Research article

Bioactive compounds, antioxidant and antibacterial properties of the pulp, peel and aril of the fruit of *Clusia quadrangula* (Clusiaceae)

Compuestos bioactivos, propiedades antioxidantes y antibacterianas de pulpa, piel y arilo de la fruta de *Clusia quadrangula* (Clusiaceae)

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Abstract:

Background and Aims: The Clusiaceae family is known for its bioactive compounds with beneficial antioxidant and anti-inflammatory properties. The objectives of this study were to 1) identify and quantify the individual polyphenol compounds in the methanolic extract from peel, pulp and aril of the fruit of *Clusia quadrangula* by UPLC-MSMS, evaluate their antioxidant properties, 2) analyze the fatty acid profile, minerals composition and 3) determine the antibacterial activity against pathogenic bacteria of the methanolic extract.

Methods: Physicochemical properties, antioxidants and bioactive compounds, fatty acid profile and mineral content were analyzed using a Microplate Spectrophotometer, Ultra High Performance Liquid Chromatography coupled with a triple quadrupole Mass Spectrometer, Gas Chromatography coupled with a Mass Spectrometer and Microwave Plasma Atomic Emission Spectroscopy.

Key results: Twenty phenolic compounds were identified and quantified plus the precursor shikimic acid (103.55 µg/g solids). Procyanidin B2 (41.56 µg/g solids), (−)-epicatechin (34.07 µg/g solids), and ellagic acid (27.58 µg/g solids) were found in greater quantity in the methanolic extract of the pulp. Palmitic and linoleic acid were the most abundant fatty acids in the pulp, peel, and aril, and linolenic acid was present in the pulp. The pulp also exhibited the highest amount of total polyphenolic compounds (24.33 mg GAE/g) and reducing power evaluated by FRAP (18.697 mg ET/g). Analysis of the minerals revealed that all fractions are rich in magnesium, potassium, sodium, and calcium. The results showed that the methanolic extract of the different parts of the fruit had antibacterial properties against *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis*.

Conclusions: These results indicate that different parts of the *C. quadrangula* fruit are a rich source of natural antioxidants and possess antibacterial properties. Therefore, it can be considered for use as ingredient or additive in the cosmetic, pharmaceutical, or food industries.

Key words: antimicrobial properties, fatty acids, minerals, polyphenols.

Resumen:

Antecedentes y Objetivos: La familia Clusiaceae es conocida por sus compuestos bioactivos con propiedades antioxidantes y antiinflamatorias beneficiosas. Los objetivos de este estudio fueron: 1) identificar y cuantificar compuestos polifenólicos individuales en el extracto metanólico de cáscara, pulpa y arilo del fruto de *Clusia quadrangula* por UPLC-MSMS, evaluar sus propiedades antioxidantes, 2) analizar el perfil de ácidos grasos, la composición mineral y 3) determinar la actividad antibacteriana contra bacterias patógenas del extracto metanólico.

Métodos: Las propiedades fisicoquímicas, antioxidantes y compuestos bioactivos, perfil de ácidos grasos y contenido de minerales fueron analizados utilizando un Espectrofotómetro de Microplaca, Cromatografía de Líquidos de Ultra Alta Resolución acoplado a un Espectrómetro de Masas triple cuadrupolo, Cromatografía de Gases acoplada a un Espectrómetro de Masas y Espectroscopia de Emisión Atómica de Plasma de Microondas.

Resultados clave: Se identificaron y cuantificaron 20 compuestos fenólicos más el precursor ácido shikímico (103.55 µg/g sólidos). Procianidina B2 (41.56 µg/g sólidos), (−)-epicatequina (34.07 µg/g sólidos) y ácido elágico (27.58 µg/g sólidos) se encontraron en mayor cantidad en el extracto metanólico de la pulpa. Los ácidos palmítico y linoleico fueron los ácidos grasos más abundantes en la pulpa, cáscara y arilo, y el ácido linolénico estuvo presente en la pulpa. La pulpa también exhibió la mayor cantidad de compuestos polifenólicos totales (24.33 mg GAE/g) y poder reductor evaluado por FRAP (18.697 mg ET/g). El análisis de los minerales reveló que todas las fracciones son ricas en magnesio, potasio, sodio y calcio. Los resultados mostraron que el extracto metanólico de las diferentes partes del fruto tenían propiedades antibacterianas contra *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, y *Enterococcus faecalis*.

Conclusión: Estos resultados indican que las diferentes partes del fruto de *C. quadrangula* son una rica fuente de antioxidantes naturales y poseen propiedades antibacterianas, por lo que se puede considerar para su uso como ingrediente o aditivo en la industria cosmética, farmacéutica o alimentaria.

Palabras clave: ácidos grasos, minerales, polifenoles, propiedades antimicrobianas.

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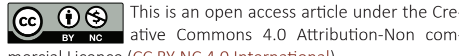

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Introduction

Clusia quadrangula Bartlett belongs to the family Clusiaceae, subfamily Clusioideae, and consists of shrubs or trees, some reaching a height of up to 15 m (Ribeiro et al., 2011). More than 300 species are known, and their occurrence is limited to tropical and subtropical regions of Central and South America (Compagnone et al., 2008; Ribeiro et al., 2011). Many of the species belonging to this genus are used as ornamentals (Compagnone et al., 2008).

Numerous studies have shown that the genus *Clusia* L. is a rich source of polyisoprenylated benzophenones and other compounds such as flavonoids and terpenes (Ribeiro et al., 2011). Many of the metabolites present in the flowers, fruits, leaves, and latex of different species of *Clusia* have antimicrobial, anti-HIV, and antioxidant activity (Huerta-Reyes et al., 2004; Ribeiro et al., 2011; Ramirez et al., 2018; Singh et al., 2020). Their use has been reported in the treatment of symptoms of weakness, constipation, and diarrhea, as well as for leprosy and scarring, and as a cough remedy (Mattos-Silva et al., 2019). The milky sap from leaves and fruits has also been used to mitigate boils and pimples (Lentz, 1993). These properties are possibly related to a broad spectrum of compounds that have antioxidant activity, such as polyphenols, which have been reported to be produced and accumulated in the epithelial cells of *Clusia* species and whose production is probably related to protection against herbivory (Machado and Emmerich, 1981).

Some other fruits of the Clusiaceae family, such as mangosteen, are a rich source of phenolic compounds, including xanthones, proanthocyanidins, and anthocyanins, of which a high correlation with their antioxidant activity has been found (Da Silva and Paiva, 2012). Similarly, it has been reported that compounds such as beta-sitosterol, stigmasterol, beta-amyrin, and epicatechin present in fruits of the Clusiaceae confer antioxidant activity (Ferreira-Oliveira et al., 2012). Clusiaceae is one of the families of great interest due to the presence of bioactive compounds, such as phenolic compounds, which give them antioxidant and anti-inflammatory properties, which is why the fruits of several Clusiaceae species are used in traditional medicine to treat dysentery, urinary disorders, cystitis, gonorrhea, inflammatory skin disorders, and wounds (Kshirsagar et al., 2022).

