

Parkia decussata Ducke: molecular networking analysis, isolation of secondary metabolites, and antioxidant and antimicrobial activities

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Abstract

The Leguminosae (Fabaceae) family contains a wide variety of secondary metabolites with biological activities; however, the chemical composition and biological potential of some species, such as *Parkia decussata* Ducke, remain unknown. The objective of this study was to investigate the phytochemical composition of *P. decussata* and evaluate its antimicrobial and antioxidant activities. The methanolic leaf extract was partitioned to yield hexane, dichloromethane, ethyl acetate, and hydromethanolic phases. The methanolic leaf extract and its respective phases were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) combined with molecular networking using the Global Natural Products Social Molecular Networking 2 (GNPS2) platform. Lupeol and a mixture of β -sitosterol and stigmasterol were isolated from the hexane fraction, while quercetin was isolated from the ethyl acetate fraction; all compounds were characterized by nuclear magnetic resonance (NMR) spectroscopy. LC-MS/MS and GNPS2 molecular networking enabled the annotation of C-glycosylated flavonoids, including isovitexin and vitexin, as well as (2S,3S,4S,5R,6R)-6-(3-benzoyloxy-2-hydroxypropoxy)-3,4,5-trihydroxyoxane-2-carboxylic acid, gallic acid, ascorbic acid, esculetin, and coumarin. In the antimicrobial assays against Gram-positive and Gram-negative bacteria, the methanolic extract and ethyl acetate fraction showed the highest inhibitory activity. Regarding antioxidant activity, evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Fe^{3+} /phenanthroline assays, the ethyl acetate and hydromethanolic fractions showed the strongest activity, suggesting that phenolic compounds may contribute to the observed effects. To the best of our knowledge, this study provides the first report of quercetin, lupeol, β -sitosterol, and stigmasterol in *P. decussata*, as well as the first comprehensive description of the chemical constituents and biological activities of this species.

1 Introduction

The Leguminosae (Fabaceae) family is one of the most diverse and widely distributed groups of angiosperms and is of considerable ecological and economic importance. Its species produce a broad range of secondary metabolites, including flavonoids, alkaloids, terpenes, saponins, and other phenolic compounds, many of which are involved in plant defence and exhibit relevant biological activities (Usman et al. 2022; Noviany et al. 2023; Maroyi 2023; Brilhante et al. 2025; Hameed et al. 2025).

Within Fabaceae, the genus *Parkia* R.Br. comprises several species whose chemical composition and biological potential remain insufficiently investigated. Phytochemical studies of *Parkia* species have reported different classes of secondary metabolites, including flavonoids, cardiac glycosides, tannins, saponins, alkaloids, steroids, and anthraquinones (Ajaiyeoba 2002; Adaramola et al. 2012; Bitchi et al. 2019; Saleh et al. 2021). *Parkia decussata* Ducke is an Amazonian species for which information on the isolation and identification of secondary metabolites and the evaluation of biological activities remains limited. In particular, its antibacterial, antioxidant, and antifungal potential has received little attention. This knowledge gap highlights the need for further phytochemical and biological investigations of the species and contributes to the valorisation of Amazonian biodiversity as a source of potentially bioactive natural products.

The biological activities reported for secondary metabolites commonly found in plants further support the investigation of *P. decussata*. Flavonoids and terpenes, for example, may exert antimicrobial effects through interactions with bacterial membranes, modulation of efflux systems, and interference with biofilm formation, while flavonoids and other phenolic compounds can act as antioxidants by scavenging reactive species and reducing oxidative stress (Dias et al. 2022; Hassanpour and Doroudi 2023; Rahaman et al. 2023; Xu et al. 2024;

Yan et al. 2024). The occurrence of such metabolites in Fabaceae and *Parkia* species therefore reinforces the potential of *P. decussata* as a source of compounds with biological relevance.

In this context, the present study aimed to investigate the phytochemical profile of leaf and branch extracts of *Parkia decussata*, with emphasis on the isolation and identification of their chemical constituents, as well as the evaluation of their antioxidant and antimicrobial activities. This approach contributes to expanding the chemical and biological knowledge of an Amazonian species that remains poorly investigated and may provide new insights into its potential as a source of bioactive natural products.

2 Materials and methods

Equipment, Materials, Solvents, and Reagents

Analytical balances (Marte, model AUW220D, and Shimadzu, model UX4200H) were used for weighing plant material, extracts, fractions, and isolated compounds. The plant material was ground using a hammer mill (Nogueira, model DPM-4) and a knife mill (Solab, model SL-31). An ultrasonic bath (UNIQUE, model USC-2800; ultrasonic frequency: 40 kHz) were used to prepare the crude extracts.

Chromatographic separations were performed using silica gel 60 (230–400 mesh; Merck®) as the stationary phase. Hexane, dichloromethane, ethyl acetate, methanol, and acetone (commercial grade, Araguaya) were distilled and used as solvents for extraction, fractionation, and chromatographic elution. Thin-layer chromatography (TLC) analyses were performed using silica gel 60 and C18 silica gel plates (Macherey-Nagel-MN®), containing a 254 nm fluorescence indicator and thicknesses of 0.20 and 0.15 mm, respectively, on aluminum supports. The chromatographic plates were visualized under UV light at 254 and 365 nm using a UV darkroom (Hipperquímica). Anisaldehyde (Vetec, 98% purity) and ferric chloride (FeCl_3 ; Vetec, 97% purity) were used as visualization reagents in the TLC analyses.

For spectroscopic analyses, deuterated chloroform (CDCl_3 ; Cambridge Isotope Laboratories, 99.8% purity) and deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$; Sigma-Aldrich, 99.9% purity) were used as solvents for NMR analyses. NMR spectra were acquired using two spectrometers: Bruker BioSpin AG Fourier 300 UltraShield spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C , equipped with an Easyprobe Dul 300 MHz S1 5 mm Z-gradient probe, Bruker BioSpin Ascend Evo 600 Fourier-transform NMR spectrometer operating at 600 MHz for ^1H and 150 MHz for ^{13}C , equipped with an Avance NEO console and a 5 mm iProbe BBFO $^1\text{H}/^{19}\text{F}/^{13}\text{C}/^2\text{H}$ probe. For UV-visible spectrophotometric analyses were performed using a Femto Cirrus 80ST spectrophotometer and a Thermo Scientific Multiskan GO 96-well plate reader.

Acetic acid (Biotec, 99.7% purity), sulfuric acid (Biotec, 98% purity), sodium acetate (Sigma-Aldrich, 99% purity), and ethyl alcohol (Biotec, 99.5% purity) were used to prepare solutions and reagents for the analytical procedures. All procedures involving volatile solvents were performed in a fume hood (Permutation). Solvent removal was performed using a rotary evaporator (Fisatom®, model 550).

Collection and botanical identification

The plant material (leaves and branches) from an individual of *Parkia decussata* was collected at the Federal University of Amazonas (Northern Sector), at the coordinates (3°31'22"S and 59°34'28"W), Manaus, Amazonas, Brazil. The species was identified by Dr Michael John Hopkins (a specialist in the taxonomy of the genus). A

voucher specimen was prepared and deposited in the Herbarium of the Botanical Coordination of INPA under deposition number 305187.

