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A Comprehensive Liquid Chromatography Mass Spectroscopy (LCMS) and Nuclear Magnetic Resonance (^{1}H-NMR) Metabolite Profiling of Methanolic Extract of *Prosopis Africana* Stem Bark

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ABSTRACT

The stem-bark of *Prosopis africana* commonly known as African mesquite or Iron tree, has been utilized in traditional medicine across Africa for its purported medicinal properties. This plant specie belongs to the Fabaceae family and is a native to Sub-Saharan Africa. Its bark has been traditionally used to treat various ailments, including malaria, fever, gastro-intestinal disorders and inflammatory conditions. This study provides a comprehensive profile of the methanolic crude extract obtained from the stem bark of *Prosopis africana* using LCMS molecular networking analysis and ^{1}H-NMR. A total of 47 metabolites were putatively identified in both positive and negative ion modes by the LCMS, while the ^{1}H-NMR spectroscopy validates the identities by providing some insights in their structures through assigning the chemical shifts that corresponds to their multiplicities. The 19 metabolites from the positive ionization were further classified as peptides, phenols, fatty acids, polyketides, steroids, lipids, amino acids, quinones, flavonoids, azaspiranes, fatty acyl glycines and acyl amino acids. On the other hand, the 28 metabolites from the negative ion mode were also categorized into different classes of metabolites which include: 6 alkaloids, 2 phenolic compounds, and four terpenoids. Other categories include: cycloalkene, polyether, triazines, anthraquinone, indole, carboxylic acid derivatives, flavonoid, glycoside and saponin. In addition, more than 26 clusters were also discovered using the MS/MS based molecular networking. The study contributes to the body of knowledge regarding the chemical constituents of *Prosopis africana* and provides a foundation for further research on its therapeutic applications in natural product-based medicine as metabolomics have never been used on *Prosopis africana* plant.

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1. Introduction

Prosopis africana commonly known as African mesquite or Iron tree, has been utilized in traditional medicine across Africa for its purported medicinal properties. This plant specie belongs to the Fabaceae family and is a native to Sub-Saharan Africa [1]. The stem-bark of this plant has been reported to contain several phytochemicals, such as, alkaloids, flavonoids, tannins and saponins among others which has been linked to its anti-inflammatory, analgesic, anti-diabetic and anti-microbial activities [2].

Prosopis africana has been used in traditional medicine for centuries due to its various therapeutic properties [3]. The leaves are used in the treatment of headache and toothache as well as various other head ailments. The leaves and bark are combined to treat rheumatism [4]. The bark is astringent. It is used in the treatment of skin diseases, caries and fevers. The bark is used to make eyewash. The roots are diuretic. They are used to treat gonorrhea, tooth and stomach-ache, dysentery and bronchitis [5].

The profiling of metabolites deals with the output of analytical techniques for qualitative and quantitative estimations of several plant metabolites. These metabolites were sequentially assessed through different statistical processes and measurements of various spectral and chromatographic peaks. These modern techniques can be utilized for comprehensive analysis of the constituents present in the plant samples [6].

The combination of NMR and MS enhances the confidence in metabolite profiling, with mass spectroscopy being highly sensitive and selective across a wide range of metabolite. The two complementary methods in metabolic analysis, targeted and untargeted, differ in their approaches. Targeted analysis, usually hypothesis-driven, identifies and quantifies selected metabolites with known chemical properties, enabling tailored sample preparation to reduce matrix effects and interference. Conversely, untargeted analysis generates hypotheses by ideally measuring all metabolites in a biological system [7].

The use of NMR in metabolic studies has a long history. ^{1}H-NMR was firstly used to monitor phosphorous-containing metabolites, such as nucleotide and sugar phosphates, including redox species, in cells and tissues. Researchers in the late 1970s optimistically stated ‘it is now possible to obtain on metabolites *in-vivo* the kinds of detailed information about structure, motion, reaction rates, and binding sites that have been obtained by NMR studies of purified biomolecules in solution [8].

Liquid chromatography – mass spectroscopy (LCMS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectroscopy (MS). Coupled chromatography – MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectroscopy provides spectral information that may help to identify each separated component [9].

LCMS has emerged as a potent technique for the swift and thorough analysis of intricate phytochemical mixtures. This method facilitates the identification and quantification of a diverse array of compounds including those present in trace amounts [10].

LCMS is a powerful analytical technique used for separation, identification, and quantification of both unknown and known compounds as well as to elucidate the structure and chemical properties of different molecules. It is very useful for analyzing small molecules and offers higher sensitivity and selectivity in the trace analysis of multicomponent containing substances [11].

2. Methodology

2.1 Collection and Identification of the Plant

Fresh stembark of *Prosopis africana* was collected at Gubi dam, within Bauchi metropolis. The plant sample was authenticated at the Department of Applied Botany, Abubakar Tafawa Balewa University Bauchi, Nigeria. The plant sample was air dried to avoid heat destruction of the active components by the sun. The dried sample was pounded into fine powder and was stored in an air tight container at room temperature.

2.2 Extraction of Prosopis Africana Stembark

A 100 g sample was extracted using the maceration method with 350 cm^3 of a solvent mixture consisting of 70% methanol and 30% water for 72 hours. The extract was then filtered, concentrated using a rotary evaporator, and air-dried at room temperature.

2.3 Preparing Samples for LCMS Analysis

To perform LCMS analysis, the following preparation of sample was made: The *Prosopis africana* stembark extract was dissolved in LCMS-grade methanol at the concentration of 2 mg/mL The solution was then subjected to ultrasonication for a duration of 10 minutes. The resulting solution was filtered through a nylon membrane with a pore size of 0.22 mm, this enables the removal of any potential precipitates. The clarified solution was collected in 2 mL screw-capped sample vial. The LCMS analysis was carried out using a Thermo-Scientific TMQ extractive TM Hybrid Quadrupole-Orbitrap mass spectrometer which was a coupled with a Dionex Ultimate 3000 UHPLC system from Thermo Fisher Scientific Inc., based in Waltham, MA, USA. Within the system, was employed an Acquity UPLC BEH C18 column from waters (Milford, MA, USA) with dimensions of 1.7 µm x 2.1 mm x 100 mm. The mobile phase consists of two components: solvent A, which was composed of 0.1 % formic acid in deionized water, and solvent B, composed of 0.1% formic acid in LCMS-grade acetonitrile. During the analysis, 5 µL injection volume was used, a 30 minutes' analysis time, and maintained at the flow rate of 0.25 mL/min. the gradient program was initiated with 10 % solvent B at 0 minutes, followed by increases 20 % at 1.00 minutes, 30 % at 2.00 minutes 70% at 7.00 minutes, 80 % at 10.00 minutes, 90 % at 12.00 minutes and reaching 100 % at 13.00 – 30 minutes. To identify the molecular ions, electrospray ionization nodes was employed, switching them and conducting a full scan ranging from m/z 100 to 1500 amu. Other mass spectrometer parameters included a collision energy of 30 eV, a spray voltage of 4.2 kV, a capillary temperature of 350 ^{0}C, a sheath gas flow rate of 50, and an auxiliary nitrogen (99 % pure) gas flow rate of 10. The mass resolution was set at 70,000 full widths at half maximum (FWHM). Also the UV detectors was utilized and set at 254, 280, 400 and 440 nm, with the PDA detector covering a range of 190-600 nm. Metabolite assignments were made using retention time, UV-vis spectra, and MS data, including accurate mass, in both positive and negative ion modes, with the assistance of Thermo X-calibur 2.0 software Thermo Fisher Scientific Inc.

In addition, the comparisons and validation of data was conducted from literature sources and freely available standard online databases such as Metabolomics workbench, Human Metabolome Database (HMDB), PubChem and MassBank. For compound fragmentation, the HighChem mass frontier 3.0 from Thermo Fisher Scientific Inc was used. The Data processing and analysis involved the used of Chenomx software (version 5.1, Edmonton, AB, Canada) and Chemdraw ultra 12.0 for NMR spectra phasing and baseline correction [12].

2.4 Preparing Samples for ^{1}H-NMR Analysis

The methanolic extract of *Prosopis africana* stembark (7:3) methanol: water was subjected to H-NMR for analysis.

Prosopis africana stem bark extract (15 ml) was combined with 1ml methanol. The mixture was vortexed for 10 minutes, sonicated for 10 minutes, centrifuged for 10 minutes, and then about 0.6 ml of the fine solution was distributed into 5 mm NMR tubes for analysis.

2.5 Molecular Networking

The Global Natural Products Social Molecular Networking (GNPS) platform, an online workflow, was employed to generate the molecular networks based on MS/MS data (https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp). Using MS convert software that was downloaded from the Proteo-wizard website (http://proteowizard.sourceforge.net/tools.shtml), subsequently, the raw MS data were transformed into MZXML format using the MS convert, before being uploaded to GNPS software at (https://filezilla-project.org). The GNPS data processing pipeline, with precursor ion mass tolerance set to 0.02 Da. A network was processed by filtering edges with cosine scores greater than 0.7 and at least six matched peaks [13]. Following processing, the output was downloaded, and the network was visualised using Cytoscape 3.7.1 software (Institute of Systems Biology, Seattle, WA, USA) and the ChemViz 1.3 plugin (available at http://www.cgl.ucsf.edu/cytoscape/chemViz/).

