Phenolic profile, antioxidant activity, and active compounds of ethanolic extract and essential oil of *Calathea lutea* leaves

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RESEARCH ARTICLE

Abstract

The leaves of *Calathea lutea* (*C. lutea*), traditionally used in food packaging, represent an untapped source for natural antioxidants, although scientific studies on their functional properties are limited. This study evaluates the phenolic content, antioxidant activity, and chemical composition of ethanolic and essential oil extracts of *C. lutea* leaves. Leaves were subjected to extraction via cold maceration, Soxhlet extraction, and steam distillation. Among the tested fractions, the dichloromethane fraction demonstrated the highest phenolic content (58.47 mg gallic acid equivalent [GAE]/g extract) and the strongest antioxidant activity, as assessed by $ABTS^{*+}$ (ABTS radical cation) and 2,2-diphenyl-1-picrylhydrazyl (DPPH*) radical scavenging assays. An advanced analytical method based on liquid chromatography coupled with mass spectrometry was used, allowing the precise identification and quantification of the compounds present in the extracts revealing a diverse polyphenolic profile, while the study of volatiles identified compounds, such as fatty acids, terpenes, phenols, hydrocarbons, esters, and heterocyclic compounds, some of which may contribute to the leaf's characteristic aroma. These findings highlight the dichloromethane fraction's potential as a natural alternative to synthetic antioxidants in food applications, with efficacy comparable to standard references. This research provides the first comprehensive chemical characterization of *C. lutea* leaves in Colombia.

Keywords: bijao; bioactive compounds; CG-MS; HPLC-QTOF-MS/MS; natural extract

Introduction

Antioxidants are widely used in the food industry (Abeyrathne *et al.*, 2022). For years, chemically synthesized preservatives have been the protagonists. However, health organizations are urging consumers to be cautious with the use of synthetic antioxidants (Ejeh *et al.*, 2023; Mitterer-Daltoé *et al.*, 2021) because of the potential health risks. One major challenge in food preservation is lipid oxidation, which compromises food quality and shelf life (Shehata *et al.*, 2021) by generating off-flavors, color and texture changes, and potentially toxic oxidized compounds (Keykhosravy *et al.*, 2022; Serra *et al.*, 2020). Such is the case of synthetic antioxidants widely used in the food industry such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are associated with harmful and dangerous health effects because of their carcinogenic potential and toxicity (Bensid *et al.*, 2022; Inanli *et al.*, 2020).

This has led to a growing interest in natural antioxidants, valued for their safety profile, efficacy, and environmental sustainability (Bensid *et al.*, 2022). Natural extracts and oils derived from plant leaves, stems, roots, peels, and seeds, rich in bioactive compounds, have shown promising antioxidant potential in food products, positioning them as possible substitutes for synthetic antioxidants (Bensid *et al.*, 2022; Gulcin, 2020; Pateiro *et al.*, 2021). Among these natural sources, *Calathea lutea* (*C. lutea*), traditionally known as bijao, is widely distributed in the Colombian rainforest (Higuera Mora *et al.*, 2020) and has gained attention for its use in wrapping and preserving traditional foods.

While plant-derived antioxidants, such as green tea (*Camellia sinensis*) (Ahwan *et al.*, 2024; Hasan *et al.*, 2024), rosemary (*Rosmarinus officinalis*) (Athanasiadis *et al.*, 2024; Hoelscher *et al.*, 2024; Villanueva-Bermejo *et al.*, 2024) and turmeric (*Curcuma longa*) (Ballester *et al.*, 2023; Visakh *et al.*, 2023), are widely studied and recognized for their bioactive properties, *C. lutea* stands out as a lesser-known yet promising source of natural antioxidants. Unlike these well-researched plants, *C. lutea*, despite its traditional use in food preservation and its potential as an antioxidant, remains an underexplored source (Aguirre *et al.*, 2010; Chandran, 2020; Tomás *et al.*, 2010). There are significant gaps in the knowledge of the specific bioactive compounds of *C. lutea* and their functionalities. However, its unique chemical profile, traditional use as a packaging for food preservation, and distinctive aroma highlight it as a novel and sustainable alternative to conventional natural antioxidants. These characteristics not only underscore its potential in the food industry but also open new opportunities to develop safe and environment-friendly solutions.

Studies indicate that *C. lutea* contains phenolic compounds with antioxidant and antimicrobial properties (Aguirre *et al.* 2010; Apagüeño Arévalo and Tamani Guerra, 2020; Chandran, 2020; Robalino Pinedo and Torres Carrión, 2021; Tomás *et al.* 2010). Phenolic compounds, such as chlorogenic acid and rutin, previously identified in this plant, are associated with anti-inflammatory, anticancer, and cardioprotective effects (Gupta *et al.*, 2022; Satari *et al.*, 2021; Singh *et al.*, 2023). However, these studies remain limited in scope, often focused on general assessments of antioxidant properties without addressing specific applications or employing advanced analytical techniques that allow for a more comprehensive bioactive profile.

The aforementioned leads on the importance of considering that extracts of *C. lutea* can be regarded as a valuable natural source for practical applications in food preservation, proving useful in meat products, edible oils, and processed foods, where lipid oxidation poses a critical challenge affecting quality and shelf life. Moreover, its volatile chemical profile, associated with its aroma and antimicrobial properties, suggests its applications in edible coatings or biodegradable packaging, which could enhance food stability while reducing plastic waste and promoting sustainable strategies in the food industry.

This study aims to expand this knowledge through a detailed characterization of the phenolic and volatile compounds of *C. lutea* using advanced analytical techniques such as high-precision liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (HPLC-QTOF-MS/MS) in order to explore its potential as a natural agent in food preservation. This is the first study to identify several new compounds in *C. lutea* that are not reported before within the *Marantaceae* family, thereby expanding knowledge of its chemical profile. Given that *C. lutea* leaves are known for their distinctive aroma and flavor, commonly used in Colombian cuisine to impart a unique sensory profile to food (Calderón Morales and Mancera, 2020), exploring its volatile profile is essential to understand its dual role as a natural flavoring and preservative.

In this way, understanding the chemical composition and biological properties of natural extracts is essential to evaluate their efficacy and safety in food applications; therefore, a detailed characterization is essential for their optimal utilization in the industry (da Silva *et al.*, 2021; Gonfa *et al.*, 2020). In this context, the aim of this research was to evaluate the total phenol content, antioxidant activity, and active compounds of ethanolic extracts and essential oils of *C. lutea* leaves. By addressing these knowledge gaps, this study provides a scientific basis for the potential use of *C. lutea* as a natural additive to enhance food safety.

Materials and Methods

Selection and conditioning of vegetal material

The leaves of *C. lutea* were acquired from commercial establishments located in Bazurto market in the city of Cartagena, Bolivar. The leaves were disinfected by immersing in 100-ppm sodium hypochlorite for 2 min (Chaves *et al.*, 2020). The size of leaves was reduced to approximately 10 cm; these were weighed, freeze-dried (Biobase freeze-dryer model BK-FD10P), and the plant material was crunched (Hamilton Beach grinder, 80350/R). Cold maceration and Soxhlet extraction were the two methods used to obtain total extract. The essential oil was obtained by steam distillation. All reagents used were of analytical grade.

Obtaining total ethanolic extract (EtOH) of *C. lutea* leaves by cold maceration

The crunched vegetal material was extracted by cold maceration with ethanol (96% v/v), using a leaf: ethanol ratio of 1:10, with intermittent shaking, at 25±3°C for 3 days in the dark (Karim *et al.*, 2020). Successive extractions (solid–liquid) and filtration (Whatman No. 1) were performed until the material was exhausted. The filtrate was concentrated (Heidolph Hei-VAP Silver 3) to constitute total EtOH extract (Lv *et al.*, 2022; Rivera *et al.*, 2019) and stored in a refrigerator for further analysis and fractional process (Figure 1).

Total ethanolic extract (EtOH) of *C. lutea* leaves obtained by Soxhlet extraction

The methodology used was that of Alara *et al.* (2018, 2019) and Hirondart *et al.* (2020), with modifications. Crunched *C. lutea* leaves, 30 g, were placed inside a cellulose thimble and transferred to Soxhlet apparatus. According to a feed–solvent ratio of 1:20, 600 mL of ethanol were utilized using a heating mantle to subject the mixture to reflux during an extraction period of 2–3 h until exhaustion of the plant material. A rotary evaporator was used to concentrate the extract. It was stored under refrigeration for further analysis and fractional process (Figure 2).

Fractionation of EtOH of C. lutea *leaves*

Both total EtOH extracts obtained by maceration and Soxhlet extraction were subjected to fractional process (liquid–liquid separation) using equal volumes of hexane, dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc), in increasing order of solvent polarity, according to the methodology used by Asuquo and Udobi (2016) and Rivera *et al.* (2019), with adjustments. The fractions were collected separately and concentrated to dryness; the last layer, termed as the residual ethanol/water fraction ($EtOH/H_2O$), was also collected and freeze-dried.

Obtaining essential oil from *C. lutea* leaves by steam distillation

Calathea lutea leaves were subjected to steam distillation for approximately 3 h to obtain a leaves–CH_2Cl_2 ratio of 1:10 (Azmir *et al.*, 2013; Peng *et al.*, 2004).

Determination of Total Phenol Content (TPC)

The Folin–Ciocalteu method was used to determine total phenol content (Del-Toro-Sánchez *et al.*, 2014; Singleton *et al.*, 1999), with some modifications. In all, 30 µL of the sample (60–500 µg/mL) and 150 µL of Folin–Ciocalteu reagent (0.1 M) were added to each well of a microplate, and a negative control and a blank were included to each sample (Table S1). After 10 min, NA_2CO_3 (7.5% w/v) was added. After incubating the plate for 2 h at 25±3°C, the optical density (DO) was determined at 620 nm using a Multiskan EX microplate reader (Thermo Fisher Scientific, MA, USA). The results were reported as

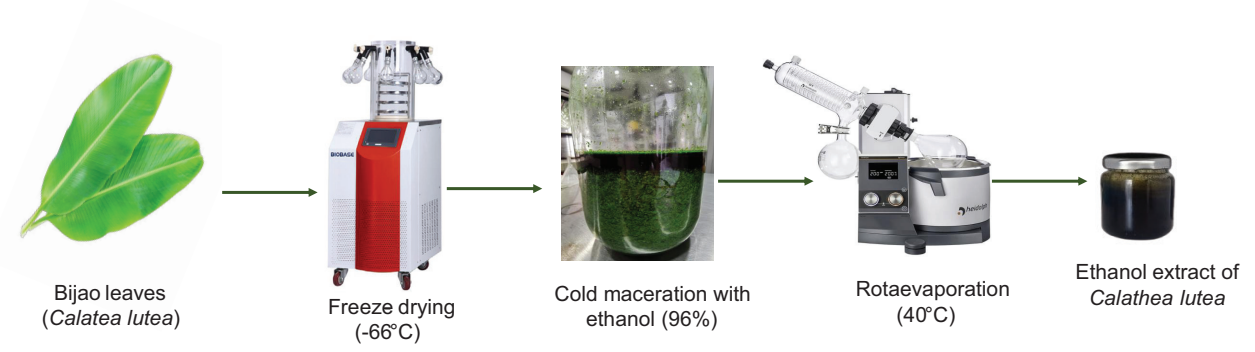

Figure 1. Total ethanolic extract (EtOH) of C. lutea leaves by cold maceration. (Author's elaboration).

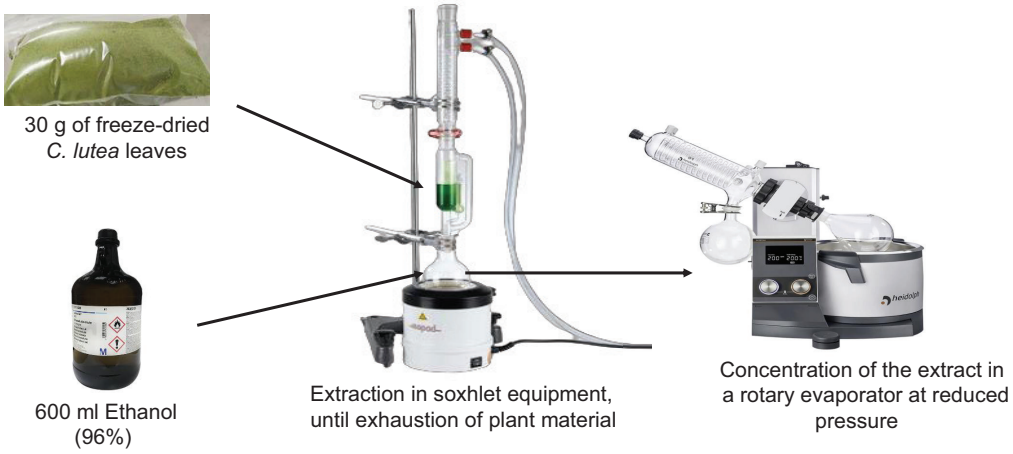

Figure 2. Total ethanolic extract (EtOH) of *C. lutea* leaves obtained by Soxhlet extraction. (Author's elaboration).

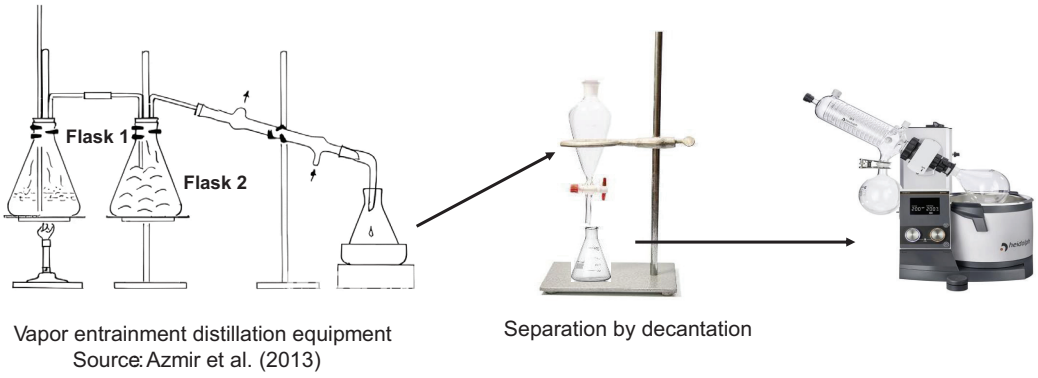

Figure 3. Essential oil from C. lutea leaves by steam distillation. (Author's elaboration).

mg gallic acid equivalent (GAE)/g extract (Rivera *et al.*, 2019). All analyses were performed in triplicate.

Determination of antioxidant activity of *C. lutea* leaf extracts, fractions, and essential oil

Free radical scavenging method ABTS+*

For this analysis, the method described by Re *et al.* (1999) was followed, with some modifications. The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{*+}$) was produced by the reaction ABTS (3.5 mM) with $K_2S_2O_8$ (1.25 mM) incubated at 25±3°C, protected from light, for 16 h. With the radical formed, we sought to obtain an absorbance value of 0.75–0.8 at 620 nm, for which the radical was diluted with ethanol. In each well of the microplate, 20 µL of the extracts, fractions, and essential oil to be evaluated (200–500 µg/mL) and 180 µL of $ABTS^{*+}$ solution were added; a negative control and a blank were also included for each sample (Table S2). The microplate was incubated, protected from light, at 25±3°C, for 30 min, after which absorbance was measured at 620 nm in a Multiskan EX (Thermo Scientific). The percentage scavenging of $ABTS^{*+}$ radical was calculated using Equation (1):

$$\%\ \text{of radical uptake ABTS}^{*+} = \frac{\text{Ab ABTS} - (\text{Ab M} - \text{Ab B})}{\text{Ab ABTS}} \times 100 \quad (1)$$

where:

Ab ABTS: negative control,
Ab M: sample absorbance,
Ab B: absorbance of the sample blank.

*Free radical scavenging method DPPH**

This analysis was performed according to the method described by Castro *et al.* (2019) and the standard described by Brand-Williams *et al.* (1995), with some modifications. In a microplate, 75 µL of the sample to be evaluated (200–500 µg/mL) and 150 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH*) solution were added to

each well, and a negative control and blank were included to each sample (Table S3). The microplate was incubated and protected from light at 25 ± 3°C for 30 min; then absorbance was measured at 515 nm in a Multiskan EX microplate reader (Thermo Scientific). The percentage scavenging of DPPH* radical was calculated according to Equation (2):

$$\% \text{ of uptake of the radical DPPH}^* = \frac{\text{Ab DPPH} - (\text{Ab M} - \text{Ab B})}{\text{Ab DPPH}} \times 100 \quad (2)$$

where:

Ab DPPH: negative control,
Ab M: sample absorbance,
Ab B: absorbance of the sample blank.

The extract, fraction, or essential oil with the highest percentage of radical scavenging was determined by determining its half-maximal inhibitory concentration (IC_{50}).

Identification of compounds present in *C. lutea* leaf extract and their active fractions by HPLC-QTOF-MS/MS

To identify the compounds present in *C. lutea* extracts, HPLC-QTOF-MS/MS was used as an analytical technique for the identification and quantification of complex compositions. This advanced method enables the separation, identification, and precise quantification of bioactive compounds in complex samples. This approach was essential for characterizing the phenolic profile of the extracts and fractions of *C. lutea* leaves.

Extracts and fractions were coded as follows:

E1: total EtOH extract, cold maceration,
E2: hexane fraction of E1),
E3: CH_2Cl_2 fraction of E1,
E4: EtOAc fraction of E1,
E5: (EtOH/H_2O residual fraction of E1,
E6: total EtOH extract, Soxhlet extraction,
E7: hexane fraction of E6,
E8: CH_2Cl_2 fraction of E6,
E9: EtOH/H_2O residual fraction of E6, and
E10: essential oil, steam distillation.

Of the total EtOH extract of *C. lutea* leaf (obtained by cold maceration [E1] and by Soxhlet extraction [E6]) and its active fractions (CH_2Cl_2 fraction [E3 and E8]), 1 mg of the sample was diluted in 1 mL of 50:50 water–acetonitrile mixture. The diluted sample was shaken until homogenized, followed by centrifugation at 16.000×g for 8 min; the supernatant was filtered through a 0.20-µm pore size membrane and transferred to an autosampler vial. A 1260 Infinity HPLC system (Agilent Technologies), coupled to a 6530-quadrupole time-of-flight (q-TOF) mass spectrometry detector, with electrospray ionization, operating in positive ionization mode, was used (Duran-Izquierdo *et al.*, 2022).

An EC-C18 column, particle size 2.7 µm, was used for separation, and a temperature of 40°C was maintained. Water (H_2O) with (A) 0.1% CH_2O_2 and (B) C_2H_3N with 0.1% CH_2O_2 was used as a mobile phase. The analysis was carried out with modifications based on a previous method (Duran-Izquierdo *et al.*, 2022) with an (A)–(B) ratio of 95:5, held for 1 min, then changed to 5:95 in 9 min, and maintained stable for 4 min; then changed to 100% C_2H_3N in 1 min and maintained stable for 3 min. Re-equilibration of the column was carried out by switching back to (A)–(B) ratio of 95:5 for 23 min and remained stable until 26 min. The flow rate was 0.3 mL/min and the injection volume was 5 µL.

The following mass detector conditions were established according to the methodology described and validated by Duran-Izquierdo *et al.* (2022): capillary voltage: +3.5 kV, nitrogen gas temperature: 320°C, drying gas flow rate: 8.0 L/min, nebulizer gas pressure: 35 psig, fragmenter voltage: 135 V, skimmer: 65 V, and optical coherence tomography radio frequency (OCT RF): 750 V. Tandem mass spectrometry (MS/MS) data acquisition mode was used to aid compound identification. The mass range in MS and MS/MS experiments was set at m/z 100–1,200 and 50–1,200 at 3 spectra/s, respectively. MS and MS/MS data were collected using the Agilent MassHunter Acquisition software (version 10.1). The obtained data were processed with Agilent MassHunter Qualitative Analysis 10.0. Peak annotations were performed using the METLIN, a database to characterize known metabolites with a mass error <10 ppm (metlin.scripps.edu) and the Global Natural Product Social Molecular Networking (GNPS) spectral library as described by Duran-Izquierdo *et al.* (2022) and Tamburini (2019).

Identification of volatile compounds of *C. lutea* leaf oil by gas chromatography–mass spectroscopy (GC-MS)

For the identification of volatile compounds, the methodology described and validated by Islam et al. (2020) and Padmini *et al.* (2020) was followed, with some modifications. A GC brand Agilent Technologies 6890 plus coupled to a MS brand Agilent Technologies 5973, with a DB-5MS column (60-m length × 0.25-mm diameter × 0.25-µm film thickness), stationary phase of 5%-phenyl-poly(methylsiloxane).

Pure helium as carrier gas (1 mL/min). 1 µL of the sample was injected in a split mode. Ionization was set at 70 eV. Temperatures of 250°C and 300°C were used for the

injector and ion source, respectively. The injector and ion source temperatures were 250°C and 300°C, respectively. The temperature ramp operated was as follows: 60°C sustained for 1 min and then increasing to 260°C at a rate of 15°C/min, with a run time 30 min.

The mass spectra of each component were analyzed by the team's database for comparison with the collection of spectra from the Wiley 7n.1 online library provided by the system. Those with a degree of correspondence >90% were selected for analysis. In addition, this tentative identification of compounds was also carried out by comparing the mass spectra with those recorded in the database of the National Institute of Standards and Technology (NIST2017) database (Harke *et al.*, 2021). For each component, the relative percentage was calculated according to the procedure described by Padmini *et al.* (2020) and Peng *et al.* (2004).

Statistical analysis

Results were reported as mean±standard deviation (SD). The statistical program IBM SPSS Statistics 25 was used; a one-way analysis was used for data analysis to establish significant differences followed by Tukey's test. For measurements, $P < 0.05$ was considered statistically significant.

Results and Discussion

Total phenolic content

The TPC of *C. lutea* extracts and fractions demonstrated significant variation depending on the solvent used, ranging from 17.27 mg GAE/g in the hexane fraction to 58.47 mg GAE/g in the CH_2Cl_2 fraction. This high TPC in the CH_2Cl_2 fraction suggested that phenolic compounds in *C. lutea* were extracted more efficiently with a moderately polar solvent, which aligned with the findings by Matrose *et al.* (2021), who reported enhanced solubility of lipophilic phenolic compounds in CH_2Cl_2 extracts of other plant species. This selective extraction ability of CH_2Cl_2 could explain the high concentration of phenols in this fraction, as it efficiently dissolves bioactive liposoluble phenolics, potentially enhancing antioxidant properties. In this regard, it is worth highlighting the study conducted by Li *et al.* (2023), who reported a high content of liposoluble diterpenoid phenols in CH_2Cl_2 extracts of *Callicarpa longissima* leaves. In addition, no significant differences in phenol content were found between cold maceration and Soxhlet maceration.

A study conducted by Oboh *et al.* (2016) on acetone extracts of *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* leaves, belonging to the same leaf family of *C. lutea*, reported lower TPC values of 12.5 mg GAE/g and 49.54 mg GAE/g, respectively. These differences suggested that *C. lutea* could possess a richer phenolic profile, particularly when extracted using CH_2Cl_2. This finding was further supported by Bhuyan *et al.* (2015), who observed similar TPC levels (58.4 mg GAE/g) in *Eucalyptus robusta* extracts using microwave-assisted extraction, which is known for effectively preserving phenolic compounds. It is worth mentioning that *Eucalyptus robusta* leaves have been recognized for having an important antioxidant activity, and their usefulness as a natural antioxidant food additive has been confirmed (Gullón *et al.*, 2019).