However, although there are several studies on other species of Clusiaceae, to our knowledge there are no studies on the identification and quantification of polyphenols and their potential antioxidant activity of the fruit of *Clusia quadrangula*. For this reason, the objectives of this work were: 1) to identify and quantify the phenolic compounds by UPLC-MSMS analysis and evaluate their antioxidant activity, 2) to analyze the fatty acid profile and mineral contents and, 3) to determine the antibacterial activity against pathogenic bacteria of methanolic extract from peel, pulp and aril of the fruit of *Clusia quadrangula*.

Materials and Methods

Chemicals

Methanol (MeOH), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonate radical cation (ABTS+), Folin-Ciocalteu's phenol reagent and gallic acid were purchased from Merck (Merck KGaA, Darmstadt, Germany). Ultrapure water was obtained with a Milli-Q Advantage A10 System apparatus (Bedford, Massachusetts, USA). Nutrient broth was purchased from Merck (Merck KGaA, Darmstadt, Germany). The bacterial strains *Staphylococcus aureus* subsp. *aureus* (Rosenbach 1945) (*S. aureus*, ATCC 25923) and *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919 (*E. coli*, ATCC 25922), *Salmonella typhi* (Schroeter 1886) Warren and Scott 1930 (*S. typhi*, ATCC 14028), and *Enterococcus faecalis* (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984 (*E. faecalis*, ATCC 19433) were cultured in the Universidad Popular Autónoma de Puebla, Mexico.

Fruit collection

Fruits of *Clusia quadrangula* were collected in El Chico, Emiliano Zapata, Veracruz, Mexico, situated at 19˚27'36''N latitude and 96˚49'48''W longitude (Fig. 1), at an elevation of 1060 m a.s.l. during August 2018 and August 2019. Fifty fruits from five trees (10 fruits per tree) were hand-harvested each year. The fruits were harvested with an optimal state of maturity with a concentration of soluble solids between 12 and 14 °Brix (Fisherbrand™ Handheld Analog Brix/Sucrose Refractometer, Fisher Scientific, Hampton, New Hampshire, USA).

Figure 1: Map of the collection site of *Clusia quadrangula* Bartlett in El Chico, Emiliano Zapata, Veracruz, Mexico.

The taxonomic identification of the fruit was confirmed by Manuel Carlos Durán Espinosa of the Instituto de Ecología, A.C. (INECOL) in Xalapa, Mexico. The voucher *N. Juárez Trujillo 3* of the reference fruit *NJuárezT2*, with herbarium barcode 149044, was deposited in the herbarium XAL of the Instituto de Ecología, A.C. (Fig. 2). The fruits of each collection period were taken to the laboratory, washed, and manually separated into their parts: pulp, aril, and peel. The fruit tissues were lyophilized (freeze dryer Labconco 121066614 E, Kansas City, Missouri, USA), milled (IKA A11 Basic, Staufen, Germany), vacuum packed (Foodsaver® FM5200, Washington, USA) and frozen (Thermo Fisher Scientific™ freezer TSX60086A, Waltham, Massachusetts, USA). The powder obtained from each collection stage was mixed to be analyzed as a single batch. The tests of each part (pulp, peel and aril) of the fruit were carried out in triplicate.

Extract preparation

The methanolic extracts of each fruit tissue were prepared using an accelerated solvent extraction system (ASE 350, Dionex, Sunnyvale, California, USA). Briefly, 3 g of each dry material was dispersed in 1 g of diatomaceous earth and placed in a 34 ml cell. The cell was filled up with MeOH up to a pressure of 1500 Psi and heated at 60 °C for 5 min. Then, the cell was washed off with 30% of cell volume. The extract was concentrated by rotary evaporation (Büchi RII, Flawil, Switzerland). Ten mg of the crude extract was re-dissolved in 1 ml of methanol with 0.1% of formic acid (both MS grade, Sigma-Aldrich, St. Louis, Missouri, USA), filtered and placed in a 1.5 ml UPLC vial (UPLC-MSMS Agilent Technologies, Santa Clara, California, USA). Samples were analyzed by triplicate.

HERBARIO
XAL
Instituto de Ecología A.C.
149044

HERBARIO XAL
INECOL
Clusia quadrangula Bartlett
Familia: Clusiaceae
Loc: El Chico
Mun: Emiliano Zapata **Edo:** Veracruz **País:** México
Lat: 19°27'36" N **Long:** 96°49'48" W **Alt:** 1061 msnm
Ecol: Solar, secundaria, suelo café con mucha materia orgánica, soleado
Descrip: Árbol, 2.5-3 m, fruto ovalado rojo, regular
Nom. Común: **Usos:** Ornamental
Col: Naida Juárez Trujillo **No.** 3 **Fecha Col:** 05/08/2018
Det: C.M. Durán E. **Fecha Det.:** /09/2018

Figure 2: Specimen of *Clusia quadrangula* Bartlett deposited in the herbarium XAL of the Instituto de Ecología, A.C. (INECOL) in Xalapa, Mexico.

Identification and quantification of individual polyphenols

Pulp, peel and aril methanolic extracts were analyzed in a liquid chromatograph (Agilent, 1290, Ultra performance liquid chromatography-tandem mass spectrometer, UPLC-MSMS, Agilent Technologies, Santa Clara, California, USA) coupled to a QqQ mass spectrometer (Agilent Technologies, model 6460, Santa Clara, California, USA) with a dynamic multiple reaction monitoring (dMRM) method for the search up to 60 compounds (Juarez-Trujillo et al., 2018). The UPLC-MSMS was equipped with a column ZORBAX SB-C18 (1.8 µm, 2.1 50 mm; Agilent Technologies, Santa Clara, California, USA) and the temperature of the column and sample were set to 40 and 15 °C, respectively. The mobile phase consisted of (A) water and (B) acetonitrile, both containing 0.1% formic acid. The gradient of the liquid phase was: 0 min 1% B, 0.1-40 min linear gradient 1-40% B, 40.1-42 min linear gradient 40-90% B, 42.1-44 min isocratic 90% B, 44.1-46 min linear gradient 90-1% B, 46.1-47 min 1% B isocratic (total run time 47 min). The flow rate was 0.3 ml/min, and 5 µl was the sample injection volume. The electrospray ionization (ESI) source was operated in positive and negative ionization modes. The desolvation temperature was 300 °C, the cone gas (N_2) flow was 5 l/min, the nebulizer pressure was 45 Psi, the sheath gas temperature was 250 °C, the sheath gas flow was 11 l/min, the capillary voltage (positive and negative) was 3500 V, and the nozzle voltage (positive and negative) was 500 V. The fragmentor voltage was 100 V and the cell accelerator voltage was 7 V for all compounds. The identity was confirmed by co-elution with authentic standards under the same analytical conditions above described for each compound. For quantitation of each phenolic compound a calibration curve in a concentration range of 1-19 µM was prepared (r^2 values ≥0.97 were considered for the linearity range). Detailed chromatographic and spectrometric information for each compound is supplied as Supplementary Table S1. The data were processed using the MassHunter Workstation Software v. B.06.00 distributed by Agilent Technologies (Santa Clara, California, USA). The results were expressed as µg/g of sample (dry weight of extract).