3. Result and Discussion

3.1 LCMS Analysis

The methanolic extract of *Prosopis africana* stembark analysed by LCMS was presented in Table 1 and 2. The total ion chromatogram of the extract is shown in Figure 1 and 2 and the observed peaks indicating different metabolites present in the extract and were labeled with numbers. Positive and negative ion switching was used to conduct the mass analysis, as larger coverage of the metabolome can be achieved by using both positive and negative ionisation modes to detect compounds than doing so with a single mode [14,15]. The chemical structures were provided by databases like HMDB, metabolomics work bench and PubChem, in which spectral interpretation software predicts and automatically creates precise fragmentation in accordance with the general principles of ionisation, fragmentation and rearrangement.

The total of 47 different metabolites have been putatively identified in both positive and negative ion mode. About 19 different metabolites was discovered at the positive ion mode while 28 metabolites were from the negative ion mode. The compounds identified, peak numbering, retention time, molecular: formula and mass, parent ion [M+H], delta value, MS/MS (m/z) and main classifications were all captured as shown in the Tables 1 and 2. Among the 19 putative components from the positive ionisation mode, which belong to different classes and categories of metabolites that include: peptides, phenols, fatty acids, polyketides, steroids, lipids, amino acids, quinones, flavonoids, azaspiranes, fatty acyl glycines and acyl amino acids. The 28 metabolites from the negative ionisation also belongs to different classes of metabolites which includes; 6 alkaloids, 2 phenolic compounds and 4 terpenoids. Other categories include; cycloalkene, polyether, triazines, anthraquinone, indole, carboxylic acid derivatives, flavonoid, glycoside and saponin.

Table 1
Putative metabolites from the positive ionisation mode of *Prosopis africana* stembark

Peak	Compound name	Retention time t_R (min)	Molecular formula	Molecular mass (g/mol)	Parent ion $[M + H]^-$	Delta value	MS/MS (*m/z*)	Class
1	Methyl(s)-2-[[(E)-4-[[(2s)-1-oxopropan-2-yl]amino]-4-oxobut-2-enoyl]amino]propanoate	1.02	$C_{12}H_{18}N_2O_6$	286.28	287.1234	0.0004	241.1184,213.3236,197.0922,186.0760(BP)	Peptide
2	Gallic acid	4.19	$C_7H_6O_5$	170.12	171.0288	0.0000	155.0212,153.0516(BP), 139.0027,135.0441	Phenolic acid
3	N-decanoyl-L-hemisterine lacton	5.30	$C_{14}H_{25}NO_3$	255.35	256.1906(BP)	0.0001	238.1801,214.1800,196.1696,192.1371	Amide derivative of fatty acid
4	2-Methoxybenzaldehyde	6.74	$C_8H_8O_2$	136.15	137.600	0.0003	136.02169(BP),128.0891,122.0362,109.0286	Benzaldehyde dervative
5	Marinoid F	7.20	$C_{14}H_{19}ClO_8$	350.75	351.0849	0.0008	261.1023,216.6132,209.1528(BP),191.1432	Polyketide
6	3-beta-Methoxypregna-5,20-diene	7.87	$C_{22}H_{34}O$	314.50	315.279(BP)	0.0037	297.2614,279.2509,267.2509,253.2354	Steroid
7	N-Palmitoylglycine	7.95	$C_{18}H_{35}NO_3$	313.50	314.2680(BP)	0.0010	296.258,278.2477,266.2476,252.2320	N-acyl amino acid
8	Dibromoacetic acid	8.05	$C_2H_2Br_2O_2$	217.84	216.6825	0.1669	212.2011(BP),201.3782, 194.2467,155.6604	Haloacetic acid
9	4-Hydroxydodecanoic acid	8.10	$C_{12}H_{24}O_3$	216.32	217.1785	0.0013	213.8751,212.2006(BP), 208.5305,194.1906	Fatty acid
10	3-{[(9Z)-3-hydroxyhexadec-9-enoyl]oxy}-4-(trimethylammonio)butanoate	8.44	$C_{23}H_{43}NO_5$	413.60	414.3210 (BP)	0.0004	396.3113,382.2449,332.2782,314.2688	Betaine lipid
11	N-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glutamic acid	8.57	$C_{27}H_{43}NO_5$	461.60	462.3218(BP)	0.0004	444.3114,426.3008,340.2846,322.2740	Acyl amino acid
12	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone	9.15	$C_8Cl_2N_2O_2$	227.00	226.9290	0.0120	213.9177(BP),211.0968, 165.5184,141.8339	Quinone
13	19,20-dihydrophomacin C	9.45	$C_{25}H_{39}NO_4$	417.6	418.2950(BP)	0.0002	400.2847,388.5383,296.2583,278.2478	Homoisoflavonoid
14	N-(9Z-hexadecenoyl)-tyrosine	9.62	$C_{25}H_{39}NO_4$	417.28	418.2949(BP)	0.0003	400.2844,296.2582,278.2477,266.2480	Acyl amino acid
15	Atiprimod	9.93	$C_{22}H_{44}N_2$	336.6	337.3593	0.0016	332.2776,313.2565,303.5558,296.2006	Azaspirane

16	Palmitoleoyl Ethanolamide	10.17	$C_{18}H_{35}NO_2$	297.5	298.2735(BP)	0.0005	280.2632,254.2379,236.2371,213.8803	N-acyl ethanolamino acid
17	1-methoxy-3-[(Z)-pentadec-10-enyl]benzene	10.28	$C_{22}H_{36}O$	316.5	317.2874(BP)	0.0035	299.2769,281.2666,263.2564,251.2553	Methoxyalkylbenzene
18	Tricosanoylglycinec	10.79	$C_{25}H_{49}NO_3$	411.7	412.3775(BP)	0.0010	394.3596,328.3233,310.3102,292.2996	Fattyacyl glycine
19	Cyclooctyne	11.57	C_8H_{12}	108.18	109.1014	0.0010	81.0338,79.0541,67.0546,67.56.9583	Cycloalkyne

Table 2
Putative metabolites from the negative ionisation mode of *Prosopis africana* stembark

Peak	Compound name	t_R (min)	Molecular formula	Molecular mass (g/mol)	Delta value	Parent ion $[M - H]^-$	MS/MS (*m/z*)	Class
1	Hemibrevetoxin B	0.70	$C_{28}H_{42}O_7$	490.6	0.0030	489.2828	442.8968,(BP),414.9019,386.9074,374.9218	Poly ether
2	Trichlorohydroquinone	0.94	$C_6H_3Cl_3O_2$	213.4	0.0035	210.9091	196.8944(BP),194.8789,180.8992,178.8836	Chlorinated phenolic compond
3	7-Hydroxy-5-hydroxymethyl-2h-benzo[1,4]thiazin-3-one	2.89	$C_9H_9NO_3S$	211.24	0.0165	210.0065(BP)	202.9967,168.0069,139.9981,138.0068	Alkaloid
4	Penialidin A	4.18	$C_{14}H_{12}O_8$	308.24	0.0001	307.0461(BP)	289.0355,277.0354,263.0576,245.0454	Polyketide
5	Scetryptoquivaline A	4.39	$C_{24}H_{22}N_4O_6S$	494.5	0.0016	493.1171(BP)	433.1011,403.0877,373.0765,331.1042	
6	4-Dodecylphenol	5.17	$C_{18}H_{30}O$	262.4	0.0112	261.2112	246.9924(BP),238.0279,212.6190,191.0559	Phenolic compound
7	Melem	5.29	$C_6H_6N_{10}$	218.18	0.0016	217.0688	213.0557(BP),194.9934,188.9682,169.0141	Triazine
8	6-Bromoindole-3-carboxaldehyde	5.32	C_9H_6BrNO	224.05	0.0290	221.9270	211.023(BP),193.0487,181.0499,167.0346	Alkaloid
9	Falconensin N	5.86	$C_{22}H_{24}Cl_2O_7$	471.3	0.0013	469.0839	463.1484(BP),431.6583,417.1780,387.9002	Polyketide
10	(2S)-2-Isopropyl-3-oxosuccinate	6.48	$C_7H_{10}O_5$	174.15	0.0007	173.0449(BP)	167.5400,155.0343,137.0239,130.0865	Dicarboxylic acid
11	Isochlorogenic acid	6.80	$C_{16}H_{18}O_9$	354.31	0.0000	353.0878(BP)	338.0640,323.0428,303.5832,289.8474	Terpenoid

