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Metabolite profiling, phytochemical studies, heavy metal determination and health risk assessment of *Entandrophragma delevoyi* De Wild in Zambia

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ABSTRACT

The bark of *Entandrophragma delevoyi De Wild* (*E. delevoyi*) is widely used in the Zambian traditional health care system as a very effective medicinal plant. Nevertheless, little is known about the phytochemicals affiliated with the ethno-pharmacological uses of the plant. Further, the heavy metals safety of consuming the plant for medicinal purposes remains unknown. In this study, metabolite profiling of the methanolic stem bark extract of *E. delevoyi* was performed using a de-replication approach by coupling HPLC-ESI-QTOF-MS with MZmine 3 based two molecular networking approaches, that is, GNPS (global natural products social molecular networking) and METLIN Gen2. Varieties of metabolites were tentatively identified for the first time in *E. delevoyi*. Health risk assessment was achieved by analysing the presence and concentration of heavy metals such as Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Si, Sn, U and Zn using inductively coupled plasma optical emission spectroscopy (ICP-OES). Further, estimated daily intake (EDI), hazard quotients (HQ), and carcinogenic risk were determined. The utilization of qualitative tests to screen metabolite classes and the molecular networking tools revealed a detailed phytochemical profile of this medicinal plant species. Qualitative metabolite screening tests revealed the presence of flavonoids, alkaloids, phenolics, saponins, anthraquinones, terpenoids, steroids, cardiac glycosides, anthocyanins, and tannins in the methanol extract. LCMS2 coupled to mzmine3.6 revealed the presence of thirty-five metabolites from MoNA database. In addition, LCMS2-GNPS revealed the presence of twenty-six metabolites, and LCMS-METLIN Gen2 revealed a further sixteen compounds. Furthermore, total polyphenolic content and antioxidant (1,1-diphenyl-2-picryl-hydrazylhydrate - DPPH) capacities were assayed. Total phenolic content was 169.46 ± 0.71 mg GAE/g and total flavonoid content was 53.14 ± 1.78 mg QE/g. The crude extract had significant total polyphenolic content and antioxidant potential (IC_{50} = 48.91 μg/mL) compared to the standard, ascorbic acid (IC_{50} = 74.47 μg/mL). This plant, full of bioactive compounds and significant antioxidant properties, offers unique health solutions and robust possibilities for future drug development and pharmacology research. Although the bark of *E. delevoyi* possesses many health-promoting bioactive metabolites, the heavy metals evaluated in the bark of *E. delevoyi* in this study have carcinogenic risk (Al, 0.096; Cd, 0.002; Cr, 1.271; Ni, 0.024). Based on the non-carcinogenic health risk evaluation, *E. delevoyi* bark may be safe. However, its safety can only be determined if the plant's health risks are fully described in extensive chronic toxicity tests. *E. delevoyi* should therefore be used with caution in medicinal preparations.

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1. Introduction

Phytochemicals are immensely significant due to their diverse applications. Natural product research mainly involves samples possessing multiple chemical constituents. The process of isolating and structural determination of the metabolites remains tedious and laborious. However, the modern and advanced performance of analytical chromatographic technologies has revolutionized the research of natural products. For example, Dereplication is one of the techniques used in drug discovery to prioritize metabolites through systematic and efficient isolation and structure determination using

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LCMS2-based molecular networking (Zhao and Yue, 2023). The speed of isolation of known and novel metabolites is increased using literature and libraries of annotated molecular networks, thereby significantly lowering the cost of the drug discovery pipeline. MZmine, GNPS, and METLIN are vital molecular networking tools used to determine the metabolite profile of LCMS2 analyzed extracts; and the current study used these molecular networking tools (Wang et al., 2016; Montenegro-Burke et al., 2020; Schmid et al., 2023). For metabolite screening, the choice for ultra-high pressure liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) has demonstrated usefulness and effectiveness in analyzing, identifying, and quantifying a broad range of metabolites (Perez et al., 2021). Further, the HRMS technique comprehensively identifies metabolites in complex samples such as plant extracts. The resulting MS2 data is obtained by matching high-quality MS2 spectra against a commercial HR-MS2 spectral library (Xu et al., 2020).

E. delevoyi is a botanically very distinctive species growing in Zambia and the Democratic Republic of Congo and has immense health benefits in the traditional healthcare system in both countries (Meerts, 2016). In Zambia, the plant is locally called Mofwe or Mofu. In folk medicine, Various species of the genus *Entandrophragma* are reported to have traditional medicinal uses in the treatment of several diseases. Specific species are traditionally utilized to manage infectious diseases such as malaria (*E. angolense, E. candollei, E. caudatum, E. congoense, E. palustre, E. utile*), dysentery (*E. cylindricum*), gonorrhoea (*E. caudatum*), syphilis (*E. caudatum*), and trypanosomiasis (*E. bussei*) (Mouthé et al., 2018). In the context of cancer treatment, *E. macrophyllum* is noted for its use against cancer (Mouthé et al., 2018). Additionally, species of *Entandrophragma* are employed in treating immune-related conditions, including rheumatism (*E. cylindricum*), gastrointestinal ulcers (*E. angolense, E. utile*), abdominal pain (E. *candollei*), and sickle cell disease *(E. utile*). Furthermore, *E. spicatum* is reported to be used as a sexual stimulant for men (Mouthé et al., 2018).

Although medicinal plant remedies are preferred because of being safe, tolerable to human cells, cheap to find and lesser or no side effects, plants tend to accumulate heavy metals to concentrations that may pose health risks to consumers. Numerous investigations have been conducted worldwide to examine and ensure the safety of herbal medicines by studying the presence of heavy metals in medicinal plants (Luo et al., 2021). Researchers have conducted exposure risk assessment, hazard quotient, and ecological risk assessment to investigate the potential negative impacts on human health and the environment (Kaouthar, et al., 2021; Bisht et al., 2022). These assessments highlight the urgent need for attention in addressing heavy metal contamination in medicinal plants, as it poses significant risks to human health. A full risk assessment of heavy metal concentration in *E. delevoyi* is hence necessary and urgent.

This study focused on the phytochemical screening of the stem bark of *E. delevoyi* methanolic extract using LCMS2 hyphenated to mass spectrometry coupled with molecular networking tools – GNPS and METLIN Gen2. This study also features qualitative tests, total polyphenolic content, and antioxidant potential determination. Further, the study determined heavy metal concentration and assessed the health risks associated with the consumption of the bark of *E. delevoyi* for medicinal purposes.

2. Materials and methods

2.1. Laboratory materials

Materials were obtained from the chemical stores of the School of Chemistry, University of Witwatersrand, South Africa: 50 mL PTFE centrifuge tubes, 2.0 mL vials, 10 mL syringes and needles, assorted glassware such as flasks and test tubes, and 0.22 μm PTFE filters (Millipore, Milford, USA).

2.2. Plant materials

Fresh plant samples were collected from the forest near Twasekela Primary School in the Mufulira district, located close to the border between the Republic of Zambia and the Democratic Republic of Congo (GPS coordinates: −12.674463195257498, 28.412723179799595). Fresh bark from the trunk of *E. delevoyi* was cut, labeled, and transported in UV-resistant plastic bags, shade-dried for 21 days at room temperature, and finely powdered using a pulverizer (DFT-200, Dahai Ltd., China). Botanical identification was certified (Voucher specimen number: BC105) by the forest department at the Kitwe District Herbarium of the Republic of Zambia's Ministry of Green Economy and Environment.

2.3. Chemicals

The following chemicals were sourced from Merck (Johannesburg, South Africa): acetonitrile (HPLC grade, ≥ 99.9 %), sodium nitrite (≥97.0 %), anhydrous sodium carbonate (≥99.5 %), and anhydrous aluminum chloride (≥ 98.0 %), sodium hydroxide (≥ 98.0 %), including the following standards: garlic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteau (FC) reagent, and primary and secondary amines (PSA). Further, the following chemicals and reagents were sourced from the chemical stores of the School of Chemistry, University of Witwatersrand, South Africa: acetic anhydride (≥ 99.5 %), chloroform (HPLC grade, ≥ 99.9 %), magnesium ribbon (≥ 99.0 %,), ferric chloride (≥ 99.99 %), hydrochloric acid (≥37 %), lead acetate (≥99 %), glacial acetic acid (≥99 %), methanol (HPLC grade, ≥ 99.9 %), Hager's reagent (1.3 %, saturated in water), ammonia (Reagent, ≥32 %), and sulphuric acid (≥ 98 %). The reagents used for metal determination was high-quality 65 % HNO_3 (Merck, RSA), 50 % Hydrogen peroxide (H_2O_2), and ultra-pure water from a purification system Milli-Q, Millipore (Merck Chemicals (PTY) Ltd. Johannesburg, RSA). A multi-element standard solution was used for ICP-OES (Spectro Genesis, Germany) calibration with a concentration ranging from 10 mg/L to 0.1 mg/L. The stock solution with a concentration of 1000 mg/L was dissolved in 5 % HNO_3 to prepare a range of calibration standards. ICP-OES utilizes ultra-high purity argon gas.

2.4. Extraction of phytochemicals for qualitative phytochemical screening

The major categories of phytochemicals in *E. delevoyi* were identified using standard qualitative testing at the Environmental Analytical Chemistry Laboratory, School of Chemistry, University of Witwatersrand, South Africa. The fresh bark of *E. devoloyi* was dried in the shade and then ground with a pulverizer. Metabolites were extracted in methanol using a method described by Chibuye et al. (2024). A 5.0 g powdered sample was soaked in 50 mL methanol (80 %), which was then placed in a 100 mL flask. The solid-solvent ratio was set at 1:10 (m/v) to achieve a high extraction yield (Chibuye et al., 2024). The menstruum was continuously agitated by placing the flasks in a water shaker with a shaking speed of 150 rpm and a set temperature of 40 °C for 24 h. The extract was cleaned through 0.22 μm PTFE filters. Finally, routine qualitative assays were used to screen the filtered extract for phytochemicals.

