL hybrid mass spectrometry. *Molecules* 19:10427–10439. <https://doi.org/10.3390/molecules190710427>
78. Taamalli A, Arráez-Román D, Abaza L, Iswaldi I, Fernández-Gutiérrez A, Zarrouk M, Segura-Carretero AJPA (2015) LC-MS-based metabolite profiling of methanolic extracts from the medicinal and aromatic species *Mentha pulegium* and *Origanum majorana*. *Phytochem Anal* 26:320–330. <https://doi.org/10.1002/pca.2566>
79. Koyama J, Takeuchi A, Morita I, Nishino Y, Shimizu M, Inoue M, Kobayashi N (2009) Characterization of emodin metabolites in Raji cells by LC-APCI-MS/MS. *Bioorg Med Chem* 17:7493–7499. <https://doi.org/10.1016/j.bmc.2009.09.024>
80. Regazzoni L, Arlandini E, Garzon D, Santagati NA, Beretta G, Facino RM (2013) A rapid profiling of gallotannins and flavonoids of the aqueous extract of *Rhus coriaria* L. by flow injection analysis with high-resolution mass spectrometry assisted with database searching. *J Pharm Biomed Anal* 72:202–207. <https://doi.org/10.1016/j.jpba.2012.08.017>
81. Álvarez-Fernández MA, Hornedo-Ortega R, Cerezo AB, Troncoso AM, GarcíaParrilla MC (2016) Determination of nonanthocyanin phenolic compounds using high-resolution mass spectrometry (UHPLC-Orbitrap-MS/MS) and impact of storage conditions in a beverage made from strawberry by fermentation. *J Agric Food Chem* 64:1367–1376. <https://doi.org/10.1021/acs.jafc.5b05617>
82. Xu M, Zhang Z, Fu G, Sun S, Sun J, Yang M, Liu A, Han J, Guo D (2007) Liquid chromatography–tandem mass spectrometry analysis of protocatechuic aldehyde and its phase I and II metabolites in rat. *J Chromatogr B* 856:100–107. <https://doi.org/10.1016/j.jchromb.2007.05.042>
83. Sobeh M, Rezaq S, Sabry OM, Abdelfattah MA, El Raey MA, El-Kashak WA, El-Shazly AM, Mahmoud MF, Wink M (2019) *Albizia anthelmintica*: HPLC-MS/MS profiling and in vivo anti-inflammatory, pain killing and antipyretic activities of its leaf extract. *Biomed Pharmacother* 1(115):108882. <https://doi.org/10.1016/j.biopha.2019.108882>
84. Maulidiani M, Abdul-Hamid NA, Abas F, Park YS, Park Y-K, Kim YM, Gorinstein S (2019) Detection of bioactive compounds in persimmon (*Diospyros kaki*) using UPLC-ESI-Orbitrap-MS/MS and fluorescence analyses. *Microchem J* 149:103978. <https://doi.org/10.1016/j.microc.2019.103978>
85. Hvattum E (2002) Determination of phenolic compounds in rose hip (*Rosa canina*) using liquid chromatography coupled to electrospray ionisation tandem mass spectrometry and diode-array detection. *Rapid Commun Mass Sp* 16:655–662. <https://doi.org/10.1002/rcm.622>
86. Perestrelo R, Lu Y, SaO S, Silvestre AJD, Neto CP, Câmara JS, Rocha SM (2012) Phenolic profile of sercial and Tinta Negra Portuguese varieties: novel phenolic compounds in *Vitis vinifera* L. grape. *Planta Med* 78:PI216. <https://doi.org/10.1055/s-0032-1320903>
87. Parejo I, Jauregui O, Sánchez-Rabaneda F, Viladomat F, Bastida J, Codina C (2004) Separation and characterization of phenolic compounds in fennel (*Foeniculum vulgare*) using liquid chromatography–negative electrospray ionization tandem mass spectrometry. *J Agric Food Chem* 52:3679–3687. <https://doi.org/10.1021/jf030813h>
88. Bakr RO, SaEH M, Ayoub N (2016) Phenolic profile of *Centaurea aegyptiaca* L. growing in Egypt and its cytotoxic and antiviral activities. *Afr J Trad Complem Alternat Med* 13:135–143. <https://doi.org/10.21010/ajtcam.v13i6.19>
