Scientific African 24 (2024) e02151

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Phytochemical profiling and bioactivity study of *Adenia panduriformis* in Zambia using UHPLC-MS/MS-MZmine3, GNPS, and METLIN Gen2

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ARTICLE INFO

Editor: DR B Gyampoh

Keywords:
Adenia panduriformis (Passifloraceae)
Antioxidant activity
Phytochemical screening
LCMS/MS
Molecular networking
Total phenolic content
Total flavonoid content

ABSTRACT

Diseases, especially degenerative illnesses, can be treated and avoided altogether, and good health can be enhanced by including health-promoting foods in the diet without side effects. *Adenia panduriformis* (Passifloraceae) is a wild vegetable consumed as part of the everyday diet in some communities in Zambia. The plant is also used in traditional medicine to effectively treat and manage many diseases, including degenerative diseases. Because of significant health benefits and medicinal attributes, this vegetable has very high merit to be investigated for its phytochemicals and bioactivities. Therefore, this study screened phytochemicals using qualitative chemical tests and Liquid chromatography-tandem mass spectrometry (LCMS/MS), identified metabolites using molecular networking tools and evaluated the total polyphenolic content and antioxidant activity of the methanolic leaf extract of the vegetable. Solvent extraction by maceration recovered metabolites for screening by qualitative chemical tests, extraction by QuEChERS (quick, easy, cheap, effective, rugged, and safe) was used to recover metabolites for screening by LCMS/MS, and tentative phytochemical constituents were identified using MZmine3, GNPS (global natural products social molecular networking) and METLIN. Total phenolic content (TPC) determination used the Folin-Ciocalteu (FC) method at 765 nm. Total flavonoid content (TFC) was evaluated using the aluminium chloride colorimetric assay at 510 nm. Antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay at 517 nm. Phytochemical screening by qualitative chemical tests revealed the presence of many classes of phytocompounds, such as alkaloids, polyphenols, terpenoids, and saponins. TPC was determined to be 122.30 ± 2.00 mg GAE/g, and TFC was 60.17 ± 0.25 mg QE/g. Screening by LCMS/MS revealed the presence of many metabolites, of which 23 phytocompounds were identified using MZmine3, 42 by GNPS, and 21 by METLIN. The crude extract exhibited high DPPH free radical quenching activity with a relatively low IC_{50} value of 53.48 μg/mL compared to the reference standard ascorbic acid IC_{50} of 74.47 μg/mL. The significantly lower IC_{50} value for the wild vegetable than that of ascorbic acid indicates a considerably high efficiency of the metabolites in the vegetable as antioxidants. The presence of many metabolites with health-promoting properties and high antioxidant activity of the leaf extract of *A. panduriformis* may be the science behind its remarkable health benefits and power to treat and manage a variety of diseases, including degenerative illnesses. This study presents results that indicate the potential

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https://doi.org/10.1016/j.sciaf.2024.e02151
Received 2 April 2023; Received in revised form 22 February 2024; Accepted 3 March 2024
Available online 4 March 2024

of *A. panduriformis* as a rich source of metabolites with brilliant health-promoting properties and high antioxidant activity, which may open exciting opportunities for further development in the fields of pharmacology and drug development.

Introduction

The expression 'Let food be your medicine and medicine be your food' was highly embraced by the father of medicine, Hippocrates, 2500 years ago [1]. Indeed, food might provide the human body with therapeutic benefits, and a good diet is essential for preventing diseases [2]. It may suffice to postulate that a better diet might be better than drugs and surgeries when fighting illness and diseases. Food may be defined as anything consumed and converted into a substance in the body. Consequently, a poor diet will harm the health of someone because once consumed, food is converted to numerous parts of the body, such as muscles and nerves [3]. This explains why food may be considered medicine to the human body. Therefore, the kind of food consumed determines their good health or lack thereof. Before resorting to surgeries and drugs, one must monitor one's diet. Consuming certain foods may prevent any diseases that may require surgery and drugs for treatment [4]. Some diseases, such as degenerative diseases, are caused mainly by free radicals. It may suffice to say that free radicals containing oxygen are termed reactive oxygen species (ROS) and are the most biologically significant free radicals [5]. Naturally, humankind has been relentlessly trying to discover healthier foods with antioxidants that ameliorate the adverse effects of free radicals and other bioactive phytocompounds with health-promoting properties [6]. As for antioxidants or free radical scavengers, they play several physiological benefits in human biological systems. Antioxidants quench free radicals, thereby preventing cell damage that might arise from chemical reactions involving oxidants. Furthermore, antioxidants stabilize or deactivate free radicals before the latter cause damage to cells and other biological targets. Thus, antioxidants are essential for maintaining optimum healthy cells and their well-being. When human cells utilize oxygen, they spontaneously form free radicals as by-products, which in turn can damage cells. Antioxidants act as "free radical scavengers," thereby preventing and repairing the damage done or may be done by free radicals. Above all, and interestingly, antioxidants may enhance the immunity of the body and reduce the risk of many infections and diseases [7–9]. Consequently, it is crucial to consume foods containing antioxidants and other therapeutic metabolites for the human body to ward off infection or treat disease naturally. Such foods may also include functional foods.

Functional foods possess some bacterial strains; they may originate from plants or animals and possess physiologically active molecules beneficial to human health and lowering the risk of chronic infections and disease [10]. Functional foods are neither pills nor capsules, but are consumed as part of the everyday diet, offering health benefits and reducing the risk of chronic disease beyond the universally recognized nutritional effects. In the Copperbelt province and elsewhere in Zambia, a medicinal wild vegetable, *Adenia panduriformis*, belonging to the family Passifloraceae, offers a myriad of health benefits and is consumed as part of the everyday diet in some communities [11,12]. Additionally, traditional healers prescribe the herb to patients with weakened immunity, such as those with HIV/AIDS. It is believed that wild vegetable is an outstanding immune booster. In addition to such health benefits, it is claimed that the leafy vegetable can heal several diseases such as diabetes, hypertension, malaria, and diarrhea. Worldwide, the woody climber plant is only found in Tanzania, Mozambique, Zambia, and Zimbabwe [11–15]. However, the plant should also grow under similar climatic conditions elsewhere globally.

Due to the amazing health benefits of this plant, it is prudent to explore the hidden secrets of this wild vegetable. The determination of the phytochemicals present in it and its antioxidation properties would be an exciting study as it will scientifically corroborate the persistent claims about the wild vegetable as a panacea for many diseases. Therefore, the current study determined the phytochemical profile of *A. panduriformis leaf extract*, its total phenolic and flavonoid content, and its antioxidant potential. Fig. 1 shows a picture of the climber plant captured in a forest in Zambia's Kalulushi District of the Copperbelt province.

Fig. 1. Leaves of *Adenia panduriformis*.

Materials

Materials

The following materials were obtained from the chemical stores of the School of Chemistry, University of Witwatersrand, South Africa: 2.0 mL vials, and various glassware such as test tubes and flasks, 50 mL polytetrafluoroethylene (PTFE) centrifuge tubes, 0.45 μm PTFE filters, and 10 mL syringes and needles.

Authentication of the plant

Fresh plant leaves and stems were collected from the bush in Kalulushi town, Copperbelt Province of the Republic of Zambia. Plant parts were taken to the Herbarium of the Ministry of the Green Economy and Environment of the Republic of Zambia for authentication. The plant was identified and authenticated as *A. panduriformis*.

Reagents

The following HPLC grade reagents were obtained from Merck (Johannesburg, South Africa): gallic acid (G7384), quercetin (Q4951), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (D9132), and acetonitrile (HPLC grade, ≥ 99.9%) Folin–Ciocalteau (FC) reagent (1,090,010,100), anhydrous sodium carbonate (≥99.5%), sodium nitrite (≥97.0%), sodium hydroxide (≥ 98.0%), and anhydrous aluminium chloride (≥ 98.0%), and primary secondary amine (PSA) (Supelclean™ PSA SPE). In addition, the following reagents were obtained from chemical stores of the School of Chemistry, University of Witwatersrand, South Africa: chloroform (HPLC grade, ≥ 99.9%), acetic anhydride (≥ 99.5%), ferric chloride (≥ 99.99%), magnesium ribbon (≥ 99.0%,), lead acetate (≥99%), hydrochloric acid (≥37%), methanol (HPLC grade, ≥ 99.9%), glacial acetic acid (≥99%), sulphuric acid (≥ 98%), Hager's reagent (1.3%, in water, saturated), and ammonia (Reagent, ≥32%).