2.5. Screening of metabolites using qualitative chemical tests

The qualitative chemical tests mentioned in the literature were used to identify the different types of metabolites found in the bark

of *E. delevoyi*. These tests include Borntrager's Test for anthraquinones, Braymer's Test for tannins, Foam Test for saponins, Shinoda Test for flavonoids, Salkowski Test for steroids, Molisch's Test for carbohydrates, Libermann's test for phenolics, Hager's Test for alkaloids, and Liebermann's Test for glycosides (Seck et al., 2021).

2.6. Extraction process for total polyphenolic content and antioxidant assay

The total polyphenol extraction and antioxidant study were conducted out using a modified method described by Pakade et al. (2013). Briefly, 5.0 g of powdered *E. delevoyi* bark was weighed in 50 mL PTFE centrifuge tubes and combined with 40 mL EtOH (80 %). The contents were then sonicated for 30 min at 30 °C, 250 W of ultrasonic power, and 40 kHz of sonication frequency. After that, the sample were centrifuged for 10 min at 5000 rpm at 20 °C. The supernatant was filtered using 0.22 μm PTFE filters, then dried under reduced pressure using a rotary evaporator at 50 °C, followed by freeze-drying at −50 °C for 48 h. For further analysis, the dried extracts were reconstituted in 10 mL methanol and stored at 4 °C. The phytochemical yield of the extract was obtained using the following equation:

$$\% \text{ Phytochemical yield} = \frac{weight\ of\ extract}{weight\ of\ sample} \text{ X } 100 \quad (1)$$

2.7. Polyphenol extraction using the QuEChERS technique

Sample preparation for LC–MS analysis was achieved using the QuEChERS extraction method as described by Chibuye et al. (2023a), and Chibuye et al. (2023b). In essence, 5.0 g of the powdered plant sample was placed in a 50-mL PTFE centrifuge tube containing 25 mL (80/20, v/v) of acetonitrile and water, respectively. The contents were then mixed thoroughly by vortex for a minute. Then, 4.0 g MgSO4 and 1.0 g NaCl were added, the mixture was vortexed for 60 s, and immediately centrifuged for 10 min at 6000 rpm. A 4.0 mL aliquot from the upper part of the extract was transferred into another 50-mL PTFE centrifuge tube containing 150.0 mg PSA and 75.0 mg $MgSO_4$. The mixture was then shaken, vortexed, and centrifuged for 10 min at 6000 rpm. The recovered 2.0 mL ACN extract was filtered using a 0.22-μm filter paper and injected into the Liquid chromatography-triple-quadrupole tandem mass spectrometer.

2.8. LC-Q-TOF/MS analysis

The bark of *E. delevoyi* was analyzed for its untargeted metabolite composition using ultra-high-performance liquid chromatography – high-resolution mass spectrometry. This method utilized a 3000 Series Rapid Resolution LC system (Ultimate 3000, Thermofisher South Africa) equipped with a vacuum degasser, autosampler, binary pump, and a diode array detector. The analysis followed a procedure described by Chibuye et al. (2024). In brief, the process involved separating metabolites using phenomenex C18 column and using formic acid and acetonitrile as mobile phases A and B, respectively. The flow rate was 0.50 mL per minute. The gradient was planned to follow the following sequence: 0 min with 5 % B, 10 min with 35 % B, 15 min with 95 % B, and 25 min with 5 % B. The UHPLC system utilized an injection volume of 10 μL. The UHPLC system was connected to a QTOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The mass spectrometer had Electrospray Ionization (ESI) capability and it was used in the negative mode. The decision was made to focus on the negative mode of acquisition as it offered better ionization and resolution, even though positive mode was also an option. Detection was carried out in the mass range of 50–1300 *m/z*, using a capillary voltage of +4000 V, and a dry gas temperature of 210 °C, with a flow rate of 8.0 L/min. Additionally, the pressure of the nebulizer was 2.0 bar, while the rate of spectra was set at 1 Hz.

2.9. Quantification of total polyphenols

Total phenolics were quantified following a modified Folin–Ciocalteu (FC) colorimetric assay by Pakade et al. (2013). In brief, 200 μL of sample extract was mixed with 10 mL distilled deionized water (DDW) and 300 μL FC reagent, and the mixture was kept for 5 min at room conditions. Thereafter, 1 mL sodium carbonate (7.5 %) was added, and 1.5 mL of DDW was added, bringing the total volume to 13 mL. The mixture was incubated for 2 h at room temperature. The absorbance was measured using a UV–vis spectrophotometer at 760 nm (UV–vis 2550, Shimadzu, Tokyo, Japan) using gallic acid as standard (0, 20, 40, 80, 120, 160, 200 μg/mL) prepared in 80 % methanol and the data were expressed as mg GAE g^{-1}. The experiment was conducted in triplicate. Total flavonoids were quantified according to the procedure outlined by Ralepele et al. (2021) with modifications (Ralepele et al., 2021). In essence, 5.0 mL sample extract was mixed with 0.5 mL sodium nitrite (5 g in 100 mL), and the solution was allowed to stand for 6 min at ambient temperature. After which 0.5 mL aluminum chloride (1:100, wt/vol) was added, and the solution was kept for 5 min at room conditions. Finally, 4 mL sodium hydroxide (1.0 M) was added, and the mixture was kept for 20 min at room temperature. Absorbance was measured using a UV–vis spectrophotometer at 510 nm (UV–vis 2550, Shimadzu, Tokyo, Japan) using quercetin as a standard, and the results were expressed as mg QE/g. The experiment was conducted in triplicate.

2.10. DPPH assay

The DPPH assay was performed in accordance with the method described by Chibuye et al. (2024). Essentially, 24 mg of DPPH was dissolved in 100 mL of methanol to make the stock solution. The stock solution was then diluted five times to form a workable solution. 3 mL of DPPH working solution was mixed with 20, 40, 60, 80, and 100 μL of plant extract. The mixes were then incubated in total darkness for 45 min before measuring absorbance at 517 nm. The percentage of antioxidant activity (RSA) was calculated using the following formula:

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

where: As—Sample absorbance, and Ac—Control absorbance

3. Feature-based molecular networking

The Bruker MS2 data files were changed to MZML format using MSConvert Software (ProteoWizard package). The MZML file was then processed using MZmine 3.3 (Chibuye et al., 2024; Schmid et al., 2023). The noise level for MS1 and MS2 was kept at 22 and 23, respectively, to detect mass. The ADAP chromatogram builder used parameters such as minimum consecutive scans of 5, minimum intensity for consecutive scans of 35.0, minimum absolute height of 45.0, and *m/z* tolerance (scan to scan) of 30.0 ppm. The local minimum resolving was set using a chromatographic threshold of 0.75, a minimum search range RT/Mobility (absolute) of 0.001, a minimum absolute height of 4000.0, a minimum ratio of peak top/edge of 1.2, a peak duration range (min/mobility) of 0.002, and a minimum of 5 scans (data points).

The deisotoping was applied with the parameters such as *m/z* tolerance (intra-sample) to be 0.01 *m/z* or 20.0 ppm, retention time tolerance of 0.1 min, Maximum charge of 2, and minimum scans (data points) to be 5. The feature rows filtering process used an *m/z*

tolerance of 5.0 ppm and a maximum charge to be 2. The feature list alignment was set to 3.0 *m/z*, 0.2 min of retention time tolerance, 2.0 g for RT, 2.0 g for mobility, and 5.0 ppm for isotope *m/z*. Gap filling was done with a 0.2 intensity tolerance, a 0.05 min retention duration, and a minimum of 3 scans (data points). Finally, the MGF precludested spectral data file and its equivalent .csv metadata file and the original MZML file were exported to the GNPS platform using the Filezilla Client 3.62.0.

3.1. Quality control and assurance, metal extraction and analysis

To ensure the accuracy and precision of the data, proper precautions were taken when handling and cleaning glass equipment. Throughout the experiment, distilled, high-purity deionized water was used for rinsing and diluting, as well as analytical grade reagents. Blank analyses were used to adjust the instrument's readings. Calibration standards were made from the stock solution of each metal to prepare the instrument for accurate measurements. The analysis was carried out repeatedly to ensure its precision and accuracy. The method was validated by performance parameters, limits of detection (LOD), quantification (LOQ), and spike recoveries (%). Essential macro-, microelements, and toxic metals were determined in the medicinal plant. To ensure quality control, a continuous calibration verification (CCV) analysis was performed for every run. The correlation coefficient values (R^2) for CCV were determined by testing different concentrations (0, 0.1, 0.5, 1, 2, 5, 10 mg/L) of a standard mixture containing all the measured metals after each set of samples.

Microwave digestion was used for heavy metal extraction using a Multiwave 3000 microwave digester (Anton Paar, Switzerland) according to the method of Thabit et al. (2021) with modifications (Thabit et al., 2021). In brief, the ground powdered medicinal plant sample was sieved using a 90-micron sieve. Then, 0.25 g of plant samples were weighed into 20 mL poly reaction vessels directly. To each reaction vessel containing the 0.25 g powdered sample was added 9 ml concentrated HNO_3 and 3 ml H_2O_2. The reaction vessels were stood for 5 min before putting them in the rota. Digestion temperature was increased from 40 °C to 180 °C in 10 min and was maintained for 30 min. After cooling, the resultant clear digests were diluted with distilled deionized water up to 50 mL in 50 ml Falcon tubes. Blanks and spikes were prepared in the same manner as the samples. For spiked samples, three replicate acid digests were performed. The concentration of heavy metals in the bark of *E. delevoyi* was determined using Inductively Coupled plasma optical emission spectroscopy (Spectro, Kleve, Germany) using a previous method described by Nupia et al. (2018).