Analysis of fatty acid profile

The fatty acid profile was determined in the pulp, peel and aril hexanic extracts of the fruit as described by López-López et al. (2001). Fatty acids were determined by converting the oil (100 µg) into methyl esters through the addition on BF_3 (1 ml). The methyl esters were extracted using 1.0 ml of hexane grade High Performance Liquid Chromatography (HPLC grade, Sigma-Aldrich, St. Louis, Missouri, USA). The hexanic extracts were dried with anhydrous sodium sulfate (Na_2SO_4) and filtered for subsequent injection into the gas chromatograph. The sample (2 µl) was analyzed in a gas chromatograph (Agilent Technologies, model 6890 N, Santa Clara, California, USA) coupled to a mass spectrometer (Agilent Technologies, model 5975 inert XL, Santa Clara, California, USA) equipped with a column DB-5, phenylmethyl polysiloxane (5%) (cat-1225082, J&W Scientific, Folsom, California, USA), 60 m long, 0.25 mm internal diameter and 0.25 µm film thickness. Mass spectra for each compound were obtained by electron impact ionization at 70 eV. The identity of each fatty acid was assigned using an external standard (FAME mix, C8:C22, No. de cat 18920-1AMP, Sigma-Aldrich, St. Louis, Missouri, USA) analyzed under the same conditions and was confirmed with the help of a library NIST Mass Spectral search program, version 2.0d (Gaithersburg, Maryland, USA). The percentages of each fatty acid in the sample were calculated by considering the individual contribution of its own area to the total area (sum of individual areas) of the fatty acids identified.

Total polyphenols, vitamin C, carotenoids, and anthocyanins content

Total polyphenols content was analyzed in the methanolic extract of each of the parts of the *C. quadrangula* fruit using Folin-Ciocalteu according to the method proposed by Padhi et al. (2016). For quantification, a calibration curve at different concentrations of gallic acid was performed. Results are reported as mg of gallic acid equivalent (GAE)/g of tissue. Vitamin C content was determined following the spectrophotometric method reported by Jacota and Dani (1982). The results were compared in a calibration curve of different concentrations of ascorbic acid. Total carotenoids content in the samples was determined in the acetone extract (50 mg sample in 10 ml solvent) as the sum of the

isochromic carotenoid fractions and analyzed using the spectrophotometric method described by Hornero-Méndez and Mínguez-Mosquera (2001). Total anthocyanin quantification was performed by the pH differential method previously reported by Giusti and Wrolstad (2001) with some modifications to adapt it to microplate conditions. Methanolic extracts from different parts of the fruit were diluted 1:150 w:v in pH 1.0 and pH 4.5 buffers, then measured at 520 and 700 nm in a UV/Vis microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific™, Waltham, Massachusetts, USA). Total anthocyanins contents were determined based on a cyanidin 3-glucoside molar extinction coefficient of 26,900 and a molecular weight of 449.2 g/mol. Results were expressed in terms of mg of cyanidin-3-glucoside/100 g of extract. Each sample was analyzed by triplicate.

Antioxidant properties

Determination of DPPH radical inhibition

The antioxidant activity of the extracts was evaluated through the percentage inhibition of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH, 0.2 mM in ethanol, Merck KGaA, Darmstadt, Germany) by the method described by Thaipong et al. (2006) with some modifications. Fifty µl of the different extracts (200 mg/ml), 50 µl of methanol and 50 µl of DPPH were mixed and placed in a microplate. Subsequently, the samples were incubated (incubator IKA® KS 3000i control, Staufen, Germany) in the dark for 30 minutes and the absorbance was determined at a wavelength of λ=517 nm, taking as a blank a mixture consisting of 50 µl of extract and 100 µl of methanol and control consisting of 50 µl of DPPH plus 100 µl of methanol. The ability to capture the DPPH radical of the extracts at different concentrations was plotted to obtain the IC_{50}. All determinations were made by triplicate.

The scavenging of DPPH radical was calculated according to the following equation:

$$DPPH\ inhibition\ (\%) = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100$$

Where: $Abs_{control}$ is the absorbance of DPPH radical plus methanol; Abs_{sample} is the absorbance of DPPH radical plus sample extract/standard.

ABTS⁺ antioxidant activity

$ABTS^+$ antioxidant activity was determined by the method of Savi et al. (2020) with some modifications. First, the $ABTS^+$ radical was prepared from the $ABTS^+$ reagent (7 mM) with potassium persulfate (2.45 mM) mixed in equal proportions. The solution was incubated (incubator IKA® KS 3000i control, Staufen, Germany) in the dark for 16 hours at room temperature. The $ABTS^+$ radical was diluted in 96% ethanol and its absorbance was measured at a wavelength of λ=734 nm until it reached a value of 0.800. Subsequently, 30 µl of each of the methanolic extracts (200 mg/ml) and 270 µl of $ABTS^+$ reagent was placed in a microplate and incubated at 25 °C for 30 min in the absence of light. Finally, absorbances were determined at λ=734 nm using 300 µl of $ABTS^+$ reagent as blank. At the same time, a calibration curve was prepared from a solution of 10 mg of trolox diluted in 5 ml of methanol and 5 ml of distilled water, and dilutions were made with known concentrations from 0.1 to 1 mg/ml. The results were expressed in mg of trolox equivalents/g of sample. All determinations were made by triplicate.

Determination of the reducing power FRAP

The reducing power was determined by the methodology proposed by Thaipong et al. (2006) with some modifications, using a standard of the Ferric Reducing Antioxidant Power Assay (FRAP) solution prepared with 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl solution and 20 mM $FeCl_3.6H_2O$ solution. Thirty µl of the different extracts were taken and mixed with 270 µl of the FRAP solution was added, incubated for 30 minutes in the dark, and absorbances were determined of the colored product (ferrous tripyridyltriazine complex) at a λ=593 nm. Finally, a standard curve with concentrations from 0.1 to 1 mg/l of trolox was performed and the results were expressed as mg of trolox equivalents/g of extract. This analysis was performed in triplicate for each sample.

Mineral analysis

Elemental composition was determined by atomic emission spectroscopy using an Agilent MP-AES MP 4100 (Agilent Technologies, Santa Clara, California, USA). One g of each of the parts of the fruit (pulp, peel and aril) was previously subjected to digestion with a solution of nitric acid (5%) in

a ratio 1:10 (w:v). The digested sample was analyzed in the equipment under the following conditions: the plasma gas flow was used at 20 l/min and the auxiliary gas flow was fixed at 1.5 l/ min. The plasma stabilization time with sample aspiration of 15 s, read time 3 s (read in triplicates), and wash time 20 s was setting for the analysis of all elements. The analysis was performed in triplicate.