However, the TPC of *C. lutea* leaves observed in this study was lower than the values reported by other studies on ethanolic extracts of *C. lutea* (Chandran, 2020; Robalino Pinedo and Torres Carrión, 2021), where TPC reached up to 976.71 mg GAE/g. This discrepancy could result from variations in factors such as geographic origin, environmental conditions, plant maturity, and extraction methodologies, all of which influence the biosynthesis and accumulation of phenolic compounds, as well as drying and extraction methods (Biniari *et al.*, 2020; Dhibi *et al.*, 2022; Simonetti *et al.*, 2020). The essential oil obtained by steam distillation (SD) had the lowest TPC (24.77 mg GAE/g), which could be related to its limited antioxidant capacity, compared to phenol-rich extracts and fractions.

It is worth mentioning that the presence of phenolic compounds in *C. lutea* leaf extracts represents an additional benefit in terms of food safety. Previous studies have shown that these compounds exhibit antimicrobial and antifungal properties (Beya *et al.*, 2021; Kalogianni *et al.*, 2020; Zamuz *et al.*, 2021) by altering bacterial membrane integrity and inhibiting adhesion to surfaces, suggesting that these compounds could improve food safety and shelf life (Zamuz *et al.*, 2021). Overall, these findings highlight *C. lutea* as a promising source of phenolic antioxidants, which may serve as natural alternatives to synthetic preservatives in food products. Future research may further investigate the stability and efficacy of these phenolic compounds in real food matrices to confirm their practical application in food preservation.

Antioxidant activity of extracts, fractions, and essential oil of *C. lutea* leaves

The antioxidant activity of the extracts, fractions, and essential oil of *C. lutea* leaves was evaluated using the DPPH* and $ABTS^{*+}$ assays, two widely accepted methods for measuring free radical scavenging capacity. The results showed a significant variation in antioxidant

Table 1. Total Phenol Content (TPC) and antioxidant capacity (percentage of ABTS^{*+} and DPPH*) of the extracts, fractions, and essential oil of *C. lutea* leaf.

Type of extract	Method of extraction	TPC (mg GAE/g extract)	Percentage of scavenging of radical ABTS^{*+}			Percentage of scavenging of radical DPPH*		
			200 (µg/mL)	350 (µg/mL)	500 (µg/mL)	200 (µg/mL)	350 (µg/mL)	500 (µg/mL)
Total extract EtOH	Cold maceration	24.68 ± 0.66^{b,c}	40.01±0.23^{d,C}	43.13±0.43^{b,B}	47.41±1.08^{c,A}	57.09±1.24^{b,c,C}	62.03±1.01^{b,B}	73.86±2.82^{c,A}
	Soxhlet extraction	26.27 ± 1.60^{b}	41.46±0.75$^{b-d,B}$	40.28±0.37^{b,c,B}	42.85±0.72^{d,A}	43.98±0.59^{d,C}	46.52±0.54^{c,B}	48.29±0.55^{d,A}
Fraction of hexane	Cold maceration	17.27 ± 1.84^{d}	25.71±0.86^{f,C}	29.04±0.77^{e,B}	35.35±0.55^{e,A}	21.3±1.34^{f,C}	24.92±0.75^{f,B}	28.36±1.03^{f,A}
	Soxhlet extraction	18.91 ± 3.92^{c,d}	37.19±0.43^{e,A}	37.11±1.64^{c,d,A}	36.67±2.18^{e,A}	34.09±0.89^{e,B}	43.25± 1.33^{d,A}	43.58±1.72^{e,A}
Fraction of CH_2Cl_2	Cold maceration	58.47 ± 3.67^{a}	46.03±1.05^{a,C}	52.1±2.79^{a,B}	71.89±1.71^{a,A}	70.8±1.23^{a,B}	83.52±1.25^{a,A}	87.18±1.97^{a,A}
	Soxhlet extraction	52.82 ± 2.25^{a}	41.62±0.55$^{b-d,B}$	44.0±0.64^{b,B}	53.26±2.63^{b,A}	60.54±2.18^{b,B}	61.91±0.51^{b,B}	79.35±1.75^{b,A}
Fraction of EtOAc	Cold maceration	28.87 ± 2.82^{b}	42.44±0.56^{b,c,C}	48.3±2.26^{a,B}	55.22±1.41^{b,A}	54.05±1.22^{a,C}	64.04±1.42^{b,B}	73.06±1.13^{c,A}
Residual frac.EtOH/H_2O	Cold maceration	27.61 ± 1.60^{b}	40.54±0.72^{c,d,B}	40.31±0.42^{b,c,B}	43.38±0.35^{c,d,A}	43.82±1.6^{d,B}	44.68±0.68^{c,d,A,B}	47.84±1.59^{d,e,A}
	Soxhlet extraction	23.65 ± 0.78^{b,c,d}	42.87±0.12^{b,A}	42.76±0.68^{b,A}	43.15±1.62^{c,d,A}	44.19±0.34^{d,B}	45.35±1.32^{c,d,B}	48.58±1.03^{d,A}
Essential oil	DAV	NA	24.77±1.01^{f,C}	33.77±0. 16^{d,B}	38.19±1.83^{e,A}	37.07±1.54^{e,B}	37.92±0.5^{e,B}	43.52±0.56^{e,A}

DAV: steam entrained distillation; GAE: gallic acid equivalents; CH_2Cl_2: dichloromethane; EtOAc: ethyl acetate; EtOH/H_2O: ethanol/water.
Results represent mean ± SD. NA: No activity (very low values could not be measured accurately).
Values with different superscript capital letters within the same row indicate significant differences ($P < 0.05$) between extract/fraction concentrations.
Values with different superscript lowercase letters within the same column indicate significant differences ($P < 0.05$) between extraction methods and solvents.

capacity among the fractions (see Table 1, and Figure S1 and S2), which is attributed to the diversity of bioactive compounds present and different polarities of the extraction solvents used (Monteiro *et al.*, 2020; Muzolf-Panek and Stuper-Szablewska, 2021).

Among the fractions analyzed, CH_2Cl_2 fraction exhibited the highest antioxidant activity in both assays, which aligned with its high TPC. This result suggested a positive correlation between TPC and the antioxidant capacity of the fraction, supporting the hypothesis that phenolics play a fundamental role in neutralizing free radicals. The superior antioxidant activity of the CH_2Cl_2 fraction may be due to its ability to extract lipophilic phenolic compounds, which typically have high antioxidant power (Erenler *et al.*, 2019; Li *et al.*, 2023; Matrose *et al.*, 2021).

However, higher percentage of radical scavenging was evidenced in the extracts, fractions, and essential oil by DPPH* assay. These discrepancies observed between the values obtained with DPPH* and ABTS^{*+} assays can be explained by differences in the reaction mechanisms involved and the affinity of each method for specific types of antioxidant compounds (Rumpf *et al.*, 2023; Wołosiak *et al.*, 2021). In this regard, previous studies, such as those done by Chaves *et al.* (2020), highlighted that the methods do not always exhibit the same sensitivity or capacity to discriminate the antioxidant activity of extracts from different plant species. The authors reported that the antioxidant activity values quantified through DPPH and reducing power (RP) assays were higher than those obtained by ABTS and Ferric Reducing Antioxidant Power (FRAP), and these values varied between species. Therefore, the ranking or categorization of these species was different depending on the method used. According to the study, the DPPH method yielded antioxidant activity values that were between 1.5 and 18.4 times higher than those obtained with the ABTS method, depending on the plant extract evaluated. In this context, the higher values obtained with DPPH, compared to ABTS, in the present study are attributed to its greater affinity for lipophilic compounds. This is particularly relevant, given that the extract studied contains a significant proportion of lipophilic antioxidants, such as terpenoids and certain antioxidant fatty acids, which appear to have reacted more efficiently with DPPH* radical compared to the $ABTS^{+\bullet}$ cation. It is worth emphasizing that these findings reinforce the importance of using multiple methods for a comprehensive evaluation of antioxidant activity.

It is observed in Table 1 that both total ethanolic extract and residual EtOH/H_2O fraction showed moderate antioxidant activity, compared to the CH_2Cl_2 and EtOAc fractions, which was consistent with the ability of ethanol to extract a mixture of phenolic and non-phenolic compounds. The lower antioxidant activity of hexane fraction

could be attributed to its low content of total phenols, since compounds extracted with hexane tended to be less polar and, in many cases, lacked antioxidant activity. The above was consistent with Ayodeji *et al.* (2016) and Hamid *et al.* (2017), who identified lower phenol content and antioxidant activity in hexane-derived extracts of *Thaumatococcus daniellii* leaves. These findings highlight the limited ability of hexane to extract antioxidant phenolic compounds because of its non-polar nature.

In this sense, as reported by Farahmandfar *et al.* (2017), who indicated that at 200-µg/mL BHA, commonly used as a synthetic antioxidant in food, scavenged approximately 76.83%, a few points above the CH_2Cl_2 fraction obtained in the present investigation (70.8%) at the same concentration. Thus, this fraction could be considered as a possible natural source of antioxidants for potential use in the food industry. This approach highlights the practical relevance of the findings by exploring natural sources as alternatives to synthetic antioxidants.

On the other hand, the essential oil of *C. lutea*, obtained by steam distillation, showed the lowest antioxidant activity among all samples. This result is consistent with the volatile nature of compounds present in natural essential oils from leaves, which usually include monoterpenes and sesquiterpenes (Kokilananthan *et al.*, 2022), which possess lower antioxidant power compared to phenols (Gutiérrez-del-Río *et al.*, 2021; Souza *et al.*, 2022). Although some essential oils show moderate antioxidant properties, the low concentration of phenols and other high molecular weight compounds in *C. lutea* essential oil could explain its limited antioxidant activity, which restricts its use in applications where significant antioxidant protection is required. No research on *C. lutea* leaf essential oil was discovered.

Overall, the results suggest that the extracts, fractions, and essential oil, especially the CH_2Cl_2 fraction, are good free radical scavengers and probably can inhibit lipid peroxidation, of utmost importance, in the food industry. It should be noted that lipid oxidation is considered one of the main causes of quality deterioration in food (Bayram and Decker, 2023; Wu *et al.*, 2022), as it is responsible for the rancidity or unpleasant taste and odor of foods, in addition to decreasing their nutritional value and reducing their shelf life (Geng *et al.*, 2023; Wang *et al.*, 2023), which ultimately makes the product unacceptable for human consumption (Ramanathan *et al.*, 2020; Wang *et al.*, 2023).

On the other hand, it is important to mention that the antioxidant activity of the extracts, fractions, and essential oil increased proportionally to the concentration. In this regard, at the highest concentration tested for the extract and its fractions (500 µg/mL), significant differences were evident between extraction methods, particularly in the total EtOH extract and the CH_2Cl_2 fraction, whose best results were observed with the cold maceration method, which may be because a prolonged increase in extraction temperature, affecting the loss of polyphenols. This is reinforced by Alara *et al.* (2019), who investigated the *in vitro* antioxidant activity of ethanolic extracts of *Vernonia cinerea* leaves, highlighting that ascorbic acid had a higher antioxidant activity than the extract, possibly because Soxhlet extraction has a localized heating effect, which could have degraded some compounds responsible for this activity in the extract (Daud *et al.*, 2022; Osorio-Tobón, 2020). However, it was evident that these extracts also possessed antioxidant activity, and therefore *V. cinerea* leaf extract can potentially be used as a natural antioxidant.

Now, considering the fact that lower IC_{50} values indicate a stronger free radical scavenging activity, the IC_{50} of CH_2Cl_2 fraction was calculated by the DPPH* radical scavenging method (Figure S3), showing a nonlinear response to concentration, with an IC_{50} of 60. 8 µg/mL (95% CI: 42.68–86.63 µg/mL), which was lower than that exhibited by the water-soluble vitamin E analogue, Trolox (IC_{50} = 35.6 µg/mL), although it was within the same order of magnitude (Figure S4), indicating good antioxidant activity of CH_2Cl_2 fraction.

It is worth noting that CH_2Cl_2 fraction showed antioxidant capacity comparable to that reported by Chandran (2020) in his study on the ethanolic extract of *C. lutea*, with an IC_{50} of 46.9 ppm. Although this value is slightly higher than that of ascorbic acid (IC_{50} of 37.1 ppm), the author emphasized that *C. lutea* possesses significant antioxidant properties and potential for further research. On the other hand, synthetic antioxidants used in the food industry, such as BHA (36.1 mg/L), BHT (35.9 mg/L), ascorbic acid (36.8 mg/L), and α-tocopherol (41.7 mg/L) (Ojekale, 2013), exhibit a higher radical scavenging capacity than the CH_2Cl_2 fraction, although within a similar range.

It is essential to highlight that excessive or inappropriate use of synthetic antioxidants, such as BHT (E No. 321) and BHA (E No. 320), common phenolic additives in the food industry, is linked to adverse effects, including carcinogenicity, cytotoxicity, oxidative stress induction, and endocrine disruptions (Xu *et al.*, 2021). In this context, several studies emphasized the importance of seeking safer, nontoxic, and environment-friendly alternatives (Esazadeh *et al.*, in press; Zhang *et al.*, 2023), with the aim of using these compounds rationally or, ideally, reducing their presence in the diet (Esazadeh *et al.*, in press).

Relationship between TPC and antioxidant capacity

A clear correlation was observed between TPC and antioxidant capacity (percentage scavenging of the $ABTS^{*+}$

and DPPH* radicals) in *C. lutea* extracts and fractions (Figure 4), especially in CH_2Cl_2 fraction, which showed both the highest TPC and the highest antioxidant activity in DPPH and ABTS assays. These findings support the role of phenolic compounds as major contributors to antioxidant activity, probably because of their ability to donate hydrogen atoms or electrons, thus neutralizing free radicals (Dehimat *et al.*, 2021; Hajlaoui *et al.*, 2022; Mejia *et al.*, 2020).

However, some variations were observed; for example, the EtOH/H_2O residual fraction showed moderate antioxidant activity despite its relatively low TPC, so it was not possible to establish a linear correlation for both assays (R^2: 0.65 for DPPH, and R^2: 0.76 for ABTS). Although several studies have reported a strong positive correlation between TPC and antioxidant activity (Dehimat *et al.*, 2021; Hajlaoui *et al.*, 2022), this trend does not always hold and may be related to the presence of non-phenolic antioxidants, such as carotenoids and ascorbic acid, among others (Quintana *et al.*, 2019).

It should also be considered that the antioxidant capacity of a compound or extract depends not only on the total phenol content but also on the composition and chemical structure of the phenols present in the sample, the number of hydroxyl groups attached to the aromatic ring and their location and orientation therein (Chen *et al.*, 2020; Mejia *et al.*, 2020; Parcheta *et al.*, 2021).

Essential oil obtained by steam distillation showed the lowest TPC and antioxidant capacity, probably because of the predominance of volatile compounds, such as monoterpenes and sesquiterpenes, which generally exhibit lower antioxidant activity than phenolic compounds. This agreed with the results of other studies on essential oils, where the limited presence of phenols was associated with lower antioxidant capacity (Molnar *et al.*, 2017). These results reinforce the idea that, while phenolic content is a significant factor in antioxidant activity, the total antioxidant profile also depends on the type and abundance of other bioactive compounds within the extract. As explained by Jerônimo *et al.* (2021), Kamal *et al.* (2022), and Masyita *et al.* (2022), the antioxidant activity of essential oil could be attributed to terpenes (such as terpinen-4-ol, thymol, and phytol, among others, detected by GC-MS); even, it has been reported that esters (also detected by GC-MS, hexadecanoate methyl ester) present in essential oils could act as antioxidants (Leblebici *et al.*, 2012).

Nonetheless, it is suggested to highlight the alternative uses of essential oil, such as its application as a flavoring agent in the food, cosmetic, or pharmaceutical industries. This proposal is supported by the presence of volatile compounds with unique aromatic and bioactive properties, which could provide added value even with a low phenolic content. These considerations not only broaden the practical implications of the findings but also emphasize the multifunctional potential of the essential oil for industrial applications and future research.

On the other hand, the positive relationship between TPC and antioxidant activity observed in *C. lutea* extracts and fractions underscores the potential of phenol-rich fractions, such as CH_2Cl_2, as natural antioxidants. The results highlight that maximizing the antioxidant potential of plant extracts involves not only the extraction of phenols but also understanding how different compounds, extraction solvents, and methods interact to enhance antioxidant properties. Future studies could explore the synergistic effects between phenols and other bioactive compounds to better elucidate their collective role in antioxidant capacity, which could broaden the applicability of *C. lutea* extracts in the food and pharmaceutical industries.

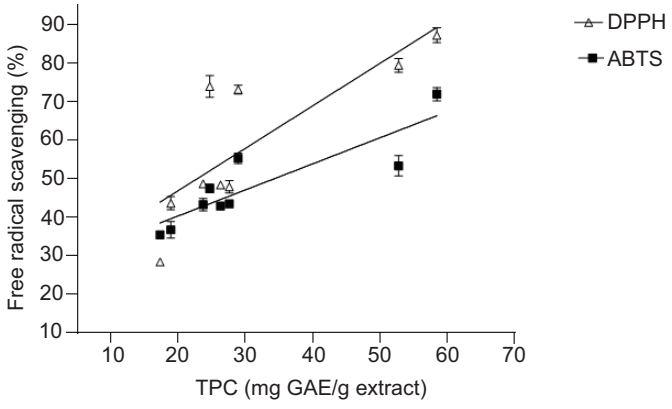

Figure 4. ABTS (■) and DPPH (Δ) free radical scavenging percentage evaluated at 500 µg/mL vs TPC of *C. lutea* extracts and their fractions.

Compounds tentatively identified in the extracts of *C. lutea* leaf and their active fractions by HPLC-QTOF-MS/MS

In this study, total EtOH extracts (E1 and E6) and CH_2Cl_2 fractions (E3 and E8) were used for analysis because of their representative antioxidant activity.

HPLC-QTOF-MS/MS analysis of *C. lutea* extracts revealed a wide diversity of compounds with potential bioactive properties, highlighting the complexity and richness of the chemical profile of this plant. Notably, several phenolic compounds, flavonoids, fatty acids, and terpenes were tentatively identified, with some fractions exhibiting a higher abundance of these compounds, correlating with their elevated antioxidant activities. This suggests that specific extraction solvents not only influence the yield of phenolic compounds but also selectively enrich bioactive compounds that contribute to the overall functionality of the extract.

As seen in Table 2, 23 compounds in *C. lutea* were annotated: alkaloid (1), terpenoids (4), phenolic acids (6), and flavonoids (12); their biological properties are widely commented on, which may justify the use of *C. lutea* in the food industry (see Table 3). The extracted ion chromatograms (EIC) for extracts and fractions obtained in positive ion mode are presented in Figures S5–S8. The results of the fragment spectra (MS/MS) are presented in Figure S9.

The results indicate that the main components of *C. lutea* leaf extract and its fractions are polyphenolic compounds, which corroborate previous findings obtained in the preliminary phytochemical analysis performed, which were also reported by Aguirre *et al.* (2010) and Tomás *et al.* (2010). It is worth mentioning that these investigations focused only on the qualitative determination of minerals and the main secondary metabolites of *C. lutea* leaf, the latter by the Cain–Bohmann method, with modifications (Tomás *et al.*, 2010). Similarly, in Peru, Aguirre *et al.* (2010) based their research only on the separation, identification, and quantification of tannins from *C. lutea* leaf.

Among the phenolic compounds identified, chlorogenic acid, rutin, and luteolin stand out. Chlorogenic acid, known for its potent antioxidant and anti-inflammatory properties, has been documented in related species within the *Marantaceae* family (Abdullah *et al.*, 2008). Rutin and luteolin, widely known for their radical scavenging and anti-inflammatory properties, further enhance the value of *C. lutea* extracts, indicating potential applications in the prevention of oxidative stress. The presence of these bioactive phenols is consistent with the strong antioxidant activity observed in DPPH and ABTS assays, especially in CH_2Cl_2 fraction, which may have selectively concentrated these hydrophobic phenols.

It is important to note that, of the 23 compounds identified in the present study, five were reported in the literature of *Calathea* and/or *Marantaceae*: chlorogenic acid and rutin (Abdullah *et al.*, 2008), cyanidin-3-glucoside (Mizuno *et al.*, 2022; Rozali *et al.*, 2016), vitexin (apigenin-8-C-glucoside), and luteolin (Saldaña, 2019; Williams and Harborne, 1977). Other compounds were not identified in the previous studies on *Calathea* and/or *Marantaceae*. Therefore, this was the first time that these are reported; however, further studies are required to confirm their presence.

It should be noted that flavonoids, luteolin, tricin, and chrysoeriol, three major peaks in the CH_2Cl_2 fraction obtained from E1 (see Figure S6) characterized by their high and recognized antioxidant activity, could be related to the outstanding radical scavenging capacity of this fraction; however, the influence of other compounds and possible synergistic mechanisms are not ruled out.

The chemical profile of *C. lutea* leaf extract, rich in antioxidant compounds, such as quinic acid, chlorogenic acid, isoschaftoside, rutin, cyanidin-3-glucoside, vitexin, narcisine, synaptic acid, ferulate, syringaldehyde, luteolin, tricin, chrysoeriol, coumarate, and syringetin-3-o-glucoside, reveals a high added value of this extract in the food industry for the development of natural antioxidants. Therefore, in addition to contributing to improve the sensory quality of the products, by retarding lipid oxidation, the leaf extract of *C. lutea* could also act as a natural preservative, prolonging the shelf life of foods and reducing the need for synthetic additives. Furthermore, *in vitro* studies suggest that several of these compounds could inhibit bacterial growth as reported in Table 3, contributing to improve the microbiological safety of foods.

Volatile compounds identified in *C. lutea* leaf essential oil by GC-MS

To date, the characterization of volatile compounds present in the leaf of *C. lutea*, possibly related to its aroma, has not yet been reported in literature. Consequently, the present report appears to be the first scientific study. Figure 5 and Table 4 summarize the compounds identified in the oil. The total ion chromatogram in Figure 5 details the retention time and the signals corresponding to the active compounds present.

GC-MS analysis of *C. lutea* leaf essential oil revealed a complex profile of volatile compounds, predominantly comprising monoterpenes, sesquiterpenes, and fatty acids. These volatile compounds not only contribute to the characteristic aroma of *C. lutea* but also enhance its applications in food preservation because of their diverse bioactivities.

Table 2. Results of tentative identification of secondary metabolites of *C. lutea* total extracts and CH_2Cl_2 fractions by HPLC-QTOF-MS/MS (ESI^+).