4. The health risk assessment of heavy metal concentration in *E. delevoyi* stem bark

The estimated daily intake (EDI), non-carcinogenic risk (HQ, HI), and carcinogenic risk (CR) were assessed to determine the potential health risks of heavy metal concentration in the bark of *E. delevoyi*. EDI was calculated using the following equation:

$$EDI = \frac{M_c \; X \; IR}{WB} \tag{2}$$

where M_c is the metal concentration (mg/kg) in the raw bark sample of *E. delevoyi*, IR (ingestion rate) refers to the daily average intake of raw plant preparations, which is assumed to be 0.06 kg/day, and WB refers to the typical weight of a human body, with the average weight of 70 kg for adults in Zambia (Miyanza et al., 2023).

Hazard Quotient (HQ) is used to assess the non-carcinogenic risk to humans from long-term exposure to heavy metals from vegetables, medicinal plants and fruits. An HQ of 1 signifies that there are potential health risks due to exposure. The HQ is calculated as shown in the following equation.

$$THQ = \frac{EDI}{RfD} \tag{3}$$

RfD means the recommended daily amount in mg/kg of body weight, which is established considering the highest safe level for orally consuming each metal for an adult. The reference doses (RfD) for various elements are listed as follows: aluminum (1.0), iron (0.7), copper (0.04), manganese (0.14), zinc (0.3), cadmium (0.001), lead (0.0035), chromium (1.5), arsenic (0.014), nickel (0.91), mercury (0.0003), uranium (soluble salts, 0.0002), tin (0.6), silver (0.005), vanadium and compounds (0.005), cobalt (0.0003), molybdenum (0.005) and selenium (0.005 mg/kg/day) (Mohammadi et al., 2019; USEPA, 2021, 2015; Miyanza et al., 2023). For a heavy metal, a THQ > 1 proposes possible health risks due to exposure to that heavy metal.

The hazard Index (HI) helps to assess the general non-carcinogenic health risk posed by multiple heavy metals to humans. Exposure to multiple heavy metals leads to an incremental impact. The HI was calculated as the sum of HQ for all tested metals (Miyanza et al., 2023).

The carcinogenic risk (CR) is a calculation of the chance that someone develops cancer as a result of being exposed to a substance that could cause cancer during their lifetime. CR was determined by applying Eq. (4) of the USEPA Region III Risk-Based Concentration Table (USEPA, 2021, 2015).

$$CR = EDI \times CPSo \tag{4}$$

CPSo is the oral slope factor for a carcinogenic metal. According to Mohammadi et al. (2019) and Miyanza et al. (2023), the value of CPSo is 6.1, 0.84, 8.5, 1.5, 0.2, and 41 for cadmium, nickel, lead, arsenic, aluminum and chromium respectively. Values larger than 0.0001 indicate a higher chance of CR occurring (Miyanza et al., 2023; Mohammadi et al., 2019). If there are multiple metals known to cause cancer, the total risk of developing cancer can be determined by adding the individual risks, assuming that their impacts on the human body are accumulated. The range of acceptable risks is between 0.000001 and 0.0001.

4.1. Statistical analysis

All experiments were repeated three times, and the findings are shown as mean ± SD. Microsoft Excel 2016 was used to evaluate numerical data and produce calibration curves.

5. Results, analysis and discussion

5.1. Metabolite screening chemical tests

Methanol was selected as the extraction solvent due to its superior dissolving capabilities for a broad range of phytochemicals, particularly polar phytochemicals such as polyphenols ((Annapurna et al., 2021; Chibuye et al., 2023a, 2023b). The bark of *E. delevoyi* is endowed with broad categories of bioactive metabolites. Phytochemical screening showed the presence of alkaloids, phenolic compounds, flavonoids, saponins, anthraquinones, steroids, terpenoids, cardiac glycosides, anthocyanins, and tannins. All these natural product classes have metabolites with immense pharmacological benefits. The pharmacological benefits might be the scientific reason for the amazing health benefits of *E. delevoyi* bark and its prevalent use in traditional medicine in Zambia, where the plant is known to be a panacea for all diseases. For example, alkaloids contain nitrogen atom(s) in their structures, and these nitrogen atoms render these metabolites alkaline. This attribute makes alkaloids possess a wide range of

Table 1
MZmine 3.6 based tentative identification of metabolites from crude bark extract of *E. delevoyi* by UHPLC–ESI-MS2 in the negative ion mode. Base peaks are indicated in bold.

ID	RT	Adduct [M-H]–	Exact Mass	MS2	Tentative compound	Formula	Cosine similarity
1	1.77	215.0350		**89.02**, 96.95, 107.02, 119.02, 179.05	(4S,5Z,6S)-4-(2-methoxy-2-oxoethyl)-5-[2-[(E)−3-phenylprop-2-enoyl]oxyethylidene]-6-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4H-pyran-3-carboxylic acid	$C_{12}H_8O_4$	1.000
2	1.78	165.0407		**75.00**, 87.00, 96.95, 128.83,	2,4-Dihydroxypteridine	$C_7H_6N_4O_2$	0.893
3	1.86	191.0561		85.02, **191.05**	D-(-)-quinic acid	$C_7H_{12}O_6$	0.992
4	1.91	152.9679	154.2080	71.01, **80.96**, 134.98	Propenyl thiosulfate (isomer of 108)	$C_3H_6O_3S_2$	0.856
5	1.95			78.95, 79.95, 80.02, **96.96**,	Phosphoric acid	H_3O_4P	0.994
6	2.10	133.01	96.9608	71.01, 72.99, 89.02, **115.00**,	Malic acid	$C_4H_6O_5$	1.000
7	2.33	191.0197		87.00**, 111.00**, 154.99	Isocitric acid; LC-tDDA; CE40	$C_6H_8O_7$	1.000
8	2.71	327.0759	326.0708	71.01, **89.02**, 101.02, 119.03, 149.04, 179.05	Bergenin	$C_{14}H_{16}O_9$	0.936
9	3.55	297.1132		92.02, 101.02, **161.07**, 179.08	Enterolactone	$C_{18}H_{18}O_4$	1.000
10	4.08	865.2300		125.02, 243.02, 287.07, 451.09, 575.11, 713.15, **865.19**	Procyanidol C1	$C_{45}H_{38}O_{18}$	0.862
11	4.16	[M+HCOO]- 347.0976	302.2790	95.01, 123.00, 138.03, 151.03, 161.04, 181.05, 193.04, **301.09**	Methoxybenzenediol + O-Hex	$C_{13}H_{18}O_8$	0.914
12	4.21	169.0143		69.03, 79.01, 97.02, 107.01, **125.02,**	Gallic acid	$C_7H_6O_5$	0.960
13	4.27	593.1353		125.02, 177.02, 305.06, 423.07, **593.13**	Tiliroside	$C_{30}H_{26}O_{13}$	0.845
14	4.39	[M + H]+ 315.1119	314.1046	81.03, 95.04, 123.04, **153.05**	Pyraclonil	$C_{15}H_{15}ClN_6$	0.985
15	4.50	451.1235		109.02, 137.02, 165.01, 203.06, 277.06, **289.07**, 313.06, 343.08	Okanin-4′-O-glucoside	$C_{21}H_{22}O_{11}$	0.904
16	4.51	433.0776		124.01, 169.01, 211.02, 271.04, 329.04, 357.08, **433.08**	Guajavarin	$C_{20}H_{18}O_{11}$	0.822
17	4.58	305.0667		81.03, 109.02, **125.02**, 179.03, 219.06, 261.08	Epigallocatechin	$C_{15}H_{14}O_7$	0.869
18	4.64	305.0667		89.03, 109.02, **125.02**, 179.03, 219.06, 261.08, 284.20	(2S,3S)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	$C_{15}H_{14}O_7$	0.915
19	4.77	577.1351		125.02, 289.07, 425.08, **577.13**	Procyanidin B1	$C_{30}H_{26}O_{12}$	0.927
20	4.93	865.1985		125.02, 289.07, 407.07, 577.13, 698.11, **865.19**	Procyanidin C1	$C_{45}H_{38}O_{18}$	0.842
21	5.07	289.0724		83.01, 109.02, 123.04, 151.04, 179.03, 203.06, 221.07, 245.07, 271.06, **289.07**	altenusin	$C_{15}H_{14}O_6$	0.956
22	5.60	[M+Na-2H]- 509.2646		119.03, 170.01, 199.13, 223.12, 253.14, 301.18, 357.17, 422.82	1-pentadecanoyl-2-hydroxy-sn-*glycero*(d5)-3-phospho-L-serine, QE	$C_{21}H_{42}NO_9P$	0.712
23	5.93	839.3090		89.02, 179.05, 327.07,	Utilin	$C_{41}H_{52}O_{17}$	1.000
24	6.02	112.9845		68.97**, 68.99**, 69.02	Acetylenedicarboxylic acid	$C_4H_2O_4$	1.000
25	6.29	[M+Na]+ 447.2720	424.2830	105.03, 133.02, 175.03, 247.08, 271.22, 345.20, 392.42, **447.27**	(*R*)-4-((3R,4R,5S,7R,8R,9S,10R,12S,13R,14S,17R)-3,4,7,12-tetrahydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid	$C_{24}H_{40}O_6$	0.895
26	6.58	329.2334		99.07, 139.11, 157.12, 171.10, 211.13, 229.14, 312.23, **329.23**	(Z)-5,8,11-trihydroxyoctadec-9-enoic acid	$C_{18}H_{34}O_5$	0.974
27	6.75	[M+Na]+ 447.2720	424.2830	109.03, 134.03, 160.01, 175.03, 233.08, 271.22, 345.20, 401.24, **447.27**	(4R)-4-((3R,5S,6S,7R,8R,9S,10R,12S,13R,17R)-3,6,7,12-tetrahydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid	$C_{24}H_{40}O_6$	0.885
28	7.87	293.1792	294.1865	148.05, **221.15**	Tetradecylsulfate	$C_{14}H_{30}O_4S$	1.000
29	8.12	467.2068		96.04, 113.05, 151.07, 229.08, 283.09, 309.12 **393.16**	Carapin	$C_{27}H_{32}O_7$	0.832
30	8.39	[M + H]+ 281.2477	280.2402	198.07, **281.24**,	Linoleic acid	$C_{18}H_{32}O_2$	1.000
31	8.57	339.1966	340.2038	119.05, **163.11**, 183.01, 205.96, 239.07, 254.09	Canrenone	$C_{22}H_{28}O_3$	0.947
32	8.75	293.2122	294.2195	107.09, 121.09, 171.10, 183.13, 211.13, 235.17, 275.19, **293.20**	9S-hydroxy-10E,12Z,15Z-octadecatrienoic acid	$C_{18}H_{30}O_3$	0.884
33	8.90	311.1136		119.04, 183.01, 225.05, 239.08, 292. 35, **311.16**	1-[2-methyl-6-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-phenyl]ethanone	$C_{15}H_{20}O_7$	0.953
34	9.28	[M + H]+ 295.2278		119.04, 171.10, 195.13, 277.21, **295.22**	Hydroxy octadecatrienoic acid	$C_{18}H_{30}O_3$	1.000
35	9.38	[M+Na]+ 507.2919		161.02, 178.02, 253.22, 271.22, 347.25, 415.24, 433.25, **507.29**	(2R,3S,4S,5S,6R)-2-[[7-hydroxy-4-[(Z)-5-hydroxy-3-methylpent-3-enyl]-4a,8,8-trimethyl-3-methylidene-2,4,5,6,7,8a-hexahydro-1H-naphthalen-2-yl]oxy]-6-(hydroxymethyl)oxane-3,4,5-triol	-	0.885