Antibacterial property and minimum inhibitory concentration (MIC)

The antimicrobial properties of the methanolic extracts were investigated by the microtiter plate assay method (Dzugan et al., 2020). The extract of each of the tissues (pulp, peel and aril) was tested on four bacteria, two Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 19433) and two Gram-negative (*Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC 14028), for which 100 µl of nutrient broth, 100 µl of microbial suspension of each bacterium adjusted to turbidity value of 0.5 according to the McFarland scale measuring optical density (OD) of the culture at 600 nm (initial absorbance) and 100 µl of different concentrations of the methanolic extract of the different parts of the fruit (0 (control), 2.5, 5, 7.5, 12.5, 25 and 50 mg/ml), were added to each well. The plate was incubated at 37 °C for 24 h and the optical density was measured at 600 nm. The percentage of inhibition was calculated from the following:

$$Inhibition\ (\%) = (\frac{A_{control} - A_{sample}}{A_{control}}) \times 100$$

Minimum inhibition concentration of extract for each bacterium was determined as the concentration of the methanolic extract that presented an inhibition percentage greater than 90% (MIC 90) (Dzugan et al., 2020) and was confirmed by the addition of 10 µl and 10 µl iodonitrotetrazolium chloride followed by incubation at 32 °C for 1 h (Kilonzo and Munisi, 2021). The test was performed in triplicate.

Statistical analyses

One way analysis of variance (ANOVA), followed by a Tukey's test with a significance level of 5% (*p<0.05*) were performed using Statistic v. 7.0 (StatSoft and TIBCO Software Group Inc., 2002).

Results and Discussion

Antioxidant properties

Table 1 shows the concentration of total polyphenols, anthocyanins, carotenoids, and vitamin C, and the evaluation of antioxidant activity using the DPPH, ABTS+, and FRAP methods for different parts of the fruit. The pulp (24.331 mg GAE/g) and peel (13.070 mg GAE/g) exhibited a concentration of total polyphenols higher than that found in the aril (8.830 mg GAE/g). Similarly, the pulp exhibited a higher concentration of anthocyanins (0.90 mg C3G/g) than that determined for the peel. All samples exhibited a total polyphenols content greater than that reported in methanolic extracts of other fruits belonging to the same family, such as *Garcinia madruno* (Kunth) Hammel (Ramirez et al., 2018). In contrast, the aril exhibited higher values of total

Table 1: Chemical compounds and antioxidant properties of methanolic extracts from different parts (pulp, peel and aril) of the fruit of *Clusia quadrangula* Bartlett. Results are expressed as the mean (*n=3*) ± SD. Different letters in the same row indicate significant differences among samples (*p<0.05*). GAE: Gallic acid equivalents, C3G: Cyanidin-3-Glucoside, AA: Ascorbic acid, TE: Trolox equivalents, "-" Not detected. DPPH: 2,2-diphenyl-1-picrylhydracil; ABTS+: 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonate radical cation; FRAP: Ferric reducing antioxidant power.

Property	Pulp	Peel	Aril
Total polyphenols (mg GAE/g)	24.331±3.405[a]	13.070±1.044[b]	8.830±2.445[c]
Total anthocyanins (mg C3G/g)	0.90±0.10[a]	0.36±0.08[b]	_
Total carotenoids (µg/g)	0.482±0.001[b]	0.333±0.001[c]	0.915±0.001[a]
Vitamin C (mg AA/g)	26.428±0.355[b]	27.447±3.100[b]	60.771±5.416[a]
DPPH (IC_{50}) (µg/ml extract)	0.750±0.001[a,b]	0.094±0.001[b]	1.065±0.001[a]
ABTS+ (mg TE/g)	182.623±19.107[b]	488.564±40.441[a]	118.836±8.445[c]
FRAP (mg TE/g)	18.697±2.709[a]	2.408±0.193[c]	6.477±0.993[b]

carotenoids (0.915 µg/g) and vitamin C (60.771 mg AA/g) compared to the pulp and peel samples. The antioxidant activity determined in each of the parts of the fruit showed that they contain bioactive compounds that act as free radical inhibitors, explaining their antioxidant activity. The antioxidant activity determined as DPPH IC_{50} values exhibited the lowest value in the peel (0.094 µg/ml) and the pulp (0.750 µg/ml), suggesting that these two fractions have higher antioxidant activity. The DPPH IC_{50} values are similar to those reported for the methanolic extract of *G. madruno* and mangosteen fruit (Ramirez et al., 2018). Consistent with this, the peel exhibited higher ABTS+ antioxidant activity values (488.564 mg TE/g), followed by the pulp (182.623 mg TE/g) and the aril (118.836 mg TE/g), suggesting that the components present in the peel contribute to antioxidant activity. The FRAP assay also showed that the pulp has a greater reducing capacity to use the ferric tripyridyl-triazine (Fe^{3+}-TPTZ) complex. The difference in antioxidant activity values might be due to different inherent components present in each part of the fruit, as antioxidant activity depends largely on the bioactive compounds that a fruit possesses, on the type of mechanisms of each reaction, on the mixture of bioactive compounds present in the extract, and the degree of interaction of the antioxidant molecules present in the extract with free radicals, among other factors (Zengin et al., 2018).

Individual polyphenol analysis

Table 2 shows the 21 chemical compounds involved in the phenolic pathway identified and quantified in the methanolic extracts from the different tissues of *C. quadrangula* fruit including two precursors, 10 phenolic acids, one phenolic aldehyde, seven flavonoids and one dihydrochalcone glucoside. Regarding phenolic precursors, shikimic acid was quantified in the pulp and peel methanolic extracts, while L-phenylalanine was only determined in the peel. Interestingly, these compounds were not found in the aril methanolic extract. The most abundant compound quantified was shikimic acid in the pulp methanolic extract. Shikimic acid is an important intermediate in the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) in plants and microorganisms (Singh et al., 2020) and it has exhibited anti-inflammatory and antiviral properties against influenza virus and HIV (Huerta-Reyes et al., 2004; Singh et al., 2020).

Phenolic acid was the most representative chemical subgroup, being quantified eight, ten, and nine compounds in the pulp, peel and aril methanolic extracts, respectively. Ellagic acid is the most abundant phenolic acid in the pulp and aril methanolic extracts, while 4-hydroxybenzoic acid is the most abundant phenolic acid in the peel methanolic extract. Ellagic acid has antioxidant activity, which can eliminate and inactivate free radicals, especially hydrogen peroxide, hydroxyl radicals, and reactive nitrogen species, which affect the redox potential and increase cellular antioxidants (Mehrzadi et al., 2018). Also, it has been shown that this compound possesses anti-inflammatory activity, attenuates testicular disruption, provides liver protection, and possesses anti-tumor activity, especially against colon cancer, esophageal cancer, and brain cancer (Zheng et al., 2019).