Methods

Extraction of phytocompounds for phytochemical screening

Preliminary phytochemical screening of *A. panduriformis* leaf extract was carried out in the Environmental Analytical Chemistry Laboratory, School of Chemistry, University of Witwatersrand, South Africa. The fresh leaves collected were dried in the shade and then reduced to powder using a mortar and pestle. Phytocompounds were extracted in 80% methanol following a method by Vieira (2022) with some modifications [16]. In brief, 5.0 g of the powdered sample was placed in 100 mL flasks, and 50 mL methanol (80%) was added. This solid-solvent ratio of 1:10 (m/v) was chosen based on its effective extraction yield for the maceration extraction of metabolites from other food matrices [17]. The flasks were sealed and subsequently placed in a water shaker with continuous agitation fixed on 150 rpm at 40°C for the extraction time of 24 hours for maximum phytochemical recovery. The recovered extracts were filtered using 0.45 μm PTFE filters. Finally, the crude extract was subjected to phytochemical screening through qualitative reactions as part of phytochemical studies to identify the various families of secondary bioactive metabolites.

QuEChERS Extraction Process for LC-MS QTOF Analysis

An adapted QuEChERS extraction procedure by Guo (2018) was used to recover metabolites from powdered leaf material [18]. The modification of the method was in terms of the amount of the sample used. In this study, a 3.0 g sample was used instead of the 5.0 g used in the previous study. This was to attain the solid-to-solvent ratio of 1:10 that assures maximum extraction [19]. Essentially, 3.0 g of plant sample was weighed and placed in 50 mL PTFE centrifuge tubes. To hydrate the plant matrix, 3 mL of double distilled water was added, and the contents were mixed well by vortex for 30 s and left to stand for 30 min. After that, 10 mL of acetonitrile was added, and the contents vortexed for 60 s. Subsequently, 4.0 g of $MgSO_4$, and 1.0 g of NaCl were added and the contents were shaken well by vortex for 60 s and immediately centrifuged at 4000 rpm for 10 min. During the clean-up stage, 5.0 mL of organic supernatant was recovered and transferred to a clean polypropylene tube, and then 150 mg PSA sorbent and 75 mg $MgSO_4$ were added. The contents were then shaken well by vortex for 60 s and promptly centrifuged for 10 min. Eventually, an aliquot of 2.0 mL supernatant was collected and used for LCMS/MS analysis. The adsorbent, PSA, was used to remove unwanted substances such as pigments, proteins, carbohydrates, organic acids, and polar interferences. Further, graphitized carbon black was used to remove chlorophylls. These unwanted substances are removed because they interfere with the LCMS analysis.

Extraction of polyphenols for total polyphenol content, and antioxidant activity determination

Polyphenols were extracted following a previous method by Ralepele, Chimuka, Nupia, and Risenga (2021) with modifications [20]. In this study, the sample–solvent ratio used was 1: 10 instead of using the 1:6 sample-solvent, due to the high phytochemical recovery for a 1:10 sample-solvent ratio [21]. Briefly, 5.0 g of powdered leaf sample were placed in a 50 mL PTFE centrifuge tube containing 45 mL of 80% methanol for 36 hours with intermittent agitation for 150 rpm in a temperature-controlled water shaker at 40°C. After spontaneous cooling, the resultant extract was centrifuged at 4000 rpm for 10 min to separate solids from the solution. The procedure was repeated and the recovered supernatants combined. A 0.45 μm PTFE filter was used to remove the solid particles. The filtered extracts were used for total polyphenolic (phenolic and flavonoids) content determination and evaluation of the antioxidant

activity.

Phytochemical screening using chemical tests

Preliminary phytochemical screening was carried out using extracts described in the earlier procedure (3.1) to identify broad categories of bioactive metabolites present in the sample using standard qualitative tests reported in previous studies, that is, Alkaloids (Hager's Test), Tannins (Braymer's Test), Saponins (Foam Test), Steroids (Salkowski Test), phenolics (Libermann's test), flavonoids (Shinoda Test), Carbohydrates (Molisch's Test), Glycosides (Liebermann's Test), and Anthraquinones (Borntrager's Test) [21–25].

LC-MS/QTOF analysis

UHPLC-MS2 screening was achieved by following a previous method by Ralepele, Chimuka, Nupia, and Risenga (2021) with modifications [20]. Essentially, 10 μL of the crude extract recovered through QuEChERS extraction was injected into and chromatographically separated on a Phenomenex C18 column. The UHPLC system was connected to a quadrupole time-of-flight (Brukinjection ver Daltonik GmbH, Bremen, Germany). Chromatographic separation was attained using a binary LC solvent system. Mobile phase A consisted of HPLC-grade formic acid and mobile phase B contained 100% acetonitrile, which was pumped at a flow rate, of 0.50 mL/min. The gradient was scheduled in the following order, that is, B increment: fixed at 5% from 0 to 10 min, raised from 5 to 100% from 10 to 25 min, and kept at 100% until 35 min. MS2 analysis was achieved through Electrospray Ionization in the negative ion mode with the following optimized operating conditions: collision energy, 20–50 eV; capillary voltage, + 4000 V; dry gas temperature, 210°C; nebulizer pressure, 2.0 bar; flow rate, 8.0 L/min; spectra rate, 1 s^{-1}; and scan mass range, 50 – 1300 m/z.

Measurement of total phenolic content (TPC)

TPC was measured using a UV–Vis spectrophotometer (Varian, Cary 50 Conc, Darmstadt, Germany) following a previously reported method by Aryal et al (2019) with some modifications [26]. Briefly, 0.2 mL of extract was added to 0.75 mL of Folin-Ciocalteau reagent (1:10 dilution with distilled deionized water (DDW)). After 3 min, 2 mL 7.5% (w/v) Na_2CO_3 solution was added, and then DDW was also added to dilute the mixture to a final volume of 10.0 mL. The contents were mixed well and left to stand in the dark at ambient conditions for 2 h. Absorbance was measured after 30 min at 765 nm using a UV–Vis spectrophotometer, and 80% MeOH was used as the blank. Measurements were carried out with gallic acid standard concentrations of 25, 50, 150, 250, 350, and 450 μg/mL prepared in 80% MeOH. Absorbance was transformed to phenolic content in mg of gallic acid equivalent (GAE) per g.

Determination of total flavonoid content (TFC)

Total flavonoids were determined following an adapted previous method by Pakade, Cukrowska, and Chimuka (2013) [27]. In essence, 0.3 mL extract was placed in a 10-mL volumetric flask containing 4.0 mL DDW. Afterward, 0.3 mL $NaNO_2$ (5 %) was added, and the constituents were shaken well by Voltex. After 5 minutes, 3.0 mL of $AlCl_3$ (1.0%) solution was added. Further, 2.0 mL of NaOH (1.0 M) was added after allowing the contents to stand for 6 min. Finally, 0.4 mL of DDW was added, bringing the total volume to 10.0 mL. The contents were mixed well by vortex, and absorbance was measured at 510 nm (Varian, Cary 50 Conc, Darmstadt, Germany). Quercetin was used to prepare the standard curve and TFC expressed as quercetin equivalent (QE) in mg per g.

Determination of DPPH antioxidant activity

A previously reported radical-scavenging assay by Aryal et al (2019) was used with modifications to determine the capacity of leaf extracts to scavenge DPPH [26]. In brief, 50.0 mg DPPH was dissolved in 100 mL of MeOH (80%) to make a stock solution, from which a working solution was made through dilution (1:5) with MeOH (80%). There were six quantities of the extract, that is, 20, 40, 60, 80, 100, and 120 μg/mL. The DPPH radical scavenging assay consisted of 0.2 – 1.2 mL extract and 1.4 mL working DPPH solution. The contents were added with MeOH (80%) to make up 2.0 mL. The mixture underwent incubation for 45 minutes in the dark before measuring the absorbance at 517 nm. Similarly, the reference, standard ascorbic acid, was prepared as follows. 2.0 mg ascorbic acid was dissolved in 2.5 mL of DDW, thereby making the concentration of stock reference solution 0.8 mg/mL. Subsequently, serial dilutions of 20, 40, 60, 80, 100, and 120 μg/mL were made. Subsequently, 1.4 mL of DPPH was added, and the contents were added with methanol to make up to 2.0 mL. The mixture underwent incubation for 45 minutes in the dark before measuring the absorbance at 517 nm. Inhibition (%) was determined using the following equation.

$$\%\ Scavenging \Big/ Inihibition = \left(\frac{ABS\ sample\ - ABS\ blank}{ABS\ control}\right) \times 100$$

Where ABS sample = sample absorbance or reference standard absorbance, ABS blank = absorbance of MeOH, and ABS control = absorbance of DPPH working solution.

Statistical Analysis

All measurements were done in triplicate and recorded as mean ± SD. Numerical data were analyzed using Microsoft Excel 2016 and calibration curves were constructed using Origin 2018.

Results and data analysis

Phytochemical screening using qualitative chemical tests

Phytochemical screening using standardized qualitative tests of the crude methanolic leaf extract revealed the presence of many groups of metabolites, as depicted in Table 1.