pharmacological benefits (Alamgir, 2018). Some alkaloids are antiarrhythmic, antigout, antitussive, analgesic, antipyretic, antimalarial, antihypertensive, antitumor, or aphrodisiac, and many other health-promoting effects. On the other hand, Saponins are good at stopping bleeding and wound healing and are anti-inflammatory (Nigussie et al., 2021).

The other scientific rationale for the effective use of *E. delevoyi* in traditional medicine is the presence of tannins in the plant. Tannins have therapeutic effects on various diseases, from their anti-inflammatory effects, bactericidal, and anticancer actions, and many other medicinal benefits (Dousari et al., 2022). In addition, *E. delevoyi* possesess terpenoids, and these metabolites possess many health benefits, including promoting transdermal absorption and possessing anti-inflammatory, antibacterial, anti-tumor, antimalarial, and antiviral effects. Further, terpenoids treat and prevent cardiovascular diseases and possess hypoglycaemic, antioxidation, neuroprotection, and antiaging effects (Yang et al., 2020). In the case of anthraquinones, they have been found to be antiulcer, diuretic, anti-inflammatory, antibacterial, antinociceptive, and anticancer (Stompor–gorący, 2021). The other medicinally active ingredients found in *E. delevoyi* bark are anthocyanins. Regular but moderate intake of these phytochemicals is associated with neuroprotection, improved weight maintenance, reduced risk of cardiovascular disease, and type 2 diabetes mellitus control (Kalt et al., 2020). Cardiac glycosides have been found to synergize with anticancer drugs, eliminate tumors, and senescent cells, thereby ameliorating a host of age-related diseases (Martin et al., 2020).

As for polyphenols, such as phenolic compounds and flavonoids, they have been found to possess a variety of anti-oxidant therapeutic effects, making them beneficial for treating and ameliorating a wide range of degenerative diseases (Lebeloane et al., 2024). Studying the health benefits of specific metabolites is even much more beneficial to medicine than studying a broad category, as singular novel metabolites are usually drug leads.

5.2. MZmine3 molecular identification

The spectral library match for MZmine 3.6 molecular networking uses the MoNA (MoNA-export-Experimental Spectra.json) library (1545052 spectra) with only MS level 2 scans, precursor *m/z* tolerance of 20.0 ppm, CCS tolerance of 0.05 %, and minimum ion density of 1.0. Molecular networking tentatively identified thirty-five (35) metabolites, shown in Table 1. Results in Fig. 1 and Tables 1 through 4 show some molecular networking, MZmine and GNPS structures of metabolites found in the bark of *E. delevoyi*.

5.3. LCMS2 – GNPS library matching

Phytochemical profiling of the bark of *E. delevoyi* using UHPLC–ESI-MS/MS coupled with GNPS library matching molecular networking revealed the presence of ninety (90) secondary metabolites as shown in Table 2 (Nothias et al., 2020; Wang et al., 2016). The results can be found online at the GNPS platform, at: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=6d3fd32d5b1e4b9dabd381957ff61944

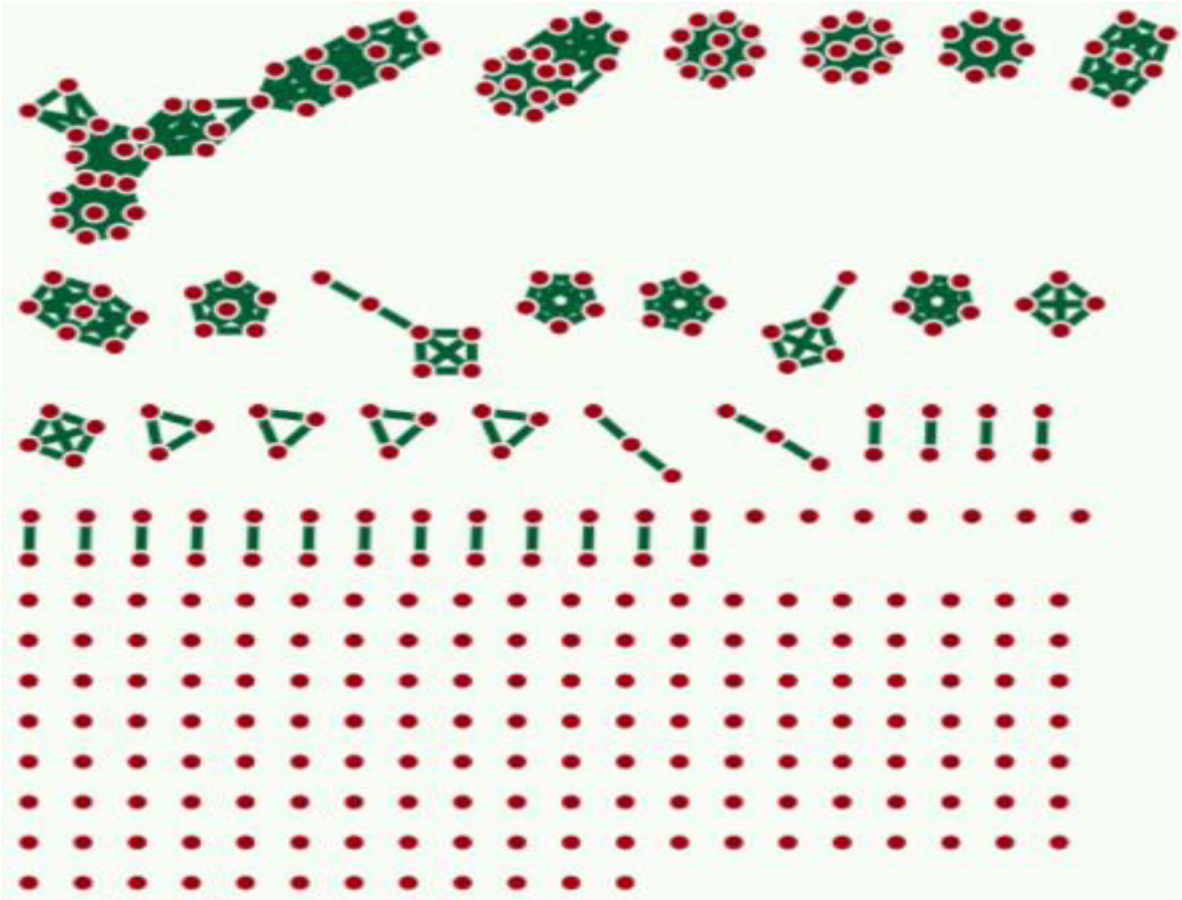


Fig. 1. Pie chart displaying the relative abundance of ions analyzed in *E. delevoyi bark* sample. The molecular network is based on the LC-MS2 data in the negative ion mode of *E. delevoyi* bark extract.

Table 2
GNPS tentative identification of phytochemicals from the crude bark extract of *E. delevoyi* by UHPLC–ESI-MS2 in the negative ion mode. Base peaks are indicated in bold.

