Vol. 16, No. 2, 2025
ISSN 2233-4203/ e-ISSN 2093-8950
ARTICLE www.msletters.org | Mass Spectrometry Letters

UHPLC-(ESI)-HRMS-Based Metabolomic to Access Chemical Diversity of *Chrozophora brocchiana* (Euphorbiaceae), an Antiparasitic Plant from Niger

Moctar Chaibou[1]*, Pierre Le Pogam-Alluard[2], Idrissa Moussa[1], and Khalid Ikhiri[1]

[1]Department of Chemistry, Abdou Moumouni University, Niamey 1062, Niger
[2]Pharmacy falculty, Université Paris-Saclay, CNRS, BioCIS, 91400 Orsay, France

Received Februrary 2, 2025, Revised May 13, 2025, Accepted June 9, 2025
First published on the web June 30, 2025; DOI: 10.5478/MSL.2025.16.2.42

Abstract : *Chrozophora brocchiana* is widely used in traditional medicine in Niger. In this study, hexanic, DCM, AcOEt, and EtOH-H_2O (70:30) fractions of *Chrozophora brocchiana* aerial parts were analyzed via ultra-high-performance liquid chromatography with high-resolution mass spectrometry (UHPLC-HRMS)-based metabolomics. We explore chemical space with an advanced Global Natural Product Social (GNPS)-based molecular networking (MN) analysis tool, *MolNetEnhancer*. As result, three superclasses were identified (phenylpropanoids and polyketides; lipids and lipid-like molecules; organoheterocyclic compounds). Flavonoids were the most dominant class, followed by tannins, lipids, and lipid-like molecules, and alkaloids. Additionally, some compounds' putative structure and molecular formula were manually determined. This novel mapping of the metabolomic landscape of *C.brocchiana* provides actionable insights into plant biochemistry and justifies certain medicinal uses.

Keywords : *Chrozophora brocchiana*; computational metabolomics, molecular networking, LC–MS/MS profiling

Introduction

Chrozophora brocchiana (Euphorbiaceae) is a shrub widely distributed in sub-Saharan countries, used as a traditional medicine.[1] Previous studies have reported that it contains organic acids, phenolics, terpenes, benzyl glycosides, and cyanogenic glycosides.[2] Numerous studies have revealed that this specie has antiplasmodial,[3] anthelminthic,[4] antimicrobial, and antioxidant activities.[5] Its phenolic content is relatively high, showing potential anti-inflammatory and antioxidant activities.[5] These accounts reflect that medicinal plants still represent a vastly rich resource for potential drug leads. In this study, the phytochemical composition of *C. brocchiana* extracts was investigated, based on Global Natural Product Social (GNPS)-based molecular networking (MN).

GNPS-based molecular networking has emerged as an efficient tool for rapid metabolite identification via mass spectrometry (MS) data in early research of natural products (NPs).[6,7] This web platform allows the organization and visualization of compounds from natural extracts by comparing annotations obtained from an MS spectral library.[8,9] Currently, ultra-high-performance liquid chromatography with high-resolution mass spectrometry (UHPLC-HRMS)-based metabolomics has been widely used to identify the different chemical constituents in various biological samples.[10,11] We mapped *C.brocchiana* extracts metabolome using a suite of computational metabolomics strategies including feature-based molecular networking, substructural discovery method (*MS2LDA*), *in silico* tools (*e.g.*, Network Annotation Propagation, *NAP*) and *MolNetEnhancer*. This study is a computational metabolomic work designed to comprehensively characterize the metabolome of *C. brocchiana*. To our knowledge, this is the first report on the metabolomic chart of this plant.

Material and methods

Extracts preparation

The aerial parts of *C. brocchiana* (Euphorbiaceae) were harvested during January 2019 in Niamey (Niger). This plant was identified in the Department of Biology (Faculty of Science and Technics at Abdou Moumouni University). Plant have been washed, dried at room temperature (37 °C) and then powdered. We prepared a crude extract by maceration of 500 g of pulverised material in 2.5 L of a mixture DCM/MeOH (50:50) for 48 hours. This crude extract was

---

*Reprint requests to Moctar Chaibou
https://orcid.org/0009-0005-6832-4153
E-mail: moctarchaibou47@gmail.com

fractionated using differents solvants with increased polarity.

We obtained hexanic extract, DCM extract, AcOEt extract and EtOH-H_2O. The various extracts were concentrated using a rotavapor. Each operation was triplicated.

Data-Dependent LC-HRMS Analysis

LC-ESI-HRMS analysis were performed on an Agilent 1290 Infinity II UHPLC coupled to a hybrid quadrupole time of flight (QTOF) mass spectrometer Agilent 6546 (Agilent Technologies) equiped with an ESI source, operating in positive ion mode. A Waters Acquity C_{18} UPLC column (2.1 × 150 mm; 1.7 µm, Agilent Technologies, Inc., Santa Clara, CA, USA) was used, with water + 0.05% formic acid (A) and acetonitrile + 0.05% (B) as mobile phase. The flow rate was set to 0.5 mL/min. A linear gradient from 5% - 100% B in 12 minutes followed by 100% B for 4 min was used. Source parameters for Agilent 6546 were set as follows: capillary temperature at 320°C, source voltage at 3500 V, sheath gas flow rate at 11 L/min. The divert valve was set to waste for the first 3 min. MS scans were operated in full-scan mode from *m/z* 100 to 1200 (0.1 s scan time) with a mass resolution of 67,000 at *m/z* 922. A MS1 scan was followed by MS2 scans of the five most intense ions above an absolute threshold of 3000 counts. Selected parent ions were fragmented at a collision energy fixed at 70 eV. The purine $[M+H]^+$ ion ($C_5H_5N_4^+$, *m/z* 121.0509) and the hexakis(1*H*,1*H*,3*H*-tetrafluoropropoxy) phosphazene $[M+H]^+$ ion ($C_{18}H_{19}F_{24}N_3O_6P_3^+$, *m/z* 922.0098) were used as internal lock masses. A permanent MS/MS exclusion list criterion was set to prevent oversampling of the internal calibrant. LC-UV and MS data acquisition and processing were performed using MassHunter Workstation software (Agilent Technologies, Massy, France).