LCMS/MS screening

Phytochemical screening of *A. panduriformis* leaf using UHPLC−ESI-MS/MS revealed the presence of exciting phytocompounds, that may cause important pharmacological effects on humans when the wild vegetable is consumed in the diet. The phytochemical LCMS/MS screening was carried out in the negative ion mode, where many metabolites were found with higher intensity.

Molecular identification

The study used tandem mass spectrometry-based tools to identify phytochemicals. Four tools used in this study included MZmine 3, GNPS library match, GNPS feature networking advance analysis, and METLIN GEen2.

MZmine3 molecular identification. Twenty-six (26) phytochemicals were identified using MZmine 3.2.8 software, forty-two (42) were identified using library matching, whereas fourteen (14) were identified by advanced feature-based analysis of GNPS [28,29]. The Bruker data were initially converted to an "mzML" file compatible with MZmine and GNPS. The file conversion was attained using the MSConvert package (Version 3.0.19330, Proteowizard Software Foundation, USA). The converted "mzML" file was initially imported to the MZmine3.2.8 platform and processed following standard MZmine 3 workflow enabling the exportation of processed data as MGF and quantification table files for further use by the GNPS platform.

The mzML data file was imported to MZmine 3.2.8. MS level (1) mass detection settings were performed by setting the noise level at 25.0 and m/z tolerance at 5.0 ppm. Mass detection level (2) was set at a noise level of 20.0 and m/z tolerance at 5.0 ppm. Chromatogram builder settings included a minimum group size in the number of scans to be 5, group intensity threshold of 35.0, a minimum highest intensity of 45.0, and scan-to-scan accuracy (m/z) at 30.0 ppm. Resolving was achieved using a retention time tolerance of 0.2 minutes, MS1 to MS2 precursor tolerance (m/z) at 10.0 ppm, the minimum merged intensity at 250, chromatographic threshold at 0.8, minimum absolute height at 4000.0, Minimum ratio of peak top/edge at1.2, and Min number of data points of 5.

13C isotope filtering parameters included m/z tolerance of 20 ppm, retention time tolerance of 0.1 minutes, and the most intense representative isotope to be considered as representative isotope. Aligning of feature list step set parameters as m/z tolerance at 20.0 ppm, retention time tolerance at 0.2 minutes, weight for RT at 2.0, and mobility weight at 2.0. Feature list filtering settings included minimum features in a row (1.0), minimum features in an isotope pattern (2), and m/z tolerance at 5.00 ppm. The parameters set in Gap filling step included intensity tolerance at 0.2, m/z tolerance at 5.0 ppm, retention time tolerance at 0.6 minutes, and minimum data points at 3.

For MZmine 3 molecular networking, spectral library match used MoNA (MoNA-export-Experimental Spectra.json) library (1,545,052 spectra) using only MS level 2 scans, precursor m/z tolerance at 20.0 ppm, CCS tolerance [%] at 0.05 and minimum ion density was 1.0. Molecular networking identified twenty-six (26) phytochemicals, as depicted in Table 2.

Table 1
Results of phytochemical screening of the leaf extract of *A. panduriformis*.

#	Metabolites	Methanolic extract
1	Phenolics	+
2	Saponins	+
3	Anthraquinones	+
4	Steroids	+
6	Terpenoids	+
7	Cardiac glycosides	+
8	Proteins	+
9	Anthocyanins	+
10	Carbohydrates	+
11	Tannins	+
12	Flavonoids	+
13	Alkaloids	+
15	Volatile oils	-
	- = absent, + = present	

Table 2
MZmine 3.2.8 based tentative identification of phytocompounds from crude extract of *A. panduriformis* leaves by UHPLC−ESI-MS/MS in the negative ion mode.

ID	RT	[M-H]−	Exact Mass	MS2	Tentative Compound	Formula	Cosine similarity
1	4.17	145.0484	146.0734	83, 99, 127 (100)	2-methylglutaric acid	$C_6H_{10}O_4$	1.000
2	4.70	327.0664	326.0722	207 (100), 234	Bergenin	$C_{14}H_{16}O_9$	0.840
3	4.72	327.0662	326.0722	192, 207 (100), 234	Bergenin isomer	$C_{14}H_{16}O_9$	0.840
4	5.59	173.0795	174.0671	111 (100), 129, 84, 83	Suberic acid	$C_8H_{14}O_4$	1.000
5	5.68	343.2122	344.4480	127, 171, 229, 325	FA 18:2+4O	$C_{18}H_{32}O_6$	0.845
6	5.93	$[M+H]^+$ 187.0965	186.0892	97, 112, 116, 125 (100), 143, 169	Gabapentin Related Compound E, 1-(carboxymethyl) cyclohexane carboxylic acid)	$C_9H_{14}O_4$	1.000
7	5.93	187.0946	188.0440	125 (100), 169	Azelaic acid	$C_9H_{16}O_4$	1.000
8	6.17	305.1190	306.0370	75, 105 (100), 129, 143, 205, 243	8,8-dimethyl-2-phenylpyrano[2,3-f]chromen-4-one	$C_{20}H_{16}O_3$	0.982
9	6.23	$[M+H]^+$ 199.0978	198.0905	130, 155 (100),	2-amino-3-methylimidazol [4,5-f] quinoline	$C_{11}H_{10}N_4$	0.998
10	6.36	$[M+H]^+$ 327.2122	326.2067	327 (100), 171, 211	Hydroquinidine	$C_{20}H_{26}N_2O_2$	0.994
11	6.47	$[M+H]^+$ 325.1956	324.1838	97, 127, 171, 211, 229, 251	(*R*)-[(2S,4S,5R)-5-ethenyl-1-azabicyclo[2.2.2] octan-2-yl]-(6-methoxyquinolin-4-yl) methanol (Quinine)	$C_{20}H_{24}N_2O_2$	1.000
12	6.47	325.1956	326.1834	171, 211, 229, 251	Quinidine	$C_{20}H_{24}N_2O_2$	1.000
13	6.59	$[M+H]^+$ 329.2275	328.2334	99, 211	(Z)-5,8,11-trihydroxyoctadec-9-enoic acid	$C_{18}H_{34}O_5$	0.952
14	6.72	137.0223	138.0253	75, 93 (100), 108	4-Hydroxybenzoic acid	$C_7H_6O_3$	
15	6.72	137.0223	138.0226	75, 93 (100), 108	Salicylic acid	$C_7H_6O_3$	1.000
16	6.76	$[M+H]^+$ 327.2117	326.2112	80, 129, 187 (100), 209	Ajmaline	$C_{20}H_{26}N_2O_2$	0.983
17	6.76	112.9846	113.9834	69.9, 70.7	Acetylenedicarboxylic acid	$C_4H_2O_4$	1.000
18	6.98	209.1145	210.1256	137, 165 (100)	Jasmonic acid	$C_{12}H_{18}O_3$	1.000
19	6.98	329.2272	328.2334	99, 199, 329 (100)	(Z)-5,8,11-trihydroxyoctadec-9-enoic acid	$C_{18}H_{34}O_5$	0.952
20	7.80	299.0506	300.0901	176, 192, 207 (100), 284	3′,5,7-Trihydroxy-4′-methoxy-flavone (Diosmetin)	$C_{16}H_{12}O_6$	0.905
21	7.86	269.1698	270.0530	148, 205, 221 (100), 236	Genistein	$C_{15}H_{10}O_5$	1.000
22	8.76	265.1422	266.1552	96, 165, 265 (100)	dodecyl hydrogen sulfate	$C_{12}H_{26}O_4S$	0.999
23	8.78	$[M+H]^+$ 293.2060	292.2110	179, 195, 223, 256,275, 293(100)	8-[3-oxo-2-[(E)-pent-2-enyl]cyclopenten-1-yl]octanoic acid	$C_{18}H_{28}O_3$	0.869
24	8.82	293.2122	294.2195	179,195, 223, 256, 275	HOTrE	$C_{18}H_{30}O_3$	0.907
25	9.10	265.1426	266.1552	96, 265 (100)	Dodecyl sulfate isomer	$C_{12}H_{26}O_4S$	1.000
26	9.84	$[M+H]^+$ 384.1728	383.1594	164, 177, 194, 237, 384 (100)	Prazosin	$C_{19}H_{21}N_5O_4$	1.000

Global natural product social molecular networking (GNPS) library matching

The UHPLC-MS/MS data for the *A. panduriformis* obtained from its QuEChERS leaf extract were subjected to GNPS library match analysis available online at: http://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=160cbd2a9f5e43edb471190f4622c1c3. The Bruker data converted "mzML" files were uploaded and submitted to the GNPS platform using Filezilla Client 3.62.0 [30,31]. Table 3 shows forty-two (42) GNPS-identified phytocompounds by UHPLC−ESI-MS/MS in the negative ion mode. Base peaks are shown in bold numbers.