Just one phenolic aldehyde (vanillin) was identified and quantified in the peel methanolic extract, while flavonoids were the second most representative chemical subgroup with seven compounds identified and quantified mainly in the pulp methanolic extract, being procyanidin B2 and (−)-epicatechin the most abundant. Only quercetin-3-glucoside was quantified in the peel and aril methanolic extracts. Some of our results coincide with what has been reported for other Clusiaceae. This is the case of flavonoids such as procyanidin, prodelphinidin, and stereoisomers of afzelechin/epiafzelechin, catechin/epicatechin, and gallocatechin/epigallocatechin which have been previously reported in mangosteen pericarps (Fu et al., 2007). (−)-Epicatechin and procyanidin B2 (PB) are flavonoids belonging to the flavan-3-ols which are recognized for their role in maintaining cellular homeostasis, potentiating the antioxidant activity of enzymes to protect against oxidative stress by regulating the endogenous cellular defense, and protecting against prostatitis in rats, which has been attributed to their ability to reduce inflammation and improve antioxidant cellular activity (Wang et al., 2017). Finally, phloridzin, a dihydrochalcone glucoside was quantified only in the pulp methanolic extract.

It has been reported that the concentration of polyphenols in the fruits depends on several factors such as climate, geographical area of cultivation, growing con-

ditions, and storage conditions (Manach et al., 2004), while their radical scavenging capacity depends on the phenolic compounds, mainly the number and position of hydroxyl groups present in the molecules that contain them (Rice-Evans et al., 1996; Manach et al., 2004; Silva-Gontijo et al., 2012). Procyanidin B2, (−)-epicatechin, and ellagic acid contain in their structure aromatic rings that have been reported to be responsible for electron delocalization, which confers their radical scavenging activity, and bioactivity which contributes to the antioxidant activity and functionality of the foods that contain them (Rice-Evans et al., 1996). The high antioxidant activity of these polyphenolic compounds is attributed to the -OH groups, which are potent hydrogen donors, producing stable delocalization of electrons in the molecule, resulting in the production of phenoxy radicals. In turn, this type of compound has greater planarity, which allows the conjugation and delocalization of electrons (Silva-Gontijo et al., 2012), favoring the formation of hydrogen bonds between -OH and C=O groups, which increases the conjugation of aromatic rings and the ability to donate hydrogens, resulting in more delocalized radicals.

Fatty acid profile

The nutritional value of the oils depends, in some respects, on the content of free fatty acids. Ten fatty acids were identified in the pulp, aril, and peel. The fatty acids varied from

Table 2: Phenolic compounds (µg/g) present in the methanolic extracts from different parts (pulp, peel and aril) of the fruit of *Clusia quadrangula* Bartlett. Results are expressed as the mean (*n=3*) ± SD. Different letters in the same row indicate significant differences among samples ($p<0.05$). "-": Not detected.

No.	Compound	Pulp	Peel	Aril
	Phenolic precursors			
1	Shikimic acid	103.55±6.04[b]	6.02±0.54[a]	-
2	L-phenylalanine	-	2.76±0.04	-
	Phenolic acids			
3	Gallic acid	-	0.23±0.01	-
4	Protocatechuic acid	1.22±0.18[a]	1.46±0.11[a]	3.98±0.47[b]
5	4-Hydroxybenzoic acid	0.57±0.01[a]	2.33±0.04[c]	1.18±0.01[b]
6	Gentisic acid	0.84±0.08[b]	0.56±0.01[a]	0.53±0.03[a]
7	4-Hydroxyphenylacetic acid	-	0.75±0.05[b]	0.44±0.02[a]
8	Vanillic acid	0.15±0.00[a]	0.29±0.08[b]	0.39±0.09[b]
9	4-Coumaric acid	0.22±0.05[a]	1.75±0.02[b]	0.29±0.05[a]
10	Salicylic acid	0.90±0.01[b]	0.53±0.05[a]	0.58±0.06[a]
11	trans-Cinnamic acid	0.13±0.00[b]	0.05±0.00[a]	0.17±0.01[c]
12	Ellagic acid	27.58±2.86[c]	1.39±0.09[a]	6.68±1.14[b]
	Phenolic aldehyde			
13	Vanillin	-	0.05±0.00	-
	Flavonoids			
14	(+)-Catechin	0.50±0.02	-	-
15	(-)-Epicatechin	34.07±0.29	-	-
16	Quercetin	0.21±0.00	-	-
17	Quercetin-3-glucoside	1.65±0.02[c]	0.02±0.00[a]	0.51±0.00[b]
18	Kaempferol-3-O-glucoside	0.84±0.01	-	-
19	Myricetin	1.13±0.04	-	-
20	Procyanidin B2	41.56±0.75	-	-
	Dihydrochalcone glucoside			
21	Phloridzin	0.52±0.01	-	-

C14:0 to C22:0 and their individual relative area percentages are shown in Table 3. The linoleic, palmitic, palmitoleic, oleic and stearic acids were most abundant fatty acids. The pulp exhibited a high content of linoleic acid (32.71%), palmitic acid (32.00%), and palmitoleic acid (10.46%), while the peel and aril exhibited a high content of palmitic acid, linoleic acid and oleic acid. In general, the content of unsaturated fatty acids (USFA) exceeded that of saturated fatty acids (SFA), being higher in the aril (1.54%) and the pulp (1.40%). The peel (30.15%) and the aril (27.01%) of the fruit exhibited higher percentages of monounsaturated fatty acids (MUFA) compared to the pulp (17.97%).

The results obtained showed that the *C. quadrangula* fruit is an important source of fatty acids. The type and proportion of fatty acids present in the different parts of the fruit are comparable to those found in seed oil from *Garcinia mangostana* L. (Ayahi et al., 2007). The balance between omega-6/omega-3 fatty acids ratio is crucial to maintain health, reduce coronary diseases and degenerative diseases (Patel et al., 2022). The ratio of linoleic to linolenic omega-6/omega-3 fatty acids in the pulp was 4.24, which suggests that it can be used as a food supplement and it can be recommended for consumption (Lupette and Benning, 2020), since the ingestion of products with polyunsaturated fatty acids (omega-3) is linked to a decrease in the presence of diseases (Patel et al., 2022). The higher percentage of monounsaturated fatty acids in the peel and the aril suggest that they have potential in the prevention of diseases (Reche et al., 2019).