LC-MS/MS Data Pretreatment and Molecular Networking Parameters

The MS^2 data file was converted from the .d standard data format to .mzML format using the MSConvert software,[12] part of the ProteoWizard package. All .mzML were then imported to MZmine 4.3.0 software.[13] The mass detection was performed on exact masses with mass level 1 and centroided masses with mass level 2 by keeping the noise level at 1.5×10^3 at MS1 and at 1×10^1 at MS2, respectively. The ADAP chromatogram builder was used to build a chromatogram with a minimum group size of scans of 3, a group intensity threshold of 4.5×10^3, a minimum highest intensity of 1×10^4, and *m/z* tolerance of 10 ppm. Chromatogram deconvolution used the local minimum search algorithm with the following settings: chromatographic threshold = 0.8, search minimum in RT/mobility range (absolute) = 0.03, minimum relative height = 0.0, minimum absolute height = 1×10^3, min ratio of peak top/edge = 1.25, and peak duration range (min) = 0.0–0.45. MS^2 scans were paired using a *m/z* tolerance range of 0.02 Da and RT tolerance range of 0.2 min. Isotopes were grouped using the isotopic peaks grouper algorithm with a *m/z* tolerance of 10 ppm and a RT tolerance of 0.15 min with the lowest peak. $[M + H – H_2O]^+$, $[M + K]^+$, $[M + NH_4]^+$, and $[M + Na]^+$ adducts were filtered with a *m/z* tolerance of 5 ppm. The resulting peak list was filtered to keep only rows with MS^2 features.

Molecular networking analysis was performed with an exported MZmine. mgf table file into the GNPS online platform using the Feature-Based Molecular Networking (FBMN) mode[9]. The following parameters were used : minimum pairs cos, 0.65 ; precursor ion mass tolerance, 0.02 Da ; fragment ion mass tolerance, 0.02 Da ; network topK, 10 ; minimum matched peaks, 6 ; minimum cluster size, 2. The spectra in the network were then searched against various GNPS spectral libraries including GNPS, COCONUT and DRUGBANK.[14]

Additionally, dereplication databases for natural products, such as ChemSpider (https://www.chemspider.com/.), PubChem (https://pubchem.ncbi.nlm.nih.gov) and Dictionary of Natural Products (http://dnp.chemnetbase.com/faces/chemical/ChemicalSearch.xhtml.) and available literature, were searched for further verification and annotation.[15]

In order to enrich chemical structural information within the generated molecular network, *in silico* structure annotations from GNPS Library Search and Network Annotation Propagation (NAP) were incorporated into the network using the GNPS MolNetEnhancer workflow. (https://ccms-ucsd.github.io/GNPSDocumentation/molnetenhancer/).

Chemical class annotations were performed using the ClassyFire chemical ontology. Substructure annotation was performed using the MS2LDA interface in GNPS.[16] Metabolite annotation was carried out at confidence level 2 to level 4 of the Metabolomics Standards Initiative (MSI).[17] Empirical formula of all matched and some unmatched nodes were generated by Masshunter software based on accurate mass or fragmentation patterns obtained from MS2 analysis. Finally, the resulting molecular networking was visualized using the Cytoscape software (v.3.7.2).[18]

Results and discussion

Chromatographically, the crude extract of *C. brocchiana* is highly complex mixtures of metabolites with a wide range of polarities (Figures 1-2). To further decode this chemical space, spectral data were mined and visualized using molecular networking strategies housed in the GNPS platform.

The metabolomic chart of *C. brocchiana* extracts

UHPLC-MS/MS-based MN is a useful tool for identifying chemical structures. This analysis method consists of comparing MS/MS spectra to cluster compounds with similar structures. In this study, MN was performed by using MS data obtained in the ESI+ following by a feature-based workflow (https://gnps.ucsd.edu/ProteoSAFe/status.jsp?

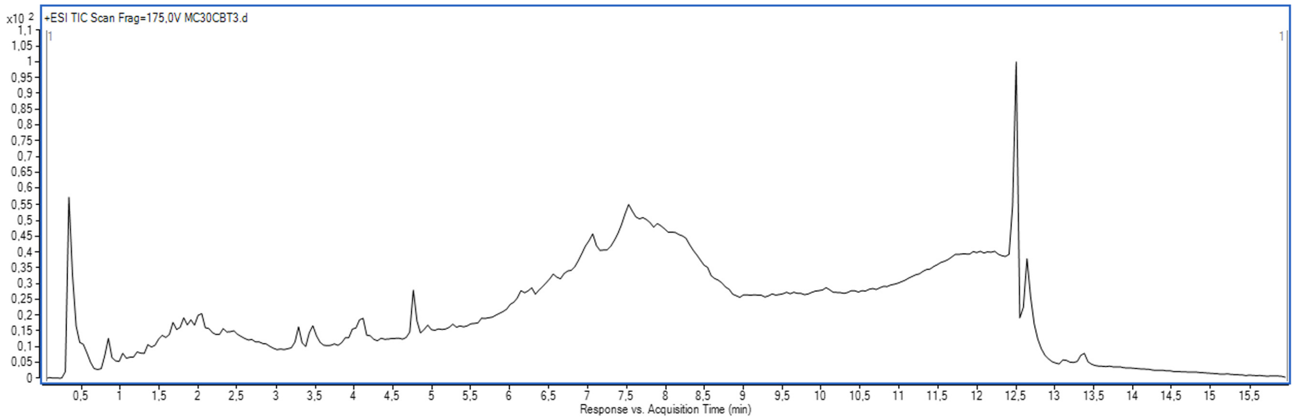

Figure 1. Total ion chromatogram of *C. brocchiana* crude extract.