12	Panosialin C	7.14	$C_{22}H_{38}O_8S_2$	494.7	0.0010	493.1925(BP)	475.1814,433.1710,317.1611, 267.0718	Arylsulfate
13	Dichotocejpin A	7.32	$C_{14}H_{14}N_2O_3S$	290.34	0.0004	289.0713(BP)	271.0621,253.0502,216.6747, 179.0341	Sesqiterpene
14	2-hydroxyemodic acid	7.83	$C_{15}H_8O_8$	316.22	0.0001	315.0148	299.9911(BP),282.9868,270.9881,244.0014	Anthraquinone
15	Yadanzioside D	8.61	$C_{23}H_{30}O_{11}$	482.5	0.0005	481.1720(BP)	456.1837,409.163,390.3805,216.6838	Triterpenoid
16	1-hydroxy-12-methoxycitromycin	9.13	$C_{14}H_{12}O_6$	276.24	0.0000	275.0561(BP)	257.0087,247.0619,234.0160, 231.0296	Microlide
17	Distemonanthin	9.39	$C_{17}H_{10}O_9$	358.3	0.0001	357.0251	342.0019(BP),339.0171,314.0054,285.0398	Alkaloid
18	Quinic acid	10.13	$C_7H_{12}O_6$	192.17	0.0006	191.0555(BP)	176.0111,170.9867,163.0004, 146.9379	Flavonoid
19	7-Ethyl-3,11-dimethyl-1,3Z,6E,10E-tridecatetraene	11.95	$C_{17}H_{28}$	232.4	0.0187	231.2305	210.9987,193.0137,190.9925(BP),182.9865	Isoprenoid
20	Diphthine	13.16	$C_{13}H_{22}N_4O_4$	298.34	0.0033	297.1535(BP)	279.1967,256.1946,2239.0753,235.2068	Amino acid
21	Tenellic acid A methyl ester	13.56	$C_{23}H_{28}O_7$	416.5	0.0038	415.1800(BP)	401.1634,394.9813,379.1589, 374.9926	Polyketide
22	Spiroterreusnoid C	13.83	$C_{28}H_{40}O_7$	488.6	0.0730	487.3431(BP)	469.3349,443.3553,425.3431, 409.3119	Polyketide
23	15R-hydroxytrametenolic acid	14.46	$C_{30}H_{48}O_4$	472.7	0.0009	471.3471(BP)	450.9937,410.9909,390.9860, 336.0826	Triterpene
24	Dichrostachine L	15.75	$C_{36}H_{40}O_8$	600.70	0.0016	599.2667	553.2992,531.3181(BP)353.3433,338.9885	Alkaloid
25	Carboxyamidotriazole	15.97	$C_{17}H_{12}Cl_3N_5O_2$	424.70	0.0207	421.9777	399.3482(BP),378.9829,353.3433,338.9885	Carboxylic acid
26	Terpendole I	16.80	$C_{27}H_{35}NO_5$	453.60	0.0026	452.2469	432.9870,412.9874,392.9872, 352.9851	Indole
27	4,5,6,7-Tetrabromo-N,N-dimethyl-1H-benzimidazol-2-amine (DMAT)	17.48	$C_9H_7Br_4N_3$	476.79	0.0194	471.7107	462.3264,442.9956,426.3682(BP),415.5177	Tryptamine alkaloid
28	(Z)-1,19-diisothiocyanatononadec-1-ene	18.20	$C_{21}H_{36}N_2S_2$	380.70	0.0049	379.2198(BP)	358.9909,338.9863,335.3324, 318.9800	Isothiocynate

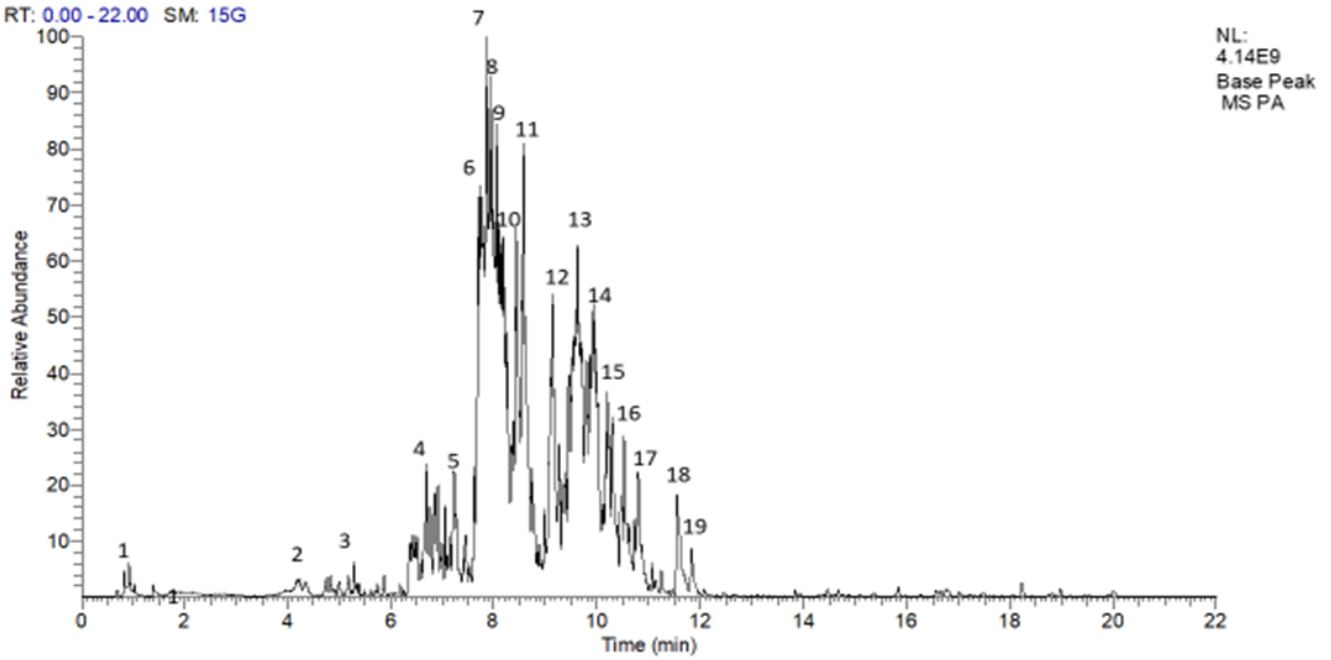

Fig. 1. Annotated LCMS chromatograph from the positive mode of *Prosopis africana* stem bark

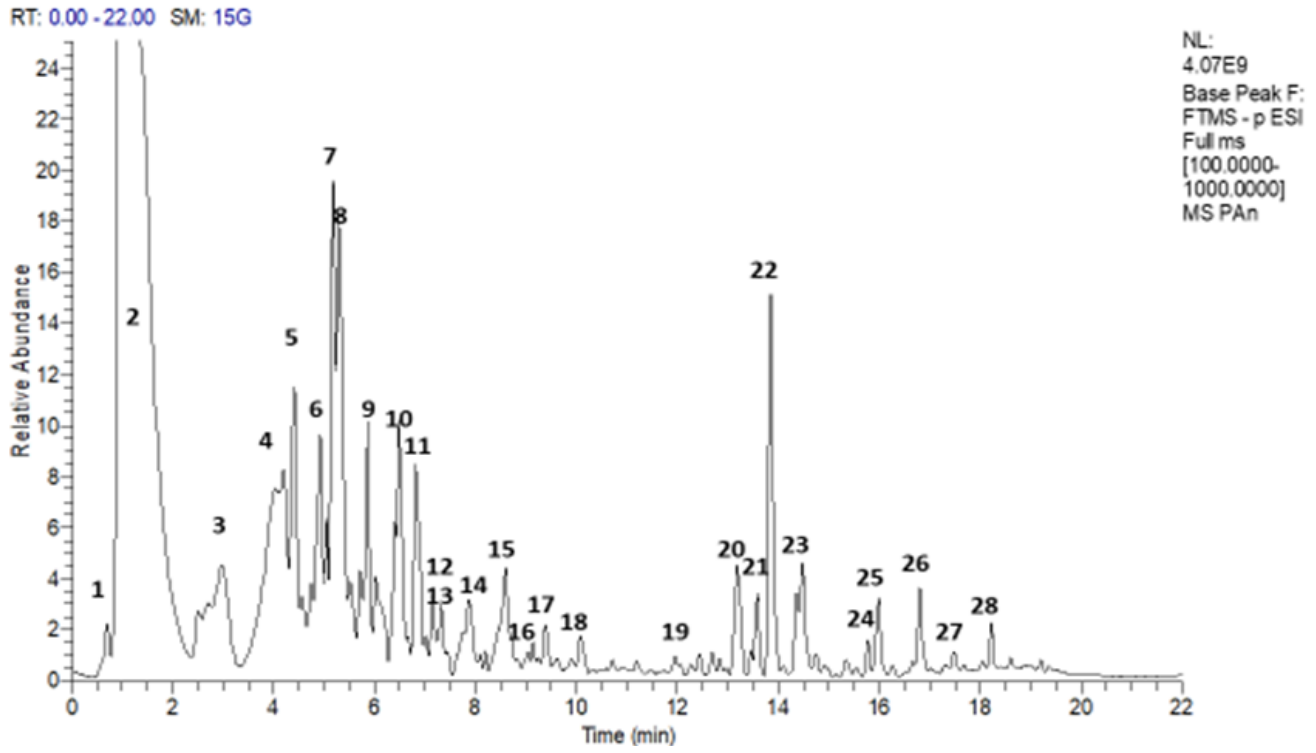

Fig. 2. Annotated LCMS chromatograph from the negative mode of *Prosopis africana* stem bark

