Bima Journal of Science and Technology, Vol. 8(1B) Apr, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i1.629

Metabolite Propile of *Cassia occidentalis* Leaves Extract Using Proton NMR (^{1}H NMR) and LC-MS Molecular Network Analysis and its Antimicrobial Activity

Zainab Abdullahi Kobi[1], Rejoice Atiko[1], Hamza Ahmed Pantami[2]*

[1]Department of Chemistry, Gombe state university, PMB 127 Gombe State Nigeria.

[2]Department of Pharmaceutical and Medicinal Chemistry, Gombe state university, PMB 127 Gombe State Nigeria.

Corresponding author: hamza3983@gsu.edu.ng

ABSTRACT

The research focuses on profiling the metabolites in *Cassia occidentalis* leaves using ^{1}H-NMR and LC-MS molecular network analysis, alongside evaluating its antimicrobial properties. The plant, known for its medicinal use in treating various diseases according to traditional herbalists, has been less explored using LC-MS molecular network analysis. The leaves were air-dried, extracted with methanol, and subjected to antibacterial activity test using various concentrations obtained from serial dilutions (200 mg/ml, 300 mg/ml, 400 mg/ml, 500 mg/ml and 600 mg/ml) and the test organisms was *escherisha coli, salmonela specie, staphylococcus aureus, proteus, klebstella pneumoneae, shegella specie, pseudomonas,* and *entrobacter aerogenosa*). The metabolite profile of *Cassia occidentalis* leaves extract was established by subjecting the sample to ^{1}H-NMR analysis and LC-MS molecular network analysis. The antimicrobial tests demonstrated positive effects against all tested organisms at different concentrations. Proton NMR and LC-MS analyses revealed the presence of secondary metabolites, including apigenin, oleic acid, linolenic acid, lignoceric acid, 5, 7-dihydroxy flavone, mannitol, arachidic acid, leucine, valine, and alanine. This study adds literature information on *C. occidentalis*, emphasizing its antimicrobial properties and providing novel insights through LC-MS molecular network analysis.

Keywords: Antibacterial activity, Cassia occidentalis, Metabolite profiling, Molecular networking, Proton nuclear magnetic resonance spectroscopy.

INTRODUCTION

Cassia occidentalis is an annual perennial plant that grows all over Nigeria. It is a medicinal plant used for the treatment of various diseases. It is used traditionally to treat malaria and typhoid fever, hypertension, diabetes, menstrual cramps and general body weakness(Vijayalakshmi *et al.*, 2013)**.** The plant *C.occidentalis* is a slender, upright short-lived annual or biennial shrub with distinguished unpleasant odor. Its compound leaf consists of 3-7 pairs of leaflets (2-10 cm long and 2-3 cm wide) with pointed tip; a mounted gland at the base of leaf stalks and with no glands between leaflets. There is a conspicuous dark colored gland near the base of the stalk of each leaf (Bagega *et al*., 2018).

Metabolite profiling in the context of drug development aims at identifying and quantifying metabolites present in a given sample including their intermediates. Such data are considered very crucial for understanding the routes of elimination, predicting drug-drug interactions and anticipating safety concerns in humans. Traditionally, human absorption, distribution, metabolism and elimination studies have been conducted at a relatively late stage of drug development to generate metabolite data, typically from plasma, faeces and urine samples. Mostly, liquid chromatography–mass spectrometry (MS) have been applied to quantify metabolites that have previously been identified invitro or in animals (Ufer *et al.*, 2017).

DOI: 10.56892/bima.v8i1.629

Although MS techniques, such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are most commonly used in metabolite profiling, NMR still enjoys a number of key advantages. In particular, NMR is nondestructive, unbiased, quantitative, does not require separation or derivatization and is amenable to compounds that are difficult to analyze by GC-MS and LC-MS (Dayrit et al., 2020). For example, GC-MS often requires derivatization of compounds, such as sugars and amines(Dayrit *et al*., 2020). LC-MS, on the other hand, generally requires sample preparation, chromatographic separation, specific experimental and ionization conditions, instrumentation and operator skill (Dayrit *et al.*, 2020). These make it difficult to standardize MS analysis. In contrast, NMR does not require elaborate sample preparation and fractionation, is highly reproducible, and is able to provide both qualitative and quantitative information on chemically diverse compounds (Dayrit *et al*., 2020). The standardization of the NMR protocol will further improve the usefulness of NMR as a tool for the profiling of natural products extracts. Even though NMR is able to detect compounds at 0.1% level, it is not suitable for the detection of trace components. NMR is less sensitive than MS, which can detect compounds down to parts per million (ppm) levels. Because of the distinct advantages of each method, NMR and MS are considered as complementary techniques. Therefore, in this study, the two techniques were applied for metabolite profiling. NMR-based metabolite profiling is best suited for comprehensive, structural identification of metabolite, while LC-MS based approaches allow for metabolite quantification with high sensitivity (Ufer *et al.*, 2017).