No.	Tentative notation	Molecular formula	Exact mass	Retention Time (min)				Type of compound
				TR_E1	TR_E3	TR_E6	TR_E8	
1.	Quinic acid	$C_7H_{12}O_6$	192.0639	3.537			3.537	Phenolic acid (shikimic acids and derivatives)
2.	Chlorogenic acid	$C_{16}H_{18}O_9$	354.0950	9.687		9.609		Phenolic acid (cinnamic acids and their derivatives)
3.	Isoschaftoside	$C_{26}H_{28}O_{14}$	564.1479	9.830			9.854	Flavonoid (flavone)
4.	(2R)-4-[(1S)-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-il]-2-butyl beta-D-glucopyranoside	$C_{19}H_{32}O_8$	388.2100	1.128	10.114	10.100		Terpenoids
5.	Routine	$C_{27}H_{30}O_{16}$	610.1534	10.285		10.225		Flavonoid (flavonols)
6.	2-(3,4-dihydroxyphenyl)-5-hydroxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(3,4,5-trihydroxy-oxan-2-il) oxymethyl]oxan-2-il] oxy-cromen-4-ona	$C_{26}H_{28}O_{15}$	580.1428	10.302		10.263	10.302	Flavonoids (flavones)
7.	Cyanidin-3-glycoside	$C_{21}H_{21}ClO_{11}$	449.1083	10.332	10.4810	10.513	10.51	Flavonoid
8.	Vitexin	$C_{21}H_{20}O_{10}$	432.1056	10.436	10.4380	10.436	10.405	Flavonoid
9.	Narcisine	$C_{28}H_{32}O_{16}$	624.1690	10.494		10.494	10.529	Flavonoid
10.	5-[6-[[3,4-dihydroxy-4-(hydroxymethyl)oxolan-2-il]oxymethyl]-3,4,5-trihydroxy-oxan-2-il] oxy-4-(3,4-dihydroxyphenyl)-7-methoxychromen-2-ona	$C_{27}H_{30}O_{15}$	594.1585	10.65		10.633	10.667	Flavonoid 4-phenylcoumarin
11.	4-(3-hydroxybutyl)-3,5,5-trimethylcyclohex-3-in-1-ol	$C_{13}H_{22}O_2$	212.1776	10.672	10.686		10.671	Terpenoids
12.	(2R,3S,4S,5R,6R)-2-(hydroxymethyl)-6-[4-(4-hydroxy-2,6,6-trimethylcyclohexen-1-il)butan-2-iloxi]oxano-3,4,5-triol	$C_{19}H_{34}O_7$	374.2305	10.749		10.791		Terpenoids
13.	4-(2,6,6-Trimethyl-4-oxo-2-cyclohexen-1-il)-2-butyl beta-D-glucopyranoside	$C_{19}H_{32}O_7$	372.2148	11.095	11.0180	11.056		Terpenoids
14.	Synaptic acid	$C_{11}H_{12}O_5$	224.0685	11.096	11.1360		11.112	Phenolic acid (cinnamic acids and their derivatives)
15.	Ferulate	$C_{10}H_{10}O_4$	194.0579	11.25	11.2490	11.173		Phenolic acid (cinnamic acids and their derivatives)
16.	Syringaldehyde	$C_9H_{10}O_4$	182.0579	11.366	11.3290	11.343	11.343	Phenolic acid (cinnamic acids and their derivatives)
17.	Luteolin	$C_{15}H_{10}O_6$	286.0477	12.018	12.0210	12.018	12.014	Flavonoid (flavonols)
18.	Tricin	$C_{17}H_{14}O_7$	330.0740	12.713	12.7150	12.616	12.72	Flavonoid (flavones)
19.	Chrysoeriol	$C_{16}H_{12}O_6$	300.0634	12.732	12.7920	12.754	12.827	Flavonoid (flavones)
20.	Ethyl beta-carboline-3-carboxylate	$C_{14}H_{12}N_2O_2$	240.0899	13.033	13.024	13.032	13.067	Alkaloids
21.	7-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-il] oxy-chromen-4-ona	$C_{23}H_{24}O_{12}$	492.1268		10.9470	10.910		Flavonoid
22.	4-Coumarate	$C_9H_8O_3$	164.0473		11.0760	11.114		Phenolic acid (cinnamic acids and their derivatives)
23.	Syringetin-3-O-glycoside	$C_{23}H_{24}O_{13}$	508.1217			10.726	10.725	Flavonoid (flavonols)

Table 3. Characteristics of secondary metabolites of total extracts of *C. lutea* and CH_2Cl_2 fractions tentatively identified by HPLC-QTOF-MS/MS.

No.	Tentative notation	Associated activity	Plants in which these are identified
1.	Quinic acid	Antioxidant (Aree, 2019; Karaman *et al.* 2021) antibacterial, antiviral, anticancer, cytotoxic anti-inflammatory, anti-inflammatory (Aree, 2019)	leaves of *Zanthoxylum bungeanum Maxim* (Yang *et al.*, 2013). Leaves of *Cupressus macrocarpa* (Attallah *et al.*, 2021)
2.	Chlorogenic acid	Antioxidant (Abdullah *et al.*, 2008; Gupta *et al.*, 2019)	Leaves of *Thaumatococcus danielli* (*Marantaceae*) (Abdullah *et al.*, 2008), leaves of *Moringa oleifera* (Alam *et al.*, 2020), leaves of *Zanthoxylum bungeanum Maxim* (Yang *et al.*, 2013)
3.	Isoschaftoside	Antioxidant (Quispe *et al.*, 2014) and antimicrobial (Li *et al.*, 2021; Serino *et al.*, 2021)	Sugarcane (Quispe *et al.*, 2014). Hojas de *Achillea Wilhelmsii* (Serino *et al.*, 2021). Fig leaves (*Ficus carica* L.) TR 13.2 (Li *et al.*, 2021)
4.	(2R)-4-[(1S)-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-il]-2-butyl beta-D-glucopyranoside	-	Leaves of *Vanda* spp. and *Cattleya* spp. (Lima *et al.*, 2022)
5.	Routine	Antioxidant (Wei *et al.*, 2022), inflammatory, anticancer, neuroprotective, antiproliferative, and antimetastatic (Abdullah *et al.*, 2008; Wadher *et al.*, 2022)	Leaves of *Calathea* (Abdullah *et al.*, 2008), *Thaumatococcus daniellii* (*Marantaceae*) (Fadahunsi *et al.*, 2021), *Moringa oleifera* (Alam *et al.*, 2020), leaves of *Zanthoxylum bungeanum Maxim* (Yang *et al.*, 2013), leaves of *Hippophae rhamnoides* y *H. tibetana* (Wei *et al.*, 2022), leaves of eucalipto (Gullón *et al.*, 2019)
6.	2-(3,4-dihydroxyphenyl)-5-hydroxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(3,4,5-trihydroxy-oxan-2-il) oxymethyl] oxan-2-il] oxy-chromen-4-ona	Related to antioxidant activity (Pires *et al.*, 2021)	Flowers of the species *Impatiens* (Pires *et al.*, 2021)
7.	Cyanidin-3-glycoside	Antioxidant (Mahnashi *et al.*, 2022; Paunović *et al.*, 2017)	Leaves of *Calathea* (Mizuno *et al.*, 2022; Rozali *et al.*, 2016), leaves of *Zea mays*, leaves of black currant (*Ribes nigrum* L.) (Paunović *et al.*, 2017)
8.	Vitexin	Antioxidant (Mahnashi *et al.*, 2022; Paunović *et al.*, 2017; Shodehinde and Oboh, 2013), and antibacterial (Attallah *et al.*, 2021)	Banana leaves (*Musa paradisiaca*) (Shodehinde and Oboh, 2013), leaves of *Cupressus macrocarpa* (Attallah *et al.*, 2021)
9.	Narcisine	Antioxidant (Liu *et al.*, 2023; Wei *et al.*, 2022)	Leaves of *Hippophae rhamnoides* y *H. tibetana* (Wei *et al.*, 2022), cassava leaves (*Manihot escylenta Crantz*), flowers of *Flos sophorae Immaturus*, leaves of *Gynura divaricata* (Liu *et al.*, 2023)
10.	5-[6-[[3,4-dihydroxy-4-(hydroxymethyl) oxolan-2-il] oxymethyl]-3,4,5-trihydroxy -oxan-2-il]oxy-4-(3,4-dihydroxyphenyl)-7-methoxychromen-2-ona	-	Leaves of *Hintonia standleyana and Hintonia latiflora* (Cristians *et al.*, 2013; National Center for Biotechnology Information [NCBI], 2023)
11.	4-(3-hydroxybutyl)-3,5,5-trimethylcyclohex-3-in-1-ol	-	-
12.	(2R,3S,4S,5R,6R)-2-(hydroxymethyl)-6-[4-(4-hydroxy-2,6,6-trimethylcyclohexen-1-il) butan-2-iloxi] oxane-3,4,5-triol	-	Leaves of *Vanda* spp. and *Cattleya* spp. (Lima *et al.*, 2022)
13.	4-(2,6,6-Trimethyl-4-oxo-2-cyclohexen-1-il)-2-butyl beta-D-glucopyranoside	Antioxidant (Prasniewski *et al.*, 2021)	Leaves of *Syzygium malaccense* (Prasniewski *et al.*, 2021)
14.	Synaptic acid	Antioxidant (Pateiro *et al.*, 2023; Pico *et al.*, 2022)	Kale leaves (Pateiro *et al.*, 2023), blueberry (Pico *et al.*, 2022), leaves of *Anacardium occidentale* (Sassi *et al.*, 2022), mint (Lin *et al.*, 2022), *Moringa oleifera* (Mumtaz *et al.*, 2021.) and strawberry (Lin *et al.*, 2020)

(*continues*)

Table 3. Continued.

No.	Tentative notation	Associated activity	Plants in which these are identified
15.	Ferulate	Antioxidant (Chen et., 2022; Zhang *et al.*, 2022) and antimicrobial (Chen *et al.*, 2022)	Leaves of *Rubus corchorifolius* (Chen et al., 2022), *Suaeda* (Wu *et al.*, 2013)
16.	Syringaldehyde	Antioxidant, antibacterial (Yancheva *et al.*, 2016), antifungal, and antiparasitic (Yancheva *et al.*, 2016)	Leaves of *Manihot esculenta* and *Magnolia officinalis* (Shahzad *et al.*, 2020)
17.	Luteolin	Antioxidant (Chau *et al.*, 2023; Malacaria *et al.*, 2022; Shimul *et al.*, 2022.) and antimicrobial (Shimul *et al.*, 2022)	Leaves of *C. lutea* (Saldaña, 2019; Williams and Harborne, 1977). Banana leaves (*Musa paradisiaca*) (Shodehinde and Oboh, 2013) oregano and thyme (Shimul *et al.*, 2022)
18.	Tricin	Antioxidant and antimicrobial activity (Bouzayani *et al.*, 2022), anticancer, and cardioprotective agents (Li *et al.*, 2022)	Wheat leaves (*Triticum dicoccum* L.) (Zheng *et al.*, 2021), bamboo leaves (Jiao *et al.*, 2007)
19.	Chrysoeriol	Antioxidant (Kim *et al.*, 2021)	*Coronopus didymus* (Muzammil *et al.*, 2022), leaves of *Capsicum chinense* (Herrera-Pool *et al.*, 2021; Oney-Montalvo *et al.*, 2020)
20.	Ethyl beta-carboline-3-carboxylate	Anticancer, inhibition of adenosine scavenging (Xie *et al.*, 2022)	Leaves of *Picrasma quassioides* (Xie *et al.*, 2022)
21.	7-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-il] oxy-chromen-4-ona	-	-
22.	4-Coumarate	Antioxidant (Shaheen, 2011)	Leaves of *Genus salvia* (Shaheen, 2011)
23.	Syringetin-3-O-glycoside	Antioxidant, anticarcinogenic, antidiabetic, antimicrobial, and anti-inflammatory (Chmiel and Stompor-Gorący, 2022).	Leaves of *Cedrus atlantica* (Belkacem *et al.*, 2021; Chmiel and Stompor-Gorący, 2022), and leaves of *Cupressus macrocarpa* (Attallah *et al.*, 2021)

The volatile compounds of C. lutea leaf oil were tentatively identified by comparison of molecular weight and mass spectra (Supplementary Figure S10) with those recorded in the literature, Wiley 7n.1 online library, and NIST2017. Sixteen compounds were tentatively observed (Table 4), corresponding to six fatty acids (37%), three terpenes (19%), three phenols (19%), two aliphatic hydrocarbons (13%), one ester (6%), and one heterocyclic compound (6%).

The main compounds tentatively identified were heneicosane (36.734%), n-hexadecanoic acid (19.607%), octadecanoic acid (8.021%), 9,12-octadecadienoic acid (Z,Z) (7.125%), and phytol (6.035%), while phenol, 2-methyl-5-(1-methylethyl)- (0.489%) was noted as a minority component. Their known applications as potential antioxidants, and anti-inflammatories, among others, as well as their possible contributions to sensory characteristics, such as odor/aroma or taste, are detailed in Table 5.

To ensure reliability of the identifications, a criterion of spectral similarity (often denoted as QUAL) ≥ 90% was established by comparing the data with Wiley 7n.1 and NIST2017 reference libraries. Only those identifications that met this criterion were considered positive. Experimental mass spectra are presented in detail in Figure S10.

While the results clearly indicate a higher presence of saturated and unsaturated fatty acids in the essential oil of *C. lutea*, contributing significantly to its characteristic aroma, interactions with minor compounds may also play a role. As noted by Starowicz (2021), the aroma of most plants arises from a complex synergy among various volatile compounds from different chemical classes. Therefore, further studies are warranted to pinpoint the specific compounds responsible for the distinctive aroma of *C. lutea*.

In this regard, although alcohols, acids, esters, terpenes, and furans identified in this study are among the primary chemical groups that contribute to the aroma of *C. lutea* leaves, interactions between these compounds and factors, such as the food matrix and processing

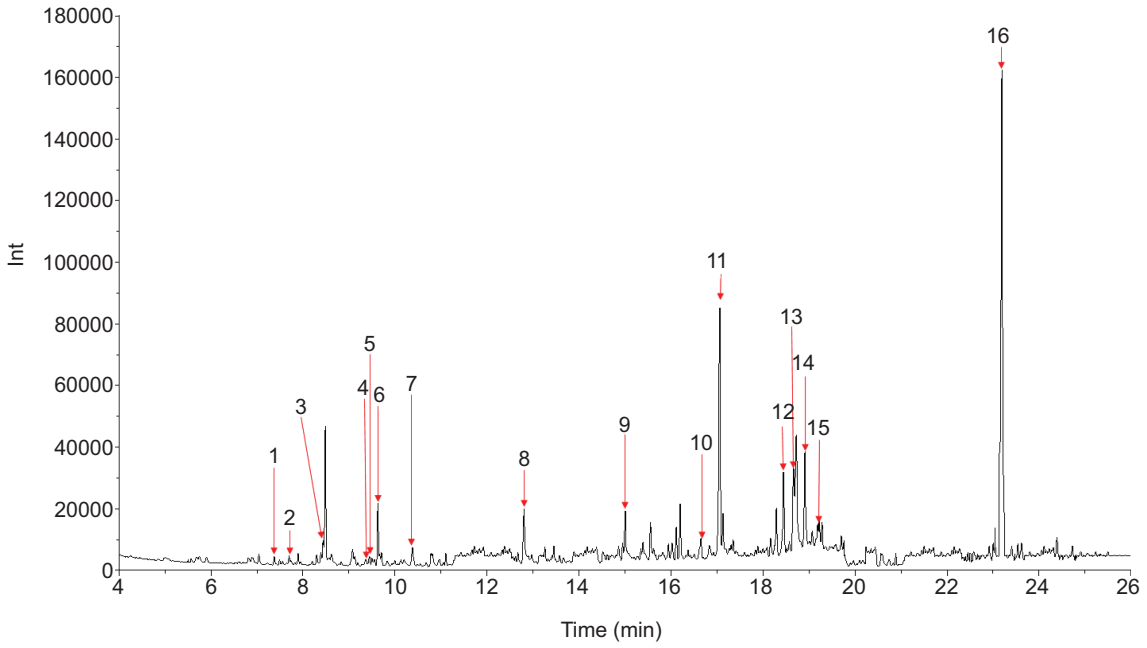

Figure 5. Extracted ion chromatogram (EIC) obtained by GC-MS of the essential oil of *C. lutea* leaves.

Table 4. Volatile compounds identified in *C. lutea* leaf essential oil by GC-MS.

No.	TR (min)	Tentative notation	Molecular formula	Molecular weight (g/mol)	Relative area (%)	Chemical class
1.	7.699	Terpinen-4-ol	$C_{10}H_{18}O$	154.2493	0.37	Terpene (Monoterpene alcohol)
2.	7.895	Terpineol	$C_{10}H_{18}O$	154.2493	0.46	Terpene (Monoterpene alcohol)
3.	8.445	Benzofuran,2,3-dihydro-	C_8H_8O	120.1485	0.97	Heterocyclic (benzofuran)
4.	9.372	Thymol	$C_{10}H_{14}O$	150.2176	0.52	Phenol (Monoterpene phenol)
5.	9.507	Phenol,2-methyl-5-(1-methylethyl)	$C_{10}H_{14}O$	150.2176	0.34	Phenol (Monoterpene phenol)
6.	9.628	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.1745	2.54	Phenol
7.	10.382	Decanoic acid	$C_{10}H_{20}O_2$	172.2646	1.14	Saturated fatty acid
8.	12.808	Dodecanoic acid	$C_{12}H_{24}O_2$	200.3178	3.14	Saturated fatty acid
9.	15.008	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709	3.06	Saturated fatty acid
10.	16.651	Methyl ester, hexadecanoic acid	$C_{17}H_{34}O_2$	270.4507	1.35	Éster
11.	17.065	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	14.08	Fatty acid (saturated fatty acid)
12.	18.452	Fitol	$C_{20}H_{40}O$	296.531	5.11	Terpene (diterpene alcohol)
13.	18.670	Ácid 9,12-octadecadienoic (Z,Z)	$C_{18}H32O_2$	280.4455	5.76	Unsaturated fatty acid
14.	18.911	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	2.23	Saturated fatty acid
15.	19.198	Octadecane	$C_{18}H3_8$	254.4943	4.33	Hydrocarbon
16.	23.192	Heneicosane	$C_{21}H_{44}$	296.5741	26.37	Hydrocarbon
		Total identified			71.77%	

RT: Retention time (min)

conditions, will ultimately shape the final sensory profile. This study underscored the importance of evaluating volatiles within food matrices, a key area of interest in food production that provided valuable insights into product quality (Starowicz, 2021).

It must be noted that none of the research reported in literature to date has addressed the relationship between phenol content and antioxidant activity of *C. lutea* leaf extract. The identification of main compounds using more precise analytical techniques, such

Table 5. Reported characteristics of volatile compounds identified in *C. lutea* leaf essential oil by GC-MS.

No.	Tentative notation	Associated activity	Associated odor/scent
1.	Terpinen-4-ol	Antioxidant (Badr *et al.*, 2022), anti-inflammatory, antifungal, antiviral, and anticarcinogenic (Yadav and Rao, 2016).	Camphor, herbal (Schmidt *et al.*, 2012)
2.	Terpineol	Antioxidant and anti-inflammatory (Sales *et al.*, 2020)	Flowers (Nakamura and Miyazawa, 2013)
3.	Benzofuran,2,3-dihydro	Antimicrobial and anti-inflammatory (Al-Tameme *et al.*, 2015)	Sweet odor (Gao *et al.*, 2021)
4.	Thymol	Antioxidant, anti-inflammatory, and antimicrobial (Escobar *et al.*, 2020)	Herbaceous, warm, and slightly spicy (Díaz-Maroto *et al.*, 2005)
5.	Phenol,2-methyl-5-(1-methylethyl)	Antioxidant, antibacterial, anti-inflammatory, antifungal, and anticancer (Suntres *et al.*, 2015).	Herbaceous, warm, and slightly spicy (Díaz-Maroto *et al.*, 2005)
6.	2-Methoxy -4-vinylphenol	Antimicrobial antioxidant, anti-inflammatory, and analgesic (Rubab *et al.*, 2020)	Earthy and fresh, similar to dried herbs (Choi, 2005)
7.	Decanoic acid	Antibacterial, insulin stimulator, and anti-inflammatory (Vellapandian, 2022)	Slightly rancid (Qian and Wang, 2005)
8.	Dodecanoic acid	Antimicrobial and anti-inflammatory (Kumar and Rajakumar, 2016).	Slightly sweet, flowery, and waxy (Zhang *et al.*, 2020).
9.	Tetradecanoic acid	Antioxidant (Henry *et al.*, 2002) and antibacterial (Patra *et al.*, 2017)	Slightly sweet, flowery, and waxy (Zhang *et al.*, 2020)
10.	Methyl ester, hexadecanoic acid	Antioxidant (Bhalla *et al.*, 2021), anti-inflammatory (Abdel-Hady *et al.*, 2018; Bhalla *et al.*, 2021), and antifungal (Abubacker and Deepalakshmi, 2013)	Flowers with fresh herbal notes have a mild odor with fatty notes (Tao *et al.*, 2014)
11.	Hexadecanoic acid	Antioxidant and anticarcinogenic (Abdel-Hady *et al.*, 2018), anti-inflammatory (Bhalla *et al.*, 2021), antibacterial (Chelliah *et al.*, 2017)	Slight greasy, sour, rancid, and pungent odor (Tao *et al.*, 2014)
12.	Fitol	Antioxidant, anticarcinogenic, antidiarrheal, anti-inflammatory, and antimicrobial (Abdel-Hady *et al.*, 2018; Bhalla *et al.*, 2021)	Fresh herbs (Zhu *et al.*, 2016)
13.	Ácid 9,12-octadecadienoic (Z,Z)	Anti-inflammatory, anticancer, anticoronary (Adeoye-Isijola *et al.*, 2018)	Slight acaeitoso-rancio odor (Rezende and Fraga, 2003)
14.	Octadecanoic acid	Anti-inflammatory, anticancer, insecticide, and anticoronary (Rao *et al.*, 2016)	Slightly greasy odor (Da Farmacopeia, 2019)
15.	Octadecane	NA	NA
16.	Heneicosane	Antimicrobial (Vanitha *et al.*, 2020)	NA

NA: Not available.

as HPLC-ESI-QTOF-MS, identifying 23 compounds, of which 18 were not reported in previous studies on *Calathea* and/or *Marantaceae*, significantly elaborate knowledge on the chemical composition and biological potential of this plant. In addition, we characterized volatile compounds, providing knowledge on the aromatic profile of this plant. Therefore, this study provides new useful information on the extracts of *C. lutea* leaves. The findings of this research are essential to better understand the biological properties of this leaf and its implications for the development of food products.

This study highlights the potential of *C. lutea* leaf as a natural source of antioxidants and other functional compounds, which could offer alternatives to synthetic preservatives in the food industry, an aspect of great relevance, because nowadays, consumers and health regulations are increasingly looking for natural ingredients (Ali and Ali, 2020; Mesías *et al.*, 2021). The results of this study suggested that C. lutea could play an important role in improving food stability and safety, especially in products susceptible to oxidation, such as oils, meat, and dairy products.

Thus, the possible future applications of these compounds include their incorporation in food formulations where the use of antioxidants is necessary to extend products' shelf life. Likewise, the volatile compounds of *C. lutea* responsible for its characteristic aroma could be used in products where sensory properties play a fundamental role, contributing to an enriched and distinctive sensory experience.