Mineral analysis

The mineral profiles of pulp, peel, and aril are shown in Table 4. The three samples contain 17 elements of the 26 analyzed. Magnesium, potassium, sodium, and calcium were the minerals found in the highest concentrations. The aril exhibited a higher mineral concentration of magnesium compared to the peel and pulp. The values of minerals are congruent with the mineral concentrations found in leaves of various species of the genus *Clusia* (Olivares and Aguiar, 1999), but in all cases they were lower than those reported for the peel, pulp, and seeds of *Garcinia humilis* (Vahl) C.D. Adams (Tome et al., 2019).

Our study revealed a higher concentration of minerals in *C. quadrangula* fruits than those reported for other fruits of the genus *Clusia* (Sayeed et al., 2020), which suggests it has therapeutic potential in several diseases. Magnesium

Table 3: Fatty acids (%) present in the hexanic extracts from different parts (pulp, peel and aril) of the fruit of *Clusia quadrangula* Bartlett. Results are expressed as the mean (*n=3*) ± SD. Different letters in the same row indicate significant differences among samples (*p<0.05*). "-": Not detected. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids. USFA: unsaturated fatty acids.

Retention Time (s)	Compound	Pulp	Peel	Aril
10.62	Tetradecanoic acid	-	2.92±0.07[b]	0.36±0.05[a]
12.89	Palmitoleic acid	10.46±0.25[b]	6.98±0.98[a]	7.13±0.35[a]
13.14	Palmitic acid	32.00±0.45[b]	28.99±0.41[a]	27.94±0.65[a]
16.23	Linoleic acid	32.71±0.75[b]	23.36±0.60[a]	33.65±0.53[b]
16.29	Oleic acid	6.64±0.42[a]	22.29±0.37[c]	18.08±0.23[b]
16.29	Linolenic acid	7.71±1.25	-	-
16.82	Stearic acid	2.51±0.09[b]	7.07±0.10[c]	1.81±0.15[a]
21.85	11-Eicosenoic Acid	0.87±0.05[a]	0.88±0.01[a]	1.80±0.02[b]
22.76	Eicosanoic acid	1.35±0.07[a]	2.19±0.18[b]	1.87±0.12[b]
33.56	Docosanoic acid	5.76±0.26[a]	5.32±0.10[a]	7.38±0.89[b]
	Total PUFA	40.42	23.36	33.65
	Total MUFA	17.97	30.15	27.01
	Total SFA	41.62	46.49	39.36
	USFA/SFA	1.40	1.15	1.54

Table 4: Mineral and trace elements (mg/100 g DW) of peel, pulp and aril of the fruit of *Clusia quadrangula* Bartlett. Results are expressed as the mean (*n=3*) ± SD. Different letters in the same row indicate significant differences among samples (*p<0.05*). "-": Not detected.

Mineral	Pulp	Peel	Aril
Al	6.31±0.85[a]	9.07±0.50[b]	15.88±0.78[c]
As	-	-	-
B	2.16±0.72[a,b]	1.25±0.55a	1.89±0.65[a,b]
Ba	1.61±0.12[a]	2.52±0.25b	2.68±0.38[b]
Be	-	-	-
Ca	46.82±0.76[a]	84.47±1.02c	56.76±1.25[b]
Cd	-	-	-
Co	0.34±0.10[a]	9.64±0.50c	5.78±0.28[b]
Cr	0.39±0.11[a]	0.67±0.20a	0.57±0.25[a]
Cu	1.09±0.15[a]	0.92±0.25a	0.88±0.32[a]
Fe	5.39±0.45[a]	11.03±0.65b	4.77±0.71[a]
K	127.68±0.98[a]	140.37±0.76b	150.76±0.58[b]
Mg	3106.38±1.77[b]	2973.60±1.42[a]	5898.99±1.89[c]
Mn	14.89±0.32[a]	20.19±0.045[b]	18.99±0.78[b]
Mo	-	-	-
Na	87.95±1.02[a]	94.89±1.0[b]	88.13±0.98[a]
Ni	0.095±0.02[a]	0.21±0.01[b]	0.09±0.01[a]
Pb	-	-	-
Sb	-	-	-
Se	-	-	-
Si	1.83±0.25[a]	14.35±0.45[b]	10.88±0.65[b]
Sr	4.94±0.46[a]	7.95±0.78[b]	5.98±0.65[a]
Ti	-	-	-
Tl	-	-	-
V	0.02±0.02[a,b]	0.02±0.00[a,b]	0.01±0.00[a]
Zn	2.06±0.09[a]	3.00±0.10[a]	5.79±0.15[b]

is one of the most important micronutrients for the human body, since it is involved in many physiological processes and is essential for the maintenance of the normal function of cells and organs, for which it has an important contribution to health (Porri et al., 2021). Calcium is essential to bone structure and function, which is an important nutritional contribution considering that children between the ages of 4 and 8 years require 1000 mg/day of calcium, as they are in a crucial stage of growth and development. This mineral is a building block for strong, healthy bones and teeth (Del Valle et al., 2011).

Antibacterial properties and minimum inhibitory concentration (MIC)

The percentage of inhibition of the methanolic extracts of the peel, pulp, and aril of the fruit of *C. quadrangula* is shown in Table 5. The methanolic extract of the peel was more effective against *S. aureus* at a concentration of 7.50 mg/ml. The percentage of inhibition at 50 mg/ml exhibited by the pulp, aril and peel for *S. typhi* was 97.75, 94.12 and 95.25%, respectively, and it decreased in a dose-dependent manner. The potent antimicrobial activity specifically against *S. typhi* helps to explain its use in diarrhea control. All extracts at a concentration of 50 mg/ml exhibited an inhibition percentage greater than 90% for the tested bacteria. The pulp extract exhibited MIC 90 value of 7.50 mg/ml against *S. typhi* and *S. aureus*, while for *E. coli* and *E. faecalis* it was 25 mg/ml, reflecting a lower effectiveness against the latter two microorganisms. The difference in the antimicrobial activity of the test bacteria can be attributed to the intrinsic tolerance of the microorganism and to the type and combination of phytochemicals present in the extract. The bacterial sensitivity among bacteria could be attributed to differences in the growth rate and reduced cell wall permeability of the pathogen. The MIC 90 values indicate that the pulp extract is more effective against *S. typhi* and *S. aureus*, while the peel and aril extracts were more effective against *S. aureus*, with MIC 90 values of 7.50 and 12.50 mg/ml, respectively. The stronger antimicrobial activity was exhibited against *S. typhi* and *S. aureus* bacteria and the values obtained were comparable to those reported for honey (Dzugan et al., 2020). The antimicrobial activity found may be associated with bioactive compounds like alkaloids and phenolics, which have been reported with antimicrobial activity (Othman et al., 2019).