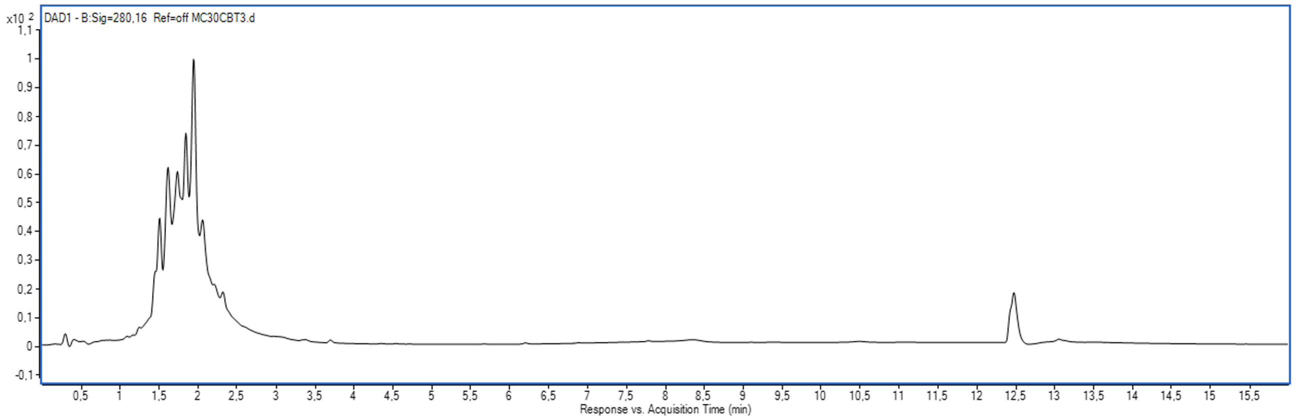

Figure 2. LC-DAD-QToF chromatogram of *C. brocchiana* crude extract at 280 nm

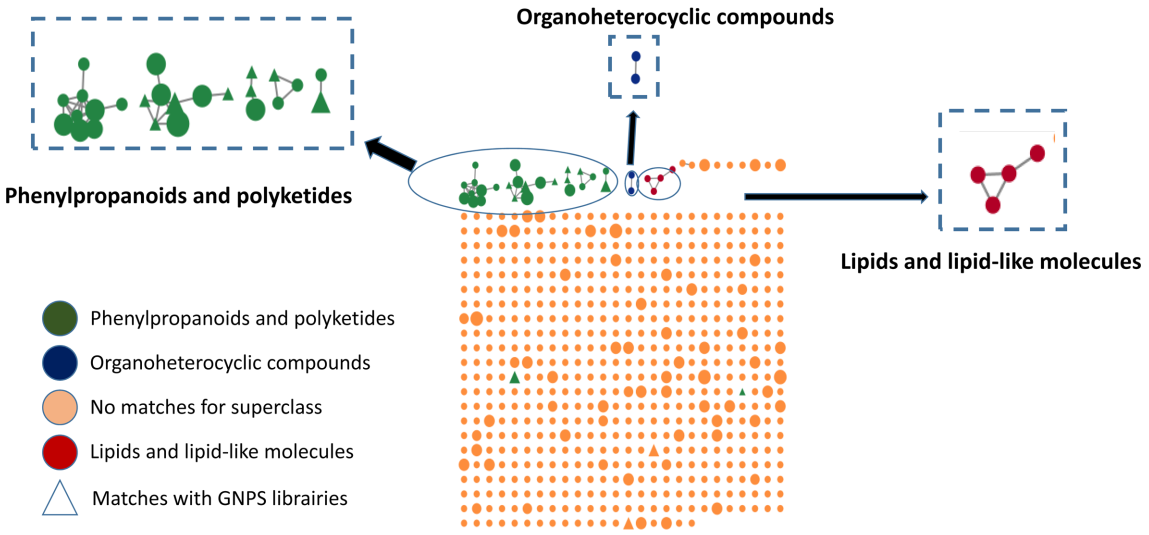

Figure 3. Superclass molecular networking from *C. brocchiana* extracts.

task=4a901d6fe2fe41f1b2596ff0f0fc83c3). Figure 3 shows the molecular network visualized using Cytoscape. This molecular networking contains 605 nodes (8 clusters) and 612 edges. Among the total nodes observed, 12 hits were matched to known metabolites present in the different databases and the others metabolites were further validated through manual confirmation by comparing the mirror spectra, mass differences and retention times. We annotated the chemical structures using *MolNetEnhancer* workflow. Figure 4 shows a total of 3 chemical superclasses, including phenylpropanoids and polyketids; organoheterocyclic compounds; lipids and lipid-like molecules.

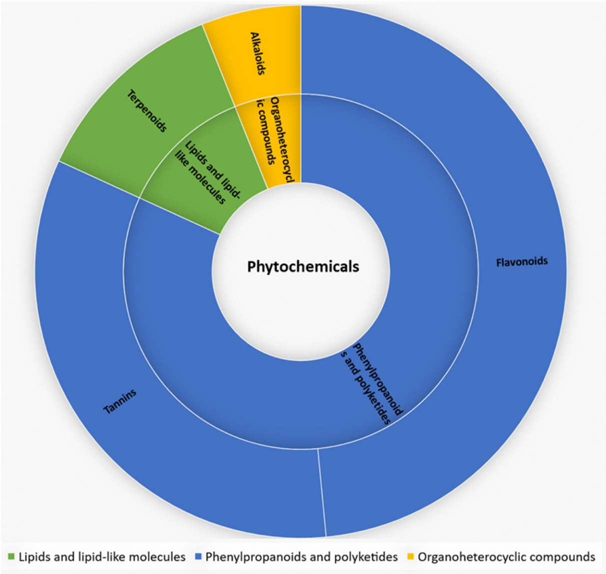

Figure 4. Sunburst plot of phytochemicals superclasses and classes.

Annotated nodes in molecular networking

The characteristic features of the mass spectra of tannins, flavonoids, alkaloids and lipids and lipid-like molecules have been studied (Figure 5). In this study, compounds' chemical structures were identified by using MN analysis and databases. Also, others structures were manually determinated by analyzing relationship between compounds from the same clusters in the MN and mass spectra.

Flavonoids identificaion

Flavonoids annotated in this study are shown in Figure 6, they included compounds matched with GNPS databases *i.e* : luteolin-4'-O-glucoside ($C_{21}H_{20}O_{11}$; *m/z* = 449.10 ; Rt = 2.14), cosmosiine ($C_{21}H_{20}O_{10}$; *m/z* = 433.11 ; Rt = 2.38), quercetine-4'-O-glucoside ($C_{21}H_{20}O_{12}$; *m/z* = 465.103 ; Rt = 2.11), peonidin-3-O-glucoside ($C_{22}H_{23}O_{11}^{+}$; *m/z* = 463.12 ; Rt = 2.47), glycitin ($C_{20}H_{20}O_{10}$; *m/z* = 447.12 ; Rt = 2.97).

In this cluster we identified manually a compound with *m/z* = 725.18 (Rt = 4.18, $[M+H]^{+}$) corresponding to $C_{33}H_{40}O_{18}$ (Figure 7). This compound structure is determinated by MN analysis. It is related to daidzeine-4',7-diglucoside (*m/z* = 579.154 ; Rt = 3.43 ; $C_{27}H_{30}O_{14}$) with a cosine score = 0.78. The difference between their masses suggests the presence of an additional pentose which turns out to be arabinose as being the most dominant pentose in the flavonosides biosynthesis.[19]

In the flavonoids cluster, another compound related to glycitin ($C_{20}H_{20}O_{10}$; *m/z*=447.12 ; Rt = 2.97) cosine score = 0.77 is putatively annoted (Figure 8). Indeed, the structural