It is noteworthy that this research establishes a basis for developing future studies on the behavior of *C. lutea* compounds in real food matrices, allowing a more specific evaluation of their efficacy and stability under practical conditions. Finally, it is worth mentioning that, one of the limitations of this study was the dependence on the extraction methods available in the laboratory, which restricts the choices of solvents and extraction techniques. This could influence the profile of compounds obtained, as some of them could be extracted more efficiently by advanced techniques that were not available in this research. In the future, the use of alternative techniques, such as supercritical extraction or ultrasound-assisted extraction, would provide a more complete profile of the bioactive compounds of *C. lutea* and optimize their applicability in food. Another limitation was that, given that some of the identified compounds contribute to the aromatic profile of the essential oil, not having the necessary equipment to perform a sensory analysis limited the contribution of more information on its applicability in food. Future research could focus on how the volatile profile of *C. lutea* can enrich sensory experience in food products.

Conclusions

This study provides a pioneering characterization of phenolic and volatile compounds in *C. lutea*, highlighting its potential as a natural source of antioxidants with applications in the food industry. In this study, the extraction efficiency of phenolic compounds from the samples varied significantly according to the solvent used. The dichloromethane fraction, with a high content of total phenols and an antioxidant capacity comparable to synthetic antioxidants, such as BHA and α-tocopherol, demonstrates that *C. lutea* can offer a viable and safe alternative in the formulation of food products that aim to improve the quality and extend the shelf life of foods.

This ability of *C. lutea* to eliminate free radicals is of great interest in the field of food quality and safety, because antioxidants can help prevent lipid oxidation, vitamin degradation, and formation of potentially toxic compounds. In addition, this work represents advancement in the valorization of a traditional Caribbean plant, opening opportunities for its industrial utilization at both regional and global levels.

From a practical perspective, *C. lutea* could be a scalable and cost-effective option for the food industry, especially in regions where this plant is abundant. Its use in food products as a natural preservative would enhance product quality and promote sustainability by reducing dependence on synthetic antioxidants.

The originality of this study lies in the use of advanced HPLC-QTOF-MS/MS techniques to identify new bioactive compounds in *C. lutea*, many of them documented for the first time in this species and in the *Marantaceae* family. This lays the foundation for future research that can explore their applications in food and cosmetic products and in the development of natural and sustainable additives. In this regard, for future research, it is recommended to evaluate the extracts in real food matrices, such as oils, sauces, or meat products, to explore their potential role as a preservative (antioxidant and antimicrobial). Studying the economic and technical feasibility of implementing the extracts in large-scale industrial processes is also suggested.

Although this study focused on the Caribbean region, the tropical climatic conditions and soil types characteristic of this region are similar to those of other tropical areas, such as Southeast Asia and Central America. Previous studies in these regions, such as those of Chandran (2020) and Robalino Pinedo and Torres Carrión (2021), have reported the presence of phenolic compounds with antioxidant properties in *C. lutea*, suggesting that our findings could be extrapolable to a broader level. However, it is critical to recognize that the chemical composition of *C. lutea* may vary due to factors such as genetic variability, soil type, altitude, and local climatic conditions.

Data Availability Statement

All data are available in this paper.

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Author Contributions

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Paternina-Sierra; writing—review and editing: Katherine Paternina-Sierra, Diofanor Acevedo-Correa, Fredyc Díaz-Castillo, Jairo Mercado-Camargo, and Jefferson José Úrsula-Ortega; visualization: Jefferson José Úrsula-Ortega; supervision: Diofanor Acevedo-Correa, Fredyc Díaz-Castillo, Jairo Mercado-Camargo; project administration: Diofanor Acevedo-Correa; and fund acquisition: Katherine Paternina-Sierra. All authors had read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declared no conflict of interest.

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Supplementary

Table S1. Preparation of negative control, blank, and *C. lutea* leaf extract sample and fractions in the microplate for TPC determination.

	Negative control (µL)	Positive control sample blank (µL)	Sample (µL)
Sample		30	30
Sample solvent	30		
Folin C solution	150		150
Distilled water		150	
NA_2CO_3 solution	120	120	120

Table S2. Preparation of negative control, blank, and sample extracts of *C. lutea* in microplate for ABTS$^{\bullet+}$ assay.

	Negative control (µL)	Positive control sample blank (µL)	Sample (µL)
Sample solvent	20		
ABTS$^{\bullet+}$ solution	180		180
Sample solution		20	20
Ethanol		180	

Table S3. Preparation of negative control, blank, and sample extracts of C. *lutea* in the microplate for DPPH* assay.

	Negative control (µL)	Positive control sample blank (µL)	Sample (µL)
Sample solvent	75		
DPPH* solution	150		150
Sample solution		75	75
Ethanol		150	

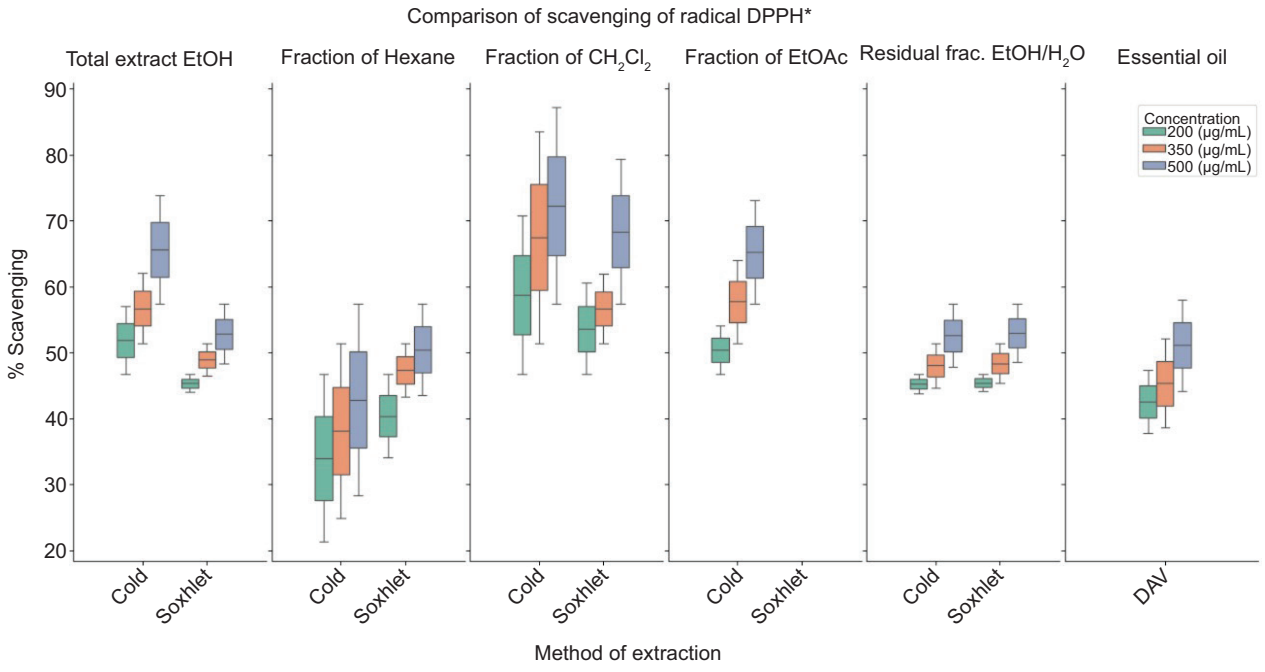


Figure S1. Percentage of DPPH* of the extracts, fractions, and essential oil of *C. lutea* leaf.

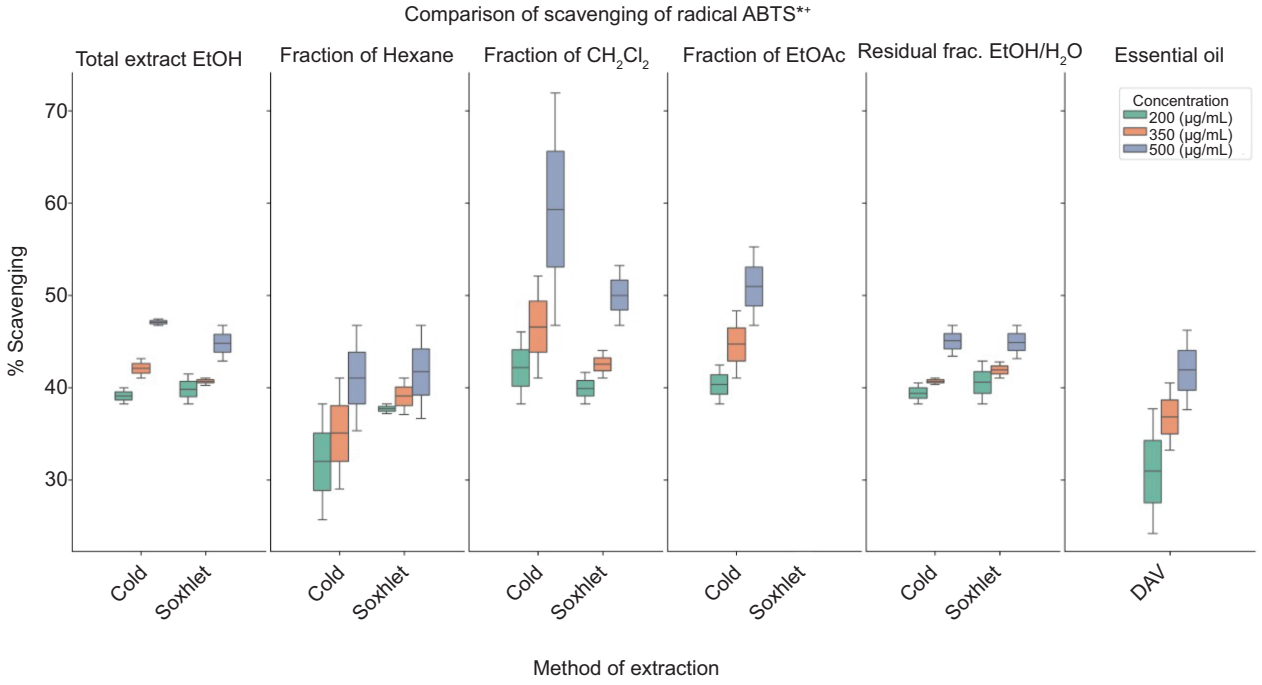


Figure S2. Percentage of ABTS^{*+} of the extracts, fractions, and essential oil of *C. lutea* leaf.

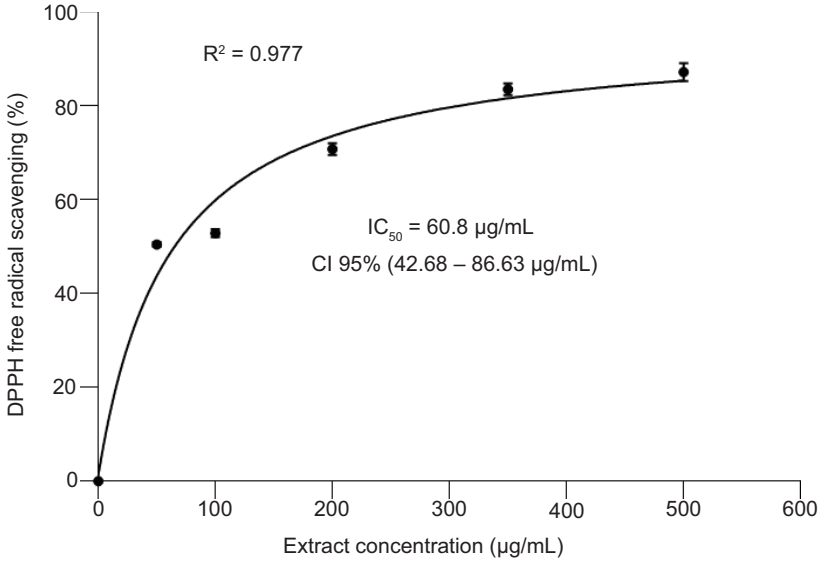


Figure S3. DPPH* radical scavenging dependent on the concentration of CH_2Cl_2 fraction of *C. lutea* leaf: value of IC_{50}.

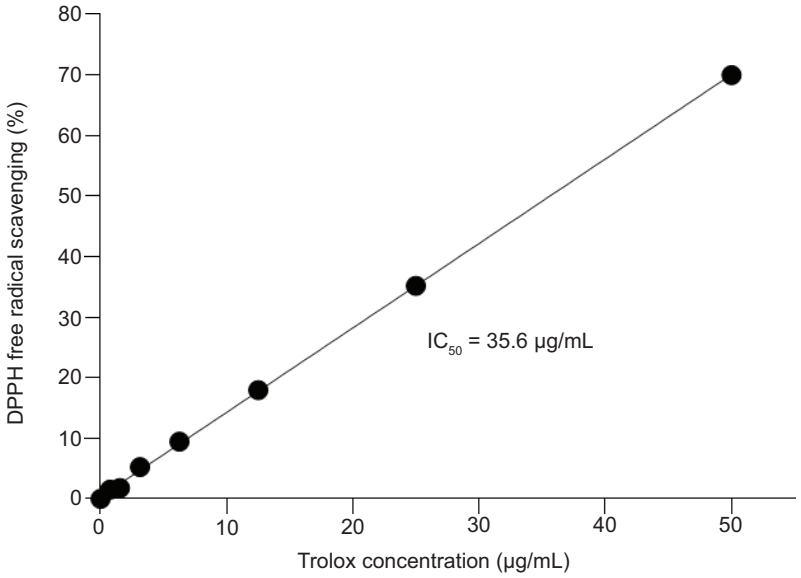


Figure S4. DPPH* free radical scavenging activity represented by the Trolox standard.

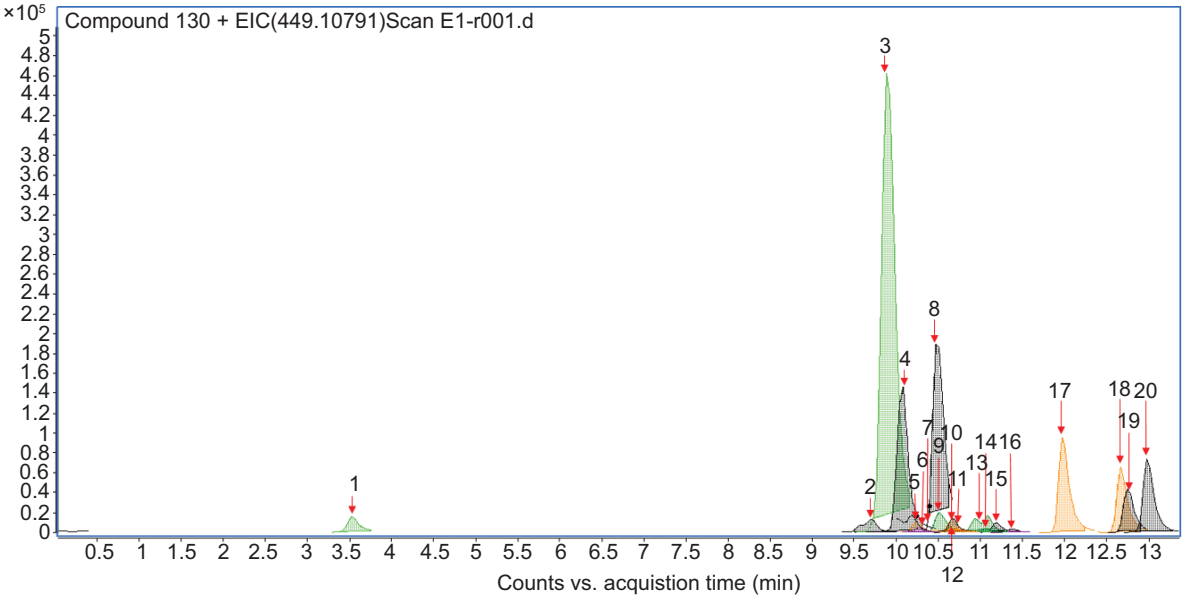


Figure S5. Extracted ion chromatogram (EIC) obtained by HPLC-ESI-QTOF in positive ion mode of total extract EtOH maceration (E1).

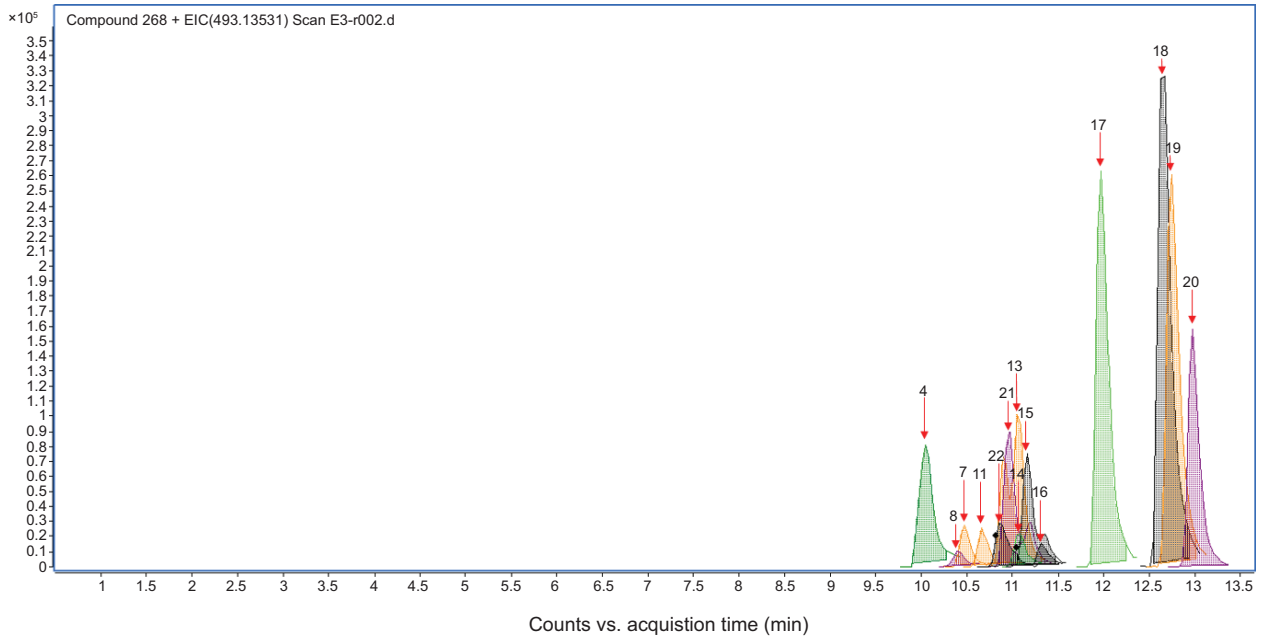


Figure S6. Extracted ion chromatogram (EIC) obtained by HPLC-ESI-QTOF in positive ion mode of CH_2Cl_2 fraction (E3).

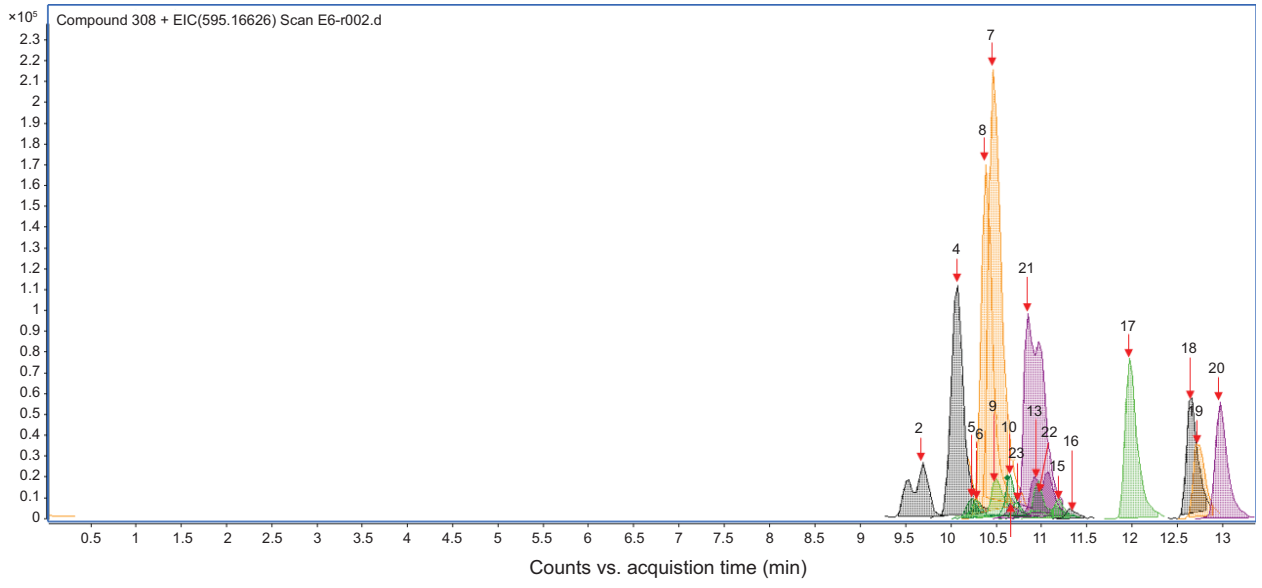


Figure S7. Extracted ion chromatogram (EIC) obtained by HPLC-ESI-QTOF in positive ion mode of the total EtOH Soxhlet extraction (E6).

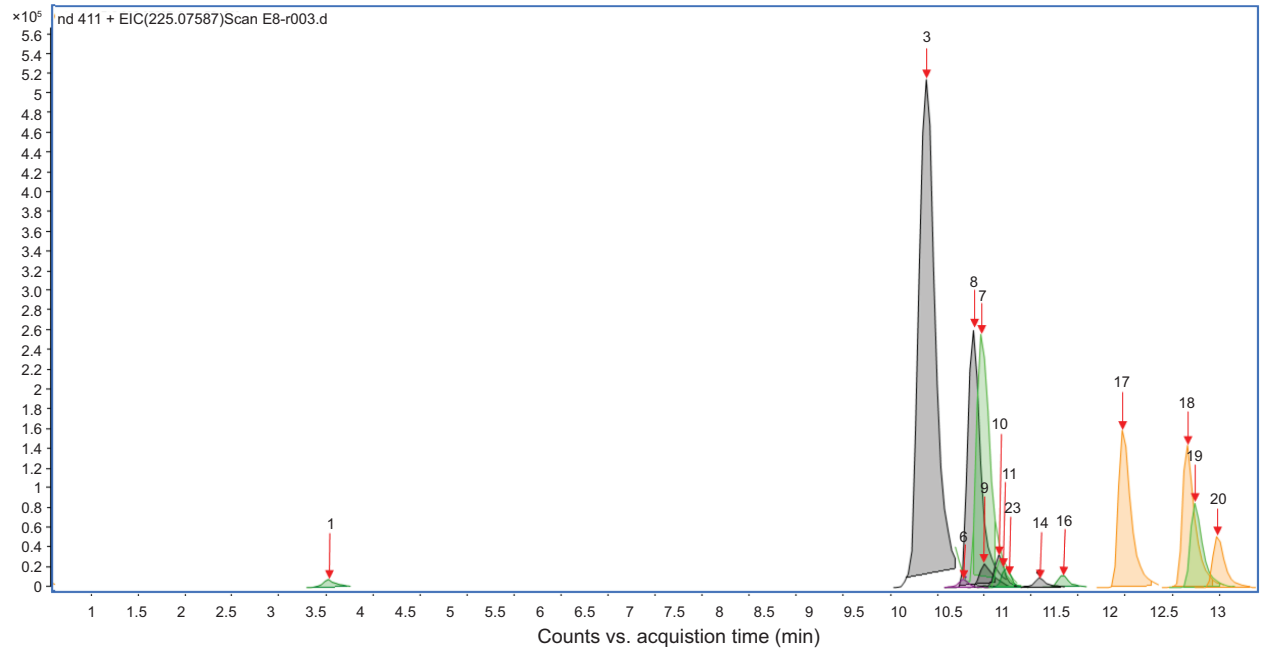


Figure S8. Extracted ion chromatogram (EIC) obtained by HPLC-ESI-QTOF in positive ion mode of CH_2Cl_2 fraction (E8).