89. Jaiswal R, Halabi EA, Karar MGE, Kuhnert N (2014) Identification and characterisation of the phenolics of *Ilex glabra* L. Gray (Aquifoliaceae) leaves by liquid chromatography tandem mass spectrometry. *Phytochem* 106:141–155. <https://doi.org/10.1016/j.phytochem.2014.07.018>
90. Grace MH, Warlick CW, Neff SA, Lila MA (2014) Efficient preparative isolation and identification of walnut bioactive components using high-speed counter-current chromatography and LC-ESI-IT-TOF-MS. *Food Chem* 158:229–238. <https://doi.org/10.1016/j.foodchem.2014.02.117>
91. Jaiswal R, Kuhnert N (2014) Identification and characterization of the phenolic glycosides of *Lagenaria siceraria* Stand. (bottle gourd) fruit by liquid chromatography–tandem mass spectrometry. *J Agric Food Chem* 62:1261–1271. <https://doi.org/10.1021/jf4053989>
92. Wu J-L, Yee LP, Jiang Z-H, Cai ZJT (2007) One single LC–MS/MS analysis for both phenolic components and tanshinones in *Radix Salviae Miltiorrhizae* and its medicinal products. *Talanta* 73:656–661. <https://doi.org/10.1016/j.talanta.2007.04.038>
93. Jaiswal R, Jayasinghe L, Kuhnert N (2012) Identification and characterization of proanthocyanidins of 16 members of the *Rhododendron* genus (Ericaceae) by tandem LC–MS. *J Mass Spectrom* 47:502–515. <https://doi.org/10.1002/jms.2954>
94. Xiong P, Qin SH, Li KL, Liu MJ, Zhu L, Peng J, Shi SL, Tang SN, Tian AP, Cai W (2021) Identification of the tannins in traditional Chinese medicine *Paenoniae Radix Alba* by UHPLC–Q-Exactive Orbitrap MS. *Arab J Chem* 14:103398. <https://doi.org/10.1016/j.arabjc.2021.103398>
95. Simirgiotis MJ, Silva M, Becerra J, Schmeda-Hirschmann G (2012) Direct characterisation of phenolic antioxidants in infusions from four Mapuche medicinal plants by liquid chromatography with diode array detection (HPLC-DAD) and electrospray ionisation tandem mass spectrometry (HPLC-ESI-MS). *Food Chem* 131:318–327. <https://doi.org/10.1016/j.foodchem.2011.07.118>
96. Kang J, Price WE, Ashton J, Tapsell LC, Johnson S (2016) Identification and characterization of phenolic compounds in hydromethanolic extracts of sorghum wholegrains by LC-ESI-MSn. *Food Chem* 211:215–226. <https://doi.org/10.1016/j.foodchem.2016.05.052>
97. Celli GB, Pereira-Netto AB, Beta T (2011) Comparative analysis of total phenolic content, antioxidant activity, and flavonoids profile of fruits from two varieties of Brazilian cherry (*Eugenia uniflora* L.) throughout the fruit developmental stages. *Food Res Int* 44:2442–2451. <https://doi.org/10.1016/j.foodres.2010.12.036>
98. Li S, Lin Z, Jiang H, Tong L, Wang H, Chen S (2016) Rapid identification and assignment of the active ingredients in *fufang banbianlian* injection using HPLC-DAD-ESI-IT-TOF-MS. *J Chromatogr Sci* 54:1225–1237. <https://doi.org/10.1093/chromsci/bmw055>
99. Singh A, Bajpai V, Kumar S, Sharma KR, Kumar B (2016) Profiling of gallic and ellagic acid derivatives in different plant parts of *Terminalia arjuna* by HPLC-ESI-QTOF-MS/MS. *Nat Prod Commun* 11:1934578X1601100227. <https://doi.org/10.1177/1934578X1601100227>