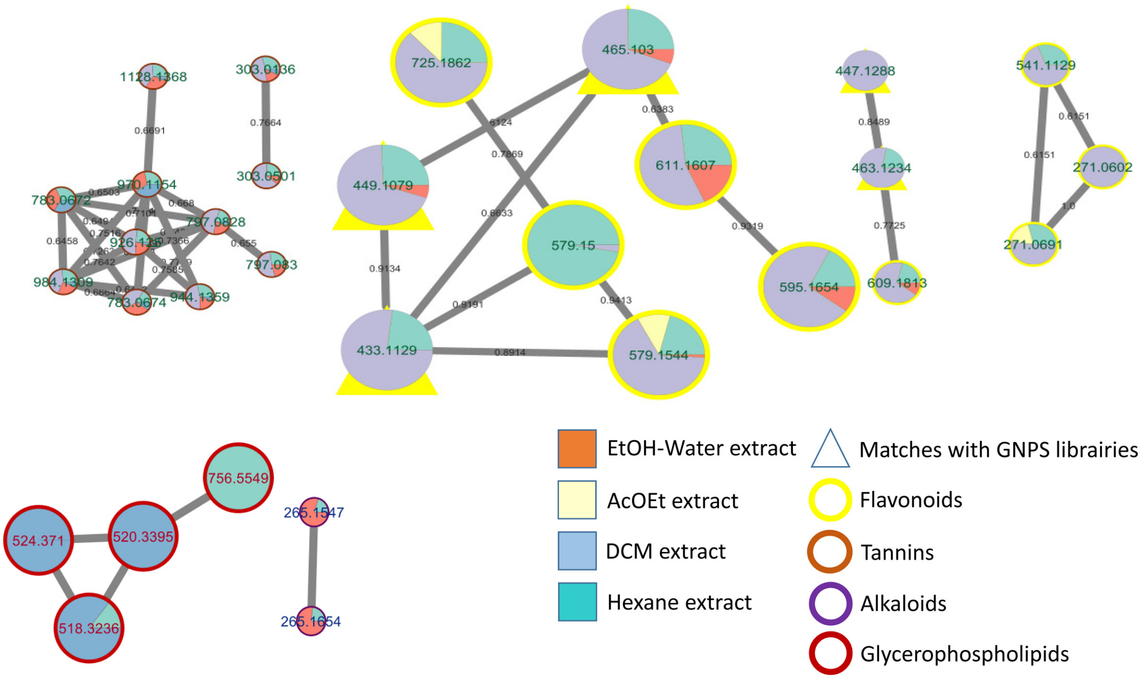

Figure 5. Phytochemicals classes molecular netwotking of *C. brocchiana* differents extracts

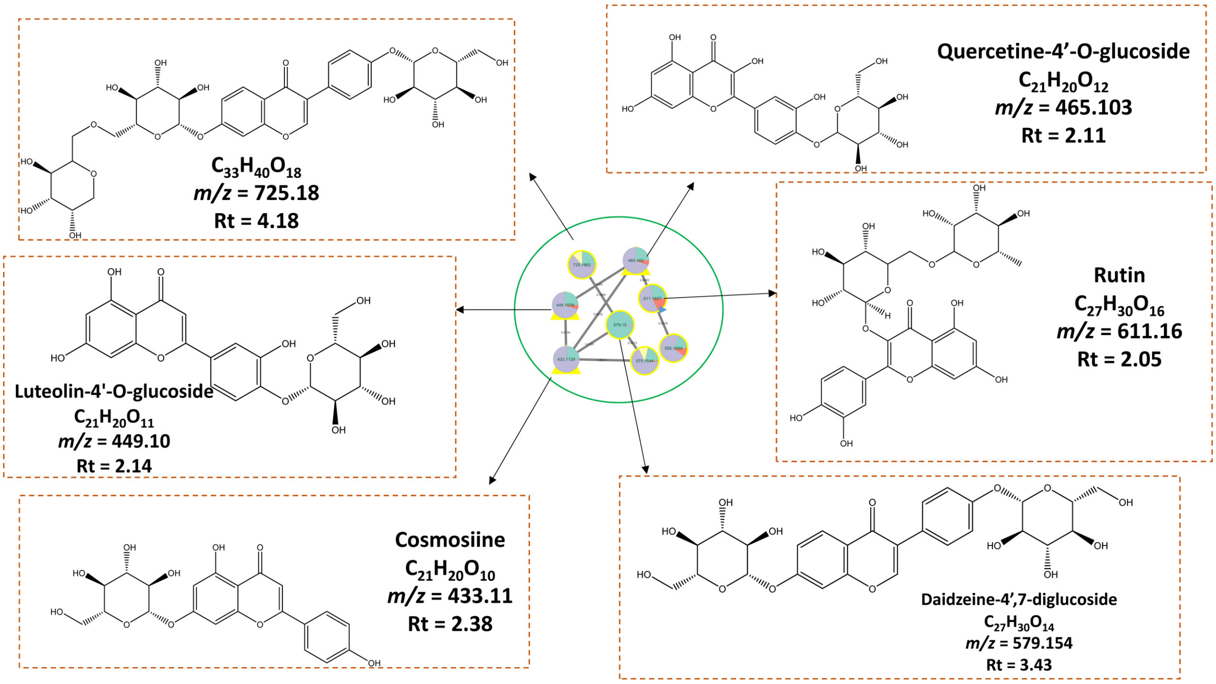

Figure 6. Flavonoids annotated cluster

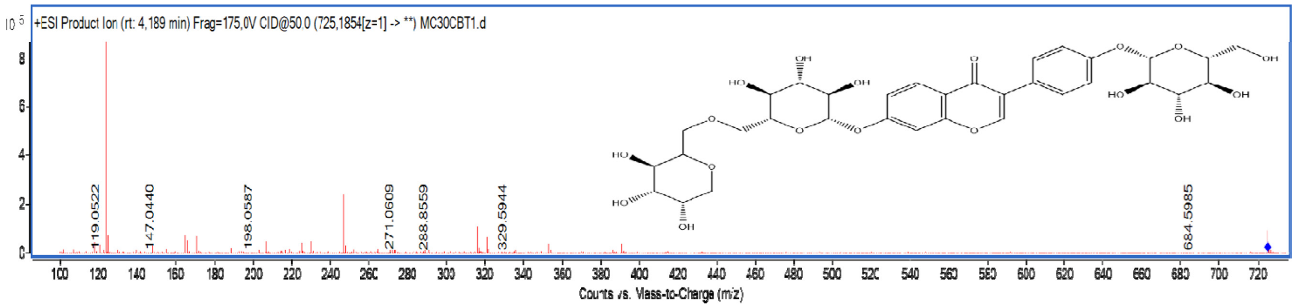

Figure 7. Typical MS/MS spectrum of compound at *m/z* = 725.18

determination of the compound at *m/z* = 609.18 ($[M+H]^+$; $C_{28}H_{32}O_{15}$; Rt = 2.44) was possible by analyzing the mass spectrum (Figure 9). Comparison of its mass spectrum with that of glycitin indicated a difference which could correspond to additional presence of a sucrose, notably arabinose. It appears that this pentose replaced a methyl group present on glycitin.