Extraction procedures

The plant material was dried in an oven at approximately 45°C for three days. After drying, the leaves were ground using a knife mill, whereas the branches were ground using a hammer mill. The dried and ground material was subjected to ultrasound-assisted extraction (40 kHz) for 20 minutes, using a ratio of 1 g of plant material to 3 mL of solvent (1:3, w/v), with three successive extractions performed.

The extractions were conducted with organic solvents in increasing order of polarity: hexane (Hex) and methanol (MeOH). After extraction, the solvents were removed using a rotary evaporator at 55 rpm and 40°C. The extracts were then kept in a fume hood and a desiccator until the residual solvents had been completely removed.

Molecular characterisation of the isolated compounds

The isolated compounds were subjected to Nuclear Magnetic Resonance (NMR) analyses of ^1H and ^{13}C , including one- and two-dimensional experiments, as well as high-resolution mass spectrometry. The NMR spectra were acquired using a Fourier 300 Ultrashield spectrometer, operating at 300 MHz and 600 MHz for ^1H and 75 MHz for ^{13}C , and processed using TopSpin v3.8.0 software.

LC-MS and GNPS Molecular Networking Analysis

The methanolic extract fractions from the leaves were analysed by liquid chromatography coupled with mass spectrometry (LC-MS). The samples were analysed using a liquid chromatograph (UFLC, LC-20AT, Shimadzu) hyphenated to a high-resolution mass spectrometer (microTOF-Q II, Bruker Daltonics), equipped with an ESI source operated in positive and negative modes.

For this purpose, 20 mg of each sample were solubilised in MeOH/H₂O (1:1) and loaded onto a Strata® C18-E cartridge (2 g/12 mL, Phenomenex) to minimise interference caused by the presence of sugars and fatty acids in the analyses. The cartridge was then eluted with 100% MeOH. After drying the samples, a concentration of 1 mg mL⁻¹ was used for the LC-MS analyses. Chromatographic separation was performed on a Kinetex C18 column (2.6 µm, 150 × 2.1 mm) at a flow rate of 0.2 mL min⁻¹. A volume of 5 µL of each sample, previously solubilised in MeOH/H₂O (1:1), was injected using an exploratory gradient with the following mobile phases: A, water containing 0.1% formic acid; and B, methanol. The chromatographic method was as follows: 0–24 min, 20–100% B; 24–26 min, 100% B; 26–28 min, 100–20% B; and 28–30 min, 20% B.

For high-resolution mass spectrum (MS1) calibration, 10 mM sodium formate was used at the end of the chromatographic run. Mass spectra (MS) and fragmentation spectra (MS2) were acquired simultaneously, with drying gas applied to the source at a flow rate of 9.0 L/min and 180°C, the capillary voltage set to 4500 V, and the collision energy set to 8.0 eV. Nitrogen (N₂) was used as the collision cell gas. Fragmentation was performed in a CID-type cell, with a scan range of m/z 50 to 1200. MS/MS analyses were conducted in automatic Multiple Reaction Monitoring (MRM) mode via LC-MS, using collision energies of 25, 30, 35, 40, 45, 50, and 70 eV.

The data were acquired and monitored using Compass DataAnalysis v4.1 software and subsequently processed using MZmine v4.7.8. For the analysis of fragmentation spectra through molecular networking, the LC-MS data

were converted to mzML format using MSConvert software (Chambers et al. 2012). The files were then submitted to classical molecular networking analysis on the GNPS2 (Global Natural Products Social Molecular Networking) platform (Wang et al. 2016) via the following link: <https://gnps2.org/homepage>. The parameters used included a minimum cosine score of 0.65 for correlation between spectra and the library, a minimum of 4 shared fragments, and a tolerance of 0.02 for both precursor ions and fragments, consistent with spectra acquired at high resolution. Finally, the annotations were manually verified to confirm metabolites derived from plant species and chemically related to *Parkia decussata*.

Finally, the molecular networks generated were exported to Cytoscape v3.10.3, where they were organised according to metabolite classes and respective samples. The compound annotations were subsequently manually reviewed for validation.

Isolation of the compounds

The methanolic leaf extract (10 g) was solubilised in a hydro-methanolic solution (1:1 v/v, 500 mL) and subjected to liquid-liquid partitioning with hexane, followed by dichloromethane and ethyl acetate, yielding four fractions: hexane (1.6 g), dichloromethane (0.4 g), ethyl acetate (2.9 g), and hydro-methanolic (5.1 g).

The ethyl acetate fraction was fractionated on an open Sephadex LH-20 column (69 × 3 cm, 100% MeOH), yielding 20 fractions. After analysis by comparative thin-layer chromatography (TLC) under UV light (254 and 365 nm) and visualisation with FeCl₃, fraction 15 (10 mg) was found to contain phenolic compounds and was fractionated on a silica gel column (17 × 0.5 cm, CHCl₃/MeOH 85:15, 8:2, 7:3, and 100% MeOH), yielding 17 fractions. Fraction 2 (1 mg) was identified as the flavonoid quercetin (**1**).

The hexane fraction was fractionated on a Strata SI-1 Silica column (Phenomenex®, 55 µm, 70 Å, 50 g/150 mL), using hexane, CHCl₃, acetone, and MeOH, yielding four fractions. Fraction 2 (1.032 g) was subjected to fractionation on a Celite column (1 × 3.8 cm, hexane, hexane/CHCl₃ (1:1), 100% CHCl₃, and 100% MeOH), yielding seven fractions. Fractions 1–2 (791 mg) were fractionated on an open silica gel column (23 × 4 cm, CHCl₃/MeOH 99:1, 98:2, 97:3, 95:5, and 9:1), yielding 47 fractions. Fraction 15 (13 mg) was identified as the triterpene lupeol (**2**). Fraction 19 (15 mg) was subjected to preparative thin-layer chromatography (Hex/EtOAc 95:05, 4 elutions), from which the resulting fraction 2 (5 mg) yielded a mixture of the steroids β-sitosterol (**3**) and stigmasterol (**4**). Compounds **1–3** reacted with sulphuric anisaldehyde reagent in TLC.

Antimicrobial activity

The antimicrobial activity of the extracts was evaluated using the broth microdilution method, according to the methodology described by the Clinical and Laboratory Standards Institute (CLSI 2015).

The following microorganisms were used: *Acinetobacter baumannii* (AB) (CBAM 0664), *Citrobacter freundii* (CF) (CBAM 0762), *Enterobacter cloacae* (ECL) (CBAM 0200), *Enterococcus faecalis* (EFL) (CBAM 0621), *Klebsiella pneumoniae* (KP) (CBAM 0051), *Morganella morganii* (MM) (CBAM 0138), *Pseudomonas aeruginosa* (PA) (CBAM 0519), *Staphylococcus aureus* (SA) (CBAM 0026), *Serratia marcescens* (SM) (CBAM 0069), and *Staphylococcus epidermidis* (STE) (CBAM 0613).