MATERIALS AND METHODS

Sample collection

Fresh leaves of *C. occidentalis* were collected from kwami local government, Gombe state, using scissors and stored in polyethene bags before taken to the laboratory.

Sample processing and extraction.

The leaves were allowed to air dried at room temperature and ground using mortar and pestle. The powdered sample was stored in aluminum foil at 20 ^{0}C to 30 ^{0}C. The leaves of *C. occidentlis* were extracted using maceration method with methanol as the solvent. The solvent was removed by rotary evaporator and allowed to completely dry at room temperature (Sadiq et al., 2011).

Sample processing for the antimicrobial test.

The test organisms for antibacterial susceptibility test were sub cultured in nutrient agar and incubated for 24 hours at 40^{0}C and later stored in nutrient agar slant (Ochie and Kolhatar 2008). The sample preparation was done by dissolving the amount of plant extract (200mg, 300mg, 400mg, 500mg, 600mg) in 1000 ml of distilled water, and the test organisms was *escherisha coli, salmonela specie, staphylococcus aureus, proteus, klebstella pneumoneae, shegella specie, pseudomonas,* and *entrobacter aerogenosa*.

NMR Analysis

A mixture of 10 mg of the crude extract, 0.7 mL deuterated methanol and chloroform (ratio 8:2) and 0.01% tetramethylsilane, TMS were vortexed for 1 min at room temperature; ultrsonicated for 15 min without heating; centrifuged for 10 min at 1300 rpm. The supernatant (0.6 ml) was transferred into 5 mm NMR tube and then ran for analysis (Safwan et al., 2020) using 500 MHz Varian INOVA NMR spectrometer (Varian Inc., Palo Alto,

DOI: 10.56892/bima.v8i1.629

California, USA), running at a frequency of 499.887 MHz at room temperature (25 °C).

LC-MS/MS Analysis

Two mg of solvent extract and 1ml of methanol LCMS grade; were sonicated for 5 min; filtered with 0.22 micron membrane filter; and then ran for LC-MS analysis a C18 Reversed-phase Hypersil GOLD aQ column (100x2.1 mm ~1.9 µm) (Thermo, Waltham, MA, USA) at 30 °C on Dionex Ultimate 3000 UHPLC with a diode-array DAD-3000 detector (Thermo Fisher Scientific,Waltham, MA, USA) and the MS analysis was done on Q-Exactive Focus Orbitrap system.

Molecular Network Analysis

The molecular networks based on MS/MS data were generated using the online workflow Global Natural Products Social Molecular Networking (GNPS) platform (http://gnps.ucsd.edu) with registered account. Prior uploading the data into GNPS, the raw MS data including blank were converted into mzXML format using MSConvert software downloaded from Proteowizard website (http://proteowizard.source-forge.net/tools.shtml). Then the converted data files were uploaded to GNPS using FileZilla software (https://filezilla-project.org/). In GNPS data analysis workflow, sample and blank data were selected as G1 and G2, respectively with precursor ion mass tolerance set to 0.02 Da and a fragment ion mass tolerance of 0.02 Da. A network was processed with edges and were filtered to have a cosine score above 0.7 and minimum 6 matched peaks (Safwan et al., 2020) Upon processing, result was downloaded and network was visualized using ChemViz 1.3 plugin (freely available at http://www.cgl.ucsf.edu/cyto scape/chemViz/) within Cytoscape software (Institute of Systems Biology Seattle, Washington D.C., USA).

Antimicrobial susceptibility test using agar well method.

The antimicrobial activity studies were carried out by using the agar well method and tested the antimicrobial activity of the leaf extract of *Cassia occidentalis* on eight (8) organisms (*E.coli, Salmonela spp, Staph aureus, Proteus,Vibro cholera, Klebstella pneumoneae, Pseudomonas aerogenosa and klebstella spp).* This was achieved by creating 6mm hole in the prepared agar (media) inside the petri dish .The organisms were then inoculated all over the surface of the petri dishes and the leaf extract (concentration from 200 mg/ml to 600 mg/ml) were also inoculated in to each hole with a control drug at the center. It was then incubated overnight at 37 °C after which the zone of bacterial growth inhibition was measured in millimeters (mm). Ofloxacin was used as a positive control.

RESULTS AND DISCUSSION

Extract Yield

The leaves of *Cassia occidentalis* were successively extracted with Methanol to give 29.8g (5.96%) of dark brown jelly solid of crude extract yield. The amount obtained from Methanol was much. This implies that most of the secondary metabolites in the leave of *Cassia occidentalis* were polar compounds.

Antimicrobial Susceptibility Test

The methanol leave extract of *Cassia occidentalis* was tested against four gram negative and four gram positive organisms. Table 1 shows the antimicrobial susceptibility results of *Cassia occidentalis* against *Escherisha coli*, *Entrobacter aerogenosa, Proteus, Klebstella Pneumoneae*, *Salmonella spp, Shegella spp* and *staphylocuccos aureus*.