Bearing in mind that there is no single analytical platform that can perform a complete identification and quantification of all molecules within a sample [16], the combination of LCMS and ^{1}H-NMR spectroscopic tools is certainly advantageous for the coverage, sensitivity and reliability of the generated results [12]. The LCMS only provides the putative metabolites in the sample, these indicate that further

validations may be required to confirm their presence and abundance in this plant part [17], therefore proton NMR was used to confirm the presence of these metabolites in this study. The peak chemical shifts for the corresponding 47 identified metabolites are summarised in Table 3 and 4. The prominent and basic peaks of the ^{1}H-NMR signals for these 47 metabolites are presented in Figure 3 and 4. The peaks in Table 3 and 4, were assigned base on the predictable spectra provided by the chemdraw 12.0 ultra 2023, comparison with previously published literatures and some standard online data base like the human metabolome database (HMDB) at http://www.hmdb.ca/ and pubchem at https://pubchem.ncbi were visited.

Table 3
^{1}HNMR profiling and validation of positive ionisation compounds of PA stembark extract

Peak	Compound name	PPM	Ref.
25	Methyl(s)-2-[[(E)-4-[[(2s)-1-oxopropan-2-yl]amino]-4-oxobut-2-enoyl]amino]propanoate	3.67 s	[18] and [22]
37		4.60 d	
81		8.00 d	
38	Gallic acid	5.00 s	[19]
60		6.99 s	
7	N-[(3s)-2-Oxotetrahydrofuran-3-Yl]decanamide	1.33 m	[20] and [22]
31		4.40 q	
25	2-Methoxybenzaldehyde	3.73 s	[21]
77		6.96 d	
68		7.43 d	
9	marinoid F	1.65 t	[22] and [31]
30		3.49 t	
57		6.64 s	
9	3beta-Methoxypregna-5,20-diene	1.63 t	[22] and [32]
18		3.24 s	
38		4.97 d	
7	N-Palmitoylglycine	1.33 m	[22] and [33]
9		1.57 m	
51	2,2-dibromoacetic acid	6.25 s	[22] and [23]
12	4-Hydroxydodecanoic acid	2.00 s	[24]
13		2.23 t	
19		3.21 d	
12	3-{[(9Z)-3-hydroxyhexadec-9-enoyl]oxy}-4-(trimethylammonio)butanoate	2.0 s	[22] and [34]
21		3.30 s	
24		3.61 d	
29		3.85 t	
38		4.81 t	
13	N-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glutamic acid	2.28 d	[22] and [35]
16		2.63 d	
33		4.46 t	
45		5.38 t	
45		5.43 t	
-	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone	-	[25]
	19,20-dihydrophomacin C		[26]
9		1.60 t	
10		1.71 s	
12		2.00 s	

24		3.62 d	
43		5.21 s	
44		5.37 s	
7	N-(9Z-hexadecenoyl)-tyrosine	1.33 m	[27]
13		2.18 t	
17		2.91 d	
37		4.85 t	
38		5.00 s	
46		5.42 s	
58		6.68 d	
7	Atiprimod	1.33 m	[28]
8		1.54 t	
13		2.25 t	
7	Palmitoleoyl Ethanolamide	1.33 m	[29]
12		2.00 s	
26		3.79 t	
46		5.42 s	
7	1-methoxy-3-[(Z)-pentadec-10-enyl]benzene	1.33 m	[22] and [36]
25		3.73 s	
54		6.59 s	
57		6.63 s	
58		6.68 d	
7	Tricosanoylglycine	1.33 m	[22] and [37]
32		4.14 s	
81		8.00 d	
7	Cyclooctyne	1.29 m	[30]

3.2 Metabolites from Positive Modes

The presence of Methyl(s)-2-[[(E)-4-[[(2S)-1-oxopropan-2-yl]amino]-4-oxobut-2-enoyl]amino]pro panoate (Table 1) was confirmed by distinctive singlets (s) at 7.40 ppm and 3.67 ppm, along with doublets (d) at 4.60 ppm and 8.00 ppm [18]. Gallic acid (Table 1) was identified based on singlets (s) at 5.00 ppm and 6.99 ppm [19].

N-[(3S)-2-Oxotetrahydrofuran-3-yl]decanamide (Table 1) was characterized by the presence of a multiplet (m) at 1.33 ppm and a quartet (q) at 4.40 ppm [20]. Similarly, 2-Methoxybenzaldehyde (Table 1) exhibited a singlet (s) at 3.73 ppm and doublets (d) at 6.96 ppm and 7.43 ppm [21]. Marinoid F (Table 1) displayed a singlet (s) at 6.64 ppm and triplets (t) at 1.65 ppm and 3.49 ppm [22], while 3β-Methoxypregna-5,20-diene (Table 1) presented a triplet (t) at 1.63 ppm, a singlet (s) at 3.24 ppm, and a doublet (d) at 4.97 ppm [22].

Additionally, N-palmitoylglycine (Table 1) was identified through multiplets (m) at 1.33 ppm and 1.57 ppm [22,33], and 2,2-dibromoacetic acid (Table 1) was confirmed by a singlet at 6.25 ppm [23]. Further metabolites detected include 4-hydroxydodecanoic acid at 2.00 ppm (s), 2.23 ppm (t), and 3.21 ppm (d) [24]; 3-{[(9Z)-3-hydroxyhexadec-9-enoyl]oxy}-4-(trimethylammonio)butanoate at 2.00 ppm (s), 2.52 ppm (t), 3.30 ppm (s), 3.61 ppm (d), 3.85 ppm (t), and 4.81 ppm (t); and N-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glutamic acid at 2.28 ppm (d), 2.63 ppm (d), 4.46 ppm (t), 5.38 ppm (t), and 5.43 ppm (t) [22].

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (Table 1) lacks proton signals due to its structural composition [25]. Meanwhile, 19,20-dihydrophomacin C exhibited peaks at 1.16 ppm (d), 1.83 ppm (t),

1.60 ppm (t), 1.71 ppm (s), 2.00 ppm (s), 3.62 ppm (d), 5.21 ppm (s), and 5.37 ppm (s) [26]. Further confirmation was provided for N-(9Z-hexadecenoyl)-tyrosine, which showed peaks at 1.33 ppm (m), 2.18 ppm (t), 2.91 ppm (d), 4.85 ppm (t), 5.00 ppm (s), 5.42 ppm (s), 6.68 ppm (d), and 6.95 ppm (d) [27]. Aniprimod was identified with peaks at 1.33 ppm (m), 1.54 ppm (t), and 2.25 ppm (t) [28].

Additionally, Palmitoleoyl Ethanolamide was confirmed with signals at 1.33 ppm (m), 1.57 ppm (m), 2.00 ppm (s), 3.79 ppm (t), and 5.42 ppm (s) [29]. Cyclooctyne was reported with peaks at 1.29 ppm (m) and 1.98 ppm (t) [30]. Other identified compounds include 1-methoxy-3-[(Z)-pentadec-10-enyl]benzene and Tricosanoylglycine, with their respective peak chemical shifts documented in the literature [8,22].

3.3 Metabolites from the Negative Modes

The 28 metabolites from the negative ionisation also belongs to different classes of compounds which includes; 6 alkaloids, 2 phenolic compounds and 4 terpenoids. In addition, cyloalkene, poly ether, triazines, anthraquinone, indole, carboxylic acid derivatives, flavonoid, glycoside and saponin are all presented in Table 2 with their various peak chemical shifts as shown in Table 4.