The microorganisms were previously activated by incubation in Müller-Hinton broth for 16–24 hours. After growth, the inocula were standardised by spectrophotometry at 625 nm, adjusting the turbidity to the 0.5 McFarland standard, corresponding to an absorbance between 0.080 and 0.100 and an approximate

concentration of 1.5×10^8 CFU/mL (colony-forming units). The suspensions were subsequently diluted (1:20) to obtain a final concentration of 5×10^4 CFU/mL for the assay. The extracts were previously solubilised in 5% DMSO and diluted in Müller-Hinton broth to an initial concentration of 1000 µg/mL. In 96-well plates, 100 µL of the solutions containing the extracts and 10 µL of the microbial inocula were added. For the positive control (PC), 100 µL of oxytetracycline (125 µg/mL) and 10 µL of inoculum were used, whereas the negative control (NC) consisted of 100 µL of Müller-Hinton broth containing 5% DMSO and 10 µL of inoculum. The samples and controls were prepared in triplicate.

After assay setup, the plates were incubated at approximately 37°C for 16–24 hours. Optical density was measured by spectrophotometry at 625 nm before and after the incubation period. Subsequently, 2,3,5-triphenyltetrazolium chloride (TTC) was used to assess cell viability. The results were expressed as percentage inhibition, mean, and standard deviation. The data were subjected to statistical analysis by one-way ANOVA, followed by Dunnett's post hoc test, comparing the samples with the negative control and considering a 95% confidence interval ($p < 0.05$). Statistical analyses were performed using GraphPad Prism software (v8.0.1).

Antioxidant activity

The antioxidant activity of the extracts was evaluated according to the methodology described by Novaes and Nunez (2008), using DPPH radical scavenging and Fe^{3+} /1,10-phenanthroline reduction assays. Both assays were performed in triplicate. To determine antioxidant activity, a calibration curve was constructed using six increasing concentrations of ascorbic acid as the antioxidant standard.

For the DPPH assay, 10 µL of the extract solutions (500 µg/mL) were added to 990 µL of DPPH solution (28 µg mL^{-1}). After 30 minutes of reaction, the absorbance was determined at 517 nm using a spectrophotometer.

For the Fe^{3+} /1,10-phenanthroline assay, 10 µL of the extract solutions (500 µg mL^{-1}), 10 µL of the Fe^{3+} standard solution, and 980 µL of 1,10-phenanthroline solution (0.25%) were added. After 1 hour of reaction, the absorbance was recorded at 515 nm.

The results were compared with the ascorbic acid standard and expressed as ascorbic acid equivalents, according to Martins et al. (2014), with values close to 1 considered indicative of antioxidant activity.

3 Results

Identification of the isolated compounds

Quercetin (1)

Quercetin was obtained as an amorphous yellow solid. When the TLC was visualised with sulphuric anisaldehyde, it showed a spot with yellow colour. Analysis of the NMR data showed the signal at 7.63 ppm for H-6', with *ortho* and *meta* coupling constants ($J = 8.4$ and 2.1 Hz), together with the signal at 7.73 ppm for H-2' ($d, J = 2.1$ Hz) and 6.88 ppm for H-5' ($d, J = 8.4$ Hz). These signals were key to establishing that ring B exhibited an ABX system characteristic of quercetin. Additionally, the correlations observed in the HMBC spectrum between the aromatic hydrogens and the quaternary carbons of ring B supported the presence of hydroxyl groups at C-4' and C-3'. In particular, correlations were observed between H-2' (7.73 ppm) and C-4' (148.7 ppm), and between H-5' (6.88 ppm) and C-3' (145.9 ppm). H-6 and H-8 of ring A appeared in the ^1H NMR spectrum at 6.18 ($d, J = 2.1$ Hz) and

6.39 (*d*, *J* = 2.1 Hz) ppm, respectively. Although it was identified in the molecular network (GNPS2) of the MeOH extracts, its isolation made it possible to distinguish it from its isomer (morina), which has different hydroxyl substitutions on the B ring.

Data observed: ¹H NMR (600 MHz, CD₃OD) in ppm: 6.18 (*d*, *J* = 2.1 Hz, 1H, H-6); 6.39 (*d*, *J* = 2.1 Hz, 1H, H-8); 7.73 (*d*, *J* = 2.1 Hz, 1H, H-2'); 6.88 (*d*, *J* = 8.4 Hz, 1H, H-5'); 7.63 (*dd*, *J* = 8.4 and 2.1 Hz, 1H, H-6'). ¹³C NMR (150 MHz, CD₃OD) in ppm: 99.0 (CH, C-6); 94.4 (CH, C-8); 154.4 (C, C-9); 104.5 (C, C-10); 124.0 (C, C-1'); 115.9 (CH, C-2'); 145.9 (CH, C-3'); 148.7 (C, C-4'); 116.2 (C, C-5'); 124.6 (CH, C-6'). *m/z* in ESI [M-H]⁻: 301.031; molecular mass: 302 u [C₁₅H₁₀O₇]. This substance was also annotated in the molecular network analysis of the MeOH extracts.

Lupeol (2)

Lupeol was obtained from the hexane fraction of the methanolic leaf extract of *P. decussata*. This compound showed a lilac colour (Fig. 1) when visualised with sulphuric anisaldehyde under heating by TLC, compared with a concentrated standard of the triterpene lupeol, with the same *R_f* (retention factor) value of 0.5 (Hex/EtOAc 8:2). Only ¹H, ¹³C, and DEPT-135° NMR analyses were required to identify this triterpene. In the ¹H NMR spectrum, the presence of seven methyl groups was confirmed, each with an integral of 3H, including the signal at 1.67 ppm, which is indicative of the exocyclic methyl group at C-30. Likewise, the multiplets at 4.56 and 4.68 ppm corresponded to H-29, with an integral of 1H for each signal. One of the diagnostic signals in triterpenes is that at 3.18 ppm, which indicates deshielding of this hydrogen caused by the presence of a β-OH group at C-3. The data indicated the presence of seven methyl carbons, eleven methylene carbons, and six methine carbons. The carbon signal at 79.1 ppm confirmed the presence of a hydroxyl group, characteristic of a β-OH group at C-3 in structures of this chemical class. Together with the signals at 151.1 ppm (C-20) and 109.4 ppm (C-29), these data confirmed the presence of a triterpene with a lupane-type skeleton. These data, together with comparison of the fraction with a lupeol standard by TLC (Fig. 1) and comparison with the scientific literature, allowed compound **2** to be identified as lupeol (Silva et al. 2023).