DOI: 10.56892/bima.v8i1.629

Table 1. Result of antimicrobial susceptibility test

S/N	Clinical Isolates	Zone of inhibition (mm)					
	Concentration (mg/ml)	200	300	400	500	600	OFL (500 mg/ml)
1	E-Coli	R	R	14	17	18	30
2	Entrobacter aerogenosa	R	9	13	15	20	28
3	Proteus	R	13	15	19	21	24
4	Klebstella Pneomoneae	R	15	18	23	28	30
5	Pseudomonas	R	R	12	15	18	22
6	Salmonella spp	R	8	12	14	17	31
7	Shegella spp	R	10	13	16	19	26
8	Staphylococus aureus	R	R	15	18	21	30

KEY: OFL = Ofloxacin, mm = millimeter, R = resistant.

The highest inhibition was shown against *Klebstella Pneumoneae* at 600mg/ml concentration which gave 28 mm as the zone of bacterial growth inhibition followed by *Staphylococus aureus* and *Proteus* which gave 21 mm zone of inhibition, then *Shegella spp* which exhibit the zone of inhibition as 19 mm, So also, *Escherisha Coli* and *Pseudomonas* exhibit a zone of inhibition of 18mm and the least inhibition was exhibited by *Salmonella spp* at 300mg/ml concentration. The amount of inhibition exhibited by the methanol leave extract of *Cassia occidentalis* at 600mg/ml concentration was a very good one to show up when compared to that of the standard drug at 500mg except for *Salmonella spp* and *Escherisha Coli* which appeared to give a considerable difference between the zone of inhibition exhibited by the control drug and that of *Cassia occidentalis*. All the organisms were resistant to the extract at 200mg/ml concentration. In addition, *E. coli*, *Pseudomonas* and *Staphylococcus aureus* resist the extract at 300mg/ml concentration. The methanol extracts shows considerable level of inhibition against all the tested organisms. This indicates the presence of some compounds or groups in the extract with similar mechanism of action to that of the standard drug used.

Assignment of Metabolites by 1D and 2D NMR Spectra of *Cassia occidentalis*

Assignment of the peak signals in the ^{1}H NMR and 2D NMR spectra were based on reference to Chenomx software database (Version 8.3, Alberta, Canada), MestreNova and previously reported literature.

Figure 1 and 2 shows the representative ^{1}H NMR spectra of *C. occidentalis*. The spectra showed the presence of different classes of metabolites, including flavonoids, alkaloids, fatty acids, amino acids and vitamins.

The peak chemical shifts for the corresponding ten (10) identified metabolites are summarized in Table 2. Further interpretation of the assigned signals was completed using ^{1}H NMR while 2D J-res and HSQC and HMBC experiments were utilized in order to increase the metabolites specificity and to minimize the overlap of the signals. The peaks were assigned by referring to previous studies (Dave & Ledwani, 2012) and by comparing with freely available online databases such as Human Metabolome Database (HMDB) and PubChem. The numbers annotated to peaks detected which were shown in Figure 1 and 2 correspond to the metabolites listed in Table 2.

The ten (10) metabolites which were identified in the methanol leaves extract of *Cassia occidentalis* are apigenin, oleic acid, linolenic acid, lignoceric acid, 5,7-dihydroxy flavone, mannitol, arachidic acid, leucine, valine, and alanin.

The up field region of the spectra exhibited signals for both saturated and unsaturated fatty acids from the appearance of characteristic terminal methyl protons at 0.88 ppm. Meanwhile, the presence of

Bima Journal of Science and Technology, Vol. 8(1B) Apr, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i1.629

apigenin was confirmed with a doublet at 6.59 ppm, triplet at 6.71 ppm, singlet at 6.65 ppm, singlet at 6.25 ppm, singlet at 5.35 ppm and the HSQC cross peak for the carbon at 5.35 ppm, 6.6 ppm and 7.5 ppm and HMBC cross peak at 5.4 ppm and at 7.0 ppm.The presence of oleic acid was confirmed by the presence of a doublet at 5.42 ppm, doublet at 2.18 ppm, doublet at 2.30 ppm, singlet at 1.29 ppm, doublet at 1.31 ppm, singlet at 0.88 ppm and a HSQC cross peak at 0.88 ppm, 2.3 ppm and at 5.3 ppm and a HMBC cross peak at 0.88 ppm and at 5.4 ppm. The presence of lignoceric acid was confirmed with a doublet at 0.88 ppm, singlet at 1.31 ppm, triplet at 2.3 ppm and a HSQC cross peak at 0.86 ppm and at 1.3 ppm and a HMBC cross peak at 0.86 ppm and at 2.31 ppm.The presence of linolenic acid was confirmed with a multiplet at 5.38 ppm, multiplet at 2.0 ppm, doublet at 2.63 ppm, singlet at 1.06 ppm, triplet at 1.52 ppm and a HSQC cross peak at 0.88 ppm, 1.29 ppm and 5.31 ppm and HMBC cross peak at 2.30 ppm and 6.2 ppm. The presence of 5,7-dihydroxy flavone was confirmed by a singlet at 5.3 ppm,, a singlet at 6.0 ppm, a singlet at 6.3 ppm, a singlet at 6.6 ppm, multiplet at 7.0 ppm, doublet at 7.8 ppm and a HSQC cross peak at 5.37 ppm and 7.6 ppm, and a HMBC cross peak at 5.64 ppm and 6.6 ppm.