Polyphenols have high antioxidant activity and confer good antimicrobial activity due to the destruction of membrane integrity (Fei et al., 2018). The pulp of *C. quadrangula* fruit exhibited a high content of shikimic acid and procyanidin B2 (Table 2). Shikimic acid has been reported with antibacterial activity against *S. aureus* ATCC 6538 with a MIC value of 2.50 mg/ml mediated by damaging cell membrane (Bai et al., 2015). In addition, this compound inhibited the formation of *S. aureus* biofilms by interfering the adhesion and decreasing the metabolic activity, motility, and viability

Table 5: Antimicrobial activity and minimum inhibitory concentration (MIC 90) of the methanol extract (mg/ml) of the pulp, peel and aril of the fruit of *Clusia quadrangula* Bartlett. Different letters indicate significant differences ($p<0.05$) within the same row. Minimum inhibitory concentration (MIC 90) is marked in gray colour.

	Extract concentration (mg/ml)					
	50	**25**	**12.50**	**7.50**	**5.00**	**2.50**
Pulp						
E. coli (ATCC 25922)	95.50±1.25[d]	90.37±1.52[b,c]	87.10±2.11[b]	88.17±3.28[b]	84.95±2.34[b]	20.00±1.25[a]
S. typhi (ATCC 14028)	97.75±3.68[c]	97.19±2.01[c]	92.71±0.84[b]	91.67±2.20[a,b]	88.54±3.01[a]	86.46±3.14[a]
S. aureus (ATCC25923)	94.15±2.80[b,c]	95.45±2.74[c]	91.26±1.89[b]	90.29±0.84[b]	84.47±2.98[a]	83.50±4.25[a]
E. faecalis (ATCC 19433)	92.35±2.69[d]	90.32±1.20[d]	86.52±2.13[c]	66.29±2.78[b]	60.67±6.25[b]	43.82±3.36[a]
Peel						
E. coli (ATCC 25922)	90.45±2.76[f]	80.14±5.43[e]	66.67±6.57[d]	33.33±4.66[c]	20.43±3.89[b]	8.60±5.23[a]
S. typhi (ATCC 14028)	95.25±3.99[d]	92.47±1.62[d]	87.50±1.24[b,c]	84.38±2.89[a,b]	83.33±4.66[a,b]	79.17±3.25[a]
S. aureus (ATCC25923)	95.70±6.83[c]	90.22±4.03[c]	93.20±5.25[c]	92.23±3.11[c]	79.61±5.88[b]	48.54±6.25[a]
E. faecalis (ATCC 19433)	95.65±6.78[d]	92.60±2.47[d]	94.38±3.22[d]	77.53±6.89[c]	47.19±3.25[b]	39.33±2.71[a]
Aril						
E. coli (ATCC 25922)	92.10±3.56[d]	90.40±1.99[d]	83.87±3.66[c]	80.65±5.54[c]	66.67±2.54[b]	13.98±3.68[a]
S. typhi (ATCC 14028)	94.12±3.69[c]	92.30±1.01[c]	88.54±1.32[b]	85.42±2.26[b]	84.382.65[a,b]	79.17±1.25[a]
S. aureus (ATCC25923)	95.43±2.54[d]	90.40±1.65[c]	91.26±1.37[c]	84.47±3.69[b]	81.55±6.53[b]	51.46±5.87[a]
E. faecalis (ATCC 19433)	90.30±3.69[c]	88.70±0.84[c]	86.52±2.25[c]	66.29±1.88[b]	60.67±4.85[b]	43.82±5.11[a]

(Bai et al., 2019). On the other hand, procyanidin B2 has been reported with strong bacteriostatic effect on different Gram-positive and Gram-negative bacteria, including *S. aureus*, *B. subtilis*, *S. pneumoniae*, and *E. faecalis* (Huang et al., 2022). The chemical composition exhibited by the methanolic extracts of the different parts of the fruit explain the antimicrobial and antioxidant activities and justify its use in the treatment of various diseases.

Conclusions

In conclusion, our study suggests that the fruit of *Clusia quadrangula* can be considered a source of bioactive phenolic compounds, especially shikimic acid, (−)-epicatechin, procyanidin B2, and ellagic acid, which confer high antioxidant capacity to the edible portions (pulp and peel), tissues that exhibited greater antioxidant activity according to the FRAP test, and DPPH and ABTS$^+$ radical scavenging ability. In turn, the hexanic extract showed that the pulp contains omega-3 and omega-6 fatty acids, which are present in a proportion suitable for human consumption. This study also proved that this fruit is a rich source of minerals including magnesium, sodium, potassium, and calcium. The methanolic extract of the fruit exhibited antimicrobial properties against *S. typhi*, *E. coli*, *S. aureus*, and *E. faecalis*. These results suggest that this fruit has a high potential for obtaining bioactive compounds that can be used in the generation of nutraceutical and pharmaceutical products.

Author contributions

MJF designed the original idea and wrote the article. BIMM and NJT carried out the experimental part and contributed to the data collection. MRML, JLMV, and JAGA contributed to the analytical experiments for the identification of bioactive compounds and data collection. All authors contributed to the analysis of the results, discussion, review, and approval of the final manuscript.

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Table S1: Chromatographic, spectrometric and quantitation conditions for each phenolic compound the fruit of *Clusia quadrangula* Bartlett. The retention time variation allowed for the search of the compounds were 2 min in each case. The cell accelerator voltage was 7 V for each compound. Dilutions were made if the concentration of some compounds were higher than the linearity range. dMRM: Dynamic Multiple Reaction Monitoring.