Data observed: ¹H NMR (300 MHz, CDCl₃) in ppm: 0.75 (s, H-24); 0.78 (s, H-28); 0.82 (s, H-25); 0.93 (s, H-23); 0.96 (s, H-27); 1.02 (s, H-26); 1.67 (s, H-30); 3.18 (*m*, *J* = 10.4 and 5.0 Hz, 1H); 4.56 (1H, *m*, *J* = 3.8 Hz); 4.68 (1H, *m*, *J* = 2.4 Hz). ¹³C (75 MHz, CDCl₃) in ppm: 38.8 (CH₂, C-1); 27.5 (CH₂, C-2); 79.1 (CH, C-3); 38.9 (C, C-4); 55.3 (CH, C-5); 18.4 (CH₂, C-6); 34.3 (CH₂, C-7); 40.9 (C, C-8); 50.5 (CH, C-9); 37.2 (C, C-10); 21.0 (CH₂, C-11); 25.2 (CH₂, C-12); 38.1 (CH, C-13); 42.9 (C, C-14); 27.5 (CH₂, C-15); 35.6 (CH₂, C-16); 43.1 (C, C-17); 48.3 (CH, C-18); 48.1 (CH, C-19); 151.1 (C, C-20); 29.9 (CH₂, C-21); 40.1 (CH₂, C-22); 28.1 (CH₃, C-23); 15.5 (CH₃, C-24); 16.2 (CH₃, C-25); 16.0 (CH₃, C-26); 14.6 (CH₃, C-27); 18.1 (CH₃, C-28); 109.4 (CH₂, C-29); 19.4 (CH₃, C-30).

Legend: Lupeol (1), lupeol standard (2), isolated lupeol + standard (3), β-sitosterol and stigmasterol (4), standards (5), and β-sitosterol and stigmasterol + standards. Hexane/EtOAc 8:2 v/v, with sulphuric anisaldehyde under heating

Figure 1 TLC analysis of the fractions containing the isolated compounds and compared to the concentrated standards available at the laboratory.

β-Sitosterol (3) and Stigmasterol (4)

As with lupeol, the steroids in the mixture were obtained from the hexane fraction of the methanolic leaf extract as white crystals. When reacted with sulphuric anisaldehyde under heating, the mixture of compounds showed a dark lilac colour. Comparison by TLC (Fig. 1) with concentrated standards indicated that they were the same constituents. The ^1H NMR spectrum showed signals consistent with the six methyl groups present in the steroid skeleton at 0.67 (s, H-19), 0.81 (d, H-27), 0.83 (d, H-26), 0.84 (d, H-29), 0.92 (d, H-21), and 1.00 (s, H-18) ppm. Additionally, H-6 of both compounds appeared at 5.34 ppm (*dl*, $J = 5.1$ Hz). H-22 and H-23 of stigmasterol were confirmed by the signals at 5.14 (*dd*, $J = 15.2$ and 8.4 Hz) and 5.00 ppm (*dd*, $J = 15.2$ and 8.7 Hz), respectively. The signal at 3.51 ppm was assigned to H-3 of both molecules, which contain a β -OH group, as in lupeol. Comparison of the NMR data with previously reported literature data confirmed the mixture of β -sitosterol and stigmasterol (Silva et al. 2023). The chemical structures of the compounds are shown in Fig. 2.

Data observed: ^1H NMR (300 MHz, CDCl_3) in ppm: 1.00 ppm (H-18); 0.67 ppm (H-19); 0.92 ppm (*d*, $J = 6.4$ Hz, H-21); 0.83 ppm (*d*, $J = 6.5$ Hz, H-26); 0.81 ppm (*d*, $J = 6.5$ Hz, H-27); 0.84 ppm (*d*, $J = 7.0$ Hz, H-29); 3.51 (*m*, H-3); 5.34 (2H, *dl*, $J = 5.1$ Hz, H-6); 5.14 (1H, *dd*, $J = 15.2$ and 8.4 Hz); 5.00 (1H, *dd*, $J = 15.2$ and 8.7 Hz).

Annotation of Other Compounds Present in the Molecular Networks

LC-MS and molecular networking analyses enabled the annotation of C-glycosylated flavonoids, coumarins, phenolic acids, as well as other flavonoids, including **compound 1**: quercetin, as shown in Table 1, Fig. 3.

The generated networks can be accessed through the following links in positive mode: <https://gnps2.org/status?task=33eabd569f6b4b1b9b749960d3d8a791> and negative mode: <https://gnps2.org/status?task=4065307c53db4e02aedc263d6f800c22>. Among the annotations obtained, those showing similarity in fragmentation spectra (MS²), the same precursor ions, and those corresponding to plant metabolites were accepted, particularly phenolic compounds occurring in the Fabaceae family.

Table 1

Compounds annotated in the MeOH extract of the leaves of *Parkia decussata* Ducke based on GNPS2 analyses in positive and negative modes

Cluster ID	Annotated compound	Cosine	Molecular formula	Rt(min)	Precursor ion	Fragments (MS2)
70	gallic acid	0.82	C ₇ H ₆ O ₅	2,3	171.025 [M + H] ⁺	153, 125, 109, 107, 81
16	coumarin	0.88	C ₉ H ₆ O ₂	1,9	121.063 [M- C ₂ H ₂ +H] ⁺	103, 91, 77
115	ascorbic acid	0.78	C ₆ H ₈ O ₆	7,2	199.055 [M + Na] ⁺	181, 140, 125, 121, 95
237	esculetin	0.63	C ₉ H ₆ O ₄	6,7	177.018 [M-H] ⁻	149, 133, 105, 89
537	(2S,3S,4S,5R,6R)-6-(3-benzoyloxy-2-hydroxypropoxy)-3,4,5-trihydroxyoxane-2-carboxylic acid	0.78	C ₁₆ H ₂₀ O ₁₀	8,7	371.093 [M-H] ⁻	311, 249, 249, 231, 189, 175, 121, 113, 99, 85
441	quercetin	0.69		16,4	301.029 [M-H] ⁻	273, 179, 151, 121, 107
676	isovitexin	0.66	C ₂₁ H ₂₀ O ₁₀	11,3	455.083 [M + Na] ⁺	437, 365, 335, 313, 283
582		0.94		11,5	431.092 [M-H] ⁻	413, 353, 341 e 311
628	vitexin	0.66	C ₂₁ H ₂₀ O ₁₀	11,2	431.092 [M-H] ⁻	431, 413, 341, 311

The compounds isovitexin and vitexin are C-glycosylated flavonoids that were found in the EtOAc fraction of the methanolic leaf extracts. In both cases, the fragmentation spectra (MS2) did not show losses of 162 or 146 u which are characteristic of the neutral losses of sugar units, indicating that they were not O-glycosylated flavonoids. Both compounds are isomers and were annotated at different retention times (Rt), differing in the position of the sugar moieties. As an attempt to use orthogonal techniques to identify these constituents, the ultraviolet spectra showed bands at λ 270 and 338 nm, which are characteristic of the aromatic rings of flavones (Fig. 4) (Mabry et al. 1970).

Antimicrobial Activity

Based on the microdilution method at a concentration of 1000 $\mu\text{g mL}^{-1}$, with results $\geq 20\%$ considered positive for inhibition, the crude leaf extracts, both hexane and methanolic, as well as the fractions of the methanolic leaf extracts, were tested and analysed (Table 2).