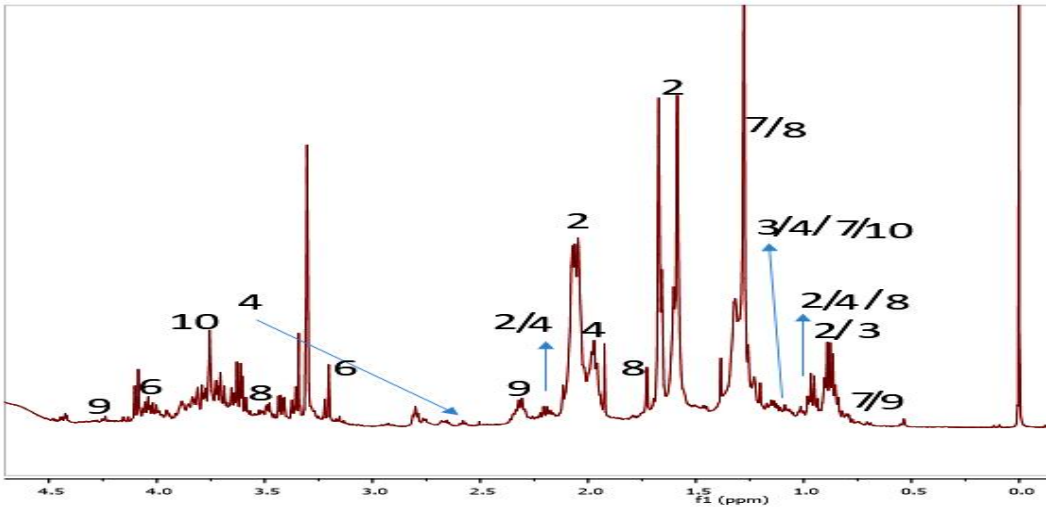

Figure 1: [1]H NMR spectra of *C. occidentalis* from chemical shift 0.0 to 4.5 ppm

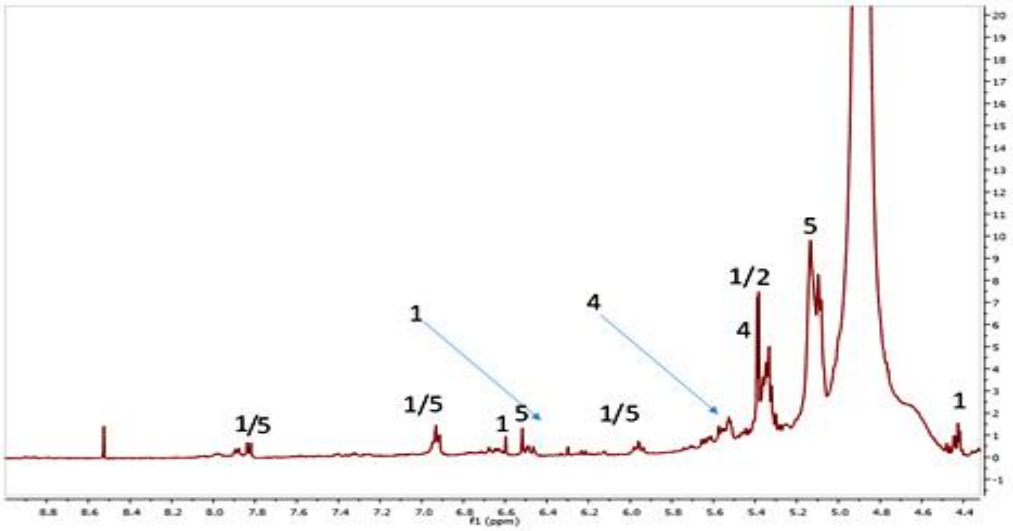

Figure 2: [1]H NMR spectra of *C. occidentalis* from chemical shift 4.4 to 8.8 ppm

DOI: 10.56892/bima.v8i1.629

Table 2. Characteristic ^{1}H NMR signals of metabolites identified in methanol extract of *Cassia occidentalis.*