#	Metabolite name	Cosine	*m/z* error (ppm)	Mass diff.	*M/Z*	MS2 fragments	Formula
1	(2S,3S)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	0.94	17	0.005	305.07	111.04, **125.02**, 137.02, 167.03, 179.03, 219.03	$C_{15}H_{14}O_7$
2	[5-acetyloxy-3-(hydroxymethyl)-2-oxo-6-propan-2-ylcyclohex-3-en-1-yl] 3-methylpentanoate	0.72	14	0.005	339.18	59.01, 61.99, 115.08, **183.01**, 197.03	$C_{18}H_{28}O_6$
3	6-Gingerol	0.82	18	0.005	293.18	57.03, 71.01, 177.09, 192.12, 220.15, **221.16**, 236.11, 274.88, 293.18	$C_{17}H_{26}O_4$
4	Catechin	0.87	21	0.006	289.07	109.03, 125.03, 179.03, 205.05, 245.08, **289.07**	$C_{15}H_{14}O_6$
5	Citrate	0.98	25	0.005	191.02	57.03, 85.03, 87.01, **111.01**, 129.02, 191.02	$C_6H_8O_7$
6	Citric acid	0.97	31	0.006	191.02	57.03, 85.03, 87.01, **111.01**, 129.02, 191.02	$C_6H_8O_7$
7	(-)-epicatechin	0.91	10	0.003	289.07	109.03, 125.02, 179.04, 205.05, 245.08, **289.07**	$C_{15}H_{14}O_6$
8	Epicatechin	0.85	21	0.006	289.07	109.03, 125.02, 137.02, 151.04, 227.07, **245.08**, 289.07	$C_{15}H_{14}O_6$
9	Gallic acid	0.87	26	0.005	169.01	81.20, 97.30, 107.00, **125.00**, 169.10	$C_7H_6O_5$
10	Gallocatechin - 30eV	0.88	20	0.006	305.07	**125.03**, 137.02, 165.02, 167.04, 219.07, 305.07	$C_{15}H_{14}O_7$
11	Canrenone	0.92	11	0.004	339.20	119.05, **183.01**, 184.02, 185.01, 197.03, 239.07	$C_{22}H_{28}O_3$
12	4-(undecan-5-yl)benzenesulfonic acid	0.86	17	0.006	311.17	79.96, 119.05, 170.00, **183.01**	$C_{17}H_{28}O_3S$
13	Catechin	0.84	21	0.006	289.07	109.03, 125.03, 179.03, 205.05, 245.08, **289.07**	$C_{15}H_{14}O_6$
14	Hydroxy-octadecatrienoic acid	0.71	4	0.001	293.21	121.11, 183.14, 275.20, **293.21**	$C_{18}H_{30}O_3$
15	trihydroxy-octadecenoic acid	0.76	10	0.004	329.23	171.10, 211.13, **329.23**	$C_{18}H_{34}O_5$
16	Citric acid	0.86	27	0.005	191.02	57.03, 85.03, 87.01, **111.01**, 129.02, 191.02	$C_6H_8O_7$
17	Massbank:PR309324 Flavanol base + 5O	0.70	20	0.006	305.06	111.04, 125.02, 137.02, 167.03, **305.06**	$C_{15}H_{14}O_7$
18	Pyrenophorol	0.85	19	0.006	311.15	79.96, **183.01**, 197.03	$C_{16}H_{24}O_6$
19	Quercetin	0.79	17	0.005	301.03	121.03, **151.00**, 179.00, 273.04	$C_{15}H_{10}O_7$
20	quinic acid	0.72	30	0.006	191.06	85.03, 127.04, **191.06**	$C_7H_{12}O_6$
21	quinic acid	0.92	29	0.006	191.06	85.03, 127.04, **191.06**	$C_7H_{12}O_6$
22	Procyanidin B2	0.89	7	0.005	577.13	125.03, 161.03, 245.07, **289.08**, 407.09, 425.10	$C_{30}H_{26}O_{12}$
23	Procyanidin B1	0.87	3	0.002	577.13	**125.03**, 161.03, 245.08, 289.07, 407.08, 425.09	$C_{30}H_{26}O_{12}$
24	Benzenesulfonic acid, 4-undecyl	0.72	13	0.004	311.16	183.01, **197.02**, 311.16	$C_{17}H_{28}O_3S$
25	Citric acid	0.97	30	0.006	191.02	57.03, 85.03, 87.01, **111.01**, 129.02, 191.02	$C_6H_8O_7$
26	Decylbenzenesulfonic acid	0.88	23	0.007	297.15	119.05, **183.01,** 197.02	$C_{16}H_{26}O_3S$

5.4. LCMS2 – GNPS advanced analysis

Metabolite profiling of the bark of *E. delevoyi* using UHPLC–ESI-MS coupled with GNPS advanced analysis molecular networking revealed the presence of sixty five (65) secondary metabolites. Out of 65 annotated metabolites, 18 were unique (Nothias et al., 2020; Wang et al., 2016). The unique metabolites tentatively identified are shown in Table 3. The detailed results can be found online on the GNPS platform, at: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=2abeb62b9e4f4f928db8f9c90695aab2

5.5. visualization of LC-MS2 – GNPS precursor ions by Cytoscape

Analysis of the GNPS advanced analysis molecular network of *E. delevoyi bark* extract revealed the presence of a total of 342 nodes equivalent to precursor ions visualized in Cytoscape 3.9.1, partially depicted in Fig. 1 (Chibuye et al., 2024; Shannon et al., 2003).

It is interesting to note that 342 nodes in the molecular network show precursor ions for bioactive phytochemicals. For example, Fig. 2 (a) and (b) represent an eight (8) node cluster obtained from Fig. 1, depicting isomeric molecular ions of two bioactive metabolites,

Table 3
Tentative Identification of unique metabolites in *E. delevoyi* bark extract via LC-MS2 GNPS advanced molecular networking analysis. Base peaks are indicated in bold.

#	Metabolite name	Cosine	*m/z* error (ppm)	Mass diff.	*M/Z*	MS2 fragments	Formula
1	(2S,3S)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	0.91	1	0.001	305.07	111.04, **125.02**, 137.02, 167.03, 179.03, 219.03	$C_{15}H_{14}O_7$
2	6-Gingerol	0.84	3	0.001	293.18	57.03, 71.01, 177.09, 192.12, 220.15, **221.16**, 236.11, 274.88, 293.18	$C_{17}H_{26}O_4$
3	Catechin gallate	0.88	0	0.000	441.08	125.02, **169.01**, 245.08, 289.07	$C_{22}H_{18}O_{10}$
4	CITRATE	0.97	6	0.001	191.02	57.03, 85.03, 87.01, **111.01**, 129.02, 191.02	$C_6H_8O_7$
5	Citric acid	0.97	0	0.000	191.02	57.03, 85.03, 87.01, **111.01**, 129.02, 191.02	$C_6H_8O_7$
6	(-)-epicatechin	0.95	2	0.002	579.15	109.03, 125.02, 137.02, 151.04, 227.07, **245.08**, 289.07	$C_{15}H_{14}O_6$
7	Epigallocatechin	0.88	0	0.000	305.07	**125.01**, 137.01, 165.01, 179.03, 219.06, 305.06	$C_{15}H_{14}O_7$
8	4-(undecan-5-yl)benzenesulfonic acid	0.88	4	0.001	311.17	79.96, 119.05, 170.00, **183.01**	$C_{17}H_{28}O_3S$
9	Catechin	0.85	0	0.000	289.07	109.03, 125.03, 179.03, 205.05, 245.08, **289.07**	$C_{15}H_{14}O_6$
10	Procyanidin trimer	0.77	2	0.003	865.20	**125.02**, 181.99, 287.06, 407.08,	$C_{45}H_{38}O_{18}$
11	Quercetin	0.72	2	0.001	301.04	121.03, **151.00**, 179.00, 273.04	$C_{15}H_{10}O_7$
12	quinic acid	0.90	3	0.001	191.06	85.03, 127.04, **191.06**	$C_7H_{12}O_6$
13	Procyanidin B2	0.91	1	0.001	577.13	125.03, 161.03, 245.07, **289.08**, 407.09, 425.10	$C_{30}H_{26}O_{12}$

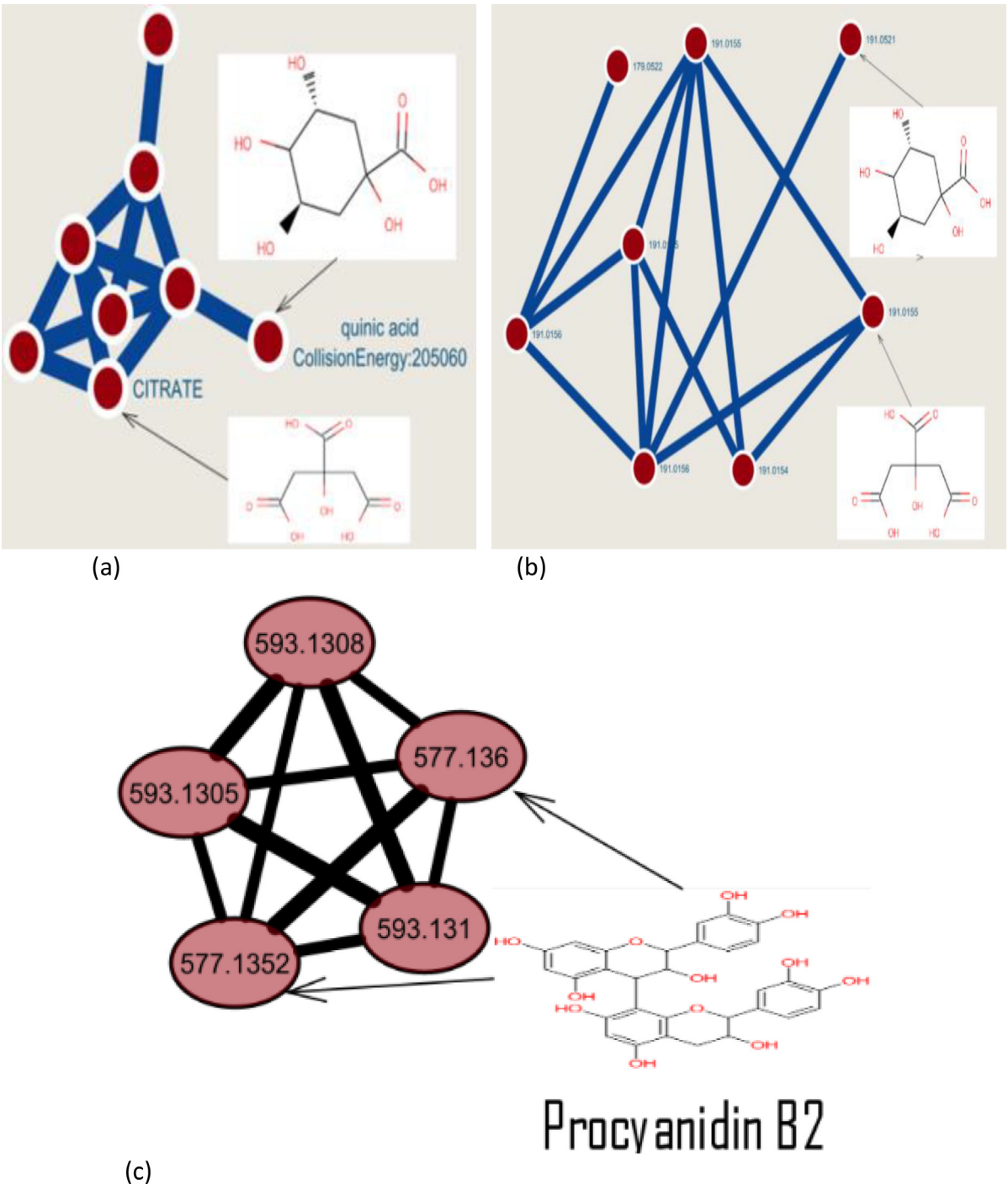


Fig. 2. Pie chart showing nodes, in red, of molecular ions of quinic acid (*m/z*, 191.0521), citrate (*m/z*, 191.0155), and procyanidin B2 (577.131, and 577.1352).

quinic acid (*m/z*, 191.0521) and citrate (*m/z*, 191.0155). Fig. 2(c) is a five (5) node cluster depicting two molecular ions for procyanidin B2 (577.131 and 577.1352), whereas the other three nodes are for molecular ions of unknown compounds.