GNPS advanced analysis

The UHPLC-MS/MS data of the *A. panduriformis* obtained from its QuEChERS leaf extract was subjected to GNPS feature networking advanced analysis tool. The GNPS analysis used mzML, MS2 MGF, and quantification table files. The Bruker data converted "mzML" files were uploaded and submitted to the GNPS platform using Filezilla Client 3.62.0 [30,31]. MGF and quantification table files derived from MZmine 3.3 were also uploaded. The results are available online at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=dc4f0f62cfd343029e412b55fd60cf88. Table 4 shows identified phytocompounds by UHPLC−ESI-MS/MS in the negative ion mode and subsequently using feature networking advanced analysis of GNPS.

It is interesting to note from Tables 2, 3, and 4 that there are some distinct compounds, such as Bergenin, Suberic acid, Azelaic acid, and (Z)-5,8,11-trihydroxyoctadec-9-enoic acid, were identified by both mzmine3 and GNPS. The GNPS analyses afforded 37 metabolites more than mzmine3. MZmine 3 identified a smaller number of molecules because of access to only one database-MoNA used in this study.

Analysis of the GNPS molecular network that was earlier obtained from the arrangement of the LC-MS/MS data of the crude leaf extract and fractions of *A. panduriformis* leaf extract revealed the presence of a total of 404 nodes equivalent to precursor ions visualized in Cytoscape 3.9.1, partially depicted in Fig. 2 [32,33]. Fig. 2 is available online at: https://gnps-cytoscape.ucsd.edu/process?task=dc4f0f62cfd343029e412b55fd60cf88%26override_path=gnps_molecular_network_iin_collapse_graphml%2F

Table 3
GNPS identification of phytocompounds from the crude extract of *A. panduriformis* leaves by UHPLC−ESI-MS/MS in the negative ion mode.

No.	RT (min)	Precursor	MS2 Fragments	Tentative Compound	Formula	Cosine	Library class	library
1	4.64	327.072	192.01, 193.01, 207.03, 234.02, 237.04, 249.04, 312.05, **327.07**	Bergenin	$C_{14}H_{16}O_9$	0.84	Bronze	Trent Northen
2	4.65	327.072	192.01, 193.01, 207.03, 234.02, 237.04, 249.04, 312.05, **327.07**	Bergenin	$C_{14}H_{16}O_9$	0.84	Bronze	Trent Northen
3	4.69	327.072	164.01, 166.03, 177.02, 192.00, 193.01, 194.02, 207.03, 234.01,249.04, 312.05, **327.07**	Bergenin	$C_{14}H_{16}O_9$	0.94	Bronze	Massbank
4	4.69	327.072	164.01, 166.02, 177.02, 190.03, 192.01, 193.01, 194.02, 207.03, 234.02, 249.04, 312.05, **327.02**	Bergenin	$C_{14}H_{16}O_9$	0.83	Bronze	Massbank
5	5.13	429.191	91.02, 135.12, 137.10, 161.10, 179.11, 205.09, **223.10**	Anziaic acid	$C_{24}H_{30}O_7$	0.71	Bronze	Jean-Luc WOLFENDER Pierre-Marie ALLARD
6	5.23	431.098	283.06, **311.06**, 312.06, 323.06, 341.06, 431.10	Isovitexin(4)	$C_{21}H_{20}O_{10}$	0.78	Bronze	Massbank
7	5.24	431.098	268.04, 283.06, **311.06**, 323.03, 341.07, 431.10	Isovitexine	$C_{21}H_{20}O_{10}$	0.81	Bronze	Stephane GREFF
8	5.57	173.0	57.30, 66.80, 81.40, 83.30, 109.20, **111.20**, 129.40, 131.00	Suberate|1,8-Octanedioic acid| Cork acid|Suberic acid| Octanedioic acid|1,6-Hexanedicarboxylic acid	$C_8H_{14}O_4$	0.86	Bronze	Massbank
9	5.90	187.098	57.03, 97.07, 123.08, **123.10**, 143.11, 169.09, 187.10	Azelaic acid (isomer)	$C_9H_{16}O_4$	0.88	Bronze	Trent Northen
10	5.91	187.097	97.07, 123.08, **125.10**, 126.10, 143.11, 187.10	Massbank:PR309132 Azelaic acid (Not validated)	$C_9H_{16}O_4$	0.91	Bronze	Massbank
11	5.92	187.098	97.06, **125.10**, 126.10, 187.10,	Azelaic acid	$C_9H_{16}O_4$	0.88	Bronze	MoNA
12	5.93	243.16	181.16, **225.15**, 143.16	Brassylic acid	$C_{13}H_{24}O_2$	0.80	Gold	PI
13	6.34	327.218	85.03, 97.07, 171.10, 183.14, 211.13, 221.12, 229.14, **327.22**	(10E,15E)-9,12,13-trihydroxyoctadeca-10,15-dienoic acid	$C_{18}H_{32}O_5$	0.89	Gold	Wolfender
14	6.36	657.437	**329.22**, 330.23	h_163_prostanozol_3′OH	$C_{20}H_{28}N_2O_2$	0.91	Gold	Mehdi Beniddir
15	6.54	659.14	217.08, 241.08, 285.07, **329.06**, 330.07	Nornotatic acid	$C_{17}H_{14}O_7$	0.72	Bronze	Joel BOUSTIE Pierre LE POGAM
16	6.56	329.231	171.10, 183.14, 211.13, 229.14, **329.23**	Massbank:PR309108 FA 18:1+3O	$C_{18}H_{34}O_5$	0.94	Bronze	Massbank
17	6.80	227.129	92.99, 142.99, 165.13, **183.14**, 184.14, 209.98, 227.13, 228.99	Spectral Match to Butanedioic acid, 2-(4,4-dimethyl-2-methylenepentyl)- from NIST14	$C_{12}H_{20}O_4$	0.90	Bronze	Data from Wiemann
18	6.81	227.129	111.08, 165.13, **83.14**, 209.12, 227.13	Traumatic Acid - 40.0 eV	$C_{12}H_{20}O_4$	0.88	Gold	PI
19	6.82	227.129	**165.13**, 183.14, 09.12, 227.13	Traumatic Acid - 30.0 eV	$C_{12}H_{20}O_4$	0.83	Gold	PI
20	6.97	329.233	99.08, 169.12, 199.13, 211.13, **329.23**	Massbank:PR309110 FA 18:1+3O	$C_{18}H_{34}O_5$	0.79	Bronze	Massbank
21	7.11	309.206	137.10, 141.08, 154.08, 171.10, 172.10, 185.11, 223.13, 247.20, 251.16, 291.20, **309.20**	Massbank:PR309084 FA 18:3+2O	$C_{18}H_{30}O_4$	0.70	Bronze	Massbank
22	7.14	329.22	127.02, 167.05, **71.08**, 195.11, 199.10, 01.09, 211.08, 227.10, 75.16, 293.17	(Z)-5,8,11-trihydroxyoctadec-9-enoic acid	$C_{18}H_{34}O_5$	0.71	Bronze	Mona
23	7.24	307.19	71.05, 97.06, 121.07, 125.09, 167.14, 1**85.12**, 209.11, 211.13, 235.13, 289.18, 307.19	Massbank:PR309076 FA 18:4+2O	$C_{18}H_{28}O_4$	0.83	Bronze	Massbank
24	7.33	881.273	325.05, 395.13, **441.14**, 881.27	ginkgolide C	$C_{20}H_{24}O_{11}$	0.82	Gold	Sang Hee SHIM Kyo Bin
25	7.83	294.146	71.01, 96.96, 220.15, **221.15**, 236.10, 293.18	1-(5,10-dioxo-2,3,5a,6,7,8-hexahydro-1H-dipyrrolo[1,2-	$C_{14}H_{21}N_3O_4$	0.78	Bronze	MoNA

(*continued on next page*)

Table 3 (*continued*)