Literature data allowed us to identify genistein ($C_{15}H_{10}O_5$; *m/z* = 271.06 ; Rt = 2.05), remikiren ($C_{15}H_{10}O_5$; *m/z* = 271.06 ; Rt = 3.11) and astragalin-7-rhamnoside ($C_{27}H_{30}O_{15}$; *m/z* = 595.16; Rt = 3.43) (Table 1). These compounds were previously isolated from many species belonging to Chrozophora genus such as *Chrozophora rottleri.*[20]

Tannins identification

In tannins cluster, 3 compounds were putatively annotated using literature and mass spectra data. They are : ellagic acid ($C_{14}H_6O_8$; *m/z* = 303.01 ; Rt = 2.09),[20] Hesperetin ($C_{16}H_{14}O_6$; *m/z* = 303.01 ; Rt = 2.12)[7] and 13-hydroxyterracinolide B ($C_{38}H_{42}O_{18}$; *m/z* = 797.08 ; Rt = 2.07) (Figures 10-13).

Compound at *m/z* = 783.06 ($C_{34}H_{22}O_{22}$; Rt = 1.58 ; Score = 98.24 ; Δppm = -0.34) was identified using the MassHunter software (table 2). It could not be tentatively identified by online literature and databases search. That suggests the presence of so far undescribed tannin compound in the Euphorbiaceae family.

Alkaloids and lipids and lipids-like molecules identification

In the alkaloids and lipids and lipid-like molecules clusters (Table 3), molecular formula generated with Masshunter software did not match with any compound in the differents databases and in the literrature. The com-

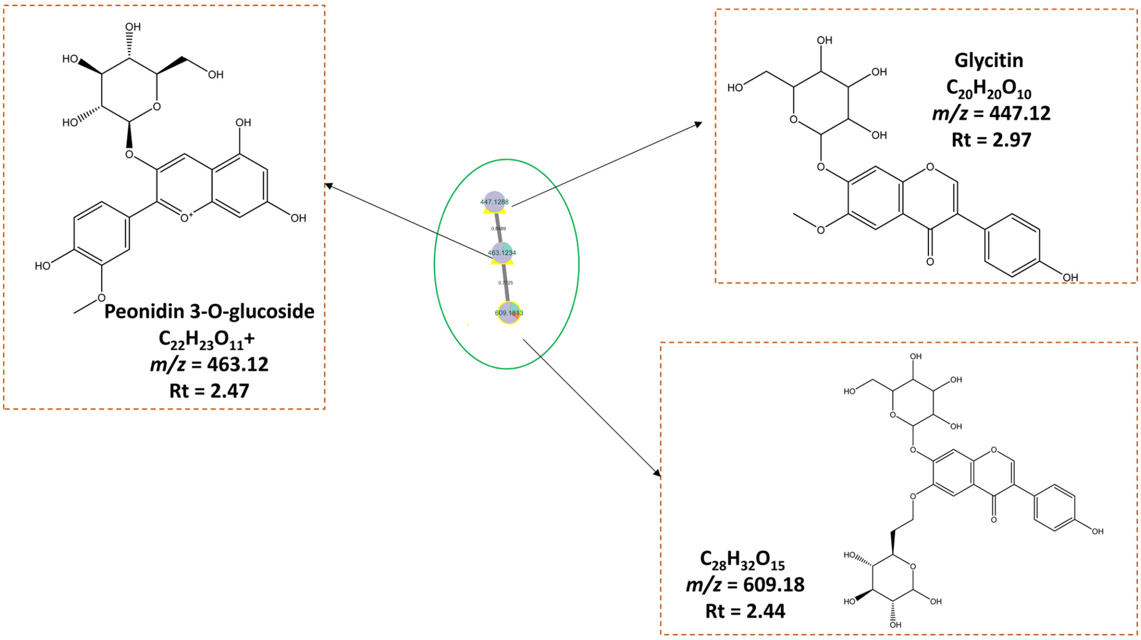

Figure 8. Glycitin cluster annotated

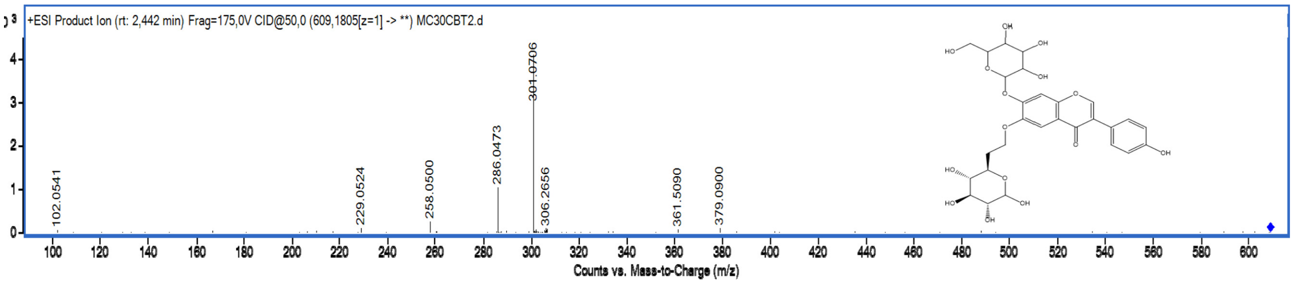

Figure 9. MS/MS spectra of compound *m/z* = 609.18

Table 1. Flavonoids identified from *C. brocchiana* extracts

Retention time	*m/z* value	Proposed formula (name)	Identification level
2.05	271.0602	$C_{15}H_{10}O_5$ (Genistein)	2
3.11	271.0691	$C_{15}H_{10}O_5$ (Remikiren)	2
2.11	303.05	$C_{14}H_6O_8$ (Quercetin)	2
2.38	433.1129	$C_{21}H_{20}O_{10}$ (Cosmosiine)	2
2.97	447.1288	$C_{20}H_{20}O_{10}$ (Glycitin)	2
2.14	449.1079	$C_{21}H_{20}O_{11}$ (Luteolin-4'-O-glucoside)	2
2.47	463.1234	$C_{22}H_{23}O_{11}^+$ (Peonidin-3-O-glucoside),	2
2.11	465.103	$C_{21}H_{20}O_{12}$ (Quercetine-4’-O-glucoside)	2
3.43	579.15	$C_{27}H_{30}O_{14}$ (Daidzeine-4’,7-diglucoside)	2
6.40	579.1544	$C_{27}H_{30}O_{14}$ (Lespenefril)	2
5.45	595.1654	$C_{27}H_{30}O_{15}$ (Astragalin 7-rhamnoside)	2
3.90	611.1607	$C_{27}H_{30}O_{16}$ (Rutin)	2
2.44	609.1813	$C_{28}H_{32}O_{15}$	3
4.18	725.1862	$C_{33}H_{40}O_{18}$	3