S/N	Metabolites	Molecular Formula	Chemical Shifts (ppm)
1	Apigenin	$C_{15}H_{10}O_5$	5.35 (t) 5.94 (s) 6.25 (s) 6.65 (d) 6.71 (s) 6.59 (d)
2	Oleic acid	$C_{18}H_{34}O_2$	0.88 (s) 1.31 (s) 1.29 (m) 1.52 (m) 2.18 (d) 2.30 (s) 5.42 (d) 11.0 (s)
3	Lignoceric acid	$C_{23}H_{47}COOH$	0.88 (t) 1.31 (s) 1.29 (s) 1.26 (m) 1.52 (s)
4	Linolenic acid	$C_{18}H_{32}O_2$	1.06 (s) 1.52 (s) 1.29 (t) 2.00 (s) 2.30 (s) 2.18 (s) 2.63 (d) 5.38 (m) 5.43 (d)
5	5,7-dihydroxy flavone	$C_{15}H_{10}O_4$	5.35 (d) 5.94 (s) 6.25 (s) 6.71 (s) 7.33 (s) 7.40 (d) 7.77 (d) 3.58 (m) 3.37 (m) 3.65 (m) 3.81 (d)
6	Mannitol	$C_6H_{14}O_6$	3.38(m) 3.58(m) 3.56(d) 3.65(d) 3.81(d)
7	Arechidic acid	$C_{20}H_{40}O_2$	0.88(s) 1.31(s) 1.29(m) 1.26(m)
8	Leucine	$C_6H_{13}NO_2$	0.91(d) 1.49(s)

DOI: 10.56892/bima.v8i1.629

			1.75(s) 3.49(s)
9	Valine	$C_5H_{11}NO_2$	0.91(d) 2.36(s) 4.29(s)
10	Alanine	$C_3H_7NO_2$	1.23(s) 3.67(s)

LC-MS Analysis

The total ion chromatogram (TIC) in Figure 3 shows the peaks detected for the methanol extract of *C.occidentalis*. The fragmentation for all compounds discussed herein was based on the pattern derived from HighChem Mass Frontier 3.0 (Thermo Fisher Scientific Inc). This spectral interpretation software predicts and automatically generates detailed fragmentation according to the general principles of ionization, fragmentation and rearrangement using chemical structure provided by databases such as HMDB and PubChem.

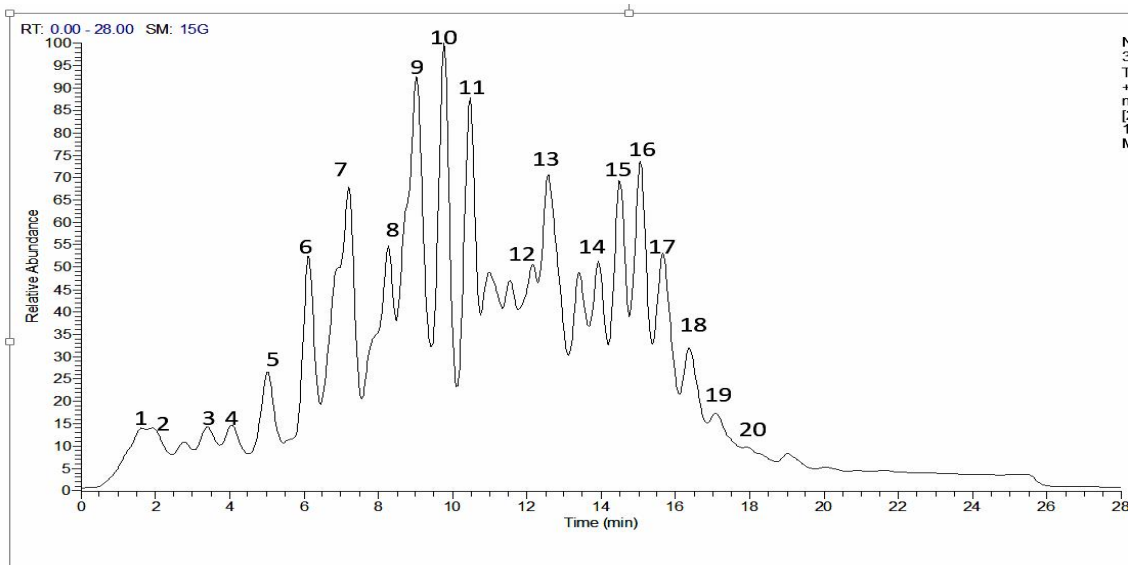

Figure 3: Total ion chromatogram of *Cassia occidentalis* for ethanol extract.