Table 4
^{1}HNMR profiling and validation of negative ionisation compounds of PA stembark extract

Peak	Compound name	PPM	Ref.
7	Hemibrevetoxin B	1.31 q	[38]
41		5.16 d	
52		6.25 q	
48		5.97 t	
31		3.99 t	
24		3.63 m	
38	Trichlorohydroquinone	5.0 s	[39]
53		6.39 s	
25	7-Hydroxy-5-hydroxymethyl-2h-benzo[1,4]thiazin-3-	3.71 s	[40]
36	one	4.79 s	
54		6.56 s	
13	Penialidin A	2.37 d	[41]
31		4.09 d	
66		7.28 s	
7	Scetryptoquivaline A	1.33 s	[22] and [42]
32		4.42 d	
79		7.50 s	
6	4-Dodecylphenol	1.29 s	[22] and [43]
58		6.68 d	
38		5.00 s	
12	Melem	2.00 s	[22] and [44]
67	6-Bromoindole-3-carboxaldehyde	7.30 s	[45]
68		7.40 d	
80		7.60 s	
10	Falconensin N	1.65 s	[22] and [46]
25		3.73 s	
2	(2S)-2-Isopropyl-3-oxosuccinate	1.01 S	[47]
55	Isochlorogenic acid	6.51 d	[22] and [48]
56		6.60 s	
38		5.00 s	
80		7.64 s	
6	Panosialin C	1.29 s	[22] and [49]
51		6.15 s	
49		6.02 s	
34	Dichotocejpin A	4.53 d	[22] and [50]
62		7.00 t	
39	2-hydroxyemodic acid	5.00 s	[22] and [51]
56		6.66 s	
5	Yadanzioside D	1.16 s	[52]
7		1.45 m	
12		2.00 s	
12	1-hydroxy-12-methoxycitromycin	2.00 s	[53]
25		3.73 s	
36		4.61 s	
38		5.0 s	
50		6.12 s	
52		6.34 s	
54		6.51 s	

26	Distemonanthin	3.79 s	[22] and [54]
38		5.00 s	
57		6.62 d	
11	Quinic acid	1.85 s	[55]
22		3.33 d	
10	7-Ethyl-3,11-dimethyl-1,3Z,6E,10E-tridecatetraene	1.71 s	[22] and [56]
14	Diphthine	2.35 t	[22] and [57]
38		4.82 t	
61		7.00 s	
3	Tenellic acid A methyl ester	1.01 d	[22] and [58]
20		3.24 s	
26		3.73 s	
28		3.88 s	
32		4.12 d	
39		5.00 s	
64		7.13 s	
5	Spiroterreusnoid C	1.11 s	[22] and [59]
31		4.06 q	
38		4.89 s	
5	15R-hydroxytrametenolic acid	1.16 s	[22] and [60]
6		1.27 s	
9		1.52 d	
12		2.01 s	
13		2.34 d	
27	Dichrostachine L	3.50 s	[22] and [61]
39		5.00 s	
48		5.95 s	
31	Carboxyamidotriazole	4. 00 s	[22] and [62]
40		4.99 s	
49		6.00 s	
61		7.05 s	
68		7.37 d	
5	(2S,3R,3aR,4aS,4bS,6aS,12bS,12cR,14aS)-	1.16 s	[63]
6	3,3a,5,6,6a,7,12,12b,12c,13,14,14a-dodecahydro-	1.26 s	
9	Terpendole I	1.67 d	
12		2.00 s	
15		2.52 s	
58		6.71 d	
58		6.86 d	
15	4,5,6,7-tetrabroMo-N,N-diMethyl-1H-	2.47 s	[22] and [64]
39	benzo[d]iMidazol-2-aMine (DMAT)	5.00 s	
7	(Z)-1,19-diisothiocyanatononadec-1-ene	1.29 m	[22] and [65]
24		3.60 t	

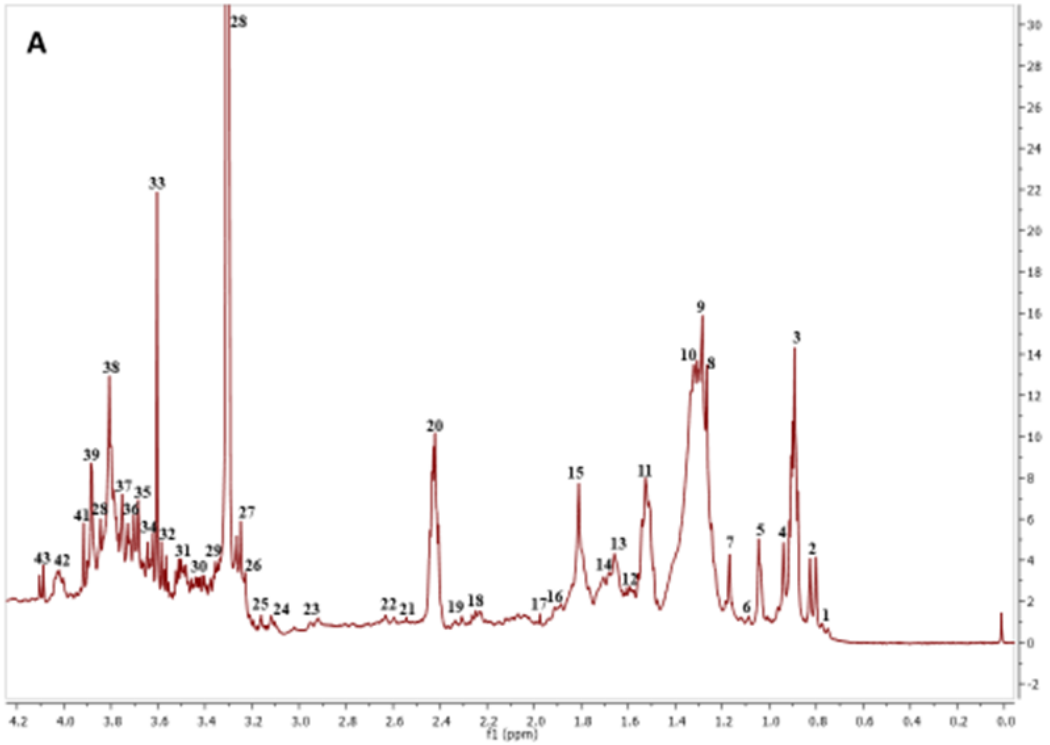

Fig. 3. Peak range from 0.0 – 4.2 ppm (A) of ^{1}H-NMR spectrum of *Prosopis africana* stem bark extract

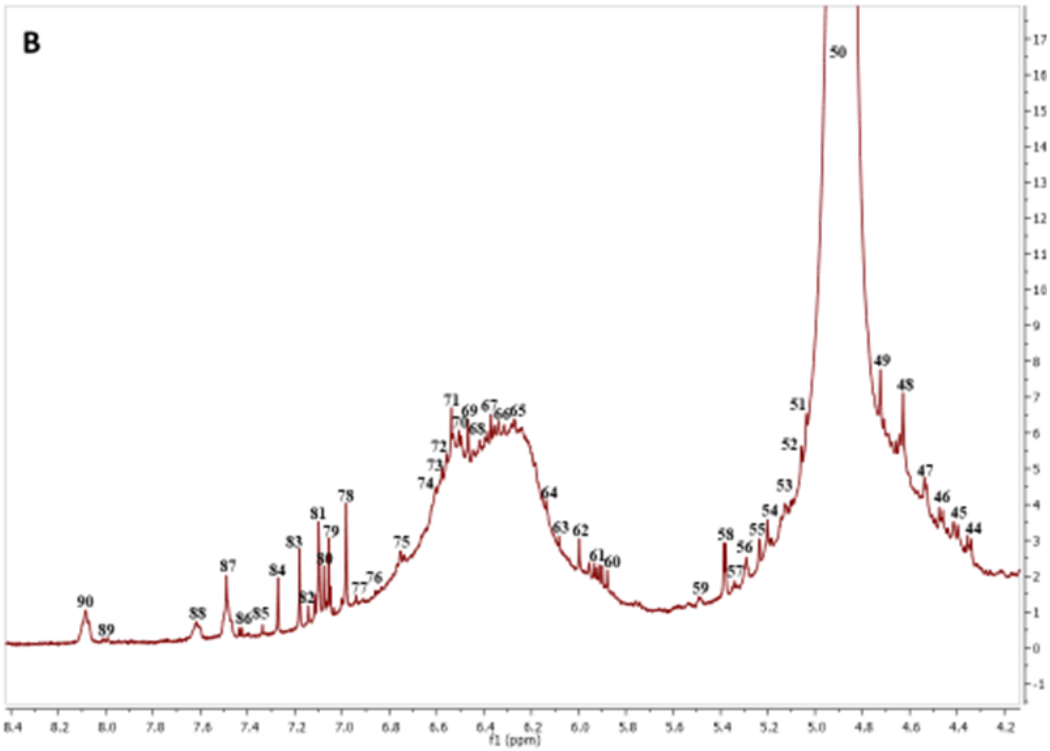

Fig. 4. Peak range from 4.2 – 8.4 ppm (B) of ^{1}H-NMR spectrum of *Prosopis africana* stem bark extract

3.4 Molecular Networking (MN)

Figure 5 shows the molecular networking of the categorised clusters identified from the LCMS results of *Prosopis africana* stembark which are represented in Table 5. The putative identified metabolites were achieved by manual dereplication matched with several external databases, namely HMDB, PubChem, LIPID MAPS, and Chemspider through metabolomics workbench platform. All clusters were classified according to the category of their various nodes, since they form similar pattern and belong to the same family. There are 27 clusters generated with more than four nodes per cluster as shown in Figure 5.

All metabolites are represented as parent ions, which are linked by the chemical fragmentation of the compound. Related compounds comprised similar parent ion fragmentation patterns, which are represented as a cosine score 1 (extremely similar fragmentation spectra) to 0 (total different parent ions) [66,67]. Therefore, the parent ion nodes are bound by edges with cosine score value, resulting in the classification of analogous or structurally related compounds in molecular clusters [12].