5.6. LCMS2 – METLIN Gen 2

The same MGF file generated using MZmine 3.3 and previously subjected to GNPS was submitted to the METLIN Gen2 platform. Sixteen (16) differently unique metabolites were identified as present in the bark of *E. delevoyi* using LCMS2 coupled with METLIN [20]. Notably, the METLIN database is one of the leading databases of metabolite MS data. Table 4 shows the attributes of the 16 metabolites tentatively identified using LCMS2 – METLIN Gen 2.

The structures of phytochemicals tentatively identified by LCMS2 – METLIN Gen 2 are presented in Fig. 3. It may suffice to state that METLIN structures are neither to be redrawn nor altered in any way.

The following six metabolites were tentatively identified by both MZmine and GNPS: quinic acid, gallic acid, epigallocatechin, (2S,3S)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol, procyanidin B1, and canrenone. The remaining

Table 4
Metabolites tentatively identified by UHPLC–ESI-MS2- METLIN Gen2 in the negative ion mode.

#	MID	PubChem	Exact mass	Metabolite's systematic name	Formula
1	1796471	1261411	385.9270	5,7-dibromo-2-methylquinolin-8-yl N,N-dimethylcarbamate	$C_{13}H_{12}Br_2N_2O_2$
2	1502751	233426	385.9390	2-[2-[[4-(4-bromophenyl)-1,3-thiazol-2-yl]amino]-2-oxoethyl]sulfanylacetic acid	$C_{13}H_{11}BrN_2O_3S_2$
3	1495678	9568632	385.9380	(E)-1-(3,5-dibromo-2-methoxyphenyl)-N-(3,5-dimethyl-1,2,4-triazol-4-yl)methanimine	$C_{12}H_{12}Br_2N_4O$
4	1465786	2938039	385.9430	2-chloro-5-nitro-N-(oxo-1,3-benzoxathiol-5-yl)benzenesulfonamide	$C_{13}H_7ClN_2O_6S_2$
5	1408823	3440,131	385.9270	5-[(4-chlorophenyl)methylsulfanyl]−4-(2,4-dichlorophenyl)thiazole	$C_{15}H_9Cl_3N_2S_2$
6	1361816	–	385.9240	5-bromo-6-chloro-N-(1H-indazol-6-yl)pyridine-3-sulfonamide	$C_{12}H_8BrClN_4O_2S$
7	1291389	3103435	385.9360	4-bromo-N-[2,2,2-trichloro-1-(propan-2-ylamino)ethyl]benzamide	$C_{12}H_{14}BrCl_3N_2O$
8	1968236	–	363.9440	N-[2-(3,4-dimethoxyphenyl)ethyl]-2-({2-methyl-[1,2,4]triazolo[1,5-c]quinazolin-5-yl}sulfanyl)acetamide	$C_{15}H_{11}BrClN_2S$
9	1968235	–	363.9440	2-({2-methyl-[1,2,4]triazolo[1,5-c]quinazolin-5-yl}sulfanyl)-N-[2-(4-sulfamoylphenyl)ethyl]acetamide	$C_{15}H_{11}BrClN_2S$
10	1968161	–	363.9440	3-benzyl-2-[(cyanomethyl)sulfanyl]-N-(3,4-dimethoxyphenyl)-4-oxoquinazoline-7-carboxamide	$C_{15}H_{11}BrClN_2S$
11	1965287	2080571	363.9610	2-(4-bromo-2-chlorophenoxy)-N-(2-cyanophenyl)acetamide	$C_{15}H_{10}BrClN_2O_2$
12	1956023	2080460	363.9610	2-(4-bromo-2-chlorophenoxy)-N-(4-cyanophenyl)acetamide	$C_{15}H_{10}BrClN_2O_2$
13	1875586	1092377	363.9614	3-bromo-5-chloro-N-[4-(cyanomethyl)phenyl]-2-hydroxybenzamide	$C_{15}H_{10}BrClN_2O_2$
14	1869679	4277735	363.9437	5-(4-bromophenyl)-3(4-chlorophenyl)-1H-imidazole-2-thione	$C_{15}H_{10}BrClN_2S$
15	1718123	46495557	499.9191	N-[3-[(2,4-dichlorobenzoyl)amino]phenyl]-5-iodofuran-2-carboxamide	$C_{18}H_{11}Cl_2IN_2O_3$
16	1,803,760	4,220,277	477.9185	1-(2,4-dibromophenyl)sulfonyl-4-(4-fluorophenyl)piperazine	$C_{16}H_{15}Br_2FN_2O_2S$

metabolites were unique to either MZmine or GNPS annotation. It is important to note that METLIN annotation identified only nitrogen-containing metabolites (alkaloids), while MZmine and GNPS primarily identified polyphenols. The identified metabolites such as bergenin, garlic acid, tiliroside, 6-gingerol, pyrenophorol, canrenone, enterolactone, catechins, quercetin and procyanidins all possess remarkable therapeutic benefits (Benali et al., 2022; Bhuia et al., 2023; Caligiuri et al., 2003; Dasiman et al., 2022; Ghisalberti, 2002; Guest and Grant, 2016; Hu et al., 2023; Ke et al., 2019; Mali et al., 2019; Xiang et al., 2020; Xing et al., 2015; Zahoor et al., 2020). The current study is part of a broader investigation into the phytochemistry of *Entandrophragma* species. From 1960 to 2018, research in this field has led to the identification of numerous phytochemicals with novel structural features, encompassing various classes of bioactive metabolites (Mouthé et al., 2018). These phytochemicals have been isolated from different parts of the plant, such as roots, bark, and leaves. Among the identified phytochemicals, limonoids and their biosynthetic precursors, protolimonoids, are the most significant, with a total of 82 limonoids and 33 protolimonoids reported. Additionally, previous research has documented 14 steroids, 11 squalene-type triterpenoids, seven sesquiterpenoids, six fatty acids, five phenolic compounds, three cyclic triterpenoids, three flavonoids, and two minor classes of compounds from *Entandrophragma* species (Mouthé et al., 2018).

5.7. Total polyphenolic content

TPC in the bark extract of *E. delevoyi* was quantified using the FC assay with gallic acid as the standard (Regression equation, $y = 0.00111x + 0.11836$, $R^2 = 0.997$), and was expressed in mg gallic acid equivalent per gram (Table 5). The extract had 169.46 ± 0.71 mg GAE/g total phenolics, as shown in Table 5. TFC was shown as quercetin equivalent (QE) in mg/g of the bark extract of *E. delevoyi*. The extract had 53.14 ± 1.78 mg QE/g total flavonoids, as shown in Table 5. Further, from Table 5, it can be deduced that the total phenolic quantity (169.46 ± 0.71 mg GAE/g) was about thrice more than the quantity of flavonoids (53.14 ± 1.78 mg QE/g). The higher phenolic concentration than flavonoids may be due to flavonoids being a subset of polyphenolic chemicals.

5.8. Antioxidant activity

The radical scavenging activity of the *E. delevoyi* bark extract was measured using the DPPH assay (Table 6).

E. delevoyi bark extract demonstrated free radical scavenging activities ranging from 20.41 % to 91.76 % compared to the standard ascorbic acid (17.30–78.92 %) at doses of 20 to 120 μg/ml. The sample extract has a lower IC_{50} value (61.27 μg/mL) than the standard ascorbic acid (74.47 μg/mL). The DPPH assay findings showed that *E. delevoyi* bark is a potential source of natural antioxidants.

5.9. Quality assurance results

All R^2 values were above 0.999. A test sample was spiked with a multi-element standard solution containing all metals to be analyzed at different concentrations (5, 0.5, 0.1, mg/L) and the percentage of recovered metals was calculated. The selection of metals was based on their significance in preserving human well-being or by taking into account their toxicity and related risks to health. To gain a thorough understanding of the metal content and accumulation in a medicinal plant, the concentrations of multiple metals were analyzed. The bark of *E. delevoyi* sample was analyzed in three replicates. Instrumental LOD, LOQ, and recovery were determined to be of multiple metals in the bark of the plant (Table 7).

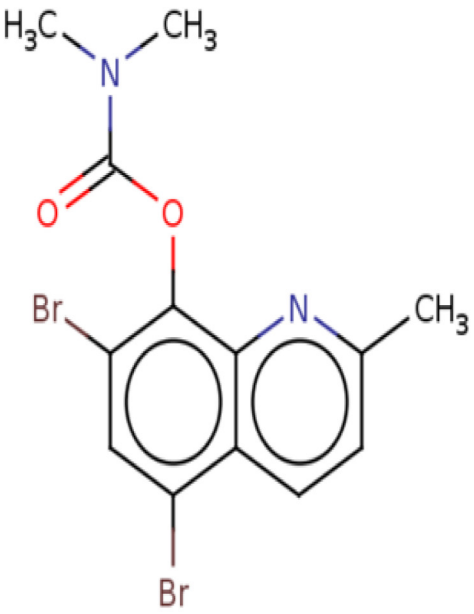
6. The health risk assessment of heavy metal contamination in the stem bark of *E. delevoyi*

The findings of this study indicated that heavy metals, arsenic, silver, aluminum, chromium, cadmium, copper, manganese, lead, cobalt, iron, nickel, silicon, and selenium were found in different amounts within the bark of *E. delevoyi*. Table 8 shows the mean, maximum, and minimum metal concentrations, and EDIs of heavy metals in the raw bark of *E. delevoyi*.