100. Hossain MB, Rai DK, Brunton NP, Martin-Diana AB, Barry-Ryan C (2010) Characterization of phenolic composition in Lamiaceae spices by

- LC-ESI-MS/MS. *J Agric Food Chem* 58:10576–10581. <https://doi.org/10.1021/jf102042g>
101. Lee RJ, Lee VS, Tzen JT, Lee MR (2010) Study of the release of gallic acid from (–)-epigallocatechin gallate in old oolong tea by mass spectrometry. *Rapid Commun Mass Sp* 24:851–858. <https://doi.org/10.1002/rcm.4442>
  102. Sun J, Liang F, Bin Y, Li P, Duan CJM (2007) Screening non-colored phenolics in red wines using liquid chromatography/ultraviolet and mass spectrometry/mass spectrometry libraries. *Molecules* 12:679–693. <https://doi.org/10.3390/12030679>
  103. Gulsoy-Toplan G, Gogger F, Yildiz-Pekoz A, Gibbons S, Sariyar G, Mat A (2018) Chemical constituents of the different parts of *Colchicum micranthum* and *C. chalconicum* and their cytotoxic activities. *Nat Prod Commun* 13:1934578X1801300506. <https://doi.org/10.1177/1934578x1801300506>
  104. Engels C, Schieber A, Gänzle MG (2012) Sinapic acid derivatives in defatted Oriental mustard (*Brassica juncea* L.) seed meal extracts using UHPLC-DAD-ESI-MS n and identification of compounds with antibacterial activity. *Eur Food Res Technol* 234:535–542. <https://doi.org/10.1007/s00217-012-1669-z>
  105. Singh D, Siew Y-Y, Chong T-I, Yew H-C, Ho SS-W, Lim CSE-S, Tan W-X, Neo S-Y, Koh H-L (2019) Identification of phytoconstituents in *Leea indica* (Burm. F.) Merr. leaves by high performance liquid chromatography micro time-of-flight mass spectrometry. *Molecules* 24:714. <https://doi.org/10.3390/molecules24040714>
  106. Li X, Zhang Y, Zeng X, Yang L, Deng Y (2011) Chemical profiling of bioactive constituents in *Sarcandra glabra* and its preparations using ultra-high-pressure liquid chromatography coupled with LTQ Orbitrap mass spectrometry. *Rapid Commun Mass Sp* 25:2439–2447. <https://doi.org/10.1002/rcm.5123>
  107. Ölschläger C, Regos I, Zeller FJ, Treutter DJP (2008) Identification of galloylated propelargonidins and procyanidins in buckwheat grain and quantification of rutin and flavanols from homostylous hybrids originating from *F. esculentum* × *F. homotropicum*. *Phytochemistry* 69:1389–1397. <https://doi.org/10.1016/j.phytochem.2008.01.001>
  108. Shakyia R, Navarre DA (2006) Rapid screening of ascorbic acid, glycoalkaloids, and phenolics in potato using high-performance liquid chromatography. *J Agric Food Chem* 54:5253–5260. <https://doi.org/10.1021/jf0605300>
  109. Simirgiotis MJ, Schmeda-Hirschmann G (2010) Direct identification of phenolic constituents in *Boldo Folium* (*Peumus boldus* Mol.) infusions by high-performance liquid chromatography with diode array detection and electrospray ionization tandem mass spectrometry. *J Chromatogr A* 1217:443–449. <https://doi.org/10.1016/j.chroma.2009.11.014>
  110. Mekam PN, Martini S, Nguetack J, Tagliazucchi D, Stefani E (2019) Phenolic compounds profile of water and ethanol extracts of *Euphorbia hirta* L. leaves showing antioxidant and antifungal properties. *S Afr J Bot* 127:319–332. <https://doi.org/10.1016/j.sajb.2019.11.001>
  111. Wang Y, Vorsa N, Harrington PDB, Chen P (2018) Nontargeted metabolomic study on variation of phenolics in different cranberry cultivars using uplc-im-hrms. *J Agric Food Chem* 66:12206–12216. <https://doi.org/10.1021/acs.jafc.8b05029>