Compound	dMRM transition			Mass spectrometric conditions			Quantification conditions		
	Precursor ion	Product ion	Retention time	Collision energy	Fragmentor	Polarity	Quantification range (µM)	Regression type	R^2
Shikimic acid	173.1	111.1	0.48	10	100	Negative	0.25 - 18	Quadratic	0.99
Gallic acid	169.0	125.2	1.17	10	100	Negative	0.25 - 18	Quadratic	0.99
L-Phenylalanine	166.1	131.0	1.85	10	100	Positive	0.25 - 18	Quadratic	0.99
Protocatechuic acid	153.0	109.1	2.23	10	100	Negative	0.25 - 18	Quadratic	0.99
4-Hydroxybenzoic acid	137.1	92.8	3.43	10	100	Negative	0.25 - 18	Quadratic	0.99
Gentisic acid	153.0	109.0	3.43	10	100	Negative	0.25 - 18	Quadratic	0.99
(-)-Epigallocatechin	305.1	125.0	4.27	20	140	Negative	0.25 - 18	Quadratic	0.99
4-Hydroxyphenylacetic acid	107.1	77.0	4.5	20	140	Positive	0.25 - 18	Quadratic	0.99
(+)-Catechin	291.0	138.9	4.58	10	100	Positive	0.25 - 18	Quadratic	0.99
Vanillic acid	169.0	93.0	4.75	10	100	Positive	0.25 - 18	Quadratic	0.99
Scopolin	355.1	193.0	4.83	20	100	Positive	0.25 - 18	Quadratic	0.99
Caffeic acid	181.0	163.0	4.90	10	100	Positive	0.25 - 18	Quadratic	0.99
Chlorogenic acid	355.1	163.0	4.90	10	100	Positive	0.25 - 18	Quadratic	0.99
Malvin	655.1	331.1	5.22	40	100	Positive	0.25 - 18	Quadratic	0.99
Kuromanin	449.0	286.9	5.6	30	100	Positive	0.25 - 18	Quadratic	0.99
Procyanidin B2	577.1	425.1	5.89	10	100	Negative	0.25 - 18	Quadratic	0.99
Vanillin	153.0	124.9	6.16	10	100	Positive	0.25 - 18	Quadratic	0.99
Keracyanin	595.2	287.1	6.18	20	100	Positive	0.25 - 18	Quadratic	0.99
(-)-Epicatechin	291.0	138.8	6.44	10	100	Positive	0.25 - 18	Quadratic	0.99
Mangiferin	423.0	302.0	6.64	10	100	Positive	0.25 - 18	Quadratic	0.99
4-Coumaric acid	165.0	147.0	6.69	10	100	Positive	0.25 - 18	Quadratic	0.99
Umbelliferone	163.0	107.0	7.16	30	100	Positive	0.25 - 18	Quadratic	0.99
(-)-Gallocatechin gallate	458.9	139.0	7.29	20	80	Positive	0.25 - 18	Quadratic	0.99
Scopoletin	193.0	133.0	7.86	10	100	Positive	0.25 - 18	Quadratic	0.99
Ferulic acid	195.1	145.0	8.1	20	100	Positive	0.25 - 18	Quadratic	0.99
Quercetin 3,4-di-O-glucoside	627.0	302.9	8.18	10	100	Positive	0.25 - 18	Quadratic	0.99
3-Coumaric acid	165.0	147.0	8.49	10	100	Positive	0.25 - 18	Quadratic	0.99
Sinapic acid	225.1	207.1	8.58	10	100	Positive	0.25 - 18	Quadratic	0.99
Salicylic acid	137.0	93	8.97	10	100	Negative	0.25 - 18	Quadratic	0.99

Table S1: Continuation.

Compound	dMRM transition			Mass spectrometric conditions			Quantification conditions		
	Precursor ion	Product ion	Retention time	Collision energy	Fragmentor	Polarity	Quantification range (µM)	Regression type	R^2
Ellagic acid	300.5	145.0	9.0	30	170	Negative	0.25 - 18	Quadratic	0.99
Epicatechin gallate	443.1	123.0	9.36	10	100	Positive	0.25 - 18	Quadratic	0.99
Myricitrin	465.0	318.9	9.38	10	100	Positive	0.25 - 18	Quadratic	0.99
Quercetin 3-D-galactoside	465.0	302.9	9.58	10	100	Positive	0.25 - 18	Quadratic	0.99
Rutin	611.0	302.9	9.74	10	100	Positive	0.25 - 18	Quadratic	0.99
Quercetin 3-glucoside	465.0	303.0	9.91	10	100	Positive	0.25 - 18	Quadratic	0.99
Luteolin 7-O-glucoside	449.0	287.0	10.24	10	100	Positive	0.25 - 18	Quadratic	0.99
p-Anisic acid	153.1	109.0	10.26	5	120	Positive	0.25 - 18	Quadratic	0.99
2,4-Dimethoxy-6-methylbenzoic acid	197.0	179.0	11.11	5	80	Positive	0.25 - 18	Quadratic	0.99
Penta-O-galloyl-B-D-glucose	771.1	153.0	11.23	20	100	Positive	0.25 - 18	Quadratic	0.99
Kaemperol 3-O-glucoside	449.0	286.9	11.27	10	100	Positive	0.25 - 18	Quadratic	0.99
Quercitrin	449.1	303.1	11.34	10	100	Positive	0.25 - 18	Quadratic	0.99
Myricetin	317.0	179.0	11.49	10	100	Negative	0.25 - 18	Quadratic	0.99
Naringin	273.0	153.0	11.89	10	120	Positive	0.25 - 18	Quadratic	0.99
trans-Resveratrol	229.1	135.1	11.94	10	100	Positive	0.25 - 18	Quadratic	0.99
Rosmarinic acid	361.1	163.0	12.35	10	100	Positive	0.25 - 18	Quadratic	0.99
Hesperidin	609.1	301.1	12.48	20	100	Negative	0.25 - 18	Quadratic	0.99
Secoisolariciresinol	363.2	137.1	12.58	20	100	Positive	0.25 - 18	Quadratic	0.99
Phloridzin	435.0	272.9	12.81	10	100	Negative	0.25 - 18	Quadratic	0.99
trans-Cinnamic acid	149.1	131.0	13.93	10	100	Positive	0.25 - 18	Quadratic	0.99
Psoralen	187.0	131.1	14.24	20	100	Positive	0.25 - 18	Quadratic	0.99
Quercetin	302.9	153.1	14.47	35	100	Positive	0.25 - 18	Quadratic	0.99
Luteolin	287.1	153.0	14.56	30	100	Positive	0.25 - 18	Quadratic	0.99
Cirsimarin	477.0	314.9	14.93	10	100	Positive	0.25 - 18	Quadratic	0.99
Angelicin	187.0	131.1	15.03	20	100	Positive	0.25 - 18	Quadratic	0.99
Naringenin	271.0	151	16.2	10	100	Negative	0.25 - 18	Quadratic	0.99
Apigenin	271.0	153.0	16.72	30	100	Positive	0.25 - 18	Quadratic	0.99
Citropten	207.0	192.0	16.92	20	100	Positive	0.25 - 18	Quadratic	0.99
Matairesinol	359.2	137.1	17.02	10	100	Positive	0.25 - 18	Quadratic	0.99
Kaempferol	287.1	153.0	17.09	30	100	Positive	0.25 - 18	Quadratic	0.99

Table S1: Continuation.

	dMRM transition			Mass spectrometric conditions			Quantification conditions		
Compound	Precursor ion	Product ion	Retention time	Collision energy	Fragmentor	Polarity	Quantification range (µM)	Regression type	R^2
Hesperetin	303.1	177.1	17.5	20	100	Positive	0.25 - 18	Quadratic	0.99
Podophyllotoxin	415.1	397.1	18.68	10	100	Positive	0.25 - 18	Quadratic	0.99
Methyl cinnamate	163.1	131.0	20.92	6	100	Positive	0.25 - 18	Quadratic	0.99
Chrysin	255.1	153.0	22.53	40	100	Positive	0.25 - 18	Quadratic	0.99
Nordihydroguaiaretic acid	303.0	193.1	22.91	10	100	Positive	0.25 - 18	Quadratic	0.99
Kaempferide	301.0	258.2	24.05	20	100	Positive	0.25 - 18	Quadratic	0.99
Emodin	269.0	225.0	27.29	20	150	Negative	0.25 - 18	Quadratic	0.99
Chrysophanol	255.1	153.0	30.89	40	100	Positive	0.25 - 18	Quadratic	0.99