The Gram-positive bacteria *Enterococcus faecalis* (EFL), *Staphylococcus aureus* (SA), and *Staphylococcus epidermidis* (STE) were inhibited by two extracts in common, namely the methanolic leaf extract and the ethyl

acetate leaf fraction. However, EFL showed greater susceptibility to inhibition, as the hexane leaf extract, hexane fraction, DCM fraction, and hydro-methanolic fraction were also active against this bacterium. The hydro-methanolic fraction was also able to inhibit STE.

The Gram-negative bacteria *Acinetobacter baumannii* (AB), *Citrobacter freundii* (CF), *Enterobacter cloacae* (ECL), *Klebsiella pneumoniae* (KP), and *Morganella morganii* (MM) were inhibited by some extracts in common. AB, CF, ECL, and MM were inhibited by the ethyl acetate fraction and, with the exception of MM, by the hydro-methanolic fraction, with KP also being inhibited by the same fraction. In addition, AB, CF, and ECL were inhibited by the hexane leaf extract, AB and CF by the methanolic leaf extract, and CF and ECL by the hexane fraction.

Table 2

Results of the antimicrobial activity analysis of the crude extracts and fractions of the MeOH leaf extract of *Parkia decussata*.

Samples	AB	CF	ECL	EFL	MM	SA	STE	KP	PA	SM
1-Hex-Leaves	100 ± 0.01	100 ± 0.02	68.53 ± 0.07	93 ± 0,00	*	*	*	*	*	*
2-MeOH-Leaves	63.27 ± 0.02	62.3 ± 0.00	45.91 ± 0.00	73,08 ± 0,02	*	77.35 ± 0.00	83.93 ± 0.00	*	*	*
3-MeOH-Leaves -Hex-phase	48.03 ± 0.02	60.44 ± 0.00	54.29 ± 0.00	93,61 ± 0,00	*	*	41.4 ± 0.01	*	*	*
4- MeOH-Leaves -DCM-phase	*	37.58 ± 0.01	*	84.44 ± 0.00	*	*	*	*	*	*
5- MeOH-Leaves - EtOAc-phase	93.16 ± 0.02	97.68 ± 0.00	97.04 ± 0.00	96.93 ± 0.00	78.52 ± 0.03	77.96 ± 0.03	100 ± 0.00	*	*	*
6- MeOH-Leaves -Hydro-phase	75.03 ± 0.04	62.08 ± 0.00	51.68 ± 0.01	57.12 ± 0.00	27.41 ± 0.02	*	64.66 ± 0.00	91.22 ± 0.22	*	*
Oxytetracycline	94.75 ± 0.01	99.35 ± 0.00	97.6 ± 0,00	99.0 ± 0.00	95.93 ± 0.00	95.41 ± 0.00	70.01 ± 0,00	96.24 ± 0,00	99.65 ± 0.00	91.0 ± 0,00

Legend

(*) indicates activity below 20%, considered inactive; values are expressed as percentages and standard deviation. AB (*Acinetobacter baumannii*), CF (*Citrobacter freundii*), ECL (*Enterobacter cloacae*), EFL (*Enterococcus faecalis*), KP (*Klebsiella pneumoniae*), MM (*Morganella morganii*), PA (*Pseudomonas aeruginosa*), SA (*Staphylococcus aureus*), SM (*Serratia marcescens*), STE (*Staphylococcus epidermidis*).

Antioxidant Activity

The antioxidant activity of the methanolic extracts of the leaves and branches, as well as the leaf fractions, was evaluated using the DPPH and Fe³⁺/phenanthroline methods. The results, obtained using a calibration curve with ascorbic acid, indicated that the EtOAc and H₂O/MeOH fractions showed greater antioxidant potential, with values ranging from 1 to 3 in ascorbic acid equivalents (Table 3).

Table 3

Results of the antioxidant activity of the MeOH extracts of the leaves and branches and the fractions of the MeOH leaf extract, expressed as mean absorbance values

Extracts	Method using DPPH■			Method using Fe ³⁺		
	ΔABS517	[AA] eq	Equiv.	[Fe ²⁺]	[AA] eq	Equiv.
MeOH Leaves	0.166	1.451	3.5	0.38	1.197	4.2
MeOH Stems	0.109	1.005	5.0	0.204	0.93	5.4
MeOH-Leaves-Hex-phase	0.052	0.566	8.9	0.131	0.819	6.1
MeOH-Leaves-DCM-phase	0.069	0.696	7.5	0.468	1.329	3.8
MeOH-Leaves-EtOAc-phase	0.41	3.356	1.5	1.546	2.961	1.7
MeOH-Leaves-Hydro-phase	0.27	2.268	2.2	0.835	1.885	2.8
Legend: AA: ascorbic acid; Equiv.: equivalence, represented by the ratio between the extract result and the standard control (ascorbic acid). Samples that showed activity are highlighted in bold						

The only samples considered active and moderately active were the EtOAc and H₂O/MeOH fractions, respectively. The EtOAc fraction was active in the DPPH assay, with an equivalence of 1.5, and showed an equivalence of 1.7 in the Fe³⁺/phenanthroline assay. The H₂O/MeOH fraction showed an equivalence of 2.2 in the DPPH assay and 2.8 in the Fe³⁺/phenanthroline assay when compared with ascorbic acid.

4 Discussion

Phytochemical composition of *Parkia decussata*

The isolation of quercetin, lupeol, β-sitosterol, and stigmasterol demonstrates the occurrence of flavonoids, triterpenes, and steroids in *P. decussata*. In addition, LC-MS/MS and molecular networking analyses enabled the annotation of C-glycosylated flavonoids, phenolic acids, and coumarins.

Quercetin was previously isolated from *P. roxburghii* by El Gizawy et al. (2021), who isolated quercetin from the plant's leaves using the EtOAc fraction of the MeOH extract. In addition, the compound was shown to inhibit acetylcholinesterase, an enzyme responsible for degrading acetylcholine, an important neurotransmitter in the nervous system.

Although the fragmentation spectra suggested differentiation based on the order of OH losses, retro-Diels-Alder fragmentation, and hydrogen rearrangement after sugar cleavage, we observed that the fragments of both compounds were very similar, particularly in negative mode, which prevented their differentiation. For unequivocal characterisation, these flavonoids could be isolated for NMR analyses to determine the coupling of the anomeric hydrogens, which would establish the position of the sugar moieties in the isolated compounds.

Regarding the coumarin compound, the fragmentation spectrum showed the absence of the [M + H]⁺ precursor ion. This may have resulted from a desorption reaction at the source, in which the molecule may have undergone

cleavage of the two aromatic CH-CH bonds, followed by ring contraction to generate the base ion m/z 121.063 $[M-C_2H_2+H]^+$, which was selected by the instrument for fragmentation. Despite this, manual analysis of the fragments of this ion and their similarity to the library enabled the putative identification of this compound.

In the case of gallic acid, a phenolic compound, although the fragmentation of this constituent showed similarity to the library, the mass spectrum could also correspond to 2,4,6-trihydroxybenzoic acid, as these compounds are isomers that differ only in the positions of the OH groups on the aromatic ring. Spectroscopic analyses are therefore required to confirm their actual positions, such as a 2D HMBC experiment. However, this compound has been reported in other *Parkia* spp., such as *P. bicolor* and *P. speciosa*. Thus, because it has been reported in the chemotaxonomy of the genus, it was annotated as gallic acid (Fotie et al. 2004; Ko et al. 2014).