  112. Paudel L, Wyzgoski FJ, Scheerens JC, Chanon AM, Reese RN, Smiljanic D, Wesdemiotis C, Blakeslee JJ, Riedl KM, Rinaldi PL (2013) Nonanthocyanin secondary metabolites of black raspberry (*Rubus occidentalis* L.) fruits: Identification by HPLC-DAD NMR, HPLC-ESI-MS, and ESI-MS/MS analyses. *J Agric Food Chem* 61:12032–12043. <https://doi.org/10.1021/jf4039953>
  113. Bakr RO, Amer RI, Fayed MA, Ragab TI (2019) A completely polyherbal conditioning and antioxidant shampoo: a phytochemical study and pharmaceutical evaluation. *J Pharm Bioallied Sci* 11:105. [https://doi.org/10.4103/jpbs.jpbs\\_214\\_18](https://doi.org/10.4103/jpbs.jpbs_214_18)
  114. Kumar S, Chandra P, Bajpai V, Singh A, Srivastava M, Mishra D, Kumar B (2015) Rapid qualitative and quantitative analysis of bioactive compounds from *Phyllanthus amarus* using LC/MS/MS techniques. *Ind Crops Prod* 69:143–152. <https://doi.org/10.1016/j.indcrop.2015.02.012>
  115. Liu Y-G, Li B, Fu Q, Zhang X-M, Ma F-Y, Hu Y (2021) Miracle fruit leaf extract: antioxidant activity evaluation, constituent identification, and medical applications. *Anal Lett* 54:2211–2226. <https://doi.org/10.1080/00032719.2020.1854277>
  116. Barroso MR, Barros L, Dueñas M, Carvalho AM, Santos-Buelga C, Fernandes IP, Barreiro MF, Ferreira IC (2014) Exploring the antioxidant potential of *Helichrysum stoechas* (L.) Moench phenolic compounds for cosmetic applications: chemical characterization, microencapsulation and incorporation into a moisturizer. *Ind Crops Prod* 53:330–336. <https://doi.org/10.1016/j.indcrop.2014.01.004>
  117. Cantos E, Espin JC, Tomás-Barberán FA (2002) Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. *J Agric Food Chem* 50:5691–5696. <https://doi.org/10.1021/jf0204102>
  118. Rodríguez-Rivera MP, Lugo-Cervantes E, Winterhalter P, Jerz G (2014) Metabolite profiling of polyphenols in peels of *Citrus limetta* Risso by combination of preparative high-speed countercurrent chromatography and LC-ESI-MS/MS. *Food Chem* 158:139–152. <https://doi.org/10.1016/j.foodchem.2014.02.077>
  119. Ziani BE, Barros L, Boumehira AZ, Bachari K, Heleno SA, Alves MJ, Ferreira ICJF (2018) Profiling polyphenol composition by HPLC-DAD-ESI/MSn and the antibacterial activity of infusion preparations obtained from four medicinal plants. *Food Funct* 9:149–159. <https://doi.org/10.1039/c7fo01315a>
  120. Kiselova-Kaneva Y, Galunska B, Nikolova M, Dincheva I, Badjakov I (2022) High resolution LC-MS/MS characterization of polyphenolic composition and evaluation of antioxidant activity of *Sambucus ebulus* fruit tea traditionally used in Bulgaria as a functional food. *Food Chem* 367:130759. <https://doi.org/10.1016/j.foodchem.2021.130759>
  121. Faria AF, Marques MC, Mercadante AZJFC (2011) Identification of bioactive compounds from jambolão (*Syzygium cumini*) and antioxidant capacity evaluation in different pH conditions. *Food Chem* 126:1571–1578. <https://doi.org/10.1016/j.foodchem.2010.12.007>
  122. Zehl M, Braunberger C, Conrad J, Crnogorac M, Krasteva S, Vogler B, Beifuss U, Krenn L (2011) Identification and quantification of flavonoids and ellagic acid derivatives in therapeutically important *Drosera* species by LC–DAD, LC–NMR, NMR, and LC–MS. *Anal Bioanal Chem* 400:2565–2576. <https://doi.org/10.1007/s00216-011-4690-3>