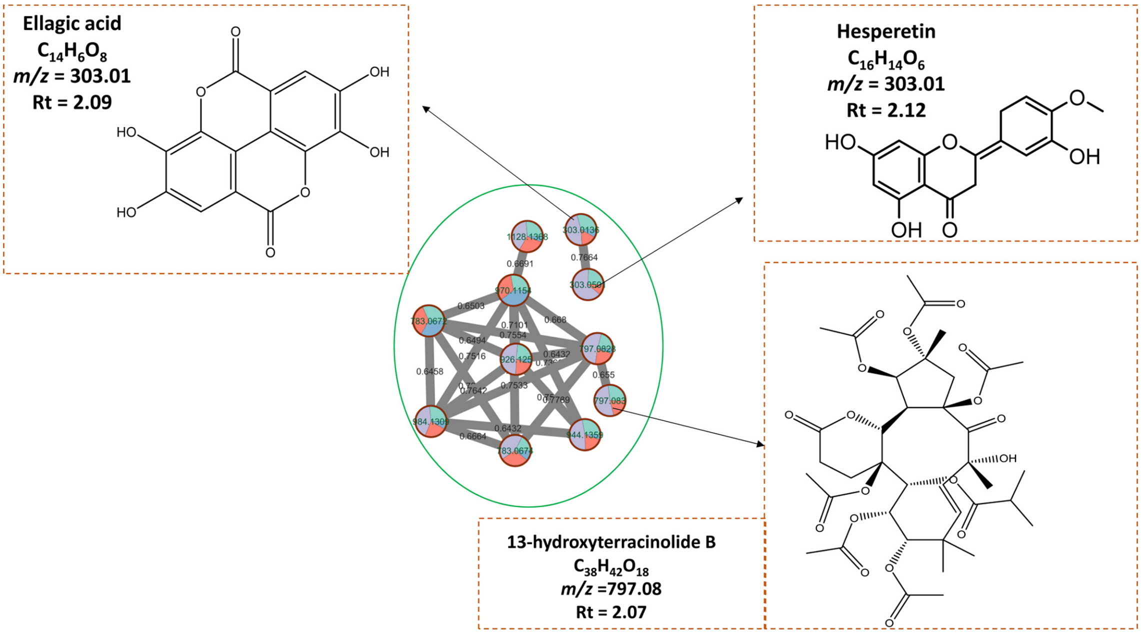

Figure 10. Tannins annotated cluster.

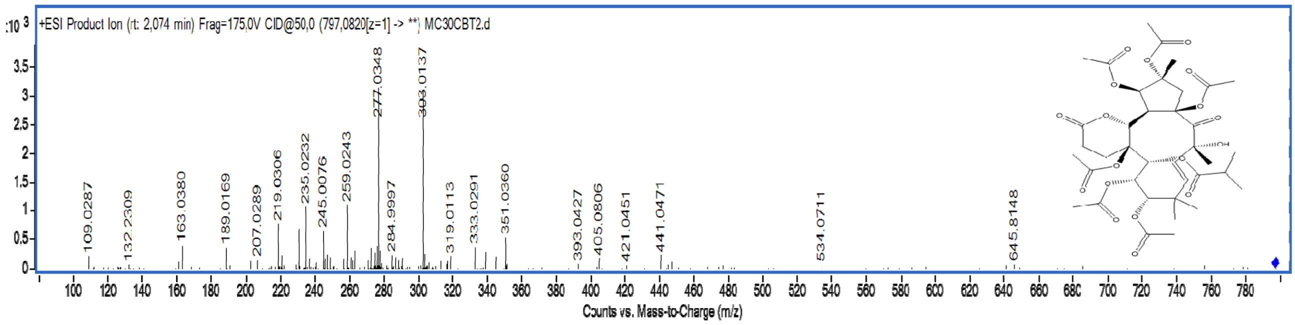

Figure 11. MS/MS spectra of 13-hydroxyterracinolide B.

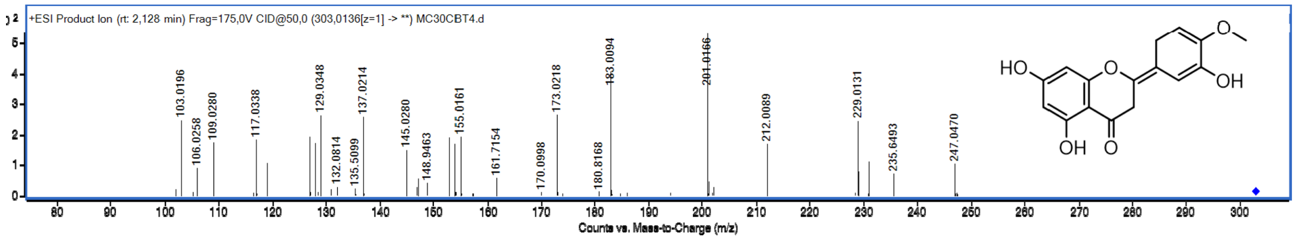

Figure 12. MS/MS spectra of Hesperetin.

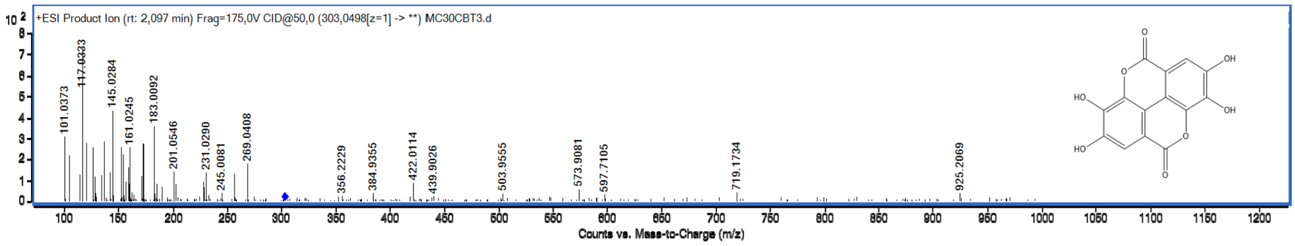

Figure 13. MS/MS of ellagic acid.

pound at $m/z = 518.32$, $[M+H]^+$ cannot be mesanaic acid G which is an isomeric terpene[21] because the relationships between compounds 518, 520 and 524 would suggest that we have a polyunsaturated compound based on their differences of masses ($\Delta m/z = 2$; $\Delta m/z = 4$ and $\Delta m/z = 6$). That suggests the presence of so far undescribed compound in the Euphorbiaceae family. Their structures must be elucidated by NMR spectroscopy after isolation and purification.

The computed feature-based molecular network (FBMN) contained nodes matched to unknown metabolites. These metabolites were further validated through manual confirmation by comparing the mirror spectra, mass differences, and retention times (Table 4), to ensure the accuracy of metabolite identification to levels 3 and 4 as classified by the Metabolomics Standard Initiative (MSI). In fact, We tentatively identified their classes using literature and databases. For those (unknown compounds), that can not be identified by this methods, we used masshunter

Table 2. Identified tannins from *C. brocchiana* extracts.