Compound 1
Retention time (min): 3.535
m/z [M+H]: 193.070
Molecular formula: $C_7H_{12}O_6$
Tentative annotation: Quinic acid

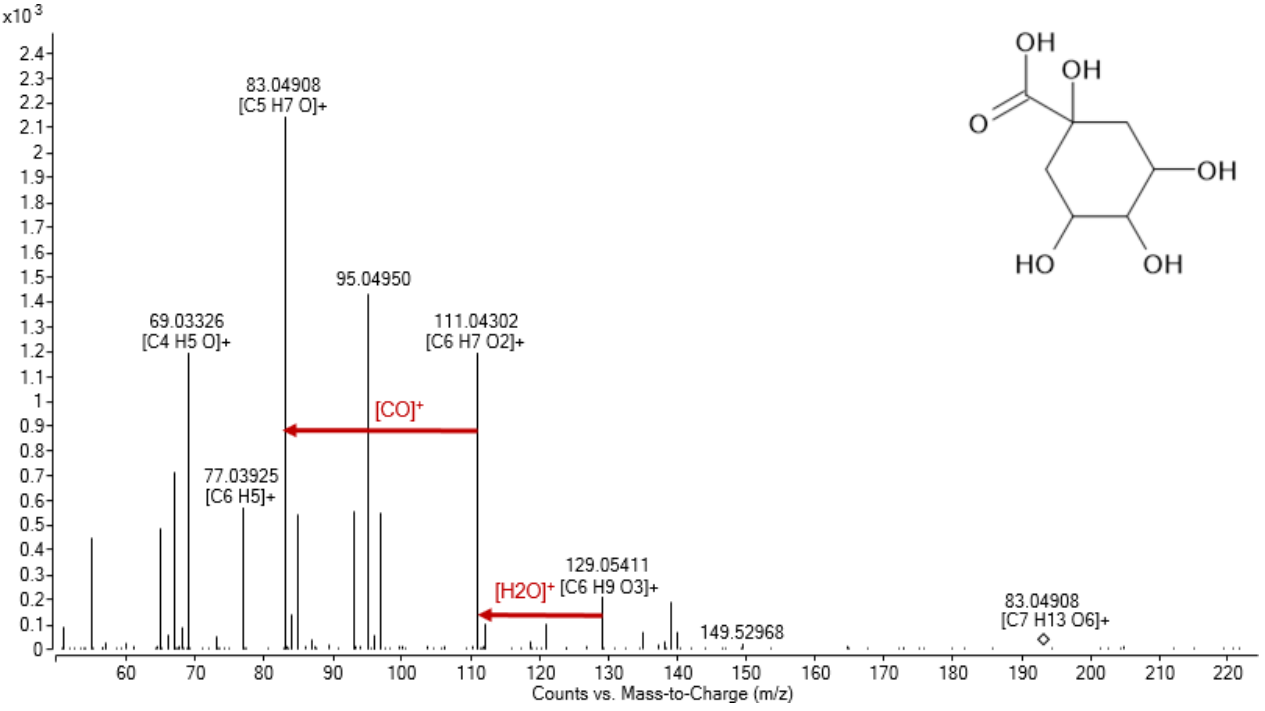
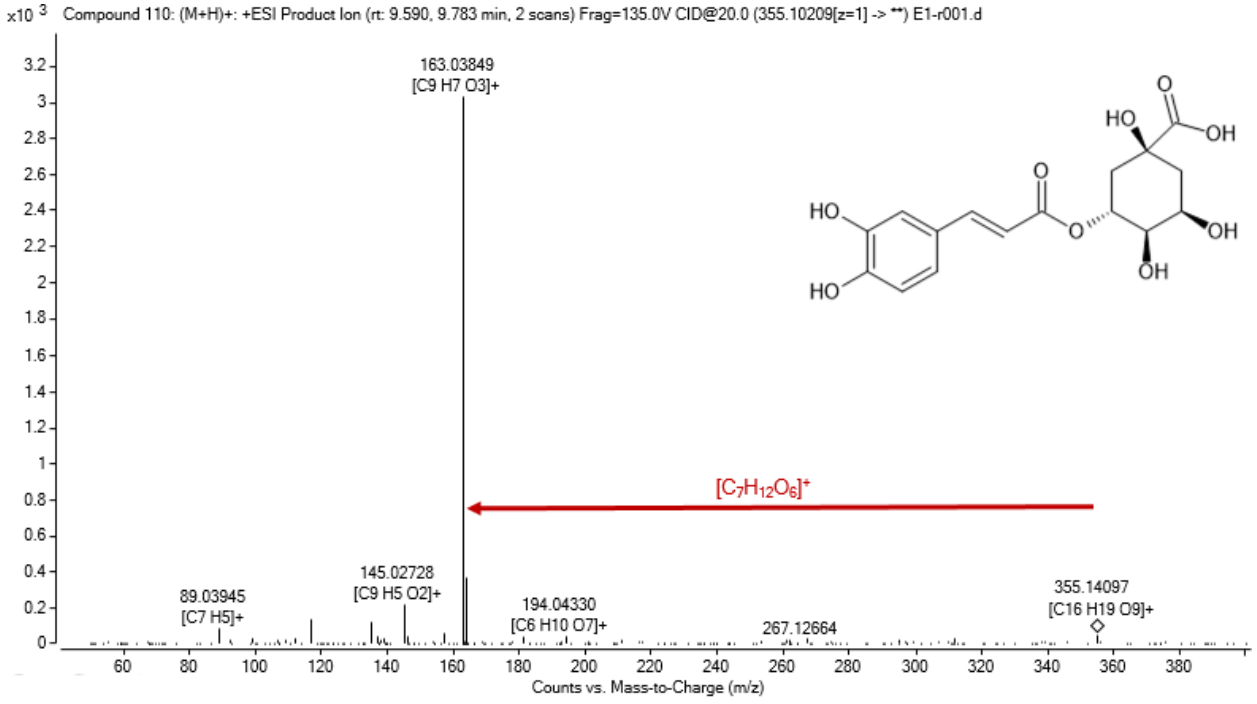


Figure S9. Compound fragment spectrum results (MS/MS).

Compound 2
Retention time (min): 9.5
m/z [M+H]: 355.10209
Molecular formula: $C_{16}H_{18}O_9$
Tentative annotation: Chlorogenic acid
Class of compound: Phenolic acid (cinnamic acids and their derivatives)



Compound 3
Retention time (min): 9.752
m/z [M+H]: 565.15609
Molecular formula: $C_{16}H_{28}O_{14}$
Tentative notation: Isoschaftoside
Class of compound: Flavonoid (flavones)

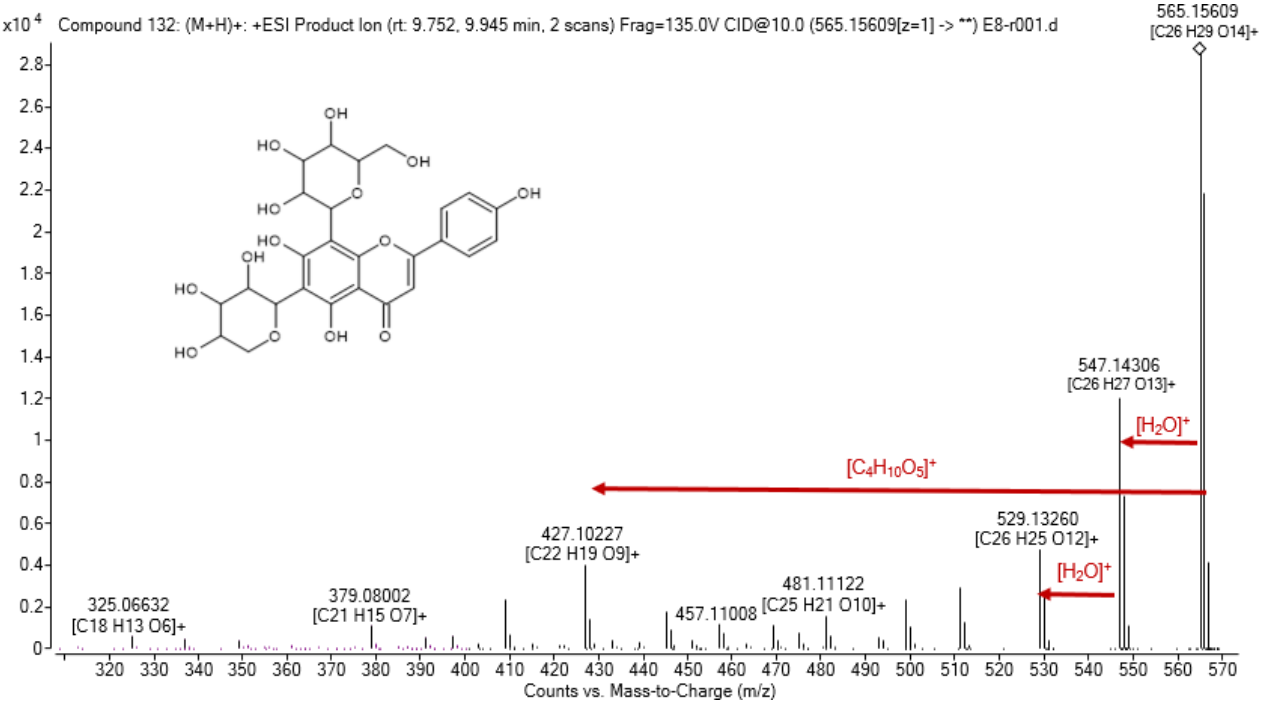
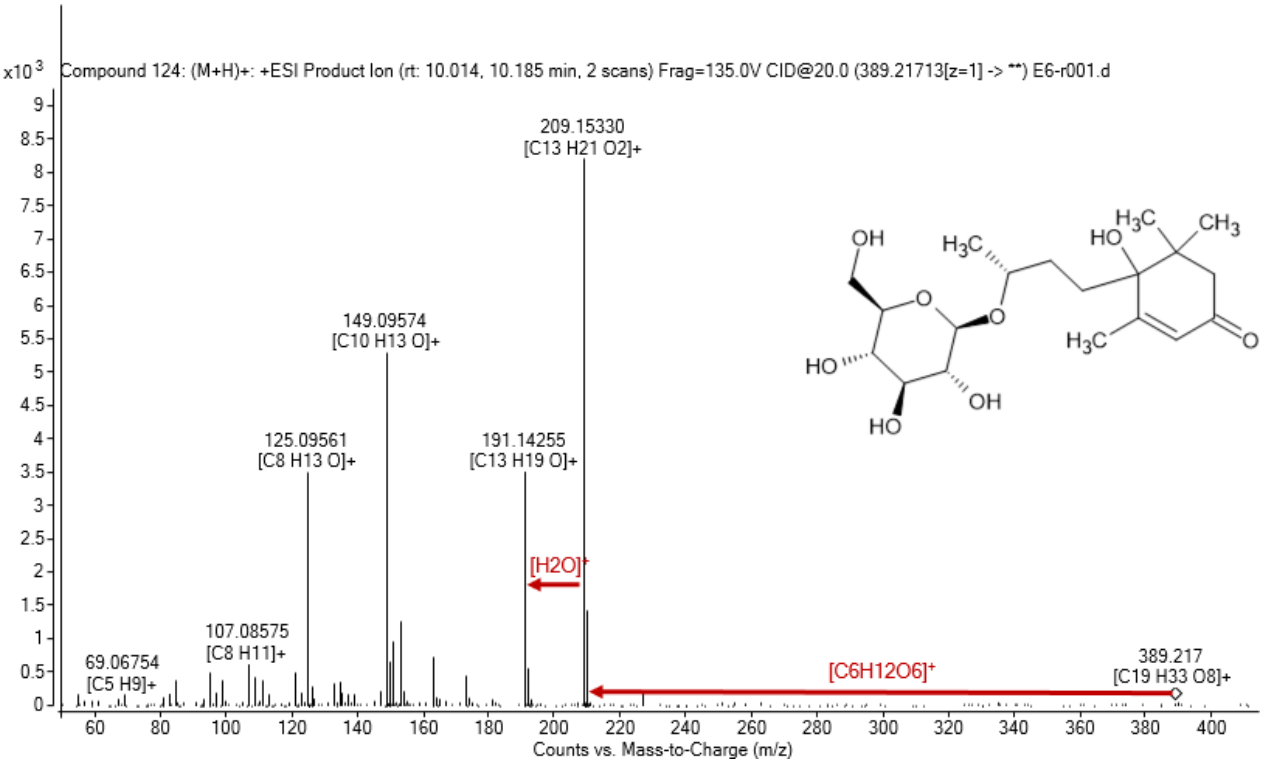


Figure S9. Continued

Compound 4
Retention time (min): 10.014
m/z [M+H]: 389.21813
Molecular formula: $C_{19}H_{32}O_8$
Tentative notation: (2R)-4-[(1S)-1-Hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-il]-2-butyl beta-D-glucopyranoside
Class of compound: Terpenoids



Compound 5
Retention time (min): 10.230
m/z [M+H]: 611.16211
Molecular formula: $C_{27}H_{30}O_{16}$
Tentative notation: Routine
Class of compound: Flavonoid (flavonols)

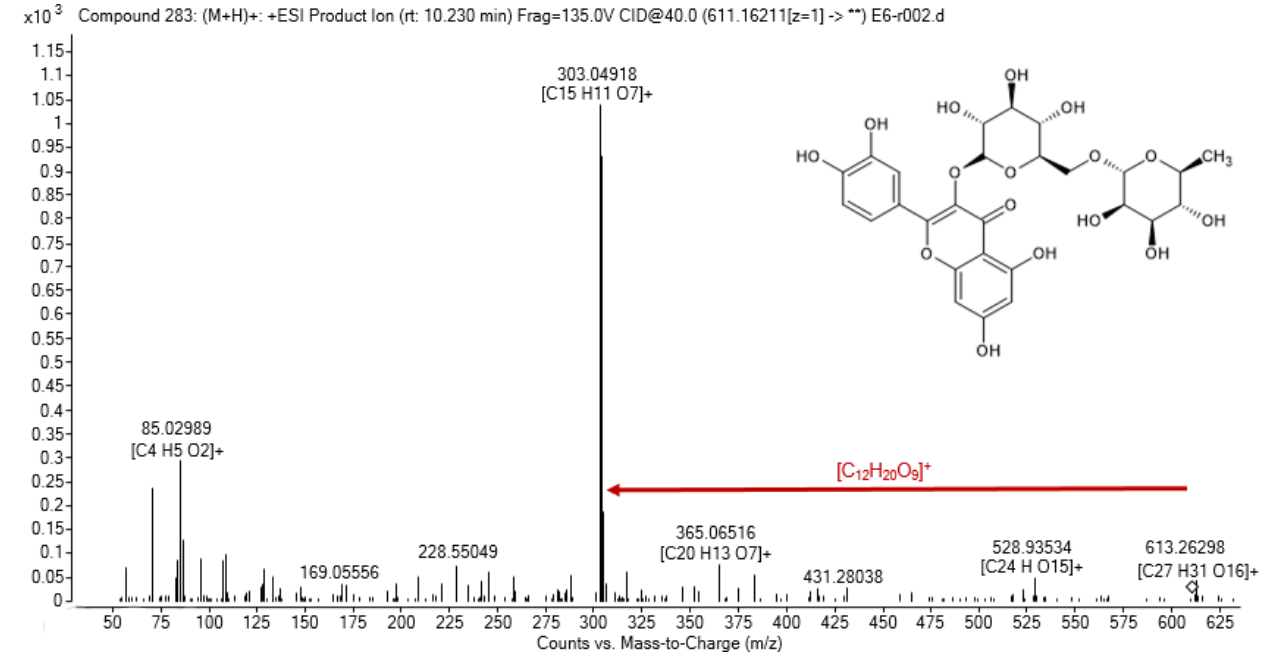
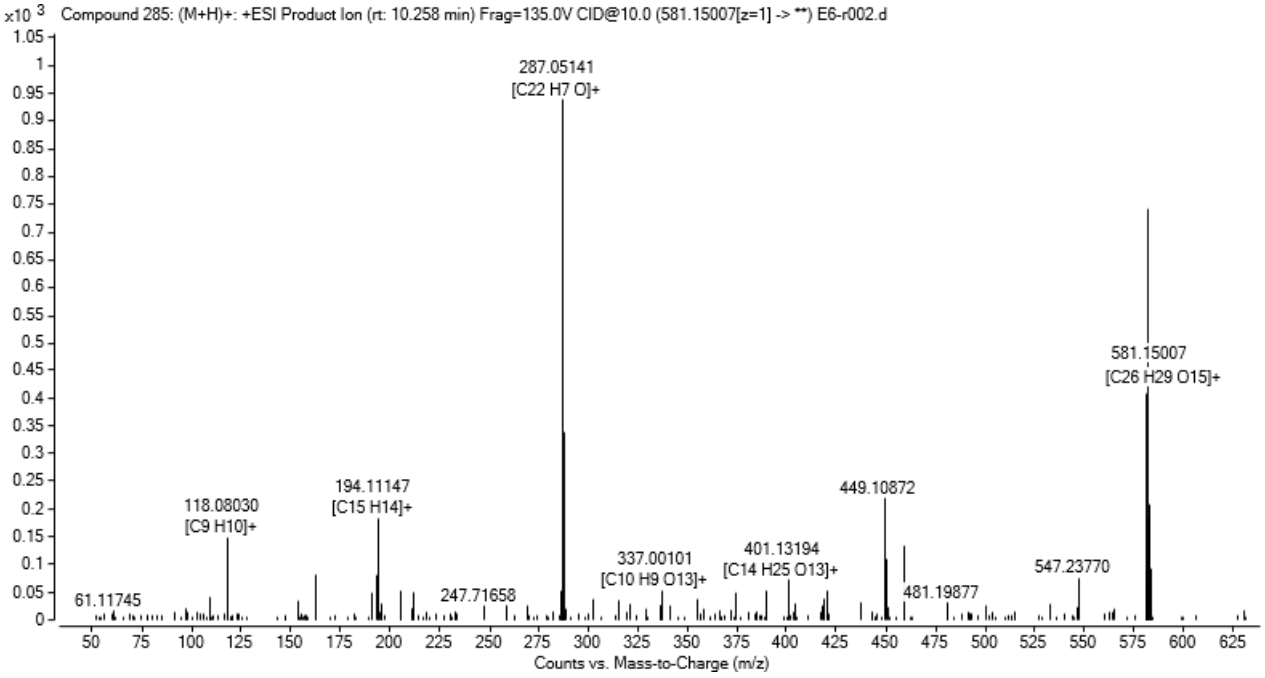


Figure S9. Continued

Compound 6
Retention time (min): 10.258
m/z [M+H]: 581.15007
Molecular formula: $C_{26}H_{28}O_{15}$
Tentative notation: trihydroxy-6-[(3,4,5-trihydroxy-oxan-2-il)oxymethyl]oxan-2-il]oxi-chromen-4-ona
Class of compound: Flavonoid (flavonols)



Compound 7
Retention time (min): 10.604
m/z [M+H]: 449.10791
Molecular formula: $C_{21}H_{21}ClO_{11}$
Tentative notation: Cyanidin-3-glycoside
Class of compound: Flavonoid

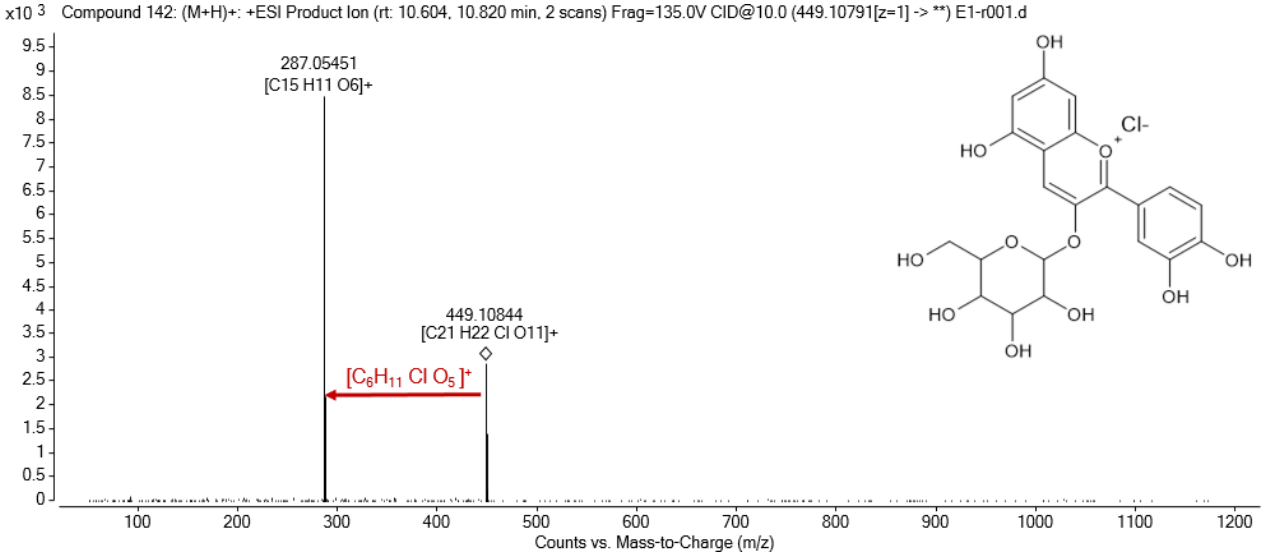
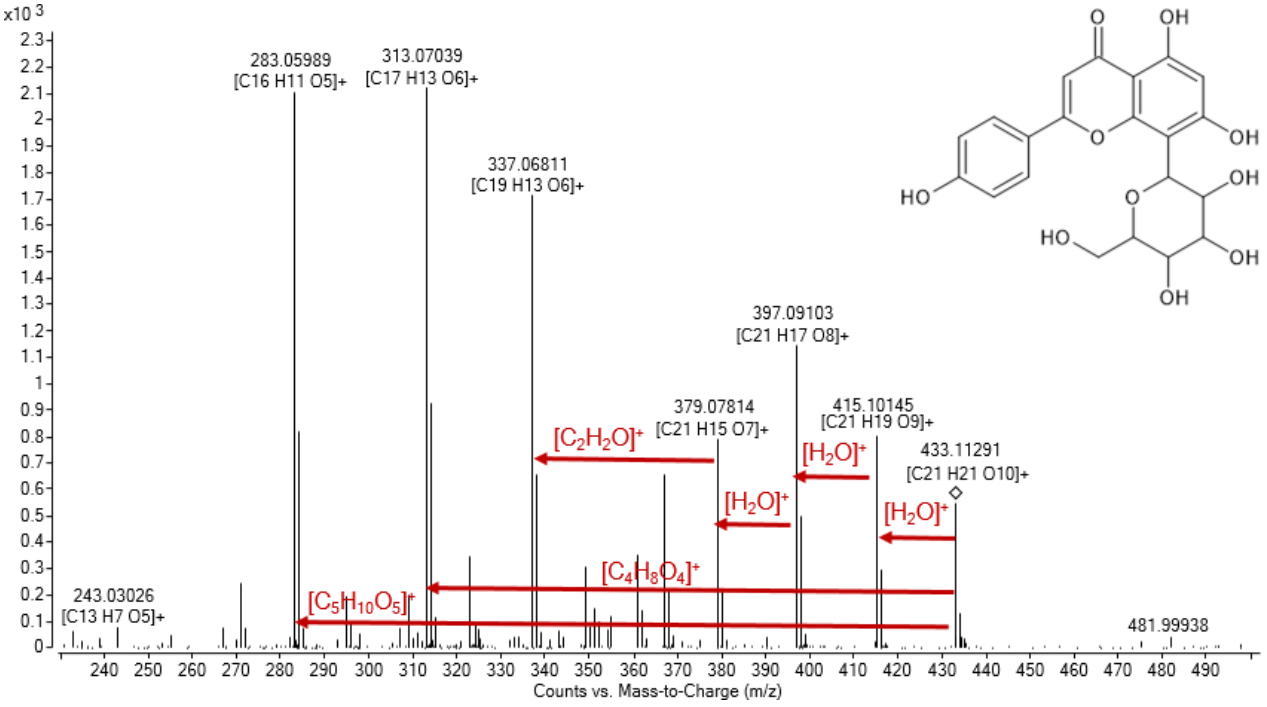