No.	RT (min)	Precursor	MS2 Fragments	Tentative Compound	Formula	Cosine	Library class	library
				d:1′,2′-f]pyrazin-10a-yl)propan-2-yl carbamate				
26	7.83	293.212	148.00, 164.08, 177.08, 192.11, 205.08, 220.10, **221.12**	13-S-hydroxy-6Z,9Z,11E-octadecatrienoic acid	$C_{18}H_{30}O_3$	0.77	Bronze	Massbank
27	7.85	293.212	195.23, 221.20, 236.15, 275.26, **293.15**	(9Z,11E,15Z)-13-Hydroxy-9,11,15-octadecatrienoic acid	$C_{18}H_{30}O_3$	0.73	Bronze	Massbank
28	7.85	293.212	97.04, 148.06, 164.03, 177.04, 205.08, **220.09**, 221.14	(9Z,11E,15Z)-13-Hydroxy-9,11,15-octadecatrienoic acid	$C_{18}H_{30}O_3$	0.82	Bronze	Massbank
29	7.86	293.176	57.03, 71.01, 177.09, 192.12, 205.12, 220.15, **221.16**, 236.11, 274.88	6-Gingerol	$C_{17}H_{26}O_4$	0.85	Bronze	Trent Northen
30	8.20	264.124	61.99, 79.96, **96.96**	Anisomycin	$C_{14}H_{19}NO_4$	0.91	Bronze	MoNA
31	8.22	313.237	99.08, 129.09, 183.13, 195.13, 246.55, 277.22, **313.23**	Massbank:PR309105 FA 18:1+2O	$C_{18}H_{34}O_4$	0.70	Bronze	Massbank
32	8.25	313.239	99.08, 129.09, 183.14, 184.14, 195.14, 77.22, 295.23, **313.24**, 314.24	Spectral Match to 12,13-DiHOME from NIST14	$C_{18}H_{34}O_4$	0.82	Bronze	Data from Wiemann
33	8.50	341.138	107.05, 123.01, 147.04, **165.02**, 179.03, 183.03	8-Prenylnaringenin|(2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)-2,3-dihydrochromen-4-one	$C_{20}H_{20}O_5$	0.90	Bronze	Massbank
34	8.53	341.139	107.05, 123.01, 137.02, 147.02, **165.02**, 183.03, 285.07	MLS001360619–01	$C_{20}H_{20}O_5$	0.87	Gold	Dorrestein
35	8.55	341.138	107.05, 123.01, 147.04, **165.02**, 179.03, 183.03	8-Prenylnaringenin|(2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)-2,3-dihydrochromen-4-one (isomer)	$C_{20}H_{20}O_5$	0.89	Bronze	Massbank
36	8.59	341.139	99.01, 123.01, 137.02, **165.02**, 183.03, 285.07	5,7-dihydroxy-2-(4-hydroxyphenyl)-6-(3-methylbut-2-enyl)-2,3-dihydrochromen-4-one		0.89	Gold	Dorrestein
37	8.64	434.931	170.01, 183.02, 217.99, 243.99, 249.96, 277.95, 317.97, **329.96**, 365.94, 398.95	Fipronil|5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile	$C_{12}H_4Cl_2F_6N_4O_S$	0.78	Bronze	Massbank EU
38	8.65	434.931	249.96, 251.95, 277.95, 317.97, 319.97, **329.96,** 331.96, 365.94	Fipronil|5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile	$C_{12}H_4Cl_2F_6N_4O_S$	0.82	Bronze	Massbank
39	8.67	434.931	249.96, 277.95, 317.97, **329.96,** 331.96, 365.94, 398.95	Fipronil|5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile	$C_{12}H_4Cl_2F_6N_4O_S$	0.83	Bronze	Massbank
40	8.71	293.212	121.11, 183.14, 235.17, 275.20, **293.21**	Massbank:PR309079 FA 18:3+1O	$C_{18}H_{30}O_3$	0.76	Bronze	Massbank
41	8.80	734.599	**294.29**, 296.30, 30.43, 448.33, 580.57, 91.62, **734.60**	Spectral Match to 1-(1Z-Octadecenyl)-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphoethanolamine from NIST14	$C_{43}H_{78}NO_7P$	0.84	Bronze	Katrina Waters; Yoshihiro Kawaoka;Richard Smith;Thomas Metz
42	8.82	293.213	97.13, 171.21, 179.19, 195.19, 196.20, 205.17, 224.18, 249.33, **275.33**	Spectral Match to 13S-Hydroxy-9Z,11E,15Z-octadecatrienoic acid from NIST14	$C_{18}H_{30}O_3$	0.77	Bronze	Data from Albert Rivas Ubach

Notably, most of the 404 nodes in the molecular network show ions for metabolites. For example, depicted in Fig. 3 is a nine (9) node cluster extracted from Fig. 2 showing four (4) isomeric molecular ions of one metabolite, bergenin, and five (5) unknown metabolites. Indeed, the information extractable from feature-based mass spectrometry for metabolites seems to be fingerprints of metabolites, analogous to information from DNA for living organisms.

Phytochemical identification using METLIN Gen2

The MGF file used in GNPS previously described was submitted to METLIN Gen2 with a tolerance set at 30 ppm [34]. Table 5 shows a list of 21 compounds generated using METLIN

The tentative structures of the metabolites from Table 5 are presented in Fig. 4.

Table 4
Phytocompounds identified by UHPLC–ESI-MS/MS in the negative ion mode and subsequent use of feature based advanced analysis of GNPS.

No.	Precursor	MS2 Fragments	Tentative Compound	Formula	Cosine	Library class	library
1	329.231	99.08, 139.11, 171.10, 183.14, 211.13, 229.14	Massbank:PR309108 FA 18:1+3O	$C_{18}H_{34}O_5$	0.93	Bronze	Massbank
2	327.072	164.01, 192.00, 194.02, 207.03, 234.01, 249.04, 312.05	Massbank:PR307403 Bergenin	$C_{14}H_{16}O_9$	0.91	Bronze	Massbank
3	327.218	85.03, 97.07, 171.10, 211.13, 229.14, 327.22,	(10E,15E)-9,12,13-trihydroxyoctadeca-10,15-dienoic acid	$C_{18}H_{32}O_5$	0.89	Gold	Wolfender
4	327.072	192.01, 193.01, 207.03, 234.02, 249.04, 312.05, 327.07	Massbank:PR307395 Bergenin	$C_{14}H_{16}O_9$	0.87	Bronze	Massbank
5	293.176	71.01, 177.09, 192.12, 205.12, 220.15, 221.16, 236.11, 274.88, 293.18	6-Gingerol	$C_{17}H_{26}O_4$	0.84	Bronze	Trent Northen
6	363.049	192.01, 193.01, 207.03, 234.02, 327.07	bergenin CollisionEnergy:205,060	$C_{14}H_{16}O_9$	0.83	Bronze	Trent Northen
7	363.049	192.01, 207.03, 234.02, 249.04, 312.05, 327.07	bergenin CollisionEnergy:102,040	$C_{14}H_{16}O_9$	0.83	Bronze	Trent Northen
8	434.931	183.02, 249.96, 277.95, 317.97, 329.96, 398.95	Fipronil/ 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile	$C_{12}H_4Cl_2F_6N_4OS$	0.83	Bronze	Massbank
9	311.092	79.96, 119.05, 170, 183.01, 184.01, 197.03	5,6,2′-Trimethoxyflavone	$C_{18}H_{16}O_5$	0.77	Bronze	MoNA
10	329.233	99.08, 169.12, 199.13, 211.13, 329.23	Massbank:PR309110 FA 18:1+3O	$C_{18}H_{34}O_5$	0.76	Bronze	Massbank
11	307.19	121.07, 185.12, 209.11, 211.13, 235.13, 307.19	Massbank:PR309076 FA 18:4+2O	$C_{18}H_{28}O_4$	0.75	Bronze	Massbank
12	265.146	97.08, 265.48	Spectral Match to Dodecyl sulfate from NIST14	$C_{12}H_{26}O_4S$	0.75	Bronze	Data from Luan
13	329.288	127.02, 171.08, 199.10, 201.09, 211.08, 293.17	(Z)-5,8,11-trihydroxyoctadec-9-enoic acid	$C_{18}H_{34}O_5$	0.75	Bronze	MoNA
14	313.239	99.08, 129.09, 183.14, 184.14, 195.14, 77.22, 295.23, 313.24, 314.24	Spectral Match to 12,13-DiHOME from NIST14	$C_{18}H_{34}O_4$	0.71	Bronze	Data from Wiemann

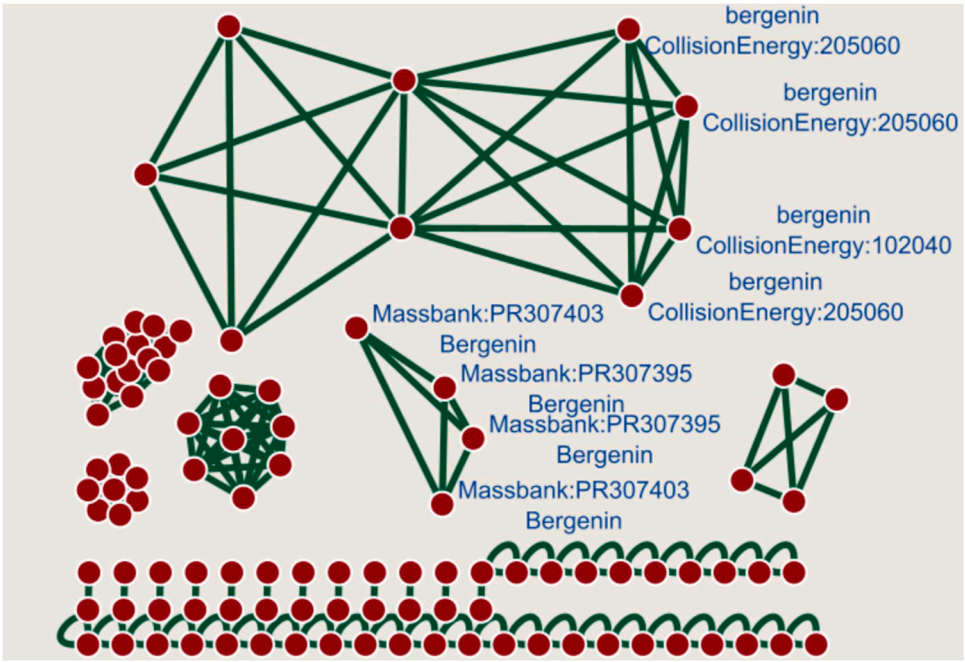

Fig. 2. Molecular network (showing clusters of metabolites of interest) based on LCMS/MS data in the negative ionization mode of *A. panduriformis* leaf extract. The network is shown as a pie chart to display the relative abundance of each ion in the analyzed sample.