The internationally established WHO /FAO permitted maximum metal concentrations in medicinal plants were obtained from the literature, (Dghaim et al., 2015; Kohzadi et al., 2019; Mohammadi et al., 2019; Nkansah et al., 2016; Sarma et al., 2011). In this study, the maximum concentration Cd and Cr were above established limit (Table 8). In addition, As, Pb, Se, and U were not detected. Further, the concentration of Cu, Fe, Mn, Ni, and Zn were below the maximum tolerable limits. Currently, there are no established tolerable maximum concentrations established by WHO / FAO in medicinal plants for Ag, Al, Co, Si, and Sn.

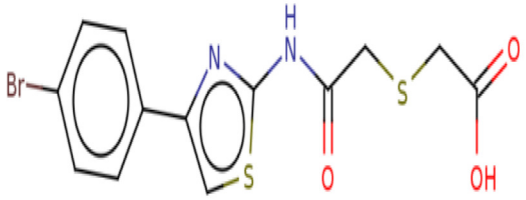
One of the most important health risk assessment techniques is an estimated daily intake (EDI) of the heavy metal pollutant. It takes into

1)



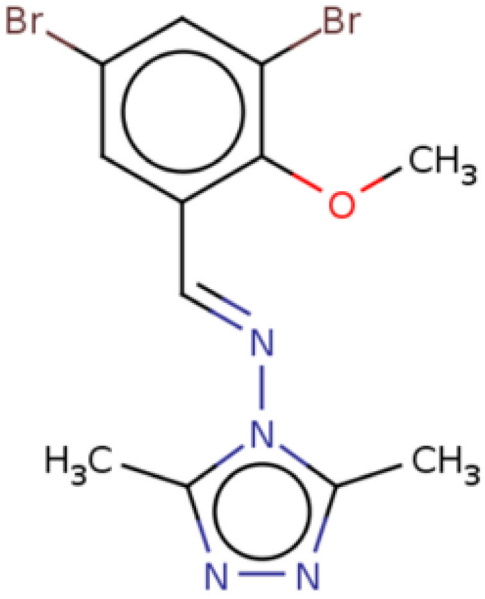
5,7-dibromo-2-methylquinolin-8-yl N,N-dimethylcarbamate

2)



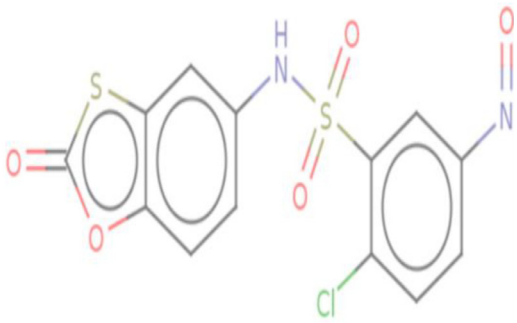
2-[2-[[4-(4-bromophenyl)-1,3-thiazol-2-yl]amino]-2-oxoethyl]sulfanylacetic acid

3)



(E)-1-(3,5-dibromo-2-methoxyphenyl)-N-(3,5-dimethyl-1,2,4-triazol-4-yl)methanimine

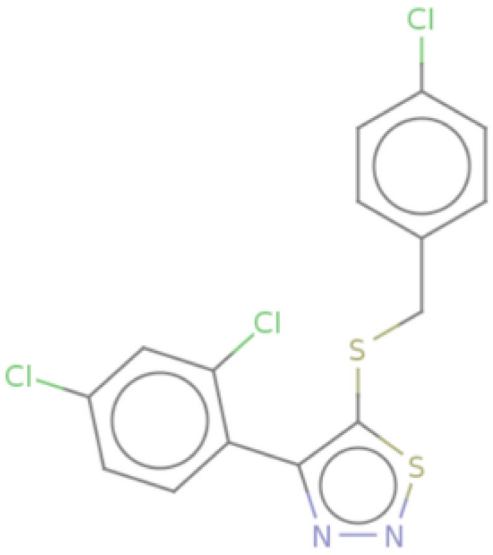
4)



2-chloro-5-nitro-N-(oxo-1,3-benzoxathiol-5-yl)benzenesulfonamide

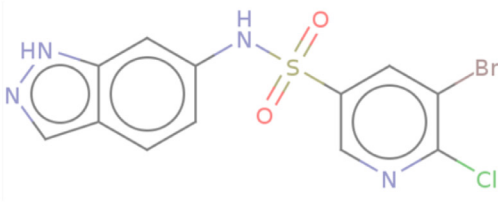
Fig. 3. Structures of phytochemicals tentatively identified in the bark extract of *E. delevoyi* by LC-MS/MS- METLIN Gen 2.

5)



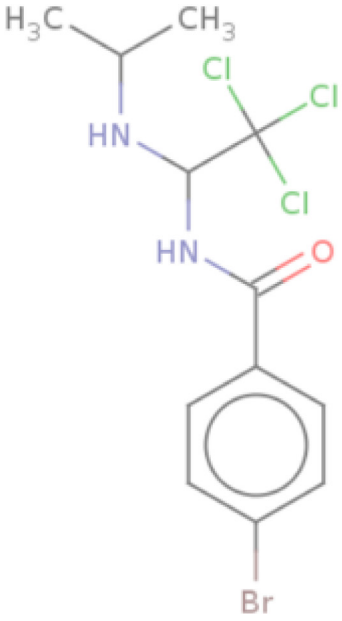
5-[(4-chlorophenyl)methylsulfanyl]-4-(2,4-dichlorophenyl)thiazole

6)



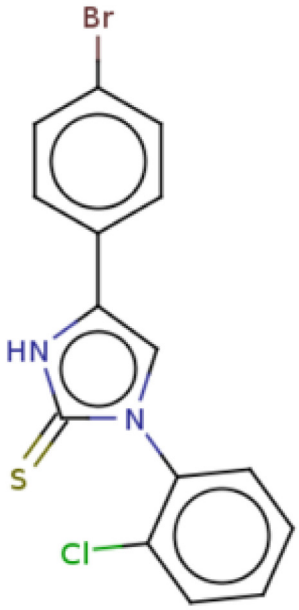
5-bromo-6-chloro-N-(1H-indazol-6-yl)pyridine-3-sulfonamide

7)



4-bromo-N-[2,2,2-trichloro-1-(propan-2-ylamino)ethyl]benzamide

8)



N-[2-(3,4-dimethoxyphenyl)ethyl]-2-({2-methyl-[1,2,4]triazolo[1,5-c]quinazolin-5-yl}sulfanyl)acetamide

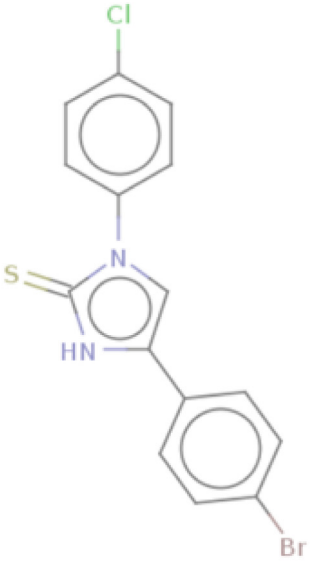
Fig. 3. Continued.

account the exposure frequency and duration, as well as the exposed individuals' body weight. The EDI for Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Sn, and Zn all fell within the upper tolerable daily intake reference ranges. This suggests that everyday consumption of *E. delevoyi* poses no short- to medium-term heavy metal health risk to the consumers.

The consumption of medicinal plant remedies does not have a significant long-term health risk related to the presence of certain metals, as long as the THQ value is below 1. However, if the THQ value is 1 or higher, it can potentially be harmful to health. The assessment of THQ for non-carcinogenic impacts evaluates the prolonged exposure to heavy metal impurities found in medicinal plant. The THQ values for Ag, Al, Cd, Cr, Cu, Fe, Mn, Ni, Sn, and Zn were all lower than 1 (Table 8). This suggests that consuming *E. delevoyi* bark poses no health risk due to these metals. However, if the HI is equal to or more than one, there is a potential health risk to consumers, and appropriate actions and protective measures must be implemented to protect consumers (Miyanza et al., 2023). The HI value (2734) was higher than one (Table 8). This implies that the cumulative effect of the heavy metal pollutants contained in *E. delevoyi* bark exceeded the permissible limit.

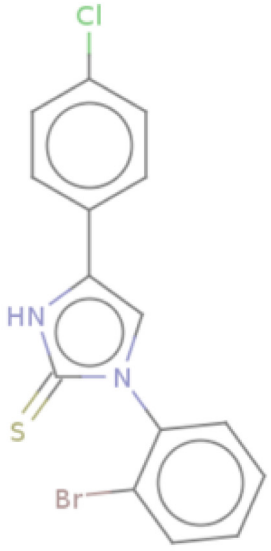
Acceptable risk levels for carcinogens range from 0.0001 (the probability of acquiring cancer in a human lifetime is one in 10,000) to 0.000001 (one in one million) (Miyanza et al., 2023). Values of CR less than 0.000001 are considered trivial, values greater than 0.0001

9)



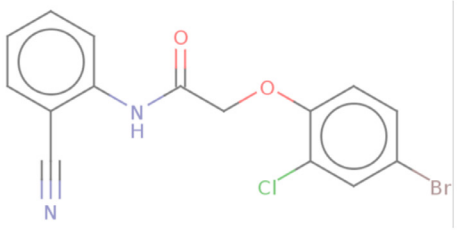
2-({2-methyl-[1,2,4]triazolo[1,5-c]quinazolin-5-yl}sulfanyl)-N-[2-(4-sulfamoylphenyl)ethyl]acetamide

10)