Figure S9. Continued

Compound 8
Retention time (min): 10.340
m/z [M+H]: 433.11343
Molecular formula: $C_{21}H_{20}O_{10}$
Tentative annotation: Vitexin
Compound class: Flavonoid



Compound 9
Retention time (min):
m/z [M+H]: 625.17657
Tentative annotation: $C_{28}H_{32}O_{16}$
Compound class: Narcisine
Compound class: Flavonoid

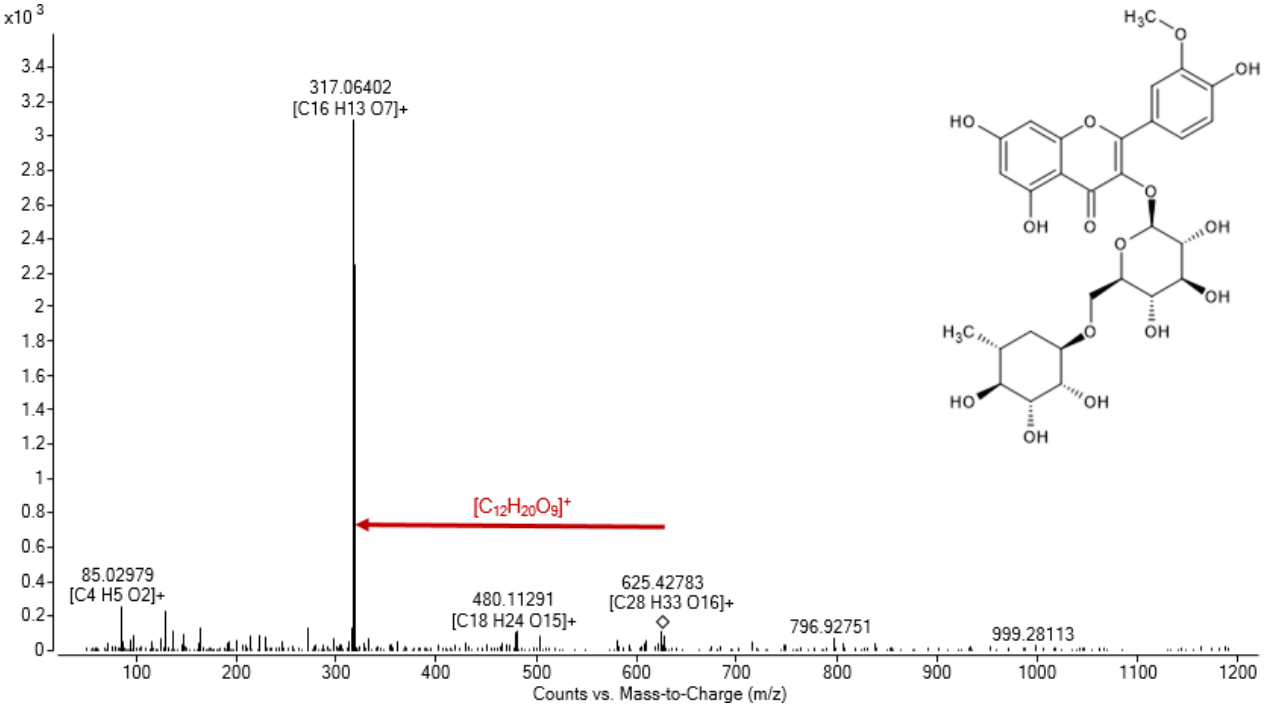
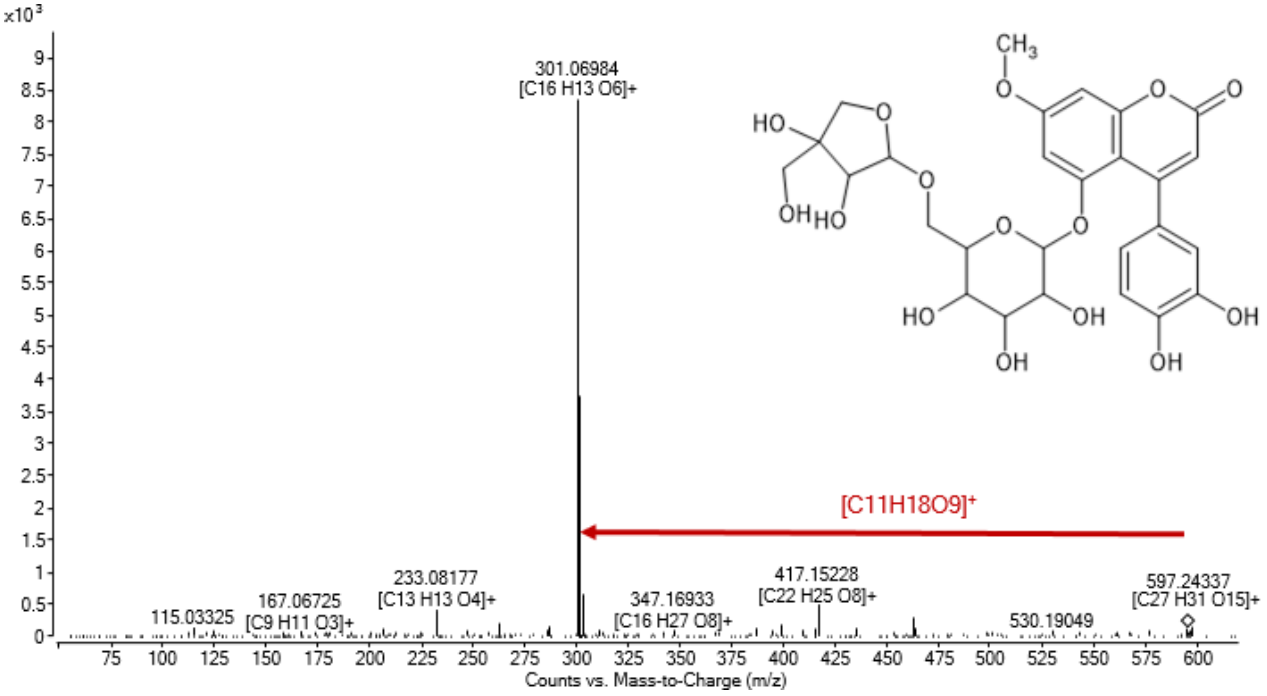


Figure S9. Continued

Compound 10
Retention time (min): 10.667
m/z [M+H]: 595.16567
Molecular formula: $C_{27}H_{30}O_{15}$
Tentative annotation: 5-[6-[[3,4-dihydroxy-4-(hydroxymethyl)oxolan-2-il]oxymethyl]-3,4,5-trihydroxy-oxan-2-il] oxi-4-(3,4-dihydroxyphenyl)-7-methoxychromen-2-ona
Compound class: Flavonoid 4-phenylcoumarin



Compound 11
Retention time (min): 10.6:43
m/z [M+H]: 10.643
Molecular formula: $C_{13}H_{24}O_2$
Tentative notation: 4-(3-hydroxybutyl)-3,5,5-trimethylcyclohex-3-en-1-ol
Class of compound: Terpenoids

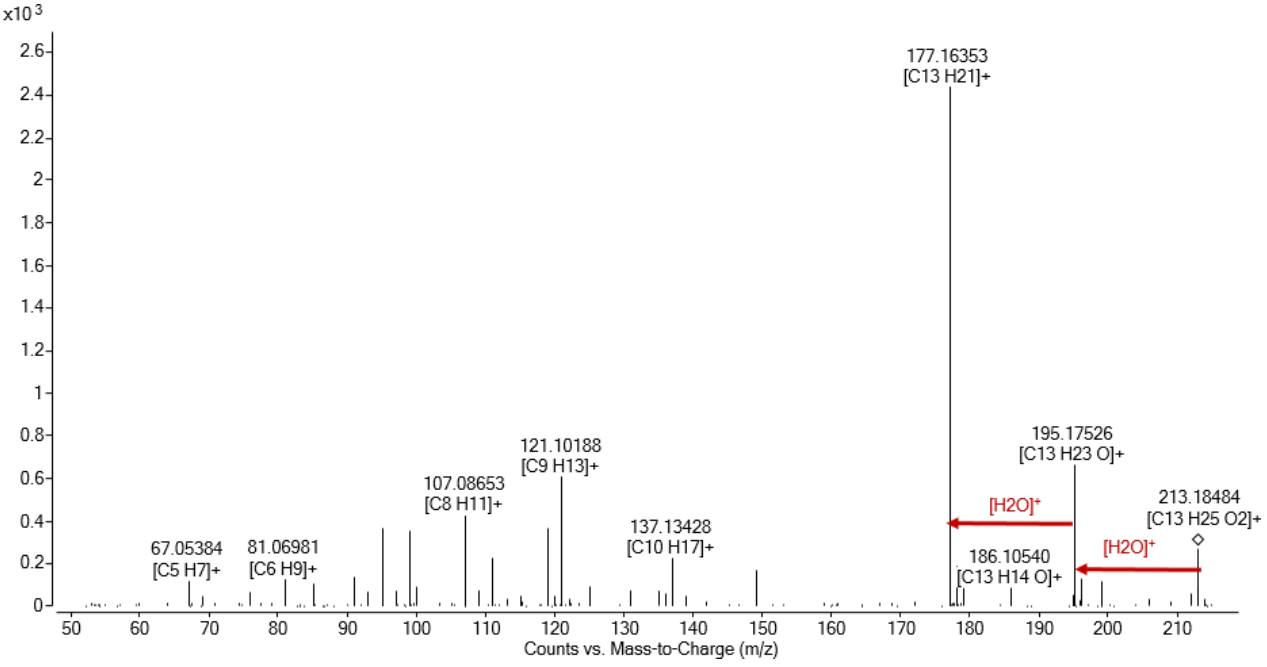
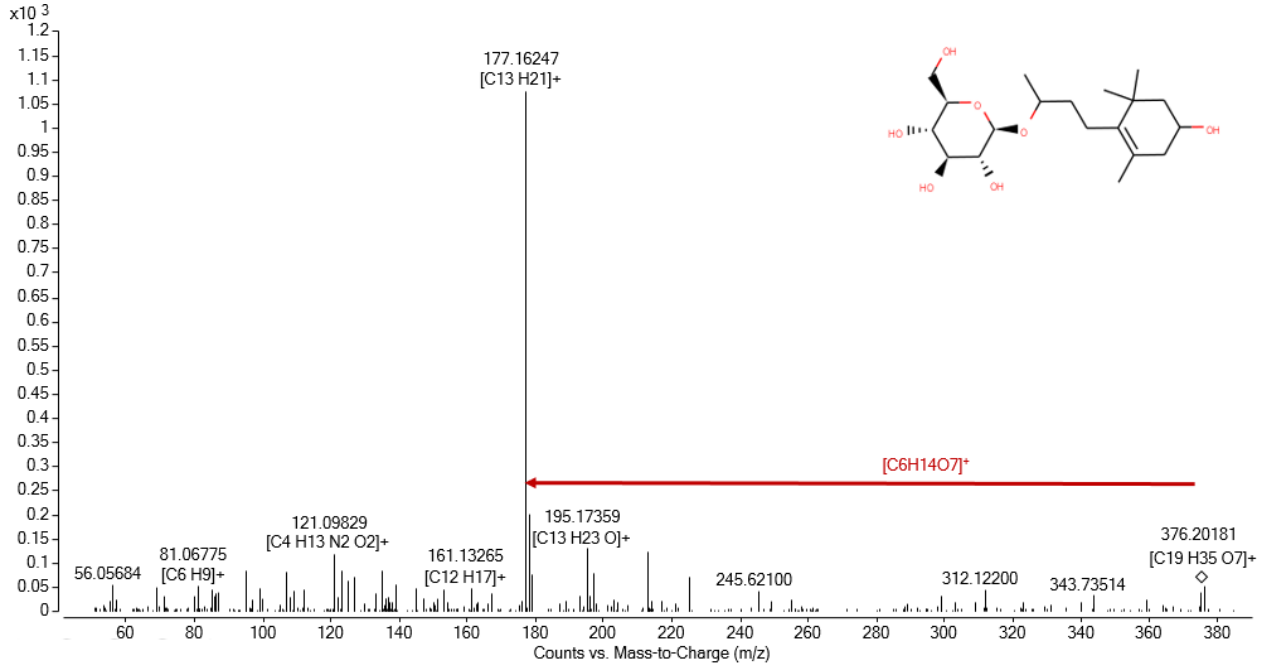


Figure S9. Continued

Compound 12
Retention time (min): 10.684
m/z [M+H]: 375.23592
Molecular formula: $C_{19}H_{34}O_7$
Tentative notation: (2R,3S,4S,5R,6R)-2-(hydroxymethyl)-6-[4-(4-hydroxy-2,6,6-trimethylcyclohexen-1-il)butan-2-iloxi]oxano-3,4,5-triol
Class of compounds: Terpenoids



Compound 13
Retention time (min): 11.056
m/z [M+H]: 373.22174
Molecular formula: $C_{19}H_{32}O_7$
Tentative notation: 4-(2,6,6-Trimethyl-4-oxo-2-cyclohexen-1-il)-2-butyl beta-D-glucopyranoside
Class of compound: Terpenoid

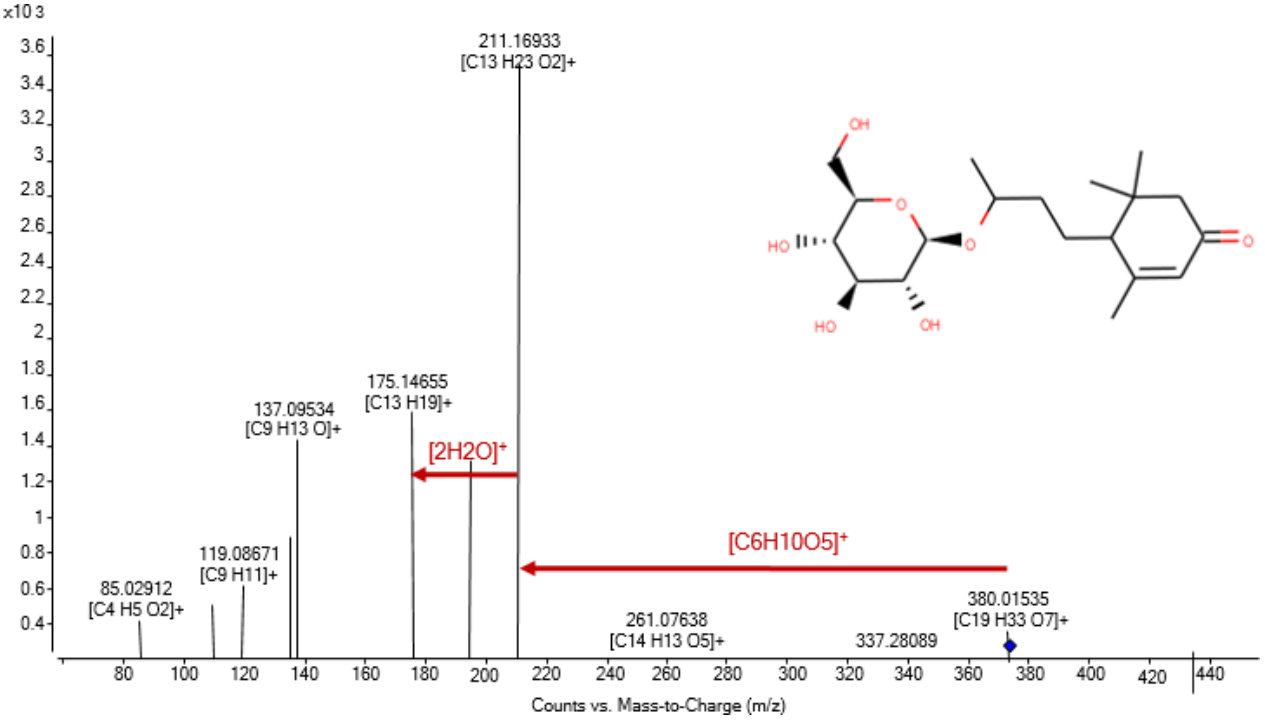
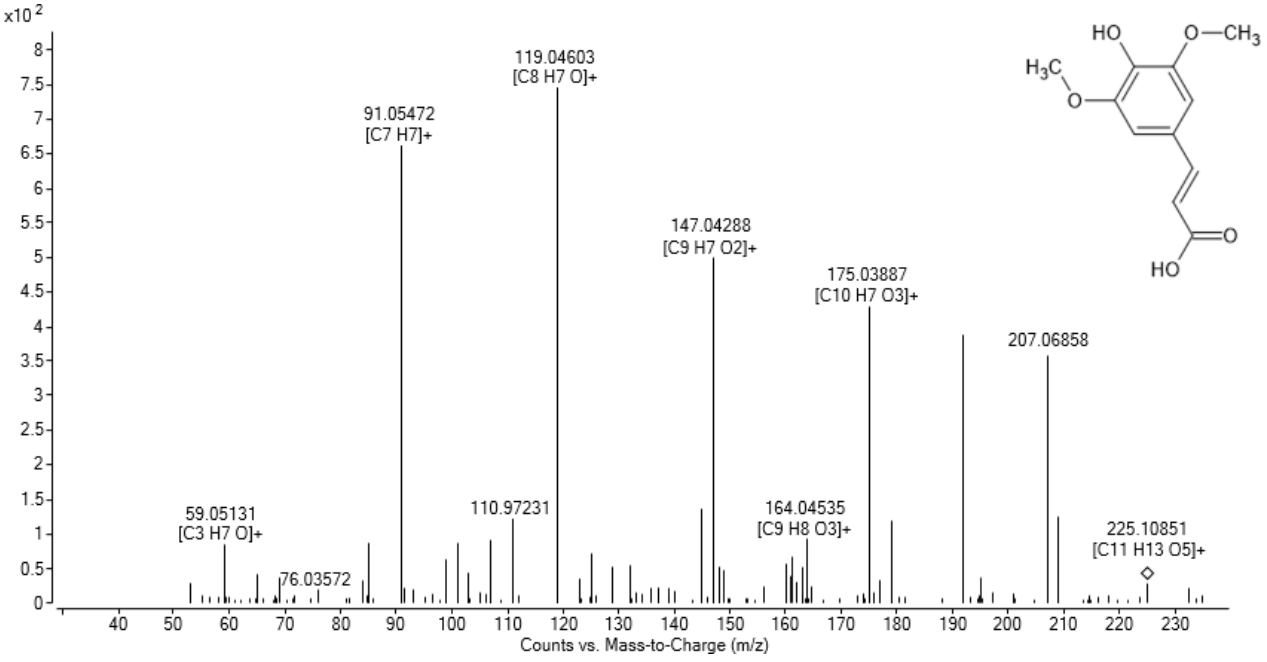


Figure S9. Continued

Compound 14
Retention time (min): 11.112
m/z [M+H]: 225.07587
Molecular formula: $C_{11}H_{12}O_5$
Tentative notation: Synapic acid
Compound type: Phenolic acid (cinnamic acids and their derivatives)



Compound 15
Retention time (min): 11.147
m/z [M+H]: 195.06583
Molecular formula: $C_{10}H_{10}O_4$
Tentative notation: Ferulato
Compound type: Phenolic acid (cinnamic acids and their derivatives)