Table 5 itemises the identified clusters and selecting just one node to represent each cluster as shown in the table. Each identified cluster was named according to the category or classification of it nodes which are: carboxylic acids and derivative, Acidic glycosphingolipids, Polypeptide, Sphingolipids, Hybrid peptides, Amino acid, peptidomimetrics, gangliosides, glycerophospholipids, organoheterocyclic compound, flavonoid, glycosides, phenylpropanoids, polyketides, glycerolipids, carbohydrate and carbohydrate derivatives, saccharolipids and benzopyrenes as shown in Table 5 and Figure 5. These classification of clusters supports the report of Yanda *et al.,* [3], about the Phytochemical composition of the stembark of *Prosopis africana*. Although this study reveals more compounds that has ever been reported in *Prosopis africana* stembark.

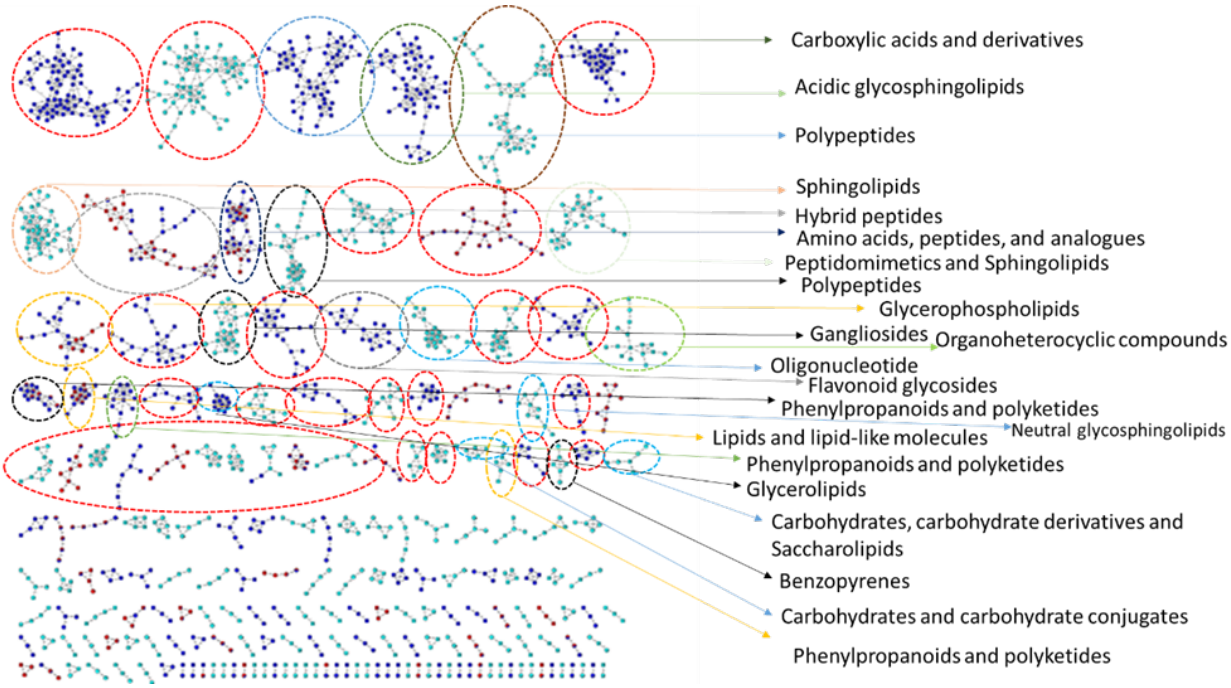

Fig. 5. Categorized clusters of annotated molecular network of *Prosopis africana* stembark

Table 5
Putative annotation of metabolites from each cluster for both positive and negative mode

No.	Compound classification	MF	*m/z*
4	**Carbohydrates and carbohydrate conjugates**		
	(4S,5R,6R)-2-[2-[[(2R,3R,4S)-6-[(4S,5R,6R)-5-acetamido-2-carboxy-2-[[(2R,3S,4S,5R)-3,4,5-trihydroxy-6-[(E)-3-hydroxy-2-(2-hydroxytetracosanoylamino)-14-methylhexadec-4-enoxy]oxan-2-yl]methoxy]-6-(1,2,3-trihydroxypropyl)oxan-4-yl]oxy-6-carboxy-4-hydroxy-2-(1,2,3-trihydroxypropyl)oxan-3-yl]amino]-2-oxoethoxy]-4-hydroxy-5-[(2-hydroxyacetyl)amino]-6-(1,2,3-trihydroxypropyl)oxane-2-carboxylic acid	$C_{80}H_{142}N_4O_{35}$	1720
5	**Phenylpropanoids and polyketides**		
	Samholide A	$C_{96}H_{160}O_{34}$	1858
7	**Benzopyrenes**		
	Heptatriacontacyclo[20.20.16.1629,32.427,57.334,36.339,41.343,45.247,50.252,55.125,58.04,44.06,46.08,48.011,49.013,51.015,53.018,54.020,56.160,64.165,66.167,68.180,84.059,62.061,89.063,91.069,83.070,71.072,82.073,74.075,81.076,78.077,92.079,90.085,94.086,95.087,96.088,93]hexanonaconta-1(42),2,4,6(46),7,9,11,13(51),14,16,18,20(56),21,23,25,27(63),28,30,32,34(76),35,37,39,41(71),43,45(68),47(67),48,50(66),52(65),53,55(64),57(60),58,61,69,72(82),73,75(81),77(92),78,80(93),83,85(94),86(95),87(96),88,90-octatetracontaene	$C_{96}H_{24}$	1177
9	**Saccharolipids**		
	2-octadecanoyl-3-O-(2S,4S,6S-trimethyl-3S-hydroxy-tetracosanoyl)-6-O-(2S,4S,6S-trimethyl-3S-hydroxy-tetracosanoyl)-2'-O-(2,4S,6S-trimethyl-2E-hexacosenoyl)-4'-O-(2,4S,6S-trimethyl-2E-hexacosenoyl)-alpha,alpha-trehalose	$C_{142}H_{268}O_{18}$	2261
10	**Sphingolipids**		
	GalNAcalpha1-3GalNAcbeta1-3(Galbeta1-3GalNAcbeta1-4)Galalpha1-4Galbeta1-4Glcbeta-Cer(d18:1/24:1(15Z))	$C_{90}H_{160}N_4O_{38}$	1906
11	**Glycerolipids**		
	1-octadecanoyl-2-(11Z-eicosenoyl)-3-heneicosanoyl-sn-glycerol	$C_{62}H_{118}O_6$	958
12	**Phenylpropanoids and polyketides**		
	[(1R,2S,19R,20R,22R)-36-[5-[[(1R,2S,19R,20S,22R)-7,8,9,12,13,14,28,29,30,33,34,35-dodecahydroxy-4,17,25,38-tetraoxo-3,18,21,24,39-pentaoxaheptacyclo[20.17.0.02,19.05,10.011,16.026,31.032,37]nonatriaconta-5,7,9,11,13,15,26,28,30,32,34,36-dodecaen-20-yl]oxycarbonyl]-2,3-dihydroxyphenoxy]-7,8,9,12,13,14,28,29,30,33,34,35-dodecahydroxy-4,17,25,38-tetraoxo-3,18,21,24,39-pentaoxaheptacyclo[20.17.0.02,19.05,10.011,16.026,31.032,37]nonatriaconta-5,7,9,11,13,15,26,28,30,32(37),33,35-dodecaen-20-yl] 3,4,5-trihydroxybenzoate	$C_{82}H_{54}O_{52}$	1869
13	**Lipids and lipid-like molecules**		
	(2R,6S)-6-[[(4R)-4-[[(2S)-2-[[(2R)-2-[(2R,3R,4R,5S,6R)-3-acetamido-5-[(2S,3R,4R,5S,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]oxy-2-[hydroxy-[hydroxy-[(2Z,6Z,10Z,14Z,18Z,22Z,26Z,30Z,34E,38E)-3,7,11,15,19,23,27,31,35,39,43-undecamethyltetratetraconta-2,6,10,14,18,22,26,30,34,38,42-undecaenoxy]phosphoryl]oxy-phosphoryl]oxy-6-(hydroxymethyl)tetrahydropyran-4-	$C_{95}H_{156}N_8O_{28}P_2$	1919