Antimicrobial activity

It was observed that all tested samples showed inhibition at a concentration of $1000\text{ }\mu\text{g mL}^{-1}$. Among the samples, the EtOAc and hydro-methanolic leaf fractions showed the highest number of inhibited bacteria. These inhibitory effects may be related to the classes of compounds found in these extracts, which may include phenolic compounds, flavonoids, terpenes, steroids, and other classes. In addition, lupeol, β -sitosterol, stigmasterol, and quercetin were isolated from the hexane and EtOAc fractions. Previous studies have shown that these compounds can inhibit certain strains.

For example, Rashed et al. (2018) reported that quercetin was one of the flavonoids capable of inhibiting *Staphylococcus aureus*, whereas Evangelina et al. (2021) showed that β -sitosterol was able to inhibit strains of *Enterococcus faecalis*. According to Tsai et al. (2016), lupeol is a widely studied triterpene due to its multiple biological activities, including anti-inflammatory, antioxidant, antidiabetic, hepatoprotective, neuroprotective, cardioprotective, and anticancer properties. Rosandy et al. (2021) reported that lupeol extracted from *Dehaasia cuneata* (Lauraceae) was evaluated for antimicrobial activity and showed inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, *Escherichia coli*, *Serratia marcescens*, and *Vibrio fluvialis*.

Studies also reinforce the pharmacological relevance of β -sitosterol and stigmasterol. The stem bark extract of *Punica granatum* showed significant antimicrobial activity, with inhibition zones of approximately 20 mm against *Salmonella typhi* and 35 mm against *Escherichia coli*, as well as MIC values of 6.25 and $12.5\text{ }\mu\text{g mL}^{-1}$, respectively. β -Sitosterol was identified as one of the main active compounds (Nweze et al. 2019). Stigmasterol, in turn, exhibits anti-inflammatory, antioxidant, anticancer, neuroprotective, and lipid-lowering activities, as demonstrated in *in vitro* and *in vivo* models (Li et al. 2025).

These findings indicate that the tested samples are sources of antibacterial compounds that could be tested individually against other microorganisms, particularly those resistant to known antibiotics. These results support further studies of *Parkia decussata* extracts aimed at isolating these active compounds and advancing preclinical assays. The annotated compounds have also been investigated previously, with studies demonstrating their antimicrobial potential.

In Tian et al. (2022), gallic acid showed activity against *Escherichia coli*, demonstrating the antimicrobial potential of this compound against a pathogen that affects the urinary and gastrointestinal tracts (Pakbin et al. 2021). This finding highlights the importance of isolating the compound to evaluate its activity against other pathogens that affect different anatomical sites, such as *E. coli*.

Coumarin was evaluated by He et al. (2022), where it inhibited the growth of *Porphyromonas gingivalis*. This finding supports the need to isolate this annotated compound, as it may also show inhibitory activity against other Gram-negative bacteria or other bacteria that affect the oral tract, such as *P. gingivalis* (Mysak et al. 2014).

Ascorbic acid was evaluated by Mirani et al. (2018) and showed inhibitory activity against *Staphylococcus aureus*, a Gram-positive bacterium that primarily affects the respiratory tract (Ahmad-Mansour et al. 2021). This finding highlights the relevance of isolating the compound so that its activity can be evaluated against other Gram-positive bacteria.

Vitexin, another annotated compound, also showed good antimicrobial activity against *Citrobacter freundii*, *Aeromonas hydrophila*, *Alcaligenes faecalis*, and *Salmonella Typhimurium* in Zai et al. (2025). These findings indicate the antimicrobial potential of this flavonoid and support the importance of its future isolation.

Antioxidant activity

The results obtained from TLC, NMR, and GNPS2 molecular networking analyses showed that the EtOAc fraction contained phenolic compounds, suggesting that the antioxidant activity was related to these compounds, which are recognised for their ability to stabilise free radicals (Silva and Gonçalves, 2010). In addition, the isolation of quercetin reinforced the association of this activity with the presence of flavonoids in the EtOAc fraction, as described by Ko et al. (2014).

In the study by Komolafe et al. (2018), extracts of *Parkia biglobosa* showed considerable antioxidant activity, particularly the free phenolic compounds from the leaves and fruits. In the DPPH assay, the extracts showed an EC_{50} (effective concentration) of $3.82 \pm 0.2 \mu\text{g mL}^{-1}$, indicating a high capacity to neutralise free radicals, in addition to relevant Fe^{3+} -reducing capacity.

These results demonstrate the antioxidant potential of the phenolic compounds in *P. decussata*, particularly those present in the EtOAc leaf fraction, as some of the isolated and annotated compounds have previously been reported to possess antioxidant activity. In this context, quercetin was previously reviewed by Xu et al. (2019), who highlighted its antioxidant properties and potential to protect against oxidative stress. Quercetin can promote the neutralisation of free radicals and contribute to cellular protection against oxidative damage, demonstrating its potential as a bioactive compound of medicinal interest.

Vitexin also exhibits antioxidant properties, acting to reduce reactive oxygen species and modulate mechanisms and enzymes involved in antioxidant defence, which demonstrates its potential as a bioactive compound in the control of processes associated with oxidative stress (Babaei et al. 2020).

Chowjarean et al. (2019) investigated the antioxidant potential of isovitexin and found that this flavonoid had a protective effect against oxidative stress induced by exposure to the pollutant. It also showed antioxidant activity in DPPH and superoxide radical assays, demonstrating its radical-scavenging capacity.

Considering the present study, it was observed that *P. decussata*, like other *Parkia* spp., can produce bioactive metabolites such as flavonoids, other phenolic compounds, terpenes, and steroids. Quercetin was isolated from the EtOAc leaf fraction, and ascorbic acid, gallic acid, isovitexin, vitexin, coumarin, and (2S,3S,4S,5R,6R)-6-(3-benzoyloxy-2-hydroxypropoxy)-3,4,5-trihydroxioxane-2-carboxylic acid were annotated. Lupeol and a mixture of the steroids β -sitosterol and stigmasterol were isolated from the hexane leaf fraction.

In the chemical and biological activity assays, the extracts and fractions showed antioxidant activity against free radicals and metal-ion-reducing potential, as well as antimicrobial activity against bacteria. These findings are novel for this species and reveal the potential of *P. decussata* as a source of bioactive compounds.

Declarations

Acknowledgments

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Author Contributions

Conceptualization: MGDCRP and CVN. Data curation, investigation and formal analysis: MGDCRP, GSC, LdCM, FGdS, DRdS, CVN. Funding acquisition: CVN. Project administration and Supervision: CVN. Resources: CVN. Writing – original draft, review & editing: MGDCRP, GSC, CVN. All authors read and approved the final version of the manuscript.

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Data availability All data will be available after request to the corresponding author.

Conflict of interest The authors declared no conflict of interest.