LCMS metabolite identification

The identity, retention time and fragment ion(s) for each detected metabolite are presented in Table 3. Twenty metabolites comprising of four carboxylic acids, three sterols, one peptidomimetic, three isoprenoids, one indole, three lipids, three glycosphingolipids and two organo oxygen were identified from the LC-MS results. All metabolites detected showed high mass accuracy with their scored mass error being less than ±0.5 *m/z* except for Fucoxanthinol 3-linoleate 3'-gondoate which has mass error of +0.9 *m/z*. The identification of Namonin with the experimental mass of *m/z* 1185.54 and theoretical mass of *m/z* 1184.5251 shows a high confidence level in the experimental mass, meanwhile the retention time of 4.05 minutes indicates a moderate polarity of the compound. The parent ion $[M+H]^+$ 1185.54 was fragmented to give a fragment with m/z 1127 resulting to a loss of 58 amu which correspond to the loss of $C_2H_2O_2$ which may be an ethanoic acid radical. Another fragment was detected with m/z 1092.49, resulting from the loss of 156 amu which corresponds to the loss of $C_6H_6O_5$. The third fragment was m/z 979.75

DOI: 10.56892/bima.v8i1.629

which results from the loss of 210 amu which corresponds to the loss of $C_{10}H_{18}O_6$.

Another identified metabolite was Pseudomonas beta ferritin which was identified at a retention time of 1.62 minutes with the parent ion $[M+H]^+$ 1172.51 was fragmented to give a fragment with m/z 1091.43 resulting to a loss of 81 amu which corresponds to the loss of C_6H_6O, followed by the loss of 147 amu to form another fragment with m/z 1025.53 which corresponds to the loss of $C_5H_{12}N_2OS$, followed by the loss of 207 amu which corresponds to the loss of $C_9H_8NO_2$ to form another fragment with m/z 965.58. Another fragment was formed with m/z 915.48 which was due to the loss of 257 amu that corresponds to the loss of $C_{10}H_{18}N_3O_3S$.

DOI: 10.56892/bima.v8i1.629

Table 3: Putative metabolites Identified in methanol extract of *Cassia occidentalis* by LCMS.

Peak	Retention Time	Putative metabolites	Exp. Mass (M+H)	Theo. Mass (M+/-H)	Molecular Ion	MS Fragments	Main Class	Molecular Formula
1	1.62	Pseudomonas beta feritin	1172.51	1171.5332	1172.5405	1091.43,1025.53,965.58,915.48,863.51,838.53,780.20,735.43,699.36, 655.39	Carboxylic acid	$C_{52}H_{77}N_{13}O_{16}S$
2	1.92	Quartromicin D3	1187.59	1186.5712	1187.5785	1138.44, 1113.59, 1068.59, 1051.62, 1025.55, 977.56, 939.53, 899.49	peptidomimetics	$C_{65}H_{86}O_{20}$
3	3.40	Fucoxanthinol 3,3'-dioleate	1173.95	1172.954	1173.9420	677,37,502.32,522.3, 496.34,468.39,460.3,424.36,390.30,380.3, 319.28,275,26	Isoprenoids	$C_{78}H_{124}O_7$
4	4.05	Namonin B	1185.54	1184.5251	1183.5324	1127.60,1029.49,979.75,945.53,897.50,845.50,829.50,594.28,615.26,593. 28,408.37,364.34, 303.29,282.28	Sterols	$C_{57}H_{84}O_{26}$
5	5.04	Fucoxanthinol 3-linoleate 3'-gondoate	1139.72	1130.6336	1131.6409	1110.93,1058.65 831.51,814.52, 666.49,601.43,502.41	Sterols	$C_{53}H_{86}N_{12}O_5$
6	6.11	Man-beta1-2-Ins-1-P-Cer(t18:0/2,3-OH-24:0)	1171.84	1170.9191	1171.9263	1122.22,971.59,876.13,739.51,699.52 597.41, 492.40	Isoprenoids	$C_{57}H_{100}N_{80}O_{15}$
7	7.21	N-(pentatriacontanoyl)-1-beta-lactosyl-docosasphinganine	1104.78	1103.7097	1104.7169	956.63, 930.62,862.64, 797.52, 700.54, 677.53,615.449	Lipids	$C_{54}H_{106}NO_{19}P$
8	8.27	N-(hexacosanoyl)-	1186.95	1185.9933	1187.6006	1161.94,1089.72,1033.77,984.66,940.72	Neutral Glycosp Ingolipids	$C_{69}H_{135}NO_{13}$