	yl]oxypropanoyl]amino]propanoyl]amino]-4-carboxy-butanoyl]amino]-2-amino-7-[[(1R)-2-[[(1R)-1-carboxyethyl]amino]-1-methyl-2-oxo-ethyl]amino]-7-oxo-heptanoic acid		
14	**Phenylpropanoids and polyketides**		
	8-bromo-6-chloro-2-(2,5-dimethoxyphenyl)chromen-4-one	$C_{17}H_{12}BrClO_4$	394
15	**Glycerophospholipids**		
	1-docosyl-2-tritriacontanoyl-sn-glycero-3-phosphoserine	$C_{61}H_{122}NO_9P$	1043
16	**Sphingolipids**		
	NeuAcalpha2-3Galbeta1-4GlcNAcbeta1-6(NeuAcalpha2-3Galbeta1-4GlcNAcbeta1-3)Galbeta1-4GlcNAcbeta1-6(NeuAcalpha2-3Galbeta1-4GlcNAcbeta1-3)Galbeta1-4GlcNAcbeta1-6(NeuAcalpha2-3Galbeta1-4GlcNAcbeta1-3)Galbeta1-4GlcNAcbeta1-3Galbeta1-4Glcbeta-Cer(d18:1/24:1(15Z))	$C_{196}H_{330}N_{12}O_{115}$	4693
17	**Flavonoids glycosides**		
	Quercetin 3-O-(2'',3''-digalloyl)-beta-D-galactopyranoside	$C_{35}H_{28}O_{20}$	767
18	**Oligonucleotide**		
	Mipomersen	$C_{230}H_{324}N_{67}O_{122}P_{19}S_{19}$	7171
19	**Organoheterocyclic compounds**		
	Pseudoceratin A	$C_{35}H_{36}Br_4N_6O_{14}$	1081
20	**Peptidomimetics and Sphingolipids**		
	Pinensin B	$C_{90}H_{132}N_{26}O_{27}S_2$	2074
	NeuAcalpha2-3Galbeta1-4GlcNAcbeta1-3(Galalpha1-3Galbeta1-4GlcNAcbeta1-6)Galbeta1-4GlcNAcbeta1-3Galbeta1-4Glcbeta-Cer(d18:1/16:0)	$C_{105}H_{183}N_5O_{56}$	2411
21	**Polypeptides**		
	Septocylindrin A	$C_{94}H_{155}N_{23}O_{25}$	2007
22	**Amino acids, peptides and analogues**		
	Tanjungide A	$C_{16}H_{16}Br_2N_4O_2S_2$	517
23	**Hybrid peptides**		
	Klebsidin	$C_{93}H_{129}N_{23}O_{27}S$	2031
24	**Sphingolipids**		
	Clarhamnoside	$C_{70}H_{132}N_2O_{24}$	1386
25	**Polypeptides**		
	Alamethicin	$C_{92}H_{150}N_{22}O_{25}$	1962
26	**Benzenoids**		
	Tokaradine A	$C_{28}H_{31}Br_4N_4O_4$	804
27	**Carboxylic acids and derivatives**		
	Roseoferin C1	$C_{60}H_{110}N_{10}O_{12}$	1164

Two among the identified clusters were illustrated at Figure 6 and 7 below. The green colour cluster node is for the positive ionisation, while blue nodes are for negative ionisation and red nodes is for solvent as shown in Figure 6 and 7. The structures of each identified compounds are attached to their various nodes with an arrow. Table 6 and 7 captured these identified metabolites from Figure 6 and 7 respectively.

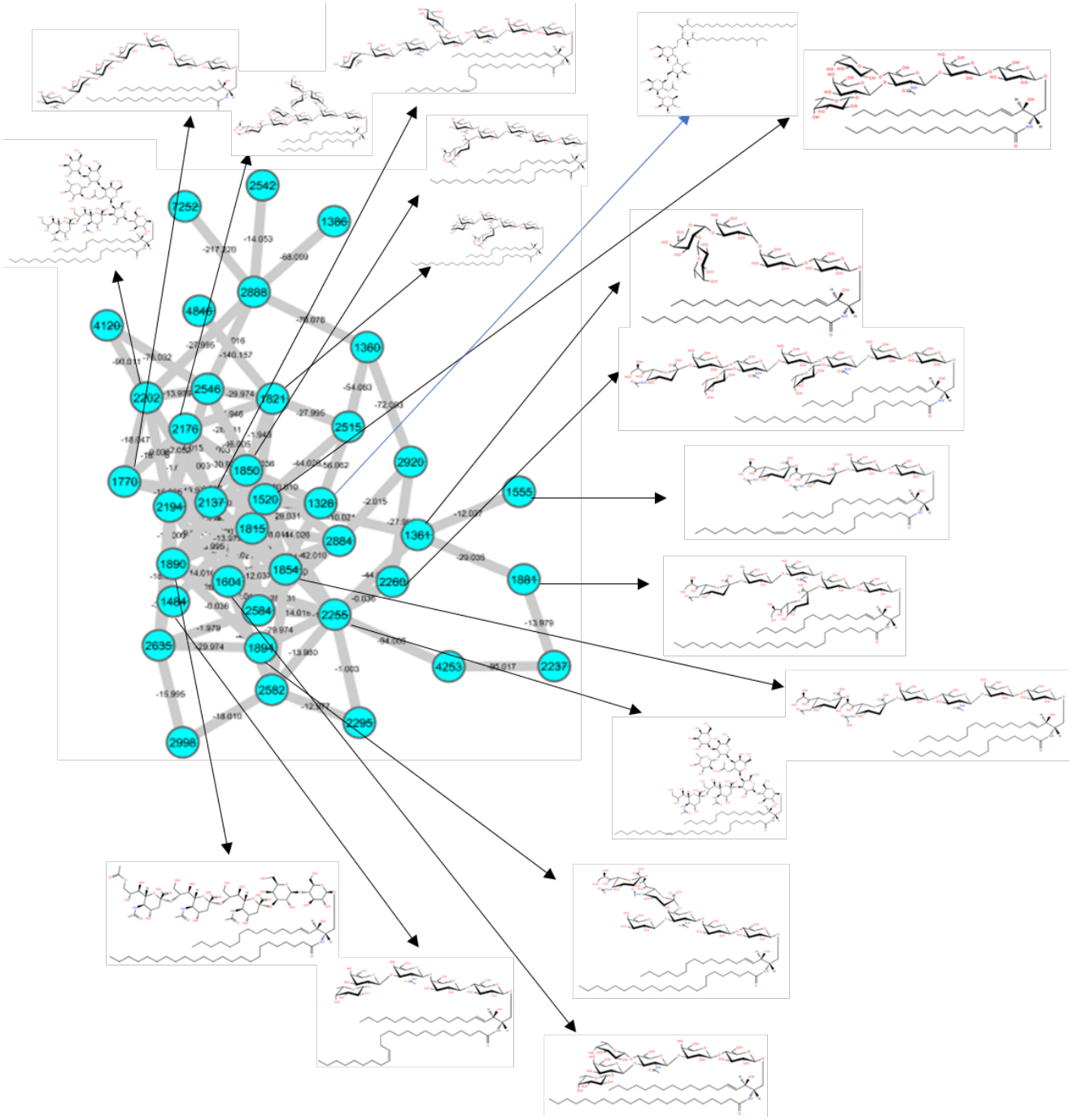

Fig. 6. An identified cluster of spingolipid from positive ionisation of molecular networking