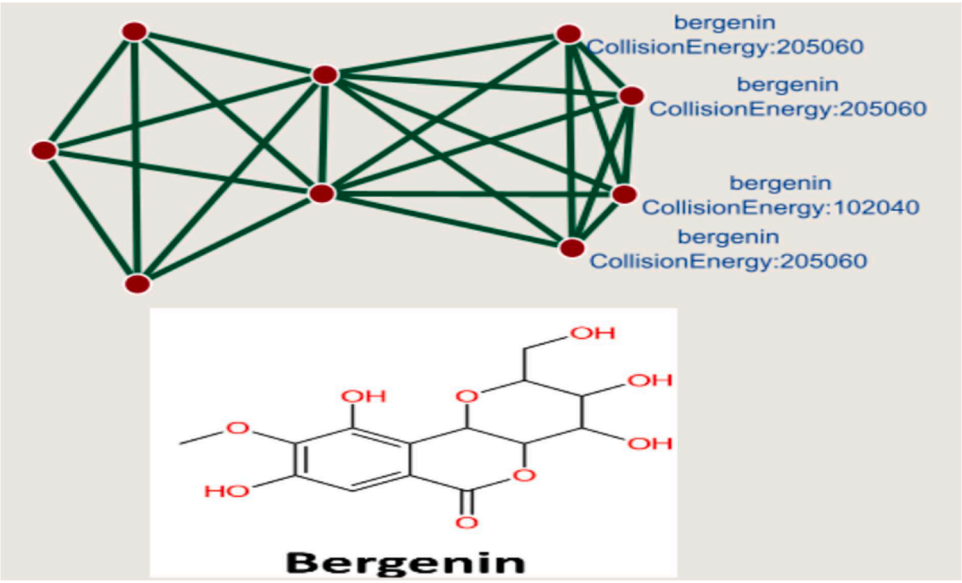

Fig. 3. Network showing a pie chart displaying nodes of molecular ions (red dots) of bergenin based on collision energies.

Table 5
Phytocompounds identified by UHPLC−ESI-MS/MS- METLIN Gen2 in the negative ion mode.

#	METLIN MID	PubChem	Name	Mass	Formula
1	1,936,754	1,398,069	5-chloro-N-[(3-methoxyphenyl)methyl]-1H-indole-2-carboxamide	580.9340	$C_{18}H_{16}Br_2F_3N_5O_2S$
2	1,794,738	3,120,608	2,5-diiodo-n-(2-methyl-5-{[1,3]-oxazolo[4,5-b]pyridine-2-yl}phenyl)benzamide	580.9100	$C_{20}H_{13}I_2N_3O_2$
3	1,814,226	1,247,375	N-(4-bromo-3-methylphenyl)-3,5-bis(methylsulfanyl)-1,2-thiazole-4-carboxamide	387.9373	$C_{13}H_{13}BrN_2OS_3$
4	1,794,988	2,819,020	N-(5-bromo-4-methoxythiophene-3-carbonyl)-2-chlorobenzohydrazide	387.9280	$C_{13}H_{10}BrClN_2O_3S$
5	1,788,386	1,037,008	Methyl 2-(5-bromo-2-chlorobenzamido)-4-methyl-1,3-thiazole-5-carboxylate	387.9280	$C_{13}H_{10}BrClN_2O_3S$
6	1,965,269	5,348,344	(2,2-diethoxyethyl)-5-methyl-8-thia-4,6-diazatricyclo[7.4.0.0˜(2,7)]trideca-1 (9),2,4,6-tetraen-3-amine	351.9610	$C_{14}H_{10}BrClN_2O_2$
7	1,965,197	5,348,748	3,5-dimethyl-4-(4-hydroxy-3,5-dimethoxyphenyl)-1-[(4-methyoxyphenyl)methyl]-4H-pyridine-3,5-dicarboxylate	351.9520	$C_{13}H_9BrN_2O_3S$
8	1,675,950	1,673,703	2-{[(2-chloro-6-fluorophenyl)methyl]sulfanyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole	387.9410	$C_{15}H_8Cl_3FN_2OS$
9	1,775,422	1,034,227	3-bromo-2-chloro-5-(4-methylpiperidin-1-ylsulfonyl)pyridine	351.9650	$C_{11}H_{14}BrClN_2O_2S$
10	1,773,801	135,460,546	2-bromo-N'-[(E)-(5-chloro-2hydroxyphenyl)methylidene)benzohydrazide	351.9610	$C_{14}H_{10}BrClN_2O_2$
11	1,678,386	22,588,545	2-(4-bromophenyl)-4H-1lambda6,2,4-benzothiadiazine-1,1,3-trione	351.9520	$C_{13}H_9BrN_2O_3S$
12	1,609,292	2,739,683	5-(4-{[(2,6-dichlorophenyl)sulfanyl]methyl}phenyl)-1,2,4-thiadiazole	351.9660	$C_{15}H_{10}Cl_2N_2S_2$
13	1,541,241	5,818,377	(2E)-N-(4-bromophenyl)-3-(5-ntrothiophen-2-yl)prop-2-enamide	351.9517	$C_{13}H_9BrN_2O_3S$
14	1,541,238	6,018,482	(2E)-N-(3-bromophenyl)-3-(5-nitrothiophen-2-yl)prop-2-enamide	351.9517	$C_{13}H_9BrN_2O_3S$
15	1,465,742	2,917,869	2-chloro-4-nitro-N-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]benzamide	351.9640	$C_{13}H_9BrN_2O_3S$
16	1,459,445	2,800,574	4-(3,4-dichlorophenyl)-3-(phenylamino)-1,3-thiazole-2-thione	351.9660	$C_{15}H_{10}Cl_2N_2S_2$
17	1,456,140	1,294,178	5-bromo-N-(2-carbamoylphenyl)-2-chlorobenzamide	351.9610	$C_{14}H_{10}BrClN_2O_2$
18	1,431,466	2,765,805	4-[(2,4-dichlorophenyl)sulfanyl]-2-(methylsulfanyl)quinazoline	351.9660	$C_{15}H_{10}Cl_2N_2S_2$
19	1,430,006	3,739,319	5-(benzylsulfinyl)-4-(2,4-dichlorophenyl)1,2,3-thiadiazole	351.9660	$C_{15}H_{10}Cl_2N_2S_2$
20	1,426,795	2,765,804	4-[(2,5-dichlorophenyl)sulfanyl]-2-(methylsulfanyl)quinazoline	351.9660	$C_{15}H_{10}Cl_2N_2S_2$
21	1,240,614	3,492,160	5-{[(2,4-dichlorophenyl)methyl]sulfanyl}-4-phenyl-1,2,3-thiadiazole	351.9660	$C_{15}H_{10}Cl_2N_2S_2$

Total polyphenolic content

Total phenolic content (TPC)

TPC in methanolic leaf extract of *A. panduriformis* was determined using the FC assay with gallic acid as standard. TPC was evaluated using the calibration curve of the regression equation, $y = 0.00111x + 0.11836$, $R^2 = 0.997$, and expressed as mg gallic acid equivalent per gram (mg GAE/g), as described in Table 6.