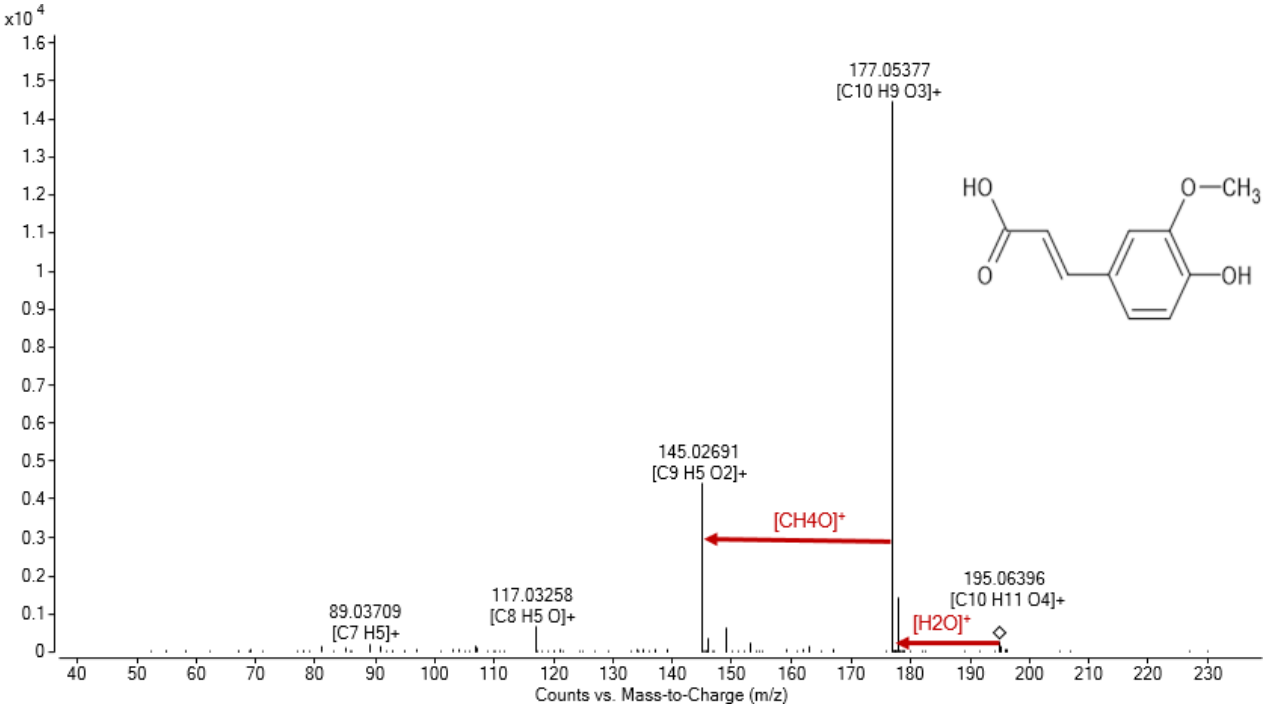
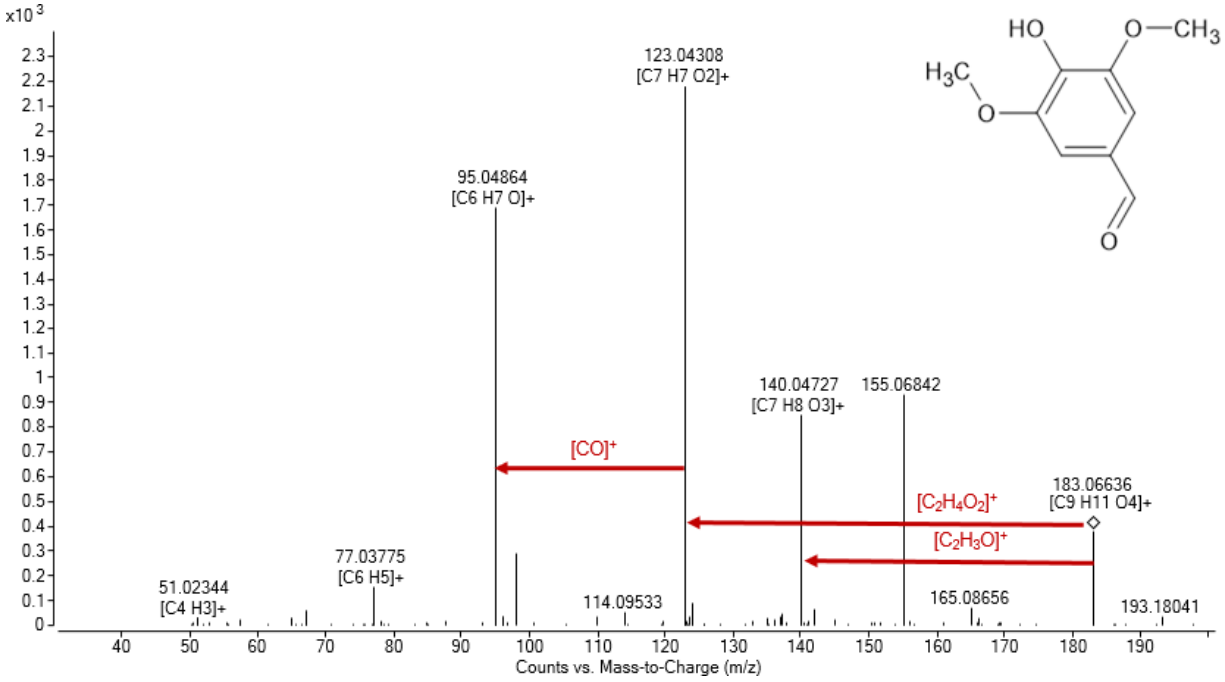


Figure S9. Continued

Compound 16
Retention time (min): 11.324
m/z [M+H]: 183.06596
Molecular formula: $C_9H_{10}O_4$
Tentative notation: Syringaldehyde
Compound type: Phenolic acid (cinnamic acids and their derivatives)



Compound 17
Retention time (min): 11.930
m/z [M+H]: 287.05571
Molecular formula: $C_{15}H_{10}O_6$
Tentative notation: Luteolin
Class of compound: Flavonoid (flavonols)

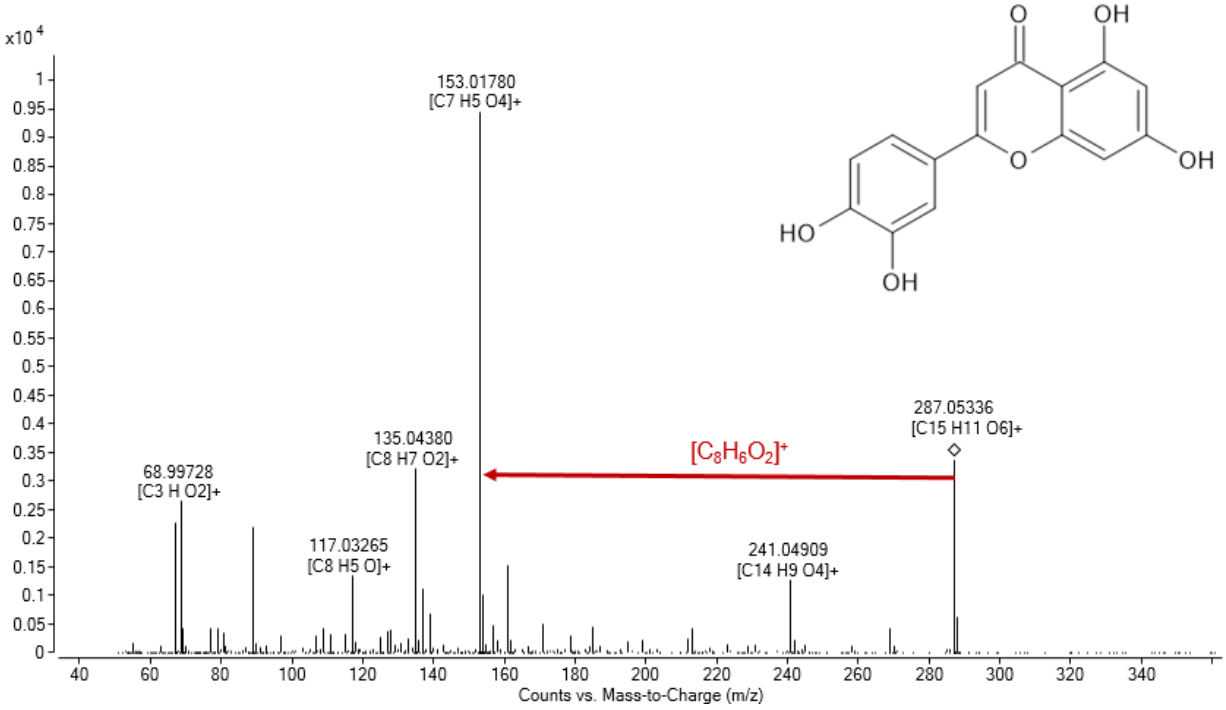
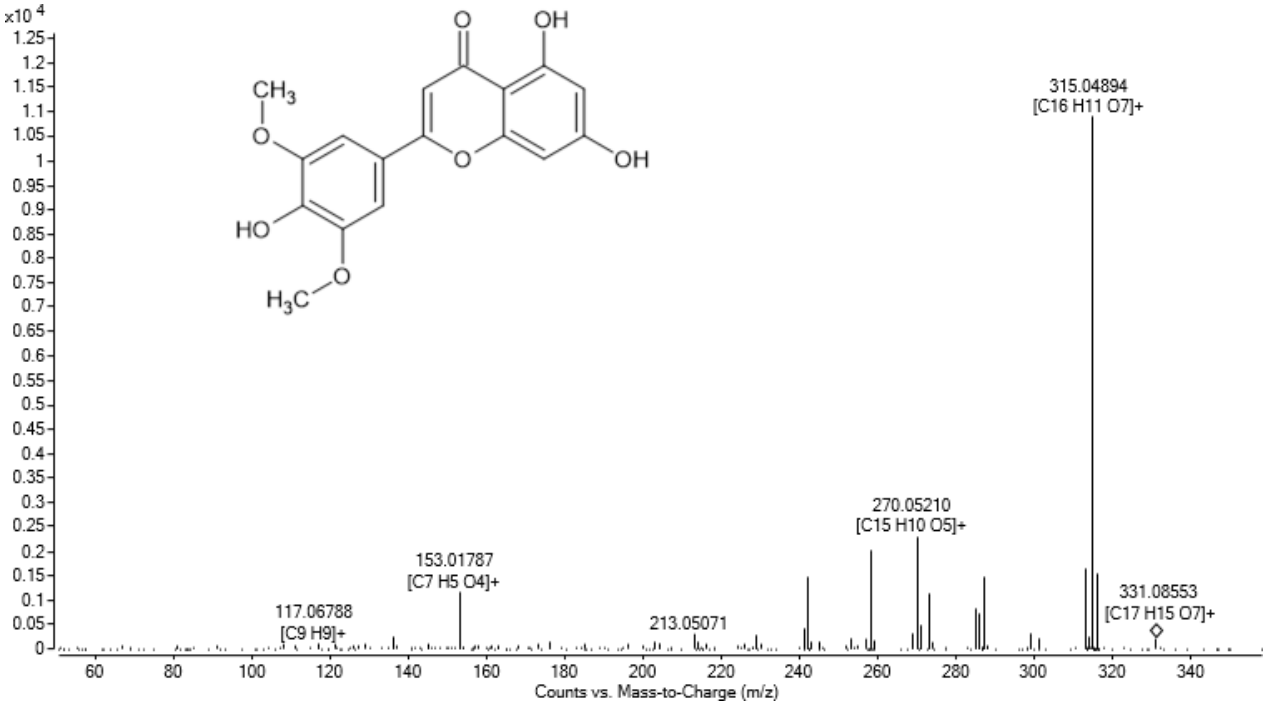


Figure S9. Continued

Compound 18
Retention time (min): 12.621
m/z [M+H]: 331.08115
Molecular formula: $C_{17}H_{14}O_7$
Tentative notation: Tricina
Class of compound: Flavonoid (flavones)



Compound 19
Retention time (min): 12.701
m/z [M+H]: 301.07152
Molecular formula: $C_{16}H_{12}O_6$
Tentative notation: Chrysoeriol
Class of compound: Flavonoid (flavones)

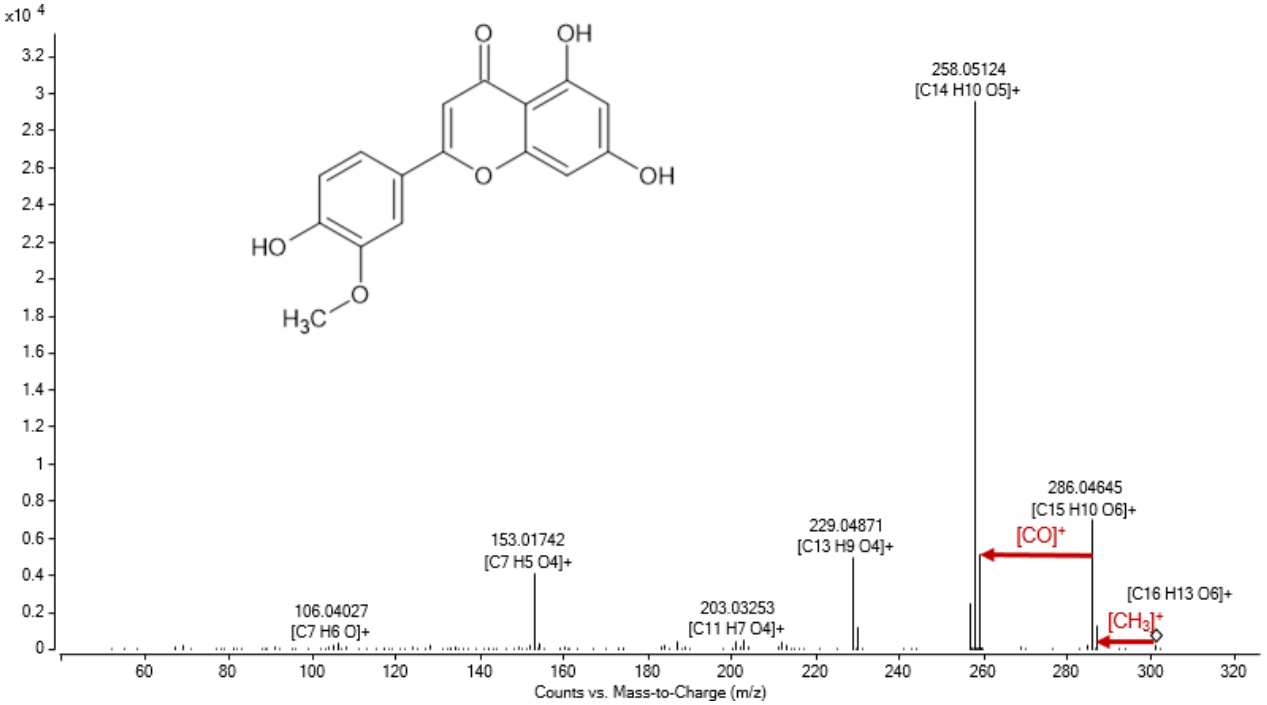
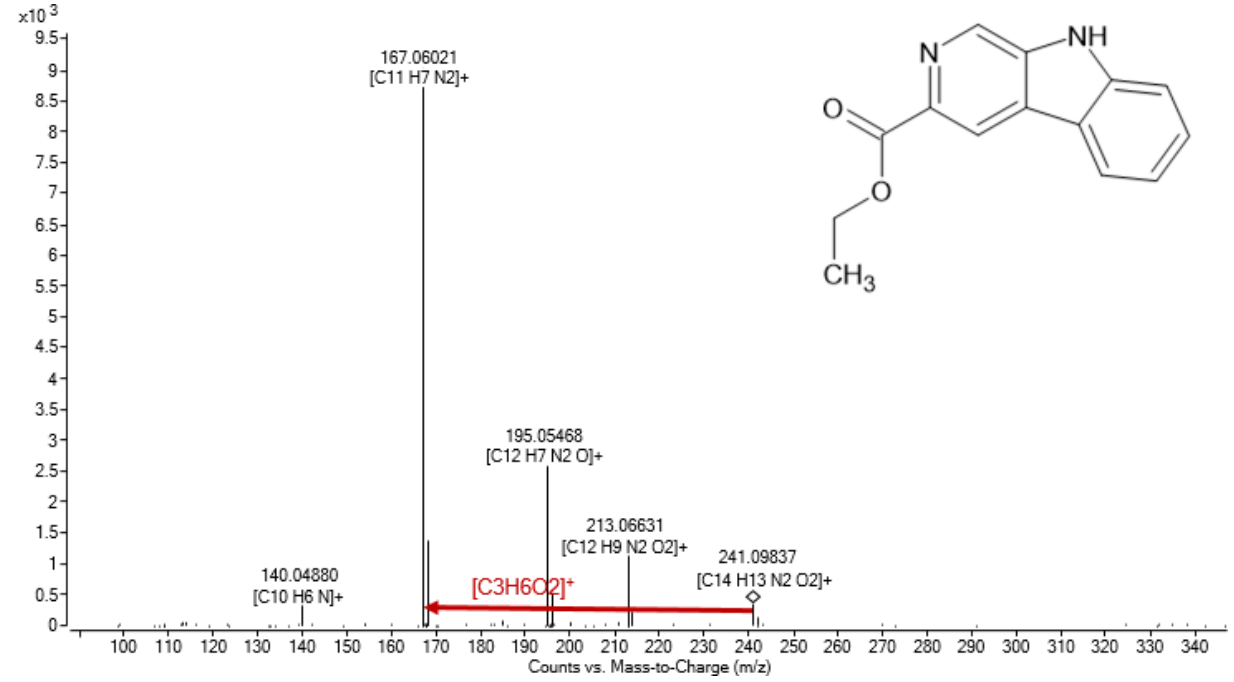


Figure S9. Continued

Compound 20
Retention time (min): 11.947
m/z [M+H]: 241.09689
Molecular formula: $C_{14}H_{12}N_2O_2$
Tentative notation: Ethyl beta-carboline-3-carboxylate
Compound class: Alkaloids



Compound 21
Retention time (min): 10.819
m/z [M+H]: 493.13417
Molecular formula: $C_{23}H_{24}O_{12}$
Tentative notation: 7-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-il] oxi-chromen-4-ona
Class of compound: Flavonoid

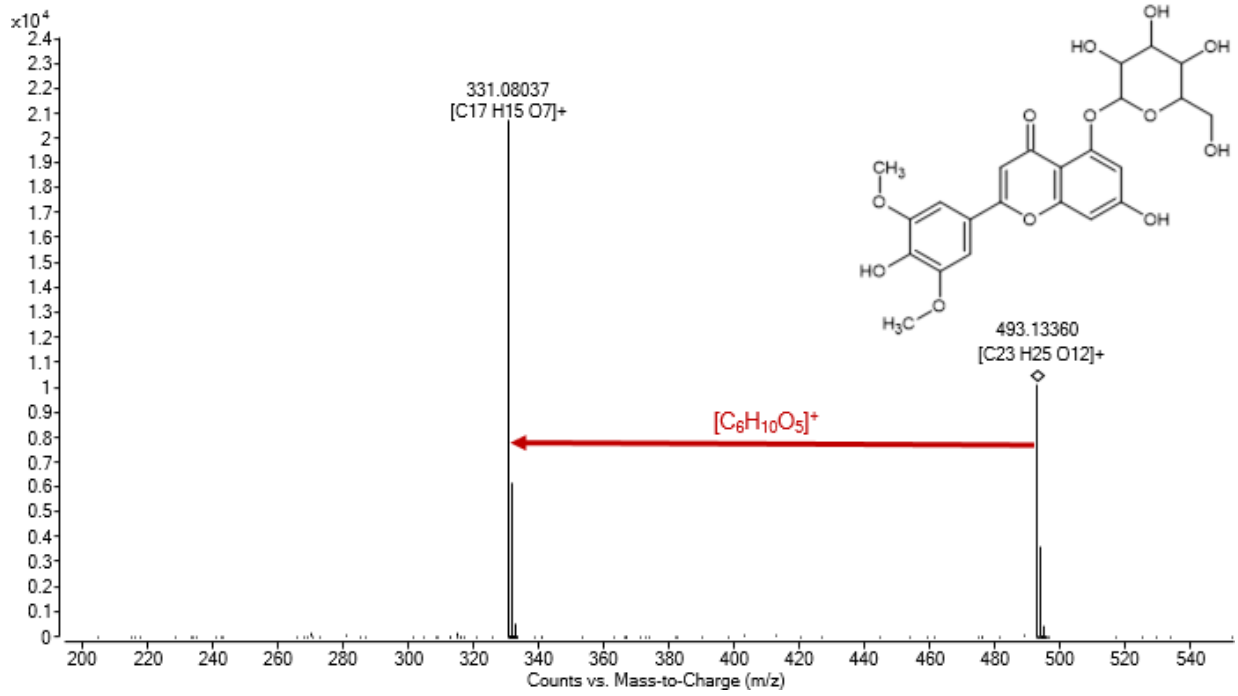
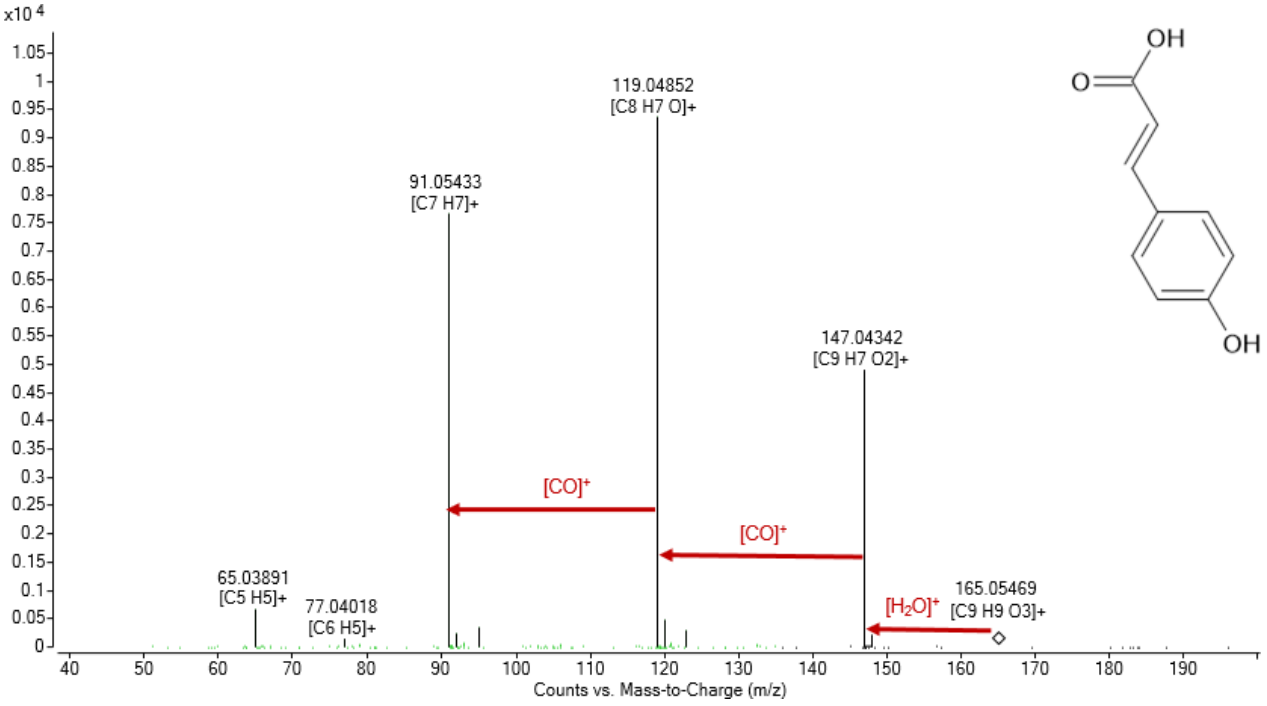


Figure S9. Continued

Compound 22
Retention time (min): 10.960
m/z [M+H]: 165.05469
Molecular formula: $C_9H_8O_3$
Tentative score: 4-Coumarate
Class of compound: Phenolic acid (cinnamic acids and their derivatives)



Compound 23
Retention time (min): 10.726
m/z [M+H]: 509.1299
Molecular formula: $C_{23}H_{24}O_{13}$
Tentative notation: Syringetin-3-O-glucoside
Class of compound: Flavonoid (flavonols)