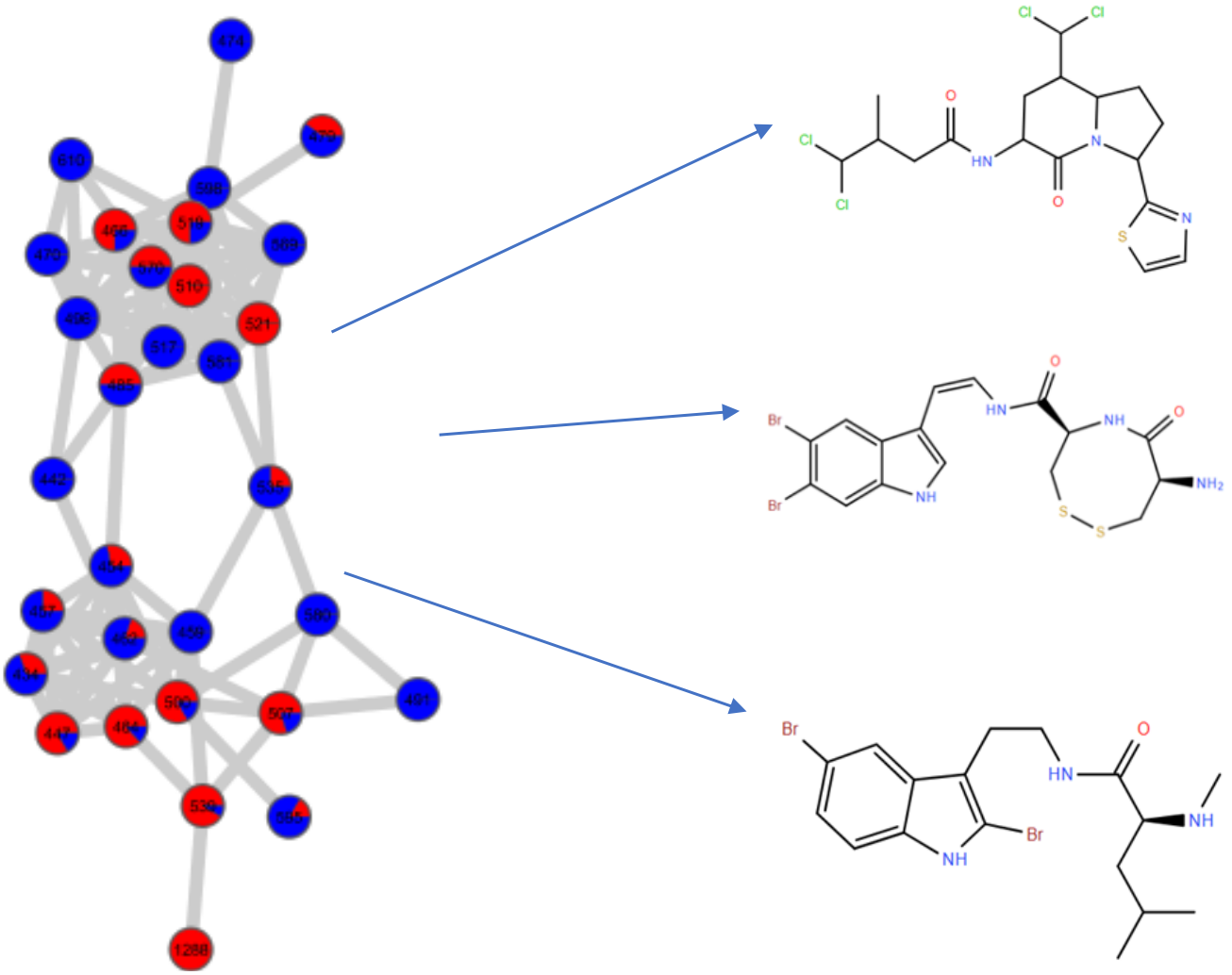

Fig. 7. An identified cluster of amino acid from negative ionisation of molecular networking

Figure 6, illustrates a cluster of the sphingolipids and identify 17 nodes as shown in the Figure. These 17 nodes correspond to 17 different types of spingolipids compounds as shown in Table 6.

Sphingolipids are important membrane components and are derivatives of the amino alcohol sphingosine, which is found in animal tissues, or phytosphingosine, which is found in plant tissues. Lipids derived from other related bases are included in this group [68]. Sphingosine is one of more than 60 long-chain amino alcohols found in animals, plants, and microorganisms. The bases commonly contain between 12 and 22 carbon atoms in the chain. A ceramide, which is an amide formed from a fatty acid and sphingosine, is the characteristic parent structure of all sphingolipids [69].

On the other hand, Figure 7, illustrates a cluster of amino acid and identifies 3 nodes as shown in Table 7. Each compound having the similar pattern of the amine functional group from amino acids. All amino acids have the same basic structure, which is; At the “center” of each amino acid is a carbon called the α carbon and attached to it are four groups - a hydrogen, an α- carboxyl group, an α-amine group, and an R-group, sometimes referred to as a side chain. The α carbon, carboxyl, and amino groups are common to all amino acids, so the R-group is the only unique feature in each amino acid.

Other clusters such as peptides, polyketides, carbohydrates, carboxylic etc. whose nodes were not fully captured might be identified if other databases are explored. This will be a time consuming task that requires thorough consideration in future studies.

Table 6
Putative annotation of metabolites from spingolipids cluster of the stembark of *Prosopis africana* extract

	Compound	MF	*m/z*	RT mean (sec)
	GD3(d18:1/24:1(15Z))	$C_{76}H_{135}N_3O_{29}$	1555	660.386
	(KDN)GD1a(d18:1/24:0)	$C_{88}H_{157}N_3O_{39}$	1881	513.129
	Galalpha1-3(Fucalpha1-2)Galbeta1-3GalNAcbeta1-4(NeuAcalpha2-8NeuAcalpha2-3)Galbeta1-4Glcbeta-Cer(d18:1/26:1(17Z))	$C_{104}H_{182}N_4O_{48}$	2255	413.402
	Sialyl dimeric Lex(d18:1/22:0)	$C_{103}H_{182}N_4O_{49}$	2260	467.454
	Fucalpha1-2Galalpha1-3Galalpha1-4Galbeta1-4Glcbeta-Cer(d18:1/18:0)	$C_{66}H_{121}NO_{27}$	1361	444.26
	Galbeta1-3(NeuAcalpha2-8NeuAcalpha2-6)GalNAcbeta1-4Galbeta1-4Glcbeta-Cer(d18:1/22:0)	$C_{88}H_{156}N_4O_{39}$	1894	385.764
	Fucalpha1-2Galbeta1-3GalNAcbeta1-4Galbeta1-4Glcbeta-Cer(d18:1/24:1(15Z))	$C_{74}H_{134}N_2O_{27}$	1484	647.624
	9-OAc-NeuAcalpha2-8NeuAcalpha2-8NeuAcalpha2-3Galbeta1-4Glcbeta-Cer(d18:1/24:0)	$C_{89}H_{156}N_4O_{38}$	1890	398.344
	Leb(d18:1/22:0)	$C_{78}H_{142}N_2O_{31}$	1604	364.127
	NeuAcalpha2-8NeuGcalpha2-3Galbeta1-4GlcNAcbeta1-3Galbeta1-4Glcbeta-Cer(d18:1/18:0)	$C_{84}H_{148}N_4O_{40}$	1854	531.537
Sphingolipids	Leb(d18:1/16:0)	$C_{72}H_{130}N_2O_{31}$	1520	510.365
	GalNAcalpha1-3Galbeta1-3GlcNAcbeta1-3(GlcNAcbeta1-6)Galbeta1-3GlcNAcbeta1-3Galbeta1-4Glcbeta-Cer(d18:1/26:1(17Z))	$C_{100}H_{177}N_5O_{43}$	2137	431.542
	GalNAcbeta1-3Galalpha1-3Galalpha1-3Galalpha1-3Galalpha1-4Galbeta1-4Glcbeta-Cer(d18:1/20:0)	$C_{82}H_{148}N_2O_{38}$	1770	421.946
	GalNAcbeta1-4(NeuGcalpha2-3)Galbeta1-3GalNAcbeta1-4Galbeta1-4Glcbeta-Cer(d18:1/24:0)	$C_{87}H_{156}N_4O_{37}$	1850	409.702
	Galbeta1-4GlcNAcbeta1-3(Galalpha1-3Galbeta1-4GlcNAcbeta1-6)Galbeta1-4GlcNAcbeta1-3Galbeta1-4Glcbeta-Cer(d18:1/20:0)	$C_{98}H_{174}N_4O_{48}$	2176	444.324
	Galalpha1-3(Fucalpha1-2)Galbeta1-3GalNAcbeta1-4(NeuAcalpha2-8NeuAcalpha2-3)Galbeta1-4Glcbeta-Cer(d18:1/22:0)	$C_{100}H_{176}N_4O_{48}$	2202	551.131
	Fucalpha1-2Galbeta1-3GalNAcbeta1-4(NeuGcalpha2-3)Galbeta1-4Glcbeta-Cer(d18:1/26:0)	$C_{87}H_{157}N_3O_{36}$	1821	673.422
	Clarhamnoside	$C_{70}H_{132}N_2O_{24}$	1386	317.036

Table 7
Putative annotation of metabolites from amino acid cluster of the stembark of *Prosopis africana* extract

Categories	Compound	MF	*m/z*	RT mean (sec)
Amino acids, peptides, and analogues	Dysideaproline C	$C_{17}H_{21}Cl_4N_3O_2S$	470	900.562
	Tanjungide A	$C_{16}H_{16}Br_2N_4O_2S_2$	517	956.941
	Altematamide C	$C_{17}H_{23}Br_2N_3O$	442	1009.28

4. Conclusion

The current study provides the comprehensive metabolites profile of *Prosopis africana* (stem bark). A total of 47 metabolites were putatively identified in both positive and negative ion modes by the LCMS, while the ^{1}H-NMR spectroscopy validates the identities by providing some insights in their molecular structures. The 19 metabolites from the positive ionization were further classified as peptides, phenols, fatty acids, polyketides, steroids, lipids, amino acids, quinones, flavonoids, azaspiranes, fatty acyl glycines and acyl amino acids. On the other hand, the 28 metabolites from the negative ion mode were also categorize into different classes of metabolites which include: 6 alkaloids, 2 phenolic compounds, and four terpenoids. Other categories include: cycloalkene, poly ether, triazines, anthraquinone, indole, carboxylic acid derivatives, flavonoid, glycoside and saponin. In addition, more than 26 clusters were generated in the molecular networking, with each cluster having more than 4 nodes per cluster.

In conclusion, the combination of LCMS and H-NMR techniques allow a thorough examination of the extract, with each method providing complementary information to its chemical profile. Importantly, several compounds detected in the extract have been reported to exhibit various pharmacological activities, suggesting the medicinal potential of *Prosopis africana* stem bark. The study contributes to the body of knowledge regarding the chemical constituents of *Prosopis africana* and provides a foundation for further research on its therapeutic applications in natural product-based medicine, as metabolomics have never been used on *Prosopis africana* plant.

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