  123. Álvarez-Fernández MA, Cerezo AB, Canete-Rodríguez AM, Troncoso AM, GarcíaParrilla MC (2015) Composition of nonanthocyanin polyphenols in alcoholic-fermented strawberry products using LC–MS (QTRAP), high-resolution MS (UHPLC–Orbitrap–MS) LC–DAD, and antioxidant activity. *J Agric Food Chem* 63:2041–2051. <https://doi.org/10.1021/jf506076n>
  124. Islam MN, Downey F, Ng C (2013) Comprehensive profiling of flavonoids in *Scutellaria incana* L. using LC–Q-TOF-MS. *Acta Chromatogr* 25:555–569. <https://doi.org/10.1556/achrom.25.2013.3.11>
  125. Larbat R, Paris C, Le Bot J, Adamowicz SJPS (2014) Phenolic characterization and variability in leaves, stems and roots of Micro-Tom and patio tomatoes, in response to nitrogen limitation. *Plant Sci* 224:62–73. <https://doi.org/10.1016/j.plantsci.2014.04.010>
  126. Han J, Ye M, Qiao X, Xu M, Wang B-R, Guo D-A (2008) Characterization of phenolic compounds in the Chinese herbal drug *Artemisia annua* by liquid chromatography coupled to electrospray ionization mass spectrometry. *J Pharm Biomed Anal* 47:516–525. <https://doi.org/10.1016/j.jpba.2008.02.013>
  127. López-Cobo A, Gómez-Caravaca AM, Cerretani L, Segura-Carretero A, Fernández-Gutiérrez A (2014) Distribution of phenolic compounds and other polar compounds in the tuber of *Solanum tuberosum* L. by HPLC-DAD-Q-TOF and study of their antioxidant activity. *J Food Compos Anal* 36:1–11. <https://doi.org/10.1016/j.jfca.2014.04.009>
  128. De Andrade NN, Stringheta PC, Gómez-Alonso S, Hermosín-Gutiérrez I (2018) Flavonols and ellagic acid derivatives in peels of different species of *Jaboticaba* (*Plinia* spp.) identified by HPLC-DAD-ESI/MSn. *Food Chem* 252:61–71. <https://doi.org/10.1016/j.foodchem.2018.01.078>
  129. Singh AP, Wilson T, Luthria D, Freeman MR, Scott RM, Bilenker D, Shah S, Somasundaram S, Vorsa N (2011) LC-MS-MS characterisation of curry leaf flavonols and antioxidant activity. *Food Chem* 127:80–85. <https://doi.org/10.1016/j.foodchem.2010.12.091>
  130. Carazzone C, Mascherpa D, Gazzani G, Papetti A (2013) Identification of phenolic constituents in red chicory salads (*Cichorium intybus*) by high-performance liquid chromatography with diode array detection and electrospray ionization tandem mass spectrometry. *Food Chem* 138:1062–1071. <https://doi.org/10.1016/j.foodchem.2012.11.060>

131. Chisté RC, Mercadante AZ (2012) Identification and quantification, by HPLC-DAD-MS/MS, of carotenoids and phenolic compounds from the Amazonian fruit *Caryocar villosum*. *J Agr Food Chem* 60:5884–5892. <https://doi.org/10.1021/jf301904f>
132. El-Sayed M, Abbas FA, Refaat S, El-Shafae AM, Fikry E (2021) UPLC-ESI-MS/MS profile of the ethyl acetate fraction of aerial parts of *Bougainvillea* Scarlett O'Hara Cultivated in Egypt. *Egypt J Chem* 64:793–806. <https://doi.org/10.21608/ejchem.2020.45694.2933>
133. Venter P, Pasch H, De Villiers A (2019) Comprehensive analysis of tara tannins by reversed-phase and hydrophilic interaction chromatography coupled to ion mobility and high-resolution mass spectrometry. *Anal Bioanal Chem* 411:6329–6341. <https://doi.org/10.1007/s00216-019-01931-x>
