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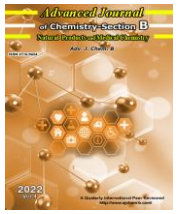

Original Research Article

Phytochemical and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Ethyl Acetate Root Extract of *Indigofera Diphylla*

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Phytochemical
Maceration

ABSTRACT

The secondary metabolite isolated from plant has been the main source of novel drugs which bears valuable therapeutic activities such as anthelmintic, antimicrobial, and antioxidant activities. *Indigofera diphylla* a semi–woody herb of dry sandy area belongs to the family fabaceae. The plant is being used as antidotes. The study was aimed at determining the chemical constituent of the extract in ethyl acetate of the root of *Indigofera diphylla* utilizing GC-MS. The plant was harvested and dried by air after that the root was separated and processed into powder. To obtain ethyl acetate extract, extraction by maceration was used. The Agilent GC-MS machine was utilized to analyze the chemical composition. There were found to be forty-five (45) different compounds from the ethyl acetate extract of root of *Indigofera diphylla*, among which 2-isopropoxyethyl propionate showed the highest peak area of 17.12% and 1-nitro-2-acetamido-1,2-dideoxy-d-mannitol showed the lowest peak area of 0.028%. The major identified compounds were methenamine, 5-fluoro-6-methyl-5-hepten-2-one, 2-(4-nitrobenzylideneamino)thiazole, hexadecanoic acid, 1,1-dimethylester, and n-hexadecanoic acid. The phytochemical result revealed that glycoside, saponins, and tannins were present. The phytochemical identified from the ethyl acetate extract of *Indigofera diphylla* may contribute to the plant's medical effectiveness and is a potential source of therapeutic medications.

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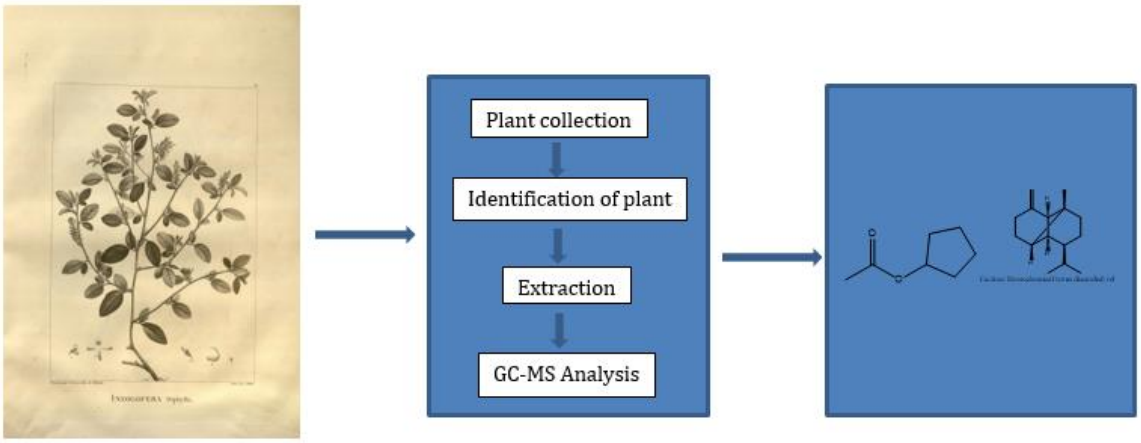
GRAPHICAL ABSTRACT



Indigofera diphylla plant *Compounds analysed from GC-MS*

1. Introduction

Herbs and plants have been used as a source of curative chemicals in traditional medical systems for the past few decades. Both traditional healthcare systems and the global herbal and pharmaceutical markets rely heavily on medicinal plants. Alkaloids, tannins, flavonoids, and phenolic chemicals, which have a distinct physiological effect on human body, are the most significant bioactive components of plants. 65 to 80 percent, according to the World Health Organization (WHO) of people worldwide who live in underdeveloped nations rely on the traditional medicine for their primary medical care and treatment. This is due to the fact that herbal remedies are affordable and come from a natural source [1].

The indigenous usage of medicinal plants in treating oral illnesses has been documented in numerous investigations in extensive and continually expanding literature on folk medicine (sometimes anecdotally amid other findings) [2]. Over time, there has been a substantial growth in interest in investigating the phytochemical and pharmacology of bioactive compounds extracted from various plant species [3].

Antiplasmodial, antibacterial, antifungal, spasmolytic, insecticidal, and antioxidant activities are only a few of the beneficial medicinal properties that the majority of phytochemicals have been shown to possess [4].