Total flavonoid content (TFC)

TFC was expressed as quercetin equivalent (QE) in mg/g of extract. The leaf extract contained 60.17 ± 0.25 mg QE/g total flavonoids, as depicted in Table 6. Table 6 shows that the total phenolic content (122.30 ± 2.00 mg GAE/g) was twice more than the

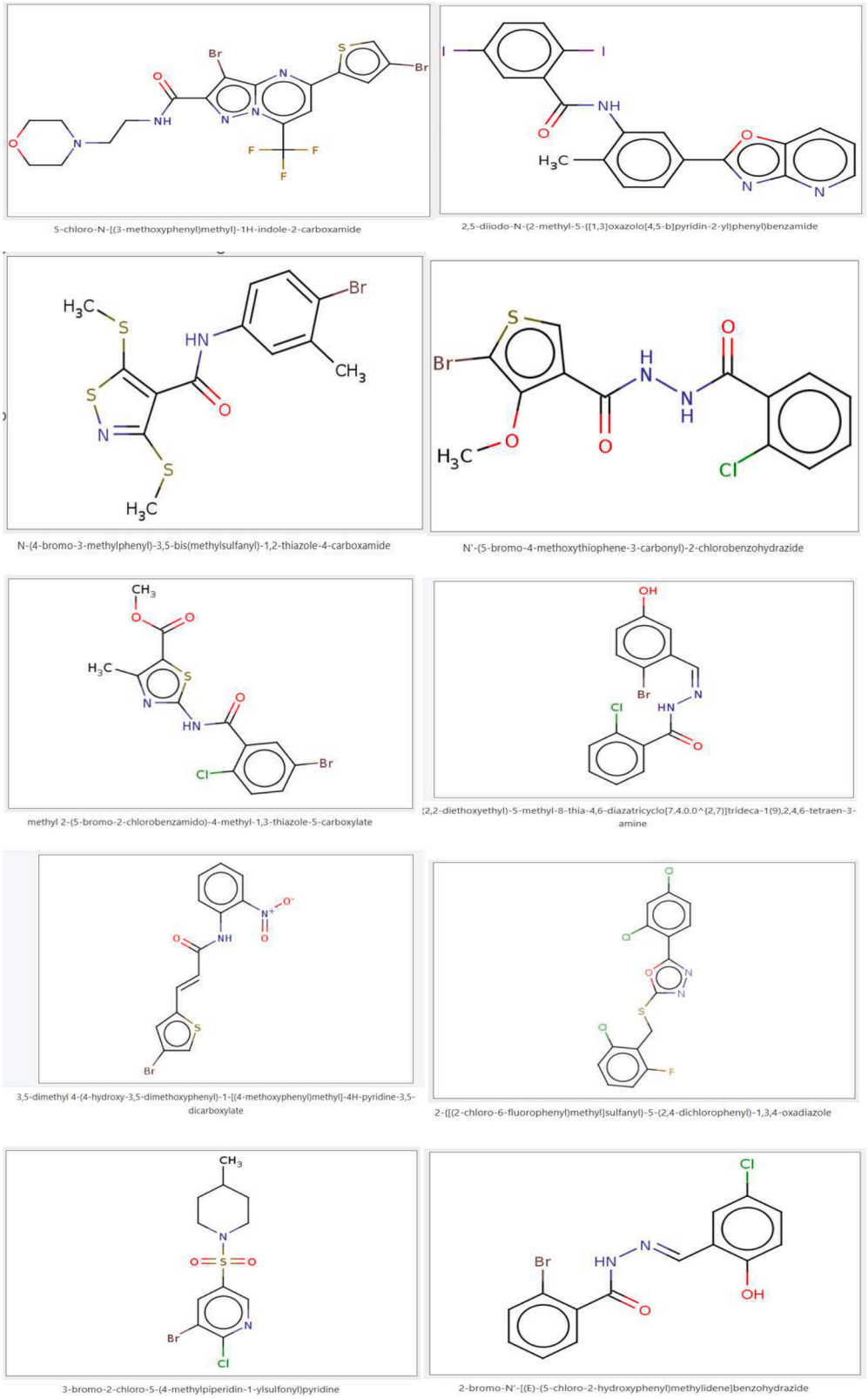

Fig. 4. The structures of metabolites tentatively identified in the leaf extract of *A. panduriformis* by LC-MS/MS- METLIN Gen 2.

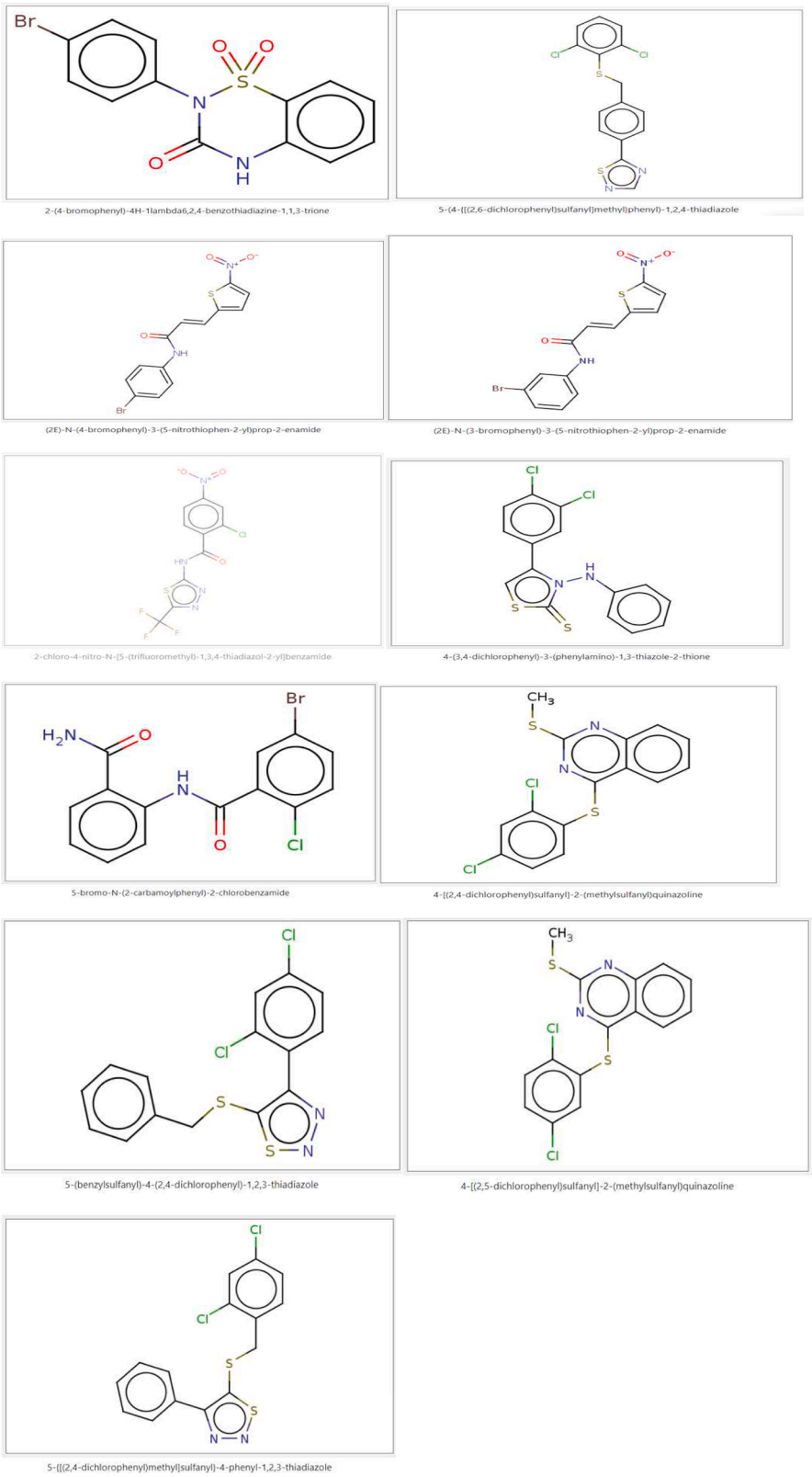

Fig. 4. (*continued*).

Table 6
TPC and TFC of *A. panduriformis* leaves (n = 3).

	Leaves
TPC (mg GAE/g)	122.30 ± 2.00
TFC (mg QE/g)	60.17 ± 0.25

amount of flavonoids (60.17 ± 0.25 mg QE/g). The higher phenolic content than flavonoids might be because flavonoids are a subset of polyphenolic compounds.

Antioxidant activity

The radical scavenging activity of the *A. panduriformis* leaf extract was evaluated using the DPPH assay, and the results are presented in Table 7.

The free radical scavenging activity of *A. panduriformis* leaf extract ranged from 26.10 % to 97.16 % relative to ascorbic acid (25.81 % to 78.92 %) at respective concentrations between 20 and 120 μg/ml. Similarly, the sample extract IC_{50} value of 53.48 μg/mL was low compared to that of ascorbic acid of 74.47 μg/mL. DPPH assay results generally indicated that *A. panduriformis* leaf extract is a potential source of antioxidants of natural origin.