DOI: 10.56892/bima.v8i1.629

		sphinganine-1-O-						
9	9.01	-[D-mannopyranosyl-alpha1-2-myo-inositol-1-phosphate]	1084.77	1083.7562	1084.7635	1033.61,955.62,904.72 764.57,663.45,680.48 637.49,615.50	Lipids	$C_{54}H_{106}NO_{19}P$
10	9.75	shishicrellastatin	1177.45	1176.4536	4609	1153.01,1053.01,962. 68,634.54,591.50	Sterols	$C_{56}H_{83}N_{93}O_{16}S_3$
11	10.48	bastadin-6-O-sulfate	1194.58	1193.6238	1194.6351	1132.49,1048.36,989.65,964.69 ,918.65,901.55 883.54,814.66,610.5422	Peptidomim Etrics,	$C_{34}H_{25}Br_6N_4NaO_{11}S$
12	12.16	Trichopolyn III	1192.84	1191.8206	1192.8279	1122.85, 1073.71, 996.79, 961. 75,910.74,908.73,846.69, 682.83	carboxylic acid	$C_{60}H_{109}N_{11}O_{13}$
13	12.58	Fucoxanthinol3-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoate) 3'-palmitate	1165.79	1164.8721	1165.8794	1128.58,1056.81,1030. 79,988.69,911.75,893 .55, 873.58, 871.57,	Isoprenoids	$C_{78}H_{116}O_{17}$
14	13.93	N-(nonatriacontanoyl)-1-beta-lactosyl-4E-sphingenine	1184.93	1183.9777	1184.9850	1129.67,1060.84, 797.84,962.82,934.78 897.69,894.74,888. 77,858.72,840.71.	Neutral Glycosphyngolipids	$C_{69}H_{133}NO_{13}$
15	14.49	erylusine	1168.77	1167.7604	1168.7677	1028.83,981.86,964.83, 899.71,896.76,894.75,868.74	organooxygyn.	$C_{58}H_{109}N_3O_{20}$
16	15.05	CMNPD23309	1164.78	1162.8305	1163.8377	1092.72,1038.87,994. 86,966.86,940.880,898.78 ,896.77,870.75	Carboxylic acid and derivative	$C_{60}H_{110}N_{10}O_{12}$

DOI: 10.56892/bima.v8i1.629

17	15.54	trichoderin A	1163.78	1162.8305	1163.8377	1130.90,1081.69,1022.89,968.86,942.85,898.78,874.782 872.77,846.75	Carboxylic acid and derivative	$C_{60}H_{110}N_{10}O_{12}$
18	16.38	N-(nonatriacontanoyl)-1-beta-lactosyl-4E-heptadecasphingenine	1170.95	1169.9620	1170.9693	1125.80,1091.92,1048.91, 984.90,942.85,907.76,905,76 902.81,900.80,874.79,848.77.	Neutral Glycosphyngolipids	$C_{66}H_{131}NO_{13}$
19	17.09	N-(nonatriacontanoyl)-1-beta-lactosyl-4E-sphingenine	1170.83	1169.8012	1170.8085	1142.95,1091.92,1062.92, 992.84,960.90,907.77 ,903.,82, 878.81,876.80.	Glycosphyngolipids	$C_{68}H_{131}NO_{13}$
20	17.93	1-eicosyl-2-nonatriacontanoyl-sn-glycero-3-phosphoinositol	1175.98	1174.9691	1175.9764	1141.94,1104.97,1052.96 1025.83,1020.88,968.86	Pyrenes	$C_{68}H_{135}O_{12}P$

Bima Journal of Science and Technology, Vol. 8(1B) Apr, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i1.629

MS based molecular networking.

Molecular networking facilitates a fast comparison of mass spectrometry profiles from complicated crude extracts for successful metabolites dereplication and exploration of novel compounds which requires high resolution of mass spectrometry data (MS/ MS) (Safwan et al., 2020). Dereplication is defined as a fast detection of defined metabolites through comparison of experimental mass spectra with libraries. All metabolites represented as parent ions are linked by the similarities in chemical fragmentation of the compounds: related compounds comprised of similar parent ion fragmentation patterns, which are represented as a cosine score from 1 (extremely similar fragmentation spectra) to 0 (totally different parent ions) (Safwan et al., 2020). Therefore, the parent ions (nodes) are bound by edges with cosine score value, resulting in the classification of analogous or structurally related compounds in molecular clusters (Safwan et al., 2020). In the present study, a classical global molecular network was generated based on LC-MS data from methanol extract with blank using GNPS platform 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 (www.metabolomicsworkbench.org) with lowest mass error since automated dereplication on the GNPS platform was limited and did not match any known compound.