Retention time	*m/z* value	Proposed formula (Name)	Identification level
2.09	303.04	$C_{14}H_6O_8$ (Ellagic acid)	2
2.12	303.01	$C_{16}H_{14}O_6$ (Hesperetin)	2
6.48	477.06	$C_{23}H_{24}O_{11}$ (Hesperetin 3'-O-glucuronide)	2
2.07	797.0828	$C_{38}H_{42}O_{18}$ (13-hydroxyterracinolide B)	2
1.58	783.06	$C_{34}H_{22}O_{22}$	4

Table 3. Alkaloids and lipids and lipid-like identified compounds.

Retention time	*m/z* values	Formula	Score	Δppm	Class	Identification level
1.36	265.15	$C_{14}H_{20}N_20_3$	99.55	0.34	Alkaloids	4
9.82	518.32	$C_{30}H_{45}0_7$	91.82	0.13	Lipids and lipid-like molecules	4
9.18	520.33	$C_{30}H_{47}0_7$	93.55	0.24	Lipids and lipid-like molecules	4
10.24	524.37	$C_{30}H_{51}0_7$	93.55	0.95	Lipids and lipid-like molecules	4

Table 4. Others compounds classes identified from *C. brocchiana* extracts.

Retention time	*m/z* value	Proposed formula (name)	Class	Identification level	References
1.96	355.23	$C_{16}H_{18}O_9$ (Cryptochlorogenic acid)	Phenolic acid	2	20
2.61	576.36	$C_{27}H_{29}NO_{13}$ (Cartormin)		2	20
6.38	625.15	$C_{29}H_{36}O_{15}$ (Isoacteoside)	Glycosides	2	20
2.77	194.05	$C_{10}H_{10}O_4$ (Isoferulic acid)	Phenolic acid	2	20
2.01	343.11	$C_{12}H_{22}O_{11}$ (Isomaltulose)	Carbohydrate	2	20
0.45	365.10	$C_{20}H_{28}O_6$ (Phorbol)	Tigliane type-diterpene	2	22
2.56	367.20	$C_{20}H_{30}O_6$ (Ingol)	Lathyrane diterpenoids	2	22
9.58	429.35	$C_{30}H_{52}O$ (Friedelinol)	Triterpenoids	2	22
10.23	347.09	$C_{20}H_{26}O_5$ (Euphelionolide)	Diterpenoids	2	22
3.26	734.45	$C_{41}H_{49}O_{12}$	Premyrsinane	2	23
7.98	782.56	$C_{45}H_{42}O_{12}$	Premyrsinane	2	24,25
1.24	146.06	$C_7H_{15}NO_2$	Amino acid	2	20
7.54	545.45	$C_{32}H_{48}O_7$	Ingenane	2	26
1.83	565.15	$C_{26}H_{28}O_{14}$	homoisoflavonoid	2	27
1.65	165.06	$C_9H_8O_3$	Unknown	4	
1.54	371.22	$C_{22}H_{42}O_4$	Unknown	4	
11.39	495.07	$C_{25}H_{18}O_{11}$	Unknown	4	
4.77	339.12	$C_{16}H_{18}O_8$	Unknown	4	
2.77	597.16	$C_{34}H_{44}O_9$	Unknown	4	
7.98	782.56	$C_{44}H_{47}NO_{12}$	Unknown	4	
1.54	371.22	$C_{22}H_{42}O_4$	Unknown	4	

software to detect their formulas. Their structures must be elucidated by NMR spectroscopy after isolation and purification.

Natural products are the richest sources of chemical compounds for improving health. However, chemical profiling of a sample remains challenging, requiring the identification of bioactive compounds from complex extracts.[28] Artificial intelligence technology in natural products research became a solution. MS/MS-based GNPS molecular networking can produce visual networks consisting of chemically associated compounds and enables the identification of known compounds, structural class and analogs.[29] The *MS2LDA* tool enables the discovery of unsupervised groups of neutral losses and mass fragments, called Mass2Motifs, which can show how compounds in the same chemical class are different in their substructures.[30] MS/MS-based GNPS approach can allow us to prioritize various extracts in the early fractionation process and improve the accuracy of target compound prediction.

Conclusion

The computational metabolomic strategies used herein helped in charting the chemical diversity of *C.brocchiana* extracts. A MN analysis based on UHPLC-QTOF-MS/MS was used to identify known metabolites through spectral library matching, and metabolites that have not been previously described were putatively annotated. The untargeted metabolomic analysis revealed diversity in the chemical family composition of extracts. Flavonoids were the main family, followed by tannins, lipids, and lipid-like molecules, and alkaloids. This novel mapping of the metabolomic landscape of *C.brocchiana* provides actionable insights into plant biochemistry and justifies certain medicinal uses. Further studies should focus on using more computational or analytical tools to discover metabolites and developing automated approaches to elucidate unknown compounds. Also, biological tests will be carried out to explore therapeutic properties of the most dominant compounds from this specie.

Conflicts of interest

Authors declare no conflicts of interest.

Acknowledgements

We acknowledge the Mass Spectrometry platform of Laboratoire de Biomolecules, conception, Isolement et Synthese (BioCIS/UMR 8076 CNRS) at Université Paris-Saclay. Specifically, we acknowledge Mehdi BENIDDIR (mehdi.beniddir@universite-paris-saclay.fr) for his help with MZmine software. The authors are indebted to Ambassade de France au Niger and Campus France for funding (grant to M.C.).