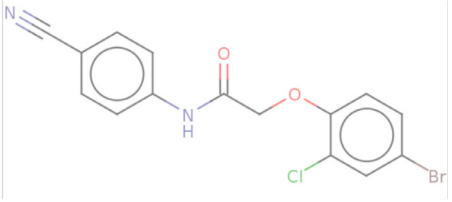
3-benzyl-2-[(cyanomethyl)sulfanyl]-N-(3,4-dimethoxyphenyl)-4-oxoquinazoline-7-carboxamide

11)



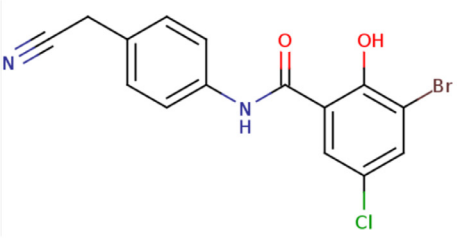
2-(4-bromo-2-chlorophenoxy)-N-(2-cyanophenyl)acetamide

12)



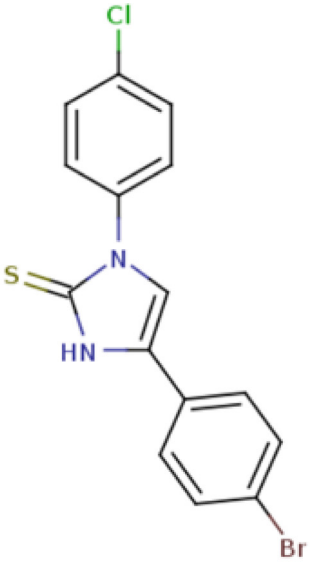
2-(4-bromo-2-chlorophenoxy)-N-(4-cyanophenyl)acetamide

13)



3-bromo-5-chloro-N-[4-(cyanomethyl)phenyl]-2-hydroxybenzamide

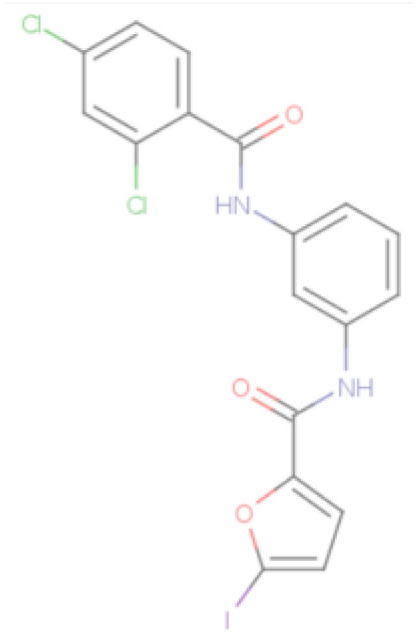
14)



5-(4-bromophenyl)-3(4-chlorophenyl)-1H-imidazole-2-thione

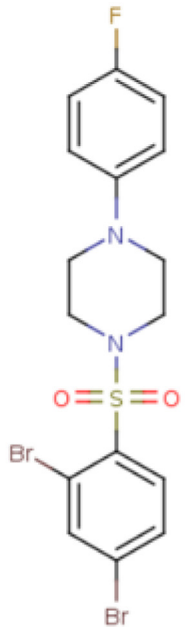
Fig. 3. Continued.

15)



N-[3-[(2,4-dichlorobenzoyl)amino]phenyl]-5-iodofuran-2-carboxamide

16)



1-(2,4-dibromophenyl)sulfonyl-4-(4-fluorophenyl)piperazine

Fig. 3. Continued.

Table 5
TPC and TFC of *E. delevoyi* bark.

	bark extract
TPC (mg GAE/g)	169.46 ± 0.71
TFC (mg QE/g)	53.14 ± 1.78

are unsatisfactory, and values between 0.000001 and 0.0001 are deemed acceptable (Miyanza et al., 2023). The cancer risk estimates for Al, Cd, Cr, and Ni found in *E. delevoyi* bark were all greater than 0.0001 and hence above acceptable limits (Table 8). The cumulative cancer risk (1.393) associated with the various carcinogenic metals present in the bark of *E. delevoyi* significantly exceeded the permissible limit. Chromium (Cr) was the predominant contributor, accounting for 92.24 % of the total cancer risk, with a specific risk value of 1.271. This observation suggests that consuming the bark of *E. delevoyi* poses a long-term cancer risk to consumers.

Table 7
Instrumental LOD, LOQ, linearity, and percent recovery of analyzed metals.

Metal	LOD (mg/L)	LOQ (mg/L)	R^2_{linear}	Spike recovery (%)
Ag	0.10	0.31	1.000	85.71
Al	0.17	0.53	0.999	101.94
As	0.30	0.92	0.999	108.61
Cd	0.06	0.18	0.999	98.87
Co	0.15	0.47	1.000	100.98
Cr	0.07	0.22	0.999	89.16
Cu	0.43	1.30	0.999	97.12
Fe	0.13	0.41	0.999	102.87
Mn	0.06	0.18	0.999	56.87
Ni	1.23	3.74	0.996	22.43
Pb	0.36	1.10	0.999	105. 98
Se	0.14	0.42	0.999	108.76
Si	0.20	0.61	0.999	8.61
Sn	0.16	0.49	0.999	16.71
Zn	0.11	0.35	0.999	99.93

Table 6
Antioxidant activity of *E. delevoyi* bark methanol extract assessed using DPPH assay.

[SAA](μg/mL)	[SE](μg/mL)	Abcont	AbSAA	AbSE	%RSA of SAA	%RSA SE	IC_{50} of SAA μg/mL	IC_{50} of SE μg/mL
20	20	0.74	0.612	0.549	17.30	20.41	74.47	61.27
40	40	0.74	0.539	0.419	27.16	37.97		
60	60	0.74	0.428	0.347	42.16	47.70		
80	80	0.74	0.356	0.238	51.89	62.43		
100	100	0.74	0.252	0.137	65.94	76.08		
120	120	0.74	0.156	0.021	78.92	91.76		

Note: Ab SAA = absorbance of standard ascorbic acid, [] = concentration, SE = sample extract, SAA = standard ascorbic acid, RSA = radical scavenging activity, Ab cont= control absorbance, Ab SE = absorbance of sample extract.

Table 8
Heavy metal concentration (Mean ± SD, n = 3), maximum, minimum (mg/kg), Estimated Daily Intake (EDI, mg/day), Hazard Quotient (HQ), and Cancer Risk (CR) of the raw bark of *E. delevoyi*.

Metal	Concentration				EDI		THQ	CR
	mean	max	min	WFL	EDI	WFL		
Ag	1.2 ± 0.2	1.2	0.8		0.001	0.025	0.200	
Al	561.0 ± 16.0	579.6	551.8		0.481	1.0	0.481	0.096
As	Nd			5.0				
Cd	0.4 ± 0.0	0.4	0.4	0.3	0.00034	0.0025	0.340	0.002
Co	4.8 ± 0.6	5.4	4.4		0.015	0.05		
Cr	36.6 ± 1.8	38.2	34.6	25.0	0.031	0.025–0.035	0.021	1.271
Cu	38.4 ± 1.2	39.8	37.4	150.0	0.033	0.5	0.825	
Fe	251.0 ± 7.2	259.2	246.4	425.5	0.215	8.0	0.307	
Mn	20.2 ± 0.2	21.0	19.8	200.0	0.017	1.8–2.3	0.121	
Ni	32.6 ± 0.4	33.0	32.4	67.9	0.028	1.0	0.031	0.024
Pb	Nd			10.0				
Se	Nd							
Si	277.0 ± 7.8	286.0	272.2		0.237			
Sn	89.6 ± 1.0	90.4	88.6		0.077	14.0	0.128	
Zn	97.8 ± 2.2	100.2	96.4	100.0	0.084	1.0	0.280	
Hazard index (HI)							2.734	
∑ CR (Al, Cd, Cr, Ni)								1.393

WFL = WHO / FAO limit, [ ] = concentration, Nd = not detected, Max = maximum, Min = minimum.

7. Conclusion

The current study has provided a sound scientific justification for the popular use of *E. delevoyi* in the traditional healthcare system in Zambia. Firstly, the phytochemical screening of the methanolic stem bark extract using standard qualitative tests of *E. delevoyi* reveals the presence of alkaloids, phenolic compounds, flavonoids, saponins, anthraquinones, steroids, terpenoids, cardiac glycosides, anthocyanins, and tannins in the plant. Literature reports have revealed many pharmacological health benefits of these bioactive natural products. Secondly, LCMS2 coupled to mzmine3.6 revealed the presence of thirty-five (35) metabolites, LCMS2-GNPS revealed twenty six (26) metabolites, and METLIN Gen 2 tentatively identified a further sixteen (16) secondary metabolites. The plant extract has high antioxidant activity, as evidenced by its low IC_{50} value of 61.27 μg/mL, which is lower than that of standard ascorbic acid of 74.47 μg/mL. Additionally, the extract contains significant amounts of phenolics (169.46 ± 0.71 mg GAE/g) and flavonoids (53.14 ± 1.78 mg QE/g). The plant's lower IC_{50} value than standard ascorbic acid, as well as its abundance of bioactive metabolites, make it a valuable medicinal resource and a prospective candidate for future pharmacological and drug development studies. Although the bark of *E. delevoyi* contains many health-promoting bioactive compounds, the heavy metals tested in this study posed a carcinogenic risk. The non-carcinogenic health risk evaluation suggests that *E. delevoyi* bark may be safe. However, its safety can only be determined if the plant's health concerns have been thoroughly defined in rigorous chronic toxicity tests. The high HI reported, on the other hand, indicates that this therapeutic plant poses greater health hazards to consumers. We thus urge that the use of *E. delevoyi* for medical reasons be done with caution. We further urge that all relevant national and international agencies recognize their responsibilities to promote public safety and global health by periodically evaluating and enforcing current laws governing the use of medicinal plants in traditional health care systems.

Declaration of competing interest

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

CRediT authorship contribution statement

Bitwell Chibuye: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Indra Sen Singh:** Writing – review & editing, Visualization, Supervision, Software, Resources, Conceptualization. **Luke Chimuka:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Kenneth Kakoma Maseka:** Supervision, Resources.

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