134. Desta KT, Lee WS, Lee SJ, Kim YH, Kim GS, Lee SJ, Kim ST, Abd El-Aty A, Warda M, Shin HC (2016) Antioxidant activities and liquid chromatography with electrospray ionization tandem mass spectrometry characterization and quantification of the polyphenolic contents of *Rumex nervosus* Vahl leaves and stems. *J Sep Sci* 39:1433–1441. <https://doi.org/10.1002/jssc.201600018>
135. Zhou A, Kikandi S, Sadik OA (2007) Electrochemical degradation of quercetin: Isolation and structural elucidation of the degradation products. *Electrochem Commun* 9:2246–2255. <https://doi.org/10.1016/j.elecom.2007.06.026>
136. Wang H, Song L, Feng S, Liu Y, Zuo G, Lai F, He G, Chen M, Huang DJM (2013) Characterization of proanthocyanidins in stems of *Polygonum multiflorum* Thunb as strong starch hydrolase inhibitors. *Molecules* 18:2255–2265. <https://doi.org/10.3390/molecules18022255>
137. Meng X, Li H, Song F, Liu C, Liu Z, Liu S (2009) Studies on triterpenoids and flavones in *Glycyrrhiza uralensis* Fisch. by HPLC-ESI-MSn and FT-ICR-MSn. *Chin J Chem* 27:299–305. <https://doi.org/10.1002/cjoc.200990048>
138. Kashchenko NI, Olennikov DN, Chirikova NK (2021) Metabolites of Siberian raspberries: LC-MS profile, seasonal variation, antioxidant activity and thermal stability of *Rubus matsumuranus* phenolome. *Plants* 10:2317. <https://doi.org/10.3390/plants10112317>
139. Zemmour H, Ammar S, Boumendjel A, Messarah M, El Feki A, Bouaziz M (2019) Chemical composition and antioxidant activity of *Borago officinalis* L. leaf extract growing in Algeria. *Arab J Chem* 12:1954–1963. <https://doi.org/10.1016/j.arabjc.2014.11.059>
140. Llorent-Martínez EJ, Fernández-De Córdoba ML, Zengin G, Bahadori MB, Aumeeruddy MZ, Rengasamy KR, Mahomoodally MF (2019) *Parentucellia latifolia* subsp. *latifolia*: a potential source for loganin iridoids by HPLC-ESI-MSn technique. *J Pharm Biomed Anal* 165:374–380. <https://doi.org/10.1016/j.jpba.2018.12.025>
141. Aghakhani F, Kharazian N, Lori Gooini Z (2018) Flavonoid constituents of *Phlomis* (Lamiaceae) species using liquid chromatography mass spectrometry. *Phytochem Anal* 29:180–195. <https://doi.org/10.1002/pca.2733>
142. Jaiswal R, Müller H, Müller A, Karar MGE, Kuhnert N (2014) Identification and characterization of chlorogenic acids, chlorogenic acid glycosides and flavonoids from *Lonicera henryi* L. (Caprifoliaceae) leaves by LC-MSn. *Phytochemistry* 108:252–263. <https://doi.org/10.1002/rcm.4639>
143. Rini Vijayan KP, Raghu A (2019) Tentative characterization of phenolic compounds in three species of the genus *Embelia* by liquid chromatography coupled with mass spectrometry analysis. *Spectrosc Lett* 52:653–670. <https://doi.org/10.1080/00387010.2019.1682013>
144. Yasir M, Sultana B, Nigam PS, Owusu-Apenten R (2016) Antioxidant and genoprotective activity of selected cucurbitaceae seed extracts and LC-ESIMS/MS identification of phenolic components. *Food Chem* 199:307–313. <https://doi.org/10.1016/j.foodchem.2015.11.138>
145. Sánchez-Rabaneda F, Jáuregui O, Casals I, Andrés-Lacueva C, Izquierdo-Pulido M, Lamuela-Raventós RMJOMS (2003) Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *J Mass Spectrom* 38:35–42. <https://doi.org/10.1002/jms.395>

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