Ethical approval Not applicable.

Consent of publication Not applicable.

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Figures

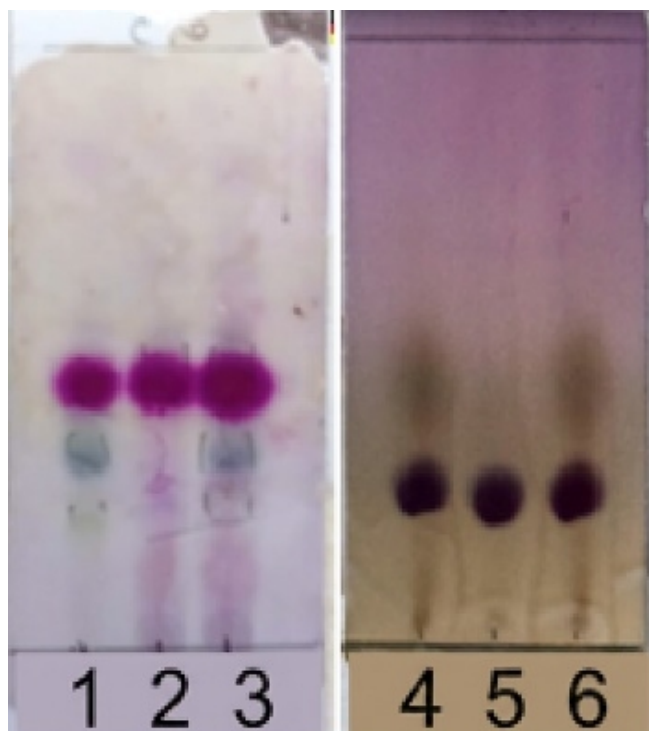
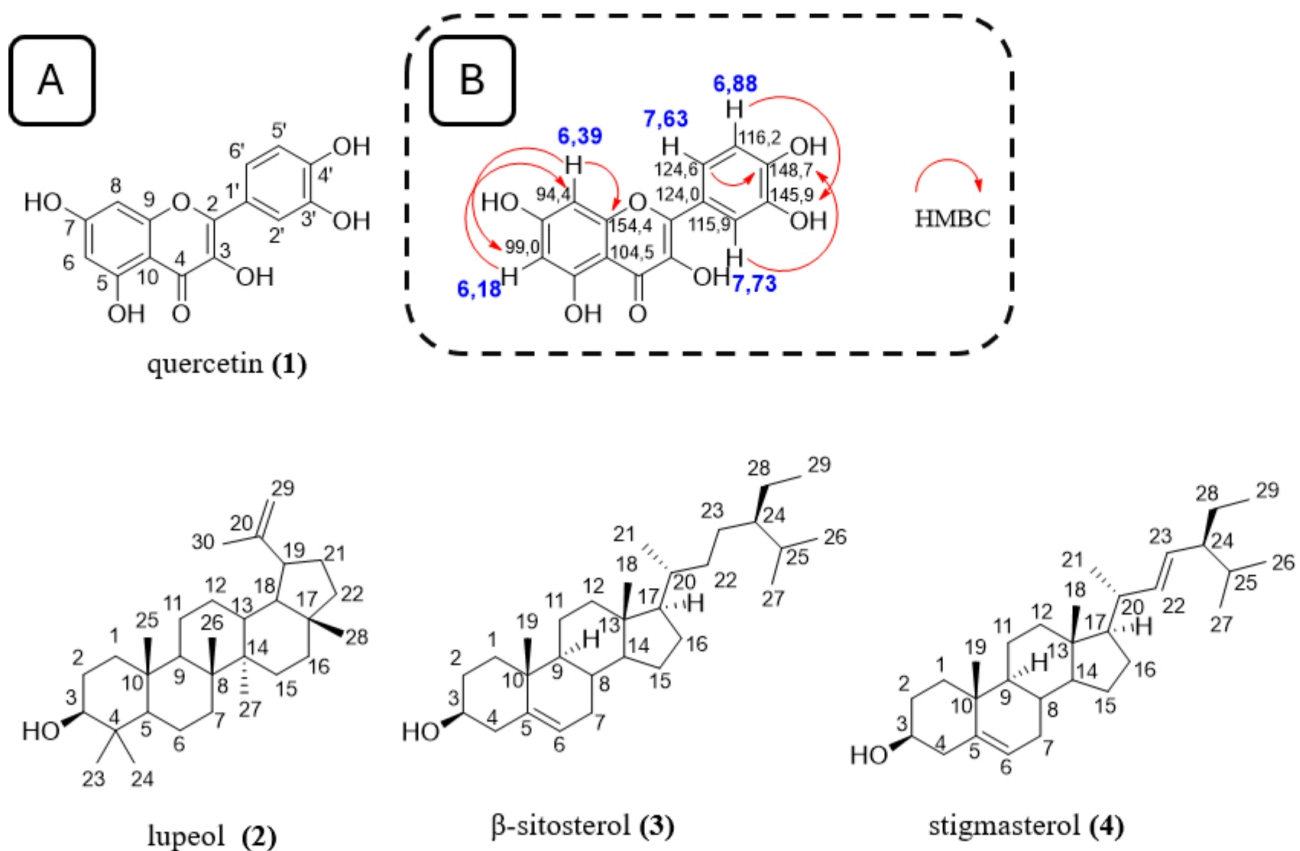


Figure 1

TLC analysis of the fractions containing the isolated compounds and compared to the concentrated standards available at the laboratory.

Legend: Lupeol (1), lupeol standard (2), isolated lupeol + standard (3), β -sitosterol and stigmasterol (4), standards (5), and β -sitosterol and stigmasterol + standards. Hexane/EtOAc 8:2 v/v, with sulphuric anisaldehyde under heating