References

1. Hussein, H.; Mariod, A.A. So*udan j. agric. res.* **2006**, 6(18) , 53. https://doi.org/10.3989/gya.0939182.
2. Manal, A. A.; Mariod, A.A. *Bangladesh J. Bot.* **2020**, 49(08) , 1. https://doi.org/10.3329/bjb.v49i1.49085.
3. Nassirou, R.S.; Ibrahim, M.L.; Ilagouma, A.T.; Aboubacar, M.; Morou, M.; Abdoulaye ,A.; Ouwe, M.O.; Ikhiri, K., *J. Appl. Biosci.* **2015**, 89(18) 8291. http://dx.doi.org/10.4314/jab.v89i1.8
4. Chaibou, M.; Habibou, H.H; Bamba, M.A.N.; Chaibou, Z.O.; Idrissa, M.; Ikhiri, K., *J. Pharmacogn. Phytochem.* **2020**, 9(1) 126. https://doi.org/10.19044/esj.2020.v16n6p126.
5. Mahamane, I.A; Bakasso, S.; Alio, S.A; Ilagouma, A.T; Chaïbou,Y.; Chaibou, M.; Adamou, I.; Kiendrebeogo, M. *J. Dis. Med. Plants.* **2020**, 06(04) 15. https://doi.org/10.11648/j.jdmp.20200604.15.
6. Beniddir, M. A.; Champy, P.; Poupon, E. *Nat. Prod.* **2019**, 10(39), 36. https://doi.org/10.1039/C9NP00006B.
7. Kim, C.-K.; Yu, J.; Lee, M. *Foods*, **2023**, 12(06), 1146. https://doi.org/10.3390/foods12061146.
8. Kim, C.K.; Ahn, J.; Yu, J.; Le, D.; Han, S.; Lee, M. *Antioxidants,* **2022,** 11(01), 989. https://doi.org/10.3390/antiox11101989.
9. Nothias, L-F., Petras, D., Schmid, R., Duhrkop, K., Rainer, J., Sarvepalli, A., Protsyuk, I., Ernst, M., Tsugawa, H., Fleischauer, F., Aksenov, A., Alka, O., Allard, P-M., Barsch, A., Cachet, X. . *Nat. Methods,* **2020,** 17(33), 905. https://doi.org/10.1038/s41592-020-0933-6.
10. Lephatsi, M.M.; Choene, M.S.; Kappo, A.P.; Madala, N.E.; Tugizimana, F. *Metabolites,* **2023**, 13(01), 1104**.** https://doi.org/10.3390/metabo13101104.
11. Pluskal, T.; Castillo, S.; Villar-Briones, A.; Oresic, M. *BMC Bioinform.* **2010**, 11(86), 395. https://doi.org/10.1186/1471-2105-11-395.
12. Yang, J. Y., Sanchez, L. M., Rath, C. M., Liu, X., Boudreau, P. D., Bruns, N., Glukhov, E., Wodtke, A., D., Felicio, R., Fenner, A., Wong, W. R., Linington, R. G., Zhang, L., Debonsi, H. M., Gerwick, W. H.. *J. Nat. Prod.* **2013,** 76(9), 1686. https://doi.org/10.1021/np400413s.
13. Schmid, R., Heuckeroth, S., Korf, A., Smirnov, A., Myers, O., Dyrlund, T. S., Bushuiev, R., Murray, K. J., Hoffmann, N., Lu, M., Sarvepalli, A., Zhang, Z., Fleischauer, M., Dührkop, K., Wesner, M., Hoogstra, S. J., Rudt, E., Mokshyna, O., Brungs, C., Pluskal, T. *Nat. Biotechnol.* **2023**, 41 (58), 447. https://doi.org/10.1038/s41587-023-01690-2.
14. Li, X.; Wang, P.; Tong, Y.;Liu, J.; Shu, G. *Molecules,* **2022,** 27(20), 6944. https://doi.org/10.3390/molecules27206944.
15. Akhona, M.; Mpho, C.; Abidemi, P.; Van Der Hooft, JJ. *Metabolomics,* **2024**, 20 (62), 2125. https://doi.org/10.1007/s11306-024-02125-y.
16. Ernst, M.; Kang, K.B.; Caraballo-Rodriguez, A.M.; Nothias, L.F.; Wandy, J.; Chen, C.; Wang, M.; Rogers, S.; Medema, M.H.; Dorrestein, P. C.. *Metabolites* **2019,**

9(07), 144. https://doi.org/10.3390/metabo9070144.

17. Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., Fan, T. W. M., Fiehn, O., Goodacre, R., Griffin, J. L., Hankemeier, T., Hardy, N., Harnly, J., Higashi, R., Kopka, J., Lane, A. N., Lindon, J. C., Marriott, P., Nicholls, A. W, M. R. *Metabolomics,* **2007**, 3(3), 113. https://doi.org/10.1007/s11306-007-0082-2.
18. Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. *Genome Res.* **2003**, 13(11), 24. https://doi.org/10.1101/gr.1239303.
19. Magozwi DK, Dinala M, Mokwana N, Siwe-Noundou X, Krause RWM, Sonopo M, McGaw LJ, Augustyn WA, T. V. *Pharm.* **2021**, 14(5), 428. https://doi.org/10.3390/ph14050428.
20. Nallapaty S, Narender M, Konidala SK, A. A. *Indian J Pharm. Educ. Res.* **2024**, 23(55), 513. https://doi.org/10.7324/JAPS.2024.155513
21. Huang H-T.; Lo I-W; Liao, G-Y; Lin, Y-C; Shen, Y-C. *Front. Chem.* **2022**, 10(03), 3356. https://doi.org/doi:10.3389/fchem.2022.1003356.
22. Mai, H.R.; Riham, A.; El Shiekh, A.; Shirley, R. *Inflammopharmacology* **2024**, 32(23) 461. https://doi.org/https://doi.org/10.1007/s10787-023-01298-7.
23. Wang, L.; Zang, Z.; Zhang, J.; Wu, X.; Huang, S.; Cao, P.; Zhao, Y. *Rec. Nat. Prod.*, **2015**, 9 (57) , 374. doi:http://doi.org/10.3390/molecules28010157.
24. Esposito, M.; Nothias, L.-F.; Retailleau, P.; Costa, J.; Roussi, F.; Neyts, J.; Leyssen, P.; Touboul, D.; Litaudon, M.; Paolini, J. *J. Nat. Prod.* **2017**, 06(44), 205. https://doi.org/https://doi.org/10.1021/acs.jnatprod.6b00644.
25. Esposito, M.; Nothias, L.-F; Pieter C.D., and Litaudon, M. *J. Nat. Prod.* **2019**, 26(41*)*, 407. https://doi.org/DOI:10.1021/acs.jnatprod.8b00916.
26. Lu, Z.-Q.; Yang, M.; Zhang, J.-Q.; Chen, G.-T.; Huang, H.-L.; Guan, S.-H.; Ma, C.; Liu, X.; Guo, D.-A. I. *Phytochemistry* **2007,** 09(13), 101. https://doi.org/https://doi.org/10.1016/j.phytochem..09.013.
27. Jadranin, M.; Savi, D.; Lupši, E.; Podolski-Reni, A.. *Plants* **2023**, 12(41) 81. https://doi.org/https:// doi.org/10.3390/plants12244181.
28. Caesar, L.K.; Kellogg, J.J.; Kvalheim, O.M.; Cech, R.A.; Cech, N. *Planta Med.* **2018***,* 05(90), 84. https://doi.org/http://doi.org/10.1055/a-0590-5223.
29. Qin, G.F.; Zhang, X.; Zhu, F.; Huo, Z.Q.; Yao, Q.; Feng, Q.; Liu, Z.; Zhang, G.M.; Yao, J.C.; Liang, H.. *Mol.* **2022**, 28(01), 154 https://doi.org/http://doi.org/10.3390/molecules28010157.
30. Cauchie, G.; N'Nang, E.O.; Van Der Hooft, J..; Le Pogam, P.; Bernadat, G.; Gallard, J.F.; Kumulungui, B.; Champy, P.; Poupon, E.; Beniddir, M.. *Org. Lett. ,* **2020**. 22(53). 21. https://doi.org/http://doi.org/10.1021/acs.orglett.0c02153.