2. Plant Description

Indigofera diphylla belong to the family fabaceae, it is a semi-woody spreading herb of dry sandy areas of the Sahel from Mauritania to Niger and Nigeria, and extending across the continent to Ethiopia. It occurs on fixed sand-dunes on the coast of northern Senegal where it assists in stabilizing them. It is a perennial plant with a height of 40 to 50 cm and prefers sandy soils. Grows in arid regions, where it remains green for the most of dry season [5,6].

The plant was reported to be used as antidotes for venomous stings, bites, etc. It was also reported to be used as fodder and land conservation (leaf) product [6]. This study was aimed at determining the chemical constituents of the root stem extract in ethyl acetate of *indigofera diphylla*. Because of its moderate polarity and low toxicity, ethyl acetate solvent was chosen for the study's extraction of both polar and non-polar phytochemicals.

3. Methods

3.1. Plant Collection

The *indigofera diphylla* was found in the Zaria Local Government Area's forest in Kaduna State, Nigeria, and Mallam Namadi Sanusi identified it. A voucher identity of ABU0329 was assigned to the plant.

Figure 1. Drawing of the flowering and fruiting stem *Photograph by: Ventenat, E.P., Choix de plantes, t. 30 (1803) [P. Bessa]*

3.2. Sample Preparation

The plant sample was cleaned to remove sand and other dirty. Distilled water was used to rinse it, and then air dried for 21 days. The dried sample was pulverized and stored in a polyethene bag until it was ready for use.

3.3. Sample Extraction

700 g of the plant sample was subsequently macerated in n-hexane, ethyl acetate, and methanol in accordance to increase in polarity of the solvents. Maceration method involve soaking the pulverized plant sample within an aspirator bottle with n-hexane (polarity =0.009). The mixture in the aspirator bottle was allowed to stand for four (4) agitated days. Following a thorough extraction with n-hexane, the process was repeated with ethyl acetate (polarity = 0.228) and methanol (polarity = 0.762). The extract concentration was done using rotary evaporator. The GC-MS of the ethyl acetate was performed to determine the chemical constituent in it.

3.4. Phytochemical Analysis

3.4.1. Test for Alkaloids

0.2 g of the extract was weighed and placed in a test tube and three drops of Mayer's reagent was added. The present of reddish-brown precipitate indicates the presence of alkaloids [7].

3.4.2. Test for Glycosides

1 ml of the extracts, 0.5 ml of con. Sulphuric acid was added and allowed to stand for 2 minutes. The presence of reddish color precipitate shows the presence of glycosides [8].

3.4.3. Test for Flavonoids

0.2 g of the extract was weighed and placed in test tube, a pinch of magnesium turning and drops of con. HCl was added in the test tube. The presence of pink color indicates the presence of flavonoids [8].

3.4.4. Test for Saponins

0.2 mg of the extract was weighed and placed in a graduated cylinder, and also was diluted with 10 ml distilled water, the mixture was shaken for 15 minutes. The foam indicates the presence of saponins and steroid [9].

3.4.5. Test for Tannins

0.2 g of the extract with 50 ml distilled water and filtered then ferric chloride reagent was added. The presence of blue-black or blue-green precipitate indicates the presence of tannins [7].

3.4. Analysis with GC-MS

GC-MS analysis of the extract of ethyl acetate from *indigofera diphylla* was executed utilizing the Agilent innovation GC-MS 7890A (USA) coupled with GC ALS as an infusion source. The samples were derivatized utilizing N-methylbis[trifluoroacetamide] in methanol and measured using 2 µL infusion volume at 325 °C and a balanced time of 0.25 min.

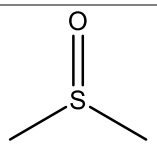
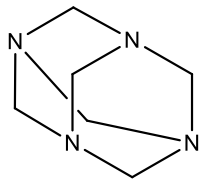
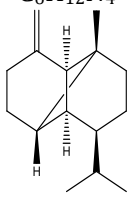
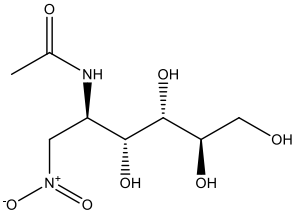
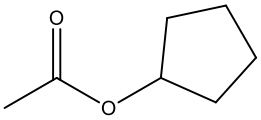
3.5. Component Identification

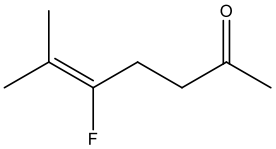