Discussions, conclusion, and Outlook

Discussion

Preliminary phytochemical screening results of the study revealed the presence of alkaloids, glycosides, tannins, saponins, steroids, polyphenols, terpenoids, and volatile oils. Interestingly, these phytocompounds provide strong scientific support for consuming *A. panduriformis*, a wild vegetable for medicinal purposes in the Zambian traditional healthcare system. For example, alkaloids might account for the analgesic properties of this wild vegetable. In this regard, codeine and morphine alkaloids can be cited to have analgesic properties [35]. Similarly, anthraquinones are present, which are reportedly anti-cancerous, laxative, and anti-arthritic [36]. Furthermore, the presence of polyphenols is remarkable, and these compounds have been associated with antioxidation properties [37]. Flavonoids present in the vegetable can offer antibacterial, antidiabetic, antifungal, and antiviral potential, as reported earlier [38,39]. The presence of such a diversity of molecules in one vegetable is significant. It may well account for its use in the traditional management of many diseases, including degenerative diseases such as cancer and diabetes mellitus in Zambia.

The presence of cardiac glycosides may be behind the effectiveness of the consumption of *A. panduriformis* in controlling hypertension in traditional healthcare. Cardiac glycosides are effective diuretics and affect the heart positively thereby aiding the heart's sturdiness and rate of shortening during contractions [21,40]. Therefore, this wild vegetable might be a possible alternative for controlling and managing cardiac diseases.

Furthermore, the wild vegetable was found to possess saponins. These compounds essentially reduce the amount of cholesterol in the blood and further assist the body in absorbing calcium [41]. Thus, *A. panduriformis* is a potential food for treating and preventing atherosclerosis, a health problem usually caused by significantly higher amounts of cholesterol. In addition, the presence of tannins may account for the plants' several medicinal properties [42]. For example, tannins are used in treating diarrheal, wounds, and catarrh and protecting the inflamed outer layer of the mouth [43,44]. Finally, the presence of steroids further validates the consumption of this vegetable for treating diarrheal in traditional herbal medicine [45].

LCMS- molecular networking tools, mzmine3 (table 2), GNPS (Tables 3 and 4), and METLIN (Table 6 and Fig. 4) results showed the presence of metabolites known to have interesting medicinal properties and nutritional value. The study, therefore, corroborates the reason for the prevalent use of the traditional wild vegetable in treating diseases in the traditional healthcare system. Consumption of this vegetable is pharmacologically significant when it comes to consuming food for medicine. For instance, quinine and its derivatives in this vegetable effectively treat malaria and illnesses such as nocturnal leg cramps, inflammation, and relieving pain [46]. In addition, Salicylic acid is an effective component in fighting acne owing to its oil-fighting capacities [47]. Further, ajmaline, an indole alkaloid, is an effective antiarrhythmic agent. Its presence in the wild vegetable renders it effective at preventing and treating a heart rhythm that may be too fast or irregular [48]. Jasmonates such as jasmonic acid are hormones in plants but exhibit anticancer and anti-inflammatory properties in humans [39]. Its presence, therefore, is pharmacologically significant.

The other metabolite present is prazosin. It is an alpha-blocker that treats high blood pressure by relaxing and widening blood vessels, thereby enabling blood to flow more easily [49]. This effect helps prevent heart attacks, kidney problems, and strokes. Further, diosmetin, a flavonoid, reduces swelling and inflammation and restores normal vein function [50]. As for bergenin, it stimulates both the anti-inflammatory messengers and protective prostaglandin secretions. Breaking down lipids is an essential function of bergenin, making it a popular constituent of thermos genic fat-burning dietary supplements. Bergenin has no adverse effects, even at large dosages [51]. Dietary suberic acid has been reported as an effective agent for the treatment of skin photoaging [50].

Many more metabolites present in this wild vegetable possess medicinal effects. They include anziaic acid that works as an antibacterial compound; isovitexin, a flavonoid, possesses multifaceted pharmacological effects, such as being anticancer, antioxidant, antidiabetic, anti-inflammatory, neuroprotective, and antinociceptive [51,39]. As for 6-gingerol, it possesses an assortment of pharmacological effects, including anti-inflammation, anticancer, and antioxidation [52]. Furthermore, anisomycin is an effective

Table 7
DPPH antioxidant activity results.

[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	25,81	74.47	53.48
40	40	0,74	0,539	0,419	27,16	43,38		
60	60	0,74	0,428	0,347	42,16	53,11		
80	80	0,74	0,356	0,238	51,89	67,83		
100	100	0,74	0,252	0,137	65,94	81,48		
120	120	0,74	0,156	0,021	78,92	97,16		

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

antibiotic molecule [53].

The preceding are some of the health benefits of some of the metabolites found in *A. panduriformis*. Of interest is to note that the individual metabolites can act singly or in concert. However, the synergistic action of therapeutic metabolites produces a more significant effect when combined than the sum of their effects if not combined [54]. The health benefits of polyphenolic compounds including their antioxidant effects are well alluded to and are well documented in the literature [55]. The results of polyphenolic content are presented in Table 4. The content of phenolic compounds and flavonoids is commensurate with the LCMS/MS screening results, which have shown several polyphenols. Further, the enhanced TPC and TFC are in tandem with the enhanced antioxidative potential of the extract, as revealed by DPPH radical quenching results. The sample IC_{50} value of 53.48 μg/mL compared to that of ascorbic acid of 74.47 μg/mL shows higher radical quenching ability and consequently desirable antioxidant potential. In recent decades, flavonoid and phenolic-rich natural diets with antioxidant activity have encouraged interest in food science and nutrition [55]. Many literature reports suggest that polyphenolic compounds affect peroxide decomposition, oxygen scavenging, free radical inhibition, or metal inactivation in biological systems and assist in averting oxidative stress disease burden [26]. It is undeniable that natural antioxidants from leafy vegetables have an important role in safeguarding from the undesirable effects of free radicals [56]. Epidemiological studies have shown that the consumption of leafy vegetables possessing polyphenolic compounds with strong antioxidant capacity is mainly associated with reduced chances of diabetes, cardiovascular diseases, neurodegenerative diseases, and cancer [27,55,56]. The results suggest that the consumption of *A. panduriformis* wild vegetable as part of the diet can help enhance human health and significantly lower the damaging effects of free radicals that foster the progression of oxidative stress-related diseases.

Conclusion

This phytochemical study and identification of metabolites using molecular networking platforms have provided good visibility, not only on the phytochemical profile of *A. panduriformis* leaf but has also revealed its importance in nutrition, traditional healthcare, and in the search for therapeutic metabolites from plant sources. Phytochemical studies revealed the presence of natural products with interesting medicinal properties. These were polyphenols (flavonoids, tannins, phenolic compounds), alkaloids, steroids, terpenoids, Glycosides, saponins, and volatile oils. Total polyphenolic content was significant (TPC = 122.30 ± 2.00 mg GAE/g, and TFC = 60.17 ± 0.25 mg QE/g), and the antioxidant potential was high with the extract's IC_{50} value of 53.48 μg/mL being lower in comparison to that of reference ascorbic acid of 74.47 μg/mL. This indicated that the wild vegetable is a potential source of antioxidants. In total, 26 metabolites were identified by mzmine3.2.8, 42 through GNPS, 21 metabolites were identified by METLIN Gen2 and previous studies confirmed the nutritional and pharmacological importance of these metabolites. Indeed, *A. panduriformis* wild vegetable, if included as part of the everyday diet, would boost health and fight and prevent infection and disease. The findings of the study will profoundly help drug developers.

Outlook

Although this study is the first investigation of the phytochemistry of *A. panduriformis* wild vegetable, the findings are very encouraging and lead to further studies, and possibly the isolation and characterization of compounds not revealed by LCMS2, which may also be responsible for the nutritional benefits as well as the use of the plant in traditional healthcare.

Notes

This work is part of the doctoral dissertation by Chibuye Bitwell and supervised by Professor Indra Sen Singh, Professor Maseka Kenneth Kakoma, and Professor Chimuka Luke. The study focussed on phytochemical studies and metal analysis of some medicinal herbs used in the traditional healthcare system in Zambia and elsewhere.

CRediT authorship contribution statement

Bitwell Chibuye: Conceptualization, Methodology, Validation, Software, Formal analysis, Investigation, Data curation, Resources, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Indra Sen Singh:** Conceptualization,

Resources, Funding acquisition, Methodology, Validation, Writing – review & editing, Supervision. **Luke Chimuka:** Conceptualization, Methodology, Validation, Investigation, Writing – review & editing, Funding acquisition, Supervision. **Kakoma Kenneth Maseka:** Conceptualization, Methodology, Supervision.

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.

Acknowledgments

The authors wholeheartedly acknowledge the support rendered by the Ministry of Education of the Republic of Zambia, Copperbelt University – Africa Centre of Excellence in Sustainable Mining (CBU – ACESM), and Mukuba University, without which this study would not have been achievable.

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