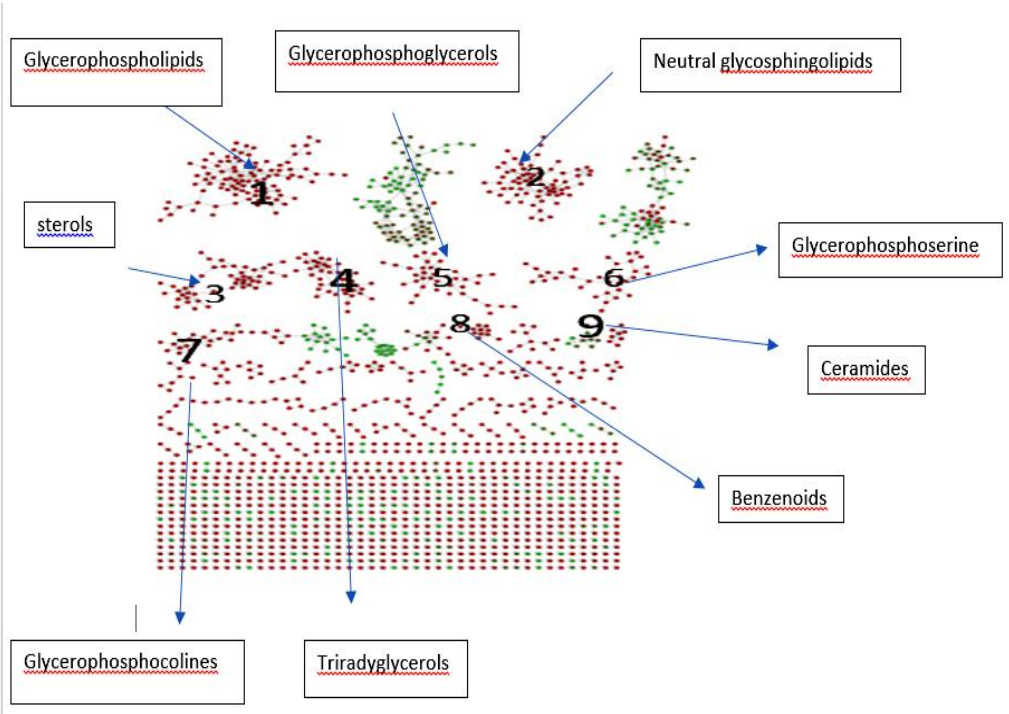

Figure 4: Representation of molecular network diagram of methanol extract of *Cassia occidentalis* leaves extract.

DOI: 10.56892/bima.v8i1.629

Table 4: Compounds identified in LCMS Molecular network.

S/N	Cluster	Class of Compound
1	1	Glycerophospholipids
2	2	Neutral glycosphingolipids
3	3	Sterols
4	4	Triradyglycerols
5	5	Glycerophosphoglycerols
6	6	Glycerphosphoserine
7	7	Glycerphosphocolines
8	8	Benzenoids
9	9	Ceramides

Figure 4 shows the representation of molecular network diagram of methanol extract of *Cassia occidentalis* leaves extract. All identified group of compounds sharing similar fragmentation patterns were represented as nodes in clusters and were numbered. The number of the different clusters in Figure 4 was named in Table 4 above.

REFERENCES

Bagega, A. I., Usman, A. A., Dankaka, S. M., Amina, M., & Mani, A. U. (2018). *Antibacterial Activity and Phytochemical Analysis of Cassia occidentalis Leaf Extract on Salmonella Typhimurium*. *BIOREMEDIATION SCIENCE AND TECHNOLOGY 6*(2), 5–8.

Dave, H. and Ledwani, L. (2012) A Review of Anthraquinones Isolated from Cassis Species and Their Applications. Indian Journal of Natural Products and Resources, 3, 291-319.

Dayrit, F. M., Dios, A. C. De, Dayrit, F. M., & Dios, A. C. De. (n.d.). *1H and 13C NMR for the Profiling of Natural Product Extracts : Theory and NMR for Applications the Profiling of Natural Product Extracts : Theory and Applications*. https://doi.org/10.5772/intechopen.71040

Sadiq, I. S., Bello, A. B., Tureta, S. G., Isah, A., & Kamaru, M. B. (2011). Phytochemistry and antimicrobial activities of Cassia occidentalis. *International Journal of Natural and Applied Sciences*, *7*(3), 331–335. http://www.ajol.info/index.php/ijonas/article/view/86517%5Cnhttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=cagh&AN=20133134849%5Cnhttp://oxfordsfx.hosted.exlibrisgroup.com/oxford?sid=OVID:caghdb&id=pmid:&id=doi:&issn=0794-4713&isbn=&

Safwan, M., Bustamam, A., Pantami, H. A., & Azizan, A. (2020). *Complementary analytical platforms of NMR spec- troscopy and LCMS analysis in the metabolite profil- ing of Isochrysis galbana*. *μ m*.

Ufer, M., Juif, P. E., Boof, M. L., Muehlan, C., & Dingemanse, J. (2017). Metabolite profiling in early clinical drug development: current status and future prospects. *Expert Opinion on Drug Metabolism and Toxicology*, *13*(8), 803–806. https://doi.org/10.1080/17425255.2017.1351944

Vijayalakshmi, S., Ranjitha, J., Devi Rajeswari, V., & Bhagiyalakshmi, M. (2013). Pharmacological profile of Cassia occidentalis L - A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(3), 29–33.

Yadav, J. P., Arya, V., Yadav, S., Panghal, M., Kumar, S., & Dhankhar, S. (2010). Cassia occidentalis L.: A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia*, *81*(4), 223–230. https://doi.org/10.1016/j.fitote.2009.09.008

Yadava, R. N., & Satnami, D. K. (2011). Chemical constituents from cassia occidentalis Linn. *Indian Journal of Chemistry - Section B Organic and Medicinal Chemistry*, *50*(8), 1112–1118.

Ochei, J. and Kolhatkar, A. (2008) Medical Laboratory Science, Theory and Practices. Tata McGraw-Hill, New York, 311-347.