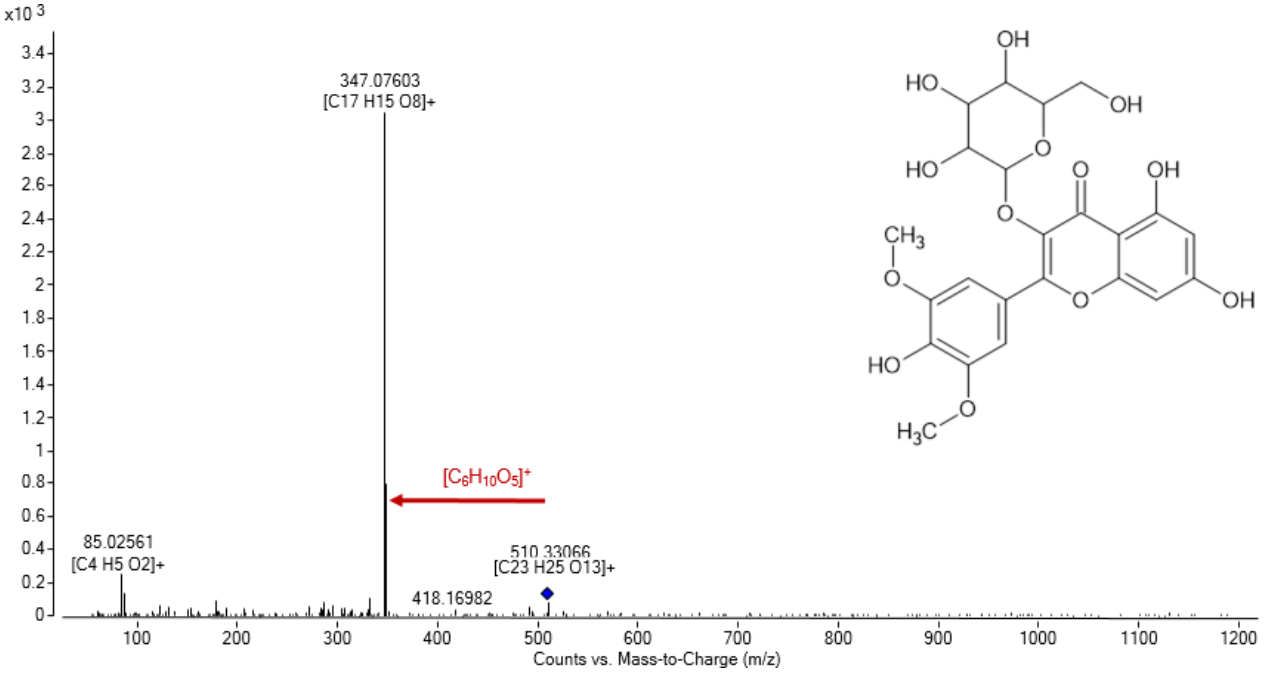
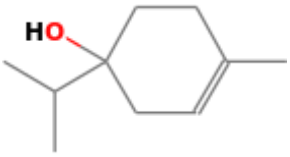
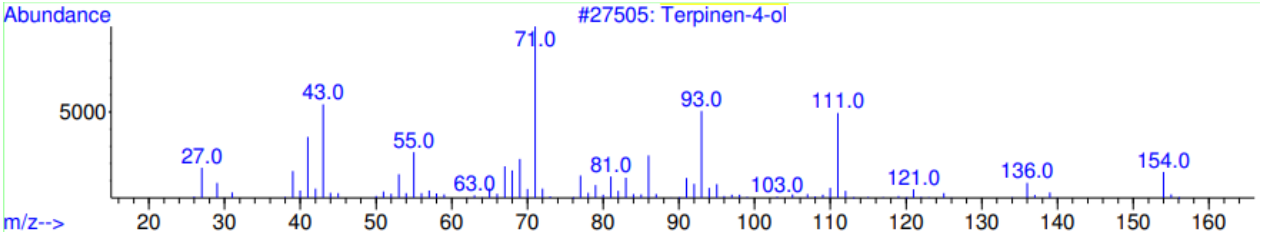


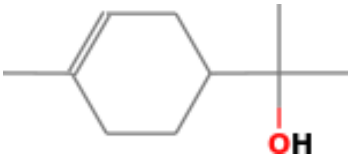
Figure S9. Continued

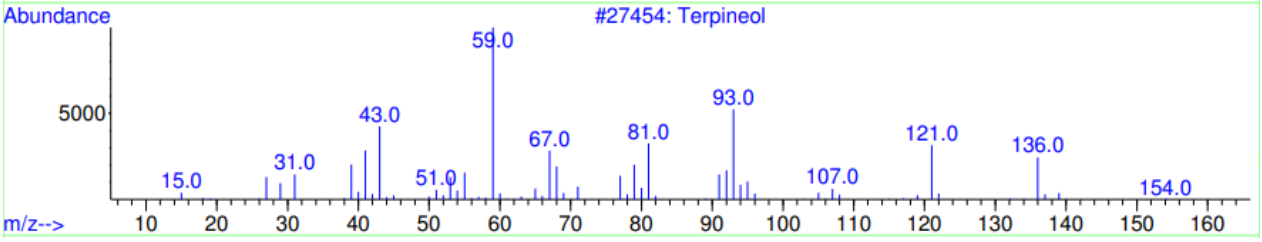
Compound 1
Compound name: Terpinen-4-ol
Retention time (min): 7.699
Molecular formula: $C_{10}H_{18}O$
Exact mass: 154.2493
Compound class: Monoterpene alcohol



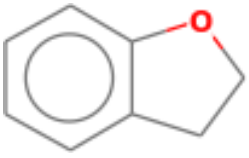


Compound 2
Compound name: Terpineol
Retention time (min): 7.895
Molecular formula: $C_{10}H_{18}O$
Exact mass: 154.2493
Compound class: Alcohol monoterpene





Compound 3
Compound name: Benzofuran,2,3-dihydro-
Retention time (min): 8.445
Molecular formula: C_8H_8O
Exact mass: 120.1485
Compound class: Heterocycle, benzofuran



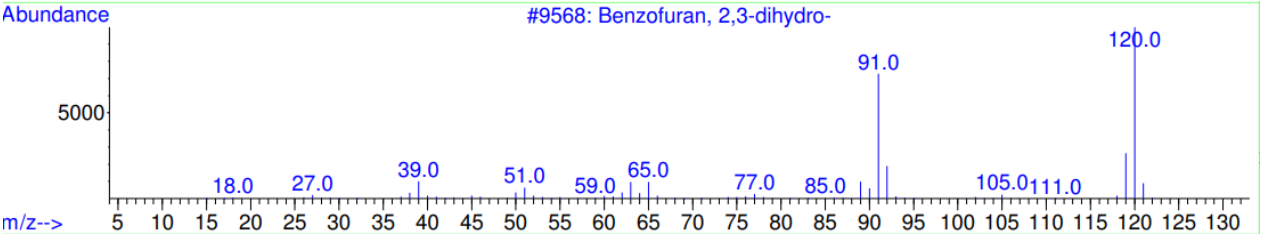
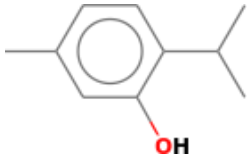
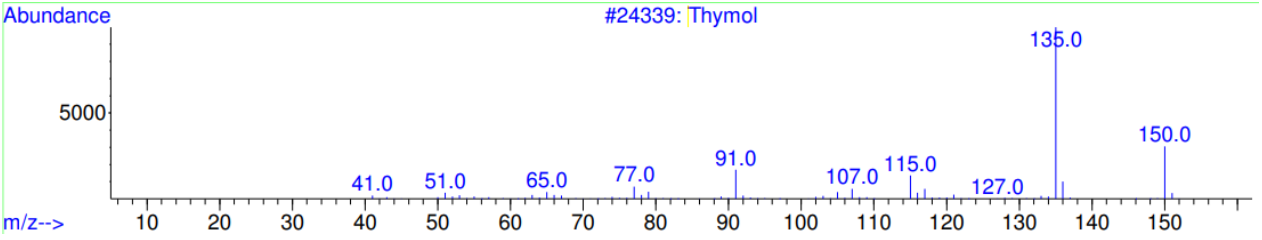


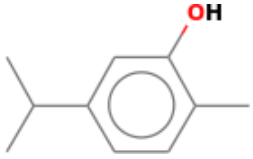
Figure S10. Compound fragment spectrum results (MS).

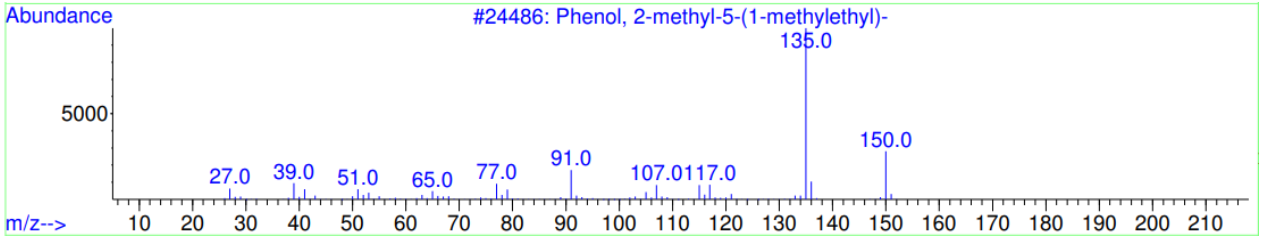
Compound 4
Compound name: Thymol
Retention time (min): 9.372
Molecular formula: $C_{10}H_{14}O$
Exact mass: 150.2176
Compound class: Monoterpene phenol



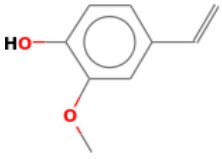


Compound 5
Compound name: Phenol,2-methyl-5-(1-methylethyl)
Retention time (min): 9.507
Molecular formula: $C_{10}H_{14}O$
Exact mass: 150.2176
Compound class: Monoterpene phenol





Compound 6
Compound name: 2-Methoxy-4-vinylphenol
Retention time (min): 9.628
Molecular formula: $C_9H_{10}O_2$
Exact mass: 150.1745
Compound class: Phenol



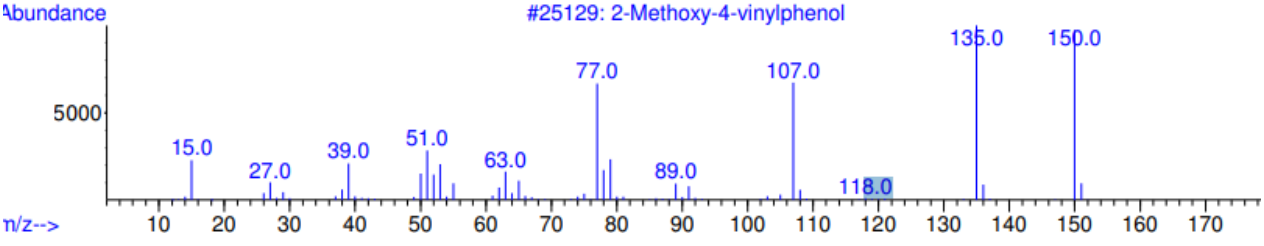
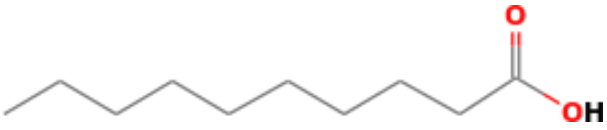
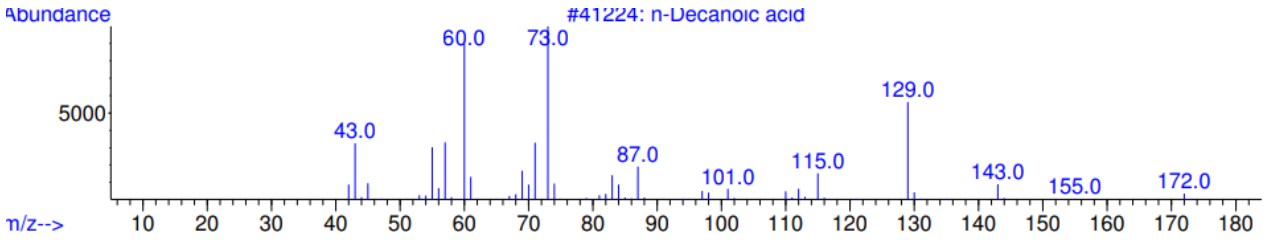


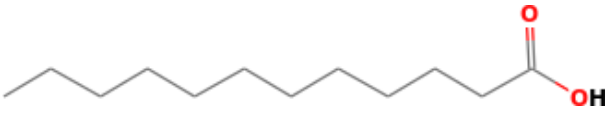
Figure S10. Conitnued.

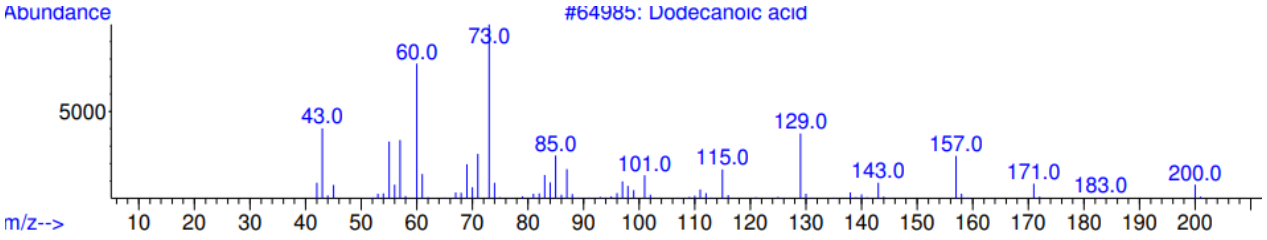
Compound 7
Compound name: Decanoic acid
Retention time (min): 10,382
Molecular formula: $C_{10}H_{20}O_2$
Exact mass: 172.2646
Compound class: Saturated fatty acid



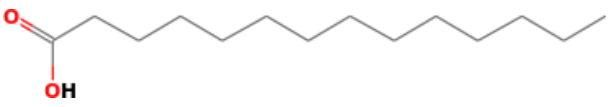


Compound 8
Compound name: Dodecanoic acid
Retention time (min): 12.808
Molecular formula: $C_{12}H_{24}O_2$
Exact mass: 200.3178
Compound class: Saturated fatty acid





Compound 9
Compound name: Tetradecanoic acid
Retention time (min): 15.008
Molecular formula: $C_{14}H_{28}O_2$
Exact mass: 228.3709
Compound class: Saturated fatty acid



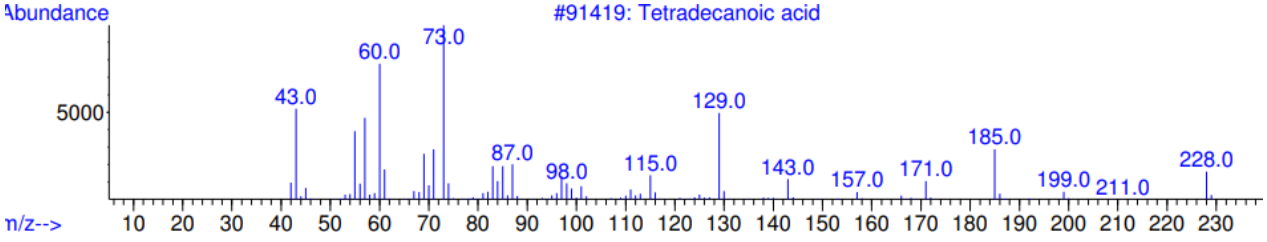
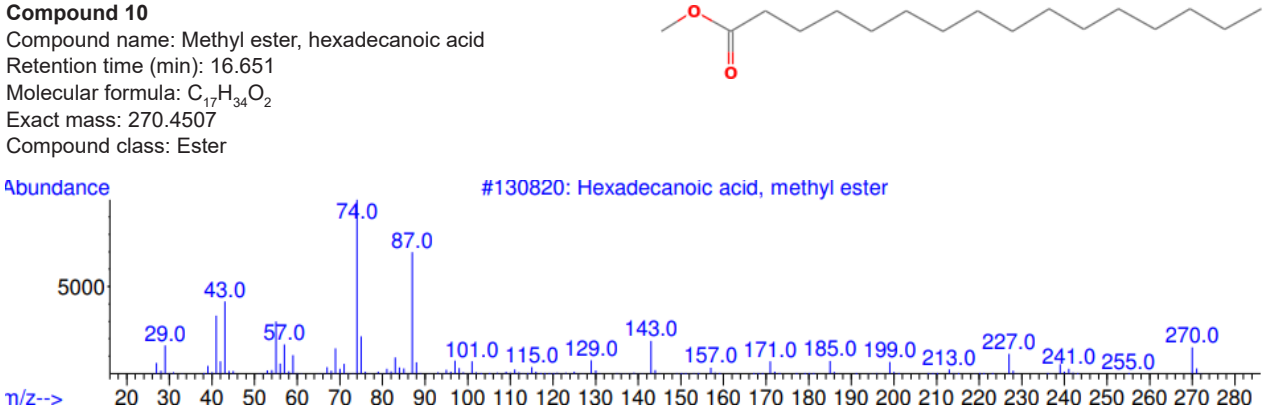
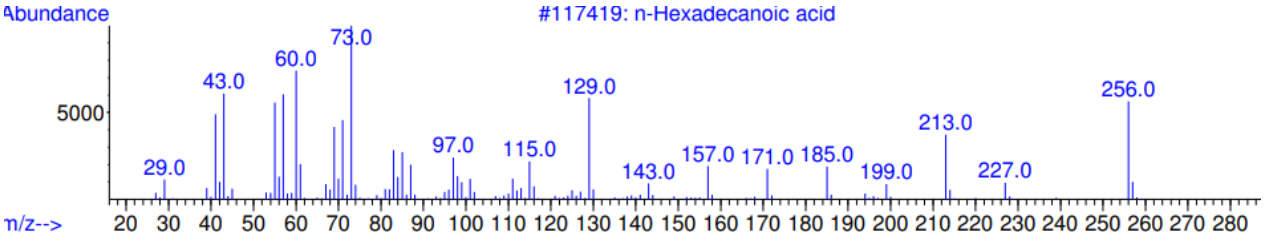


Figure S10. Conitnued.

Compound 10
Compound name: Methyl ester, hexadecanoic acid
Retention time (min): 16.651
Molecular formula: $C_{17}H_{34}O_2$
Exact mass: 270.4507
Compound class: Ester



Compound 11
Compound name: Hexadecanoic acid
Retention time (min): 17.065
Molecular formula: $C_{16}H_{32}O_2$
Exact mass: 256.4241
Compound class: Saturated fatty acid



Compound 12
Compound name:Phytol
Retention time (min): 18.452
Molecular formula: $C_{20}H_{40}O$
Exact mass: 296.5310
Compound class: Diterpenic alcohol

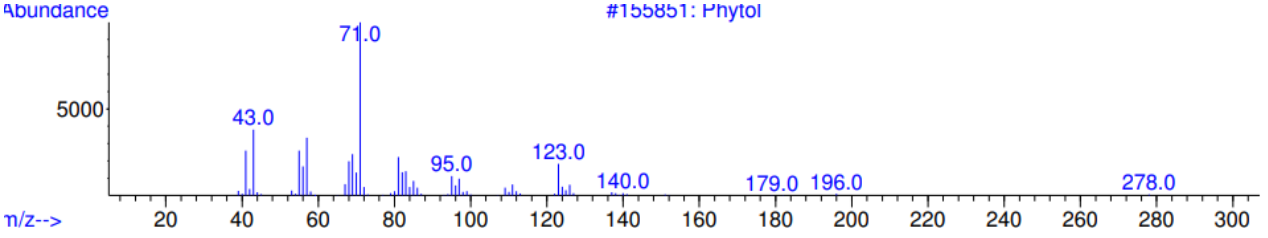
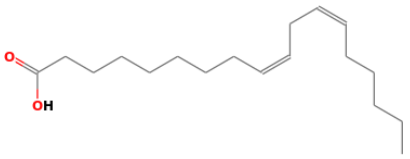
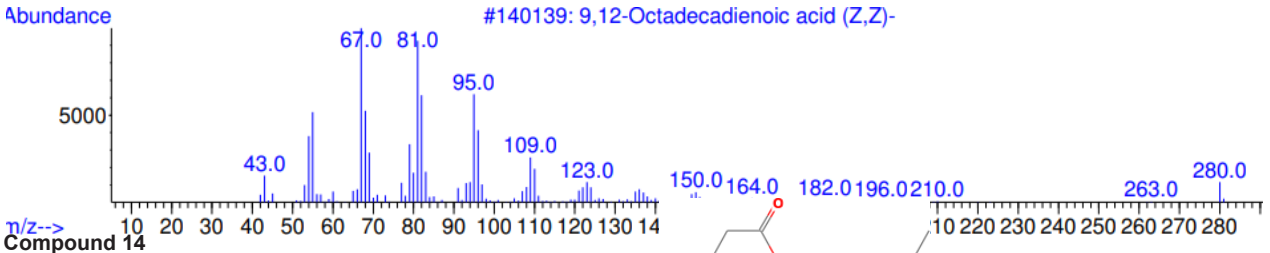


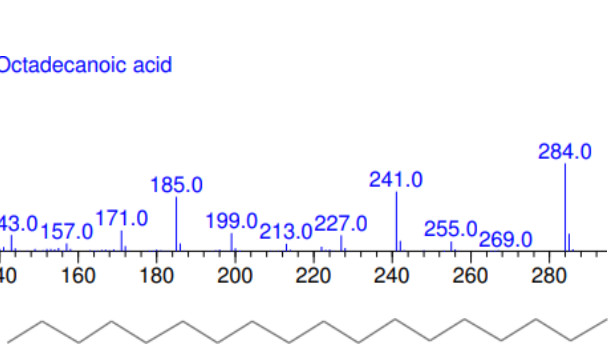
Figure S10. Conitnued.

Compound 13
Compound name: 9,12-octadecadienoic acid (Z,Z)
Retention time (min): 18.670
Molecular formula: $C_{18}H_{32}O_2$
Exact mass: 280.4455
Compound class: Unsaturated fatty acid

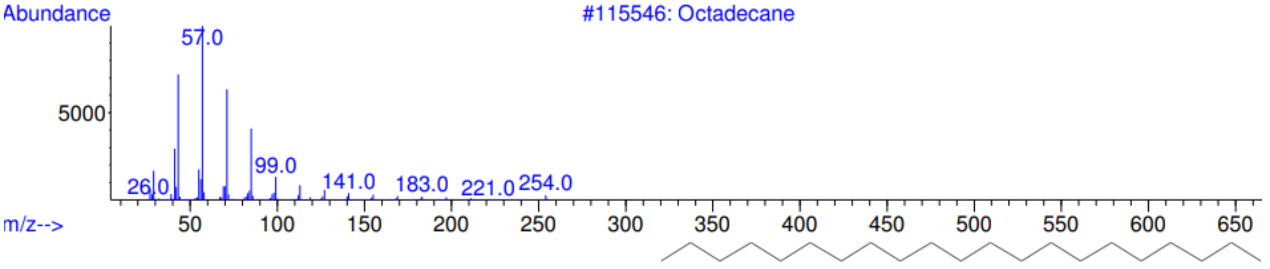




Compound 14
Compound name: Octadecanoic acid
Retention time (min): 18.911
Molecular formula: $C_{18}H_{36}O_2$
Exact mass: 284.4772
Compound class: Fatty acid



Compound 15
Compound name: Octadecane
Retention time (min): 19.198
Molecular formula: $C_{18}H_{38}$
Exact mass: 254.4943
Compound class: Aliphatic hydrocarbon



Compound 16
Compound name: Heneicosane
Retention time (min): 23.192
Molecular formula: $C_{21}H_{44}$
Exact mass: 296.5741
Compound class: Aliphatic hydrocarbon

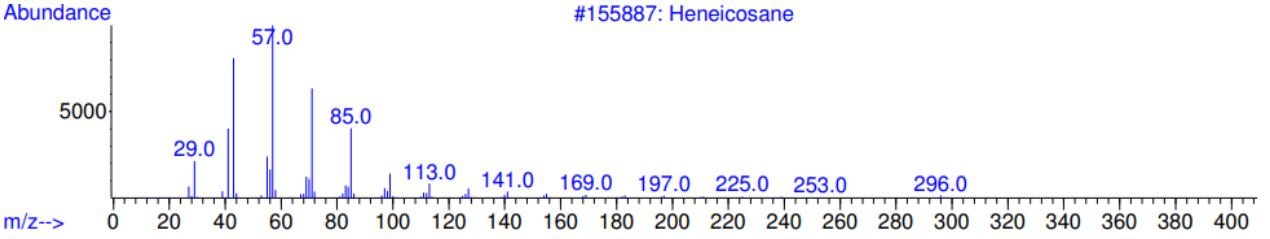


Figure S10. Conitnued.