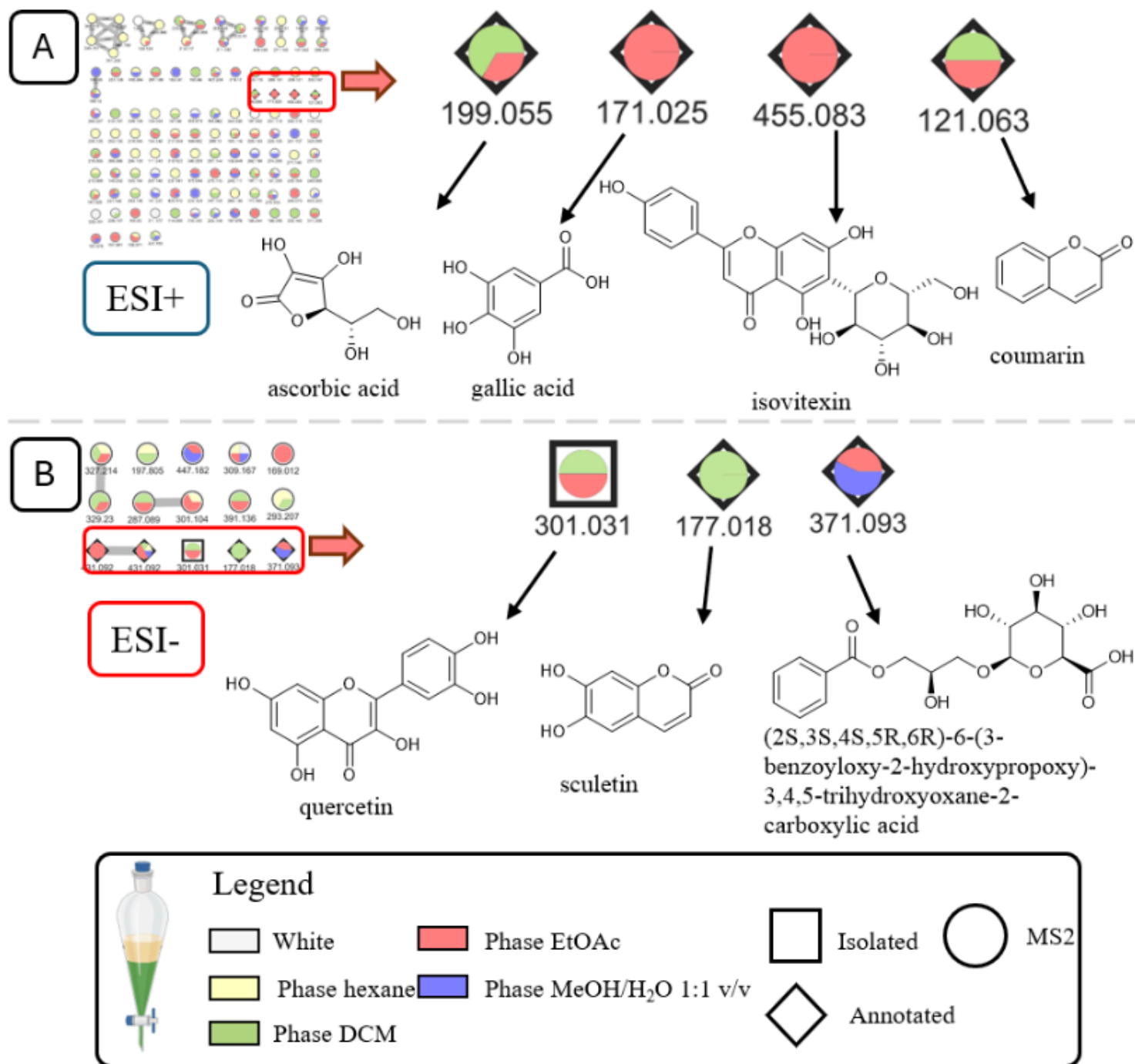


Figure 3

Molecular network of the MeOH extracts of *Parkia decussata* leaves, showing the annotated compounds. The nodes were organised according to the fractions obtained from partitioning of the MeOH extract of the leaves. A) compounds annotated in positive mode; B) compounds annotated in negative mode

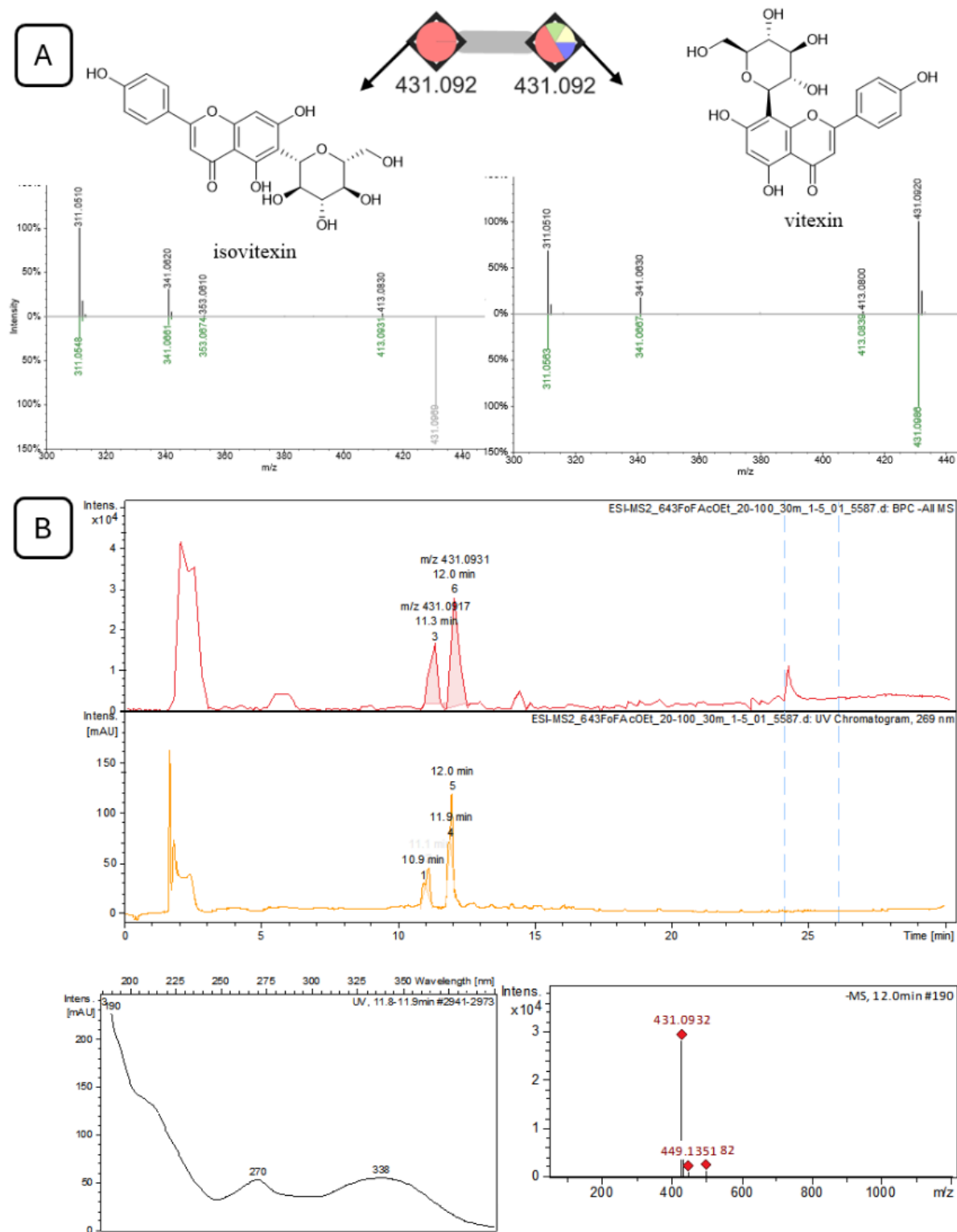


Figure 4

C-glycosylated flavonoids annotated in negative mode in the MeOH extract of *P. decussata* leaves. A) Comparison of the fragmentation spectra (MS2 in ESI) of isovitexin and vitexin using the GNPS2 library Mirror plot. B) Total ion and ultraviolet chromatograms of the EtOAc fraction containing these flavonoids, showing the bands at λ 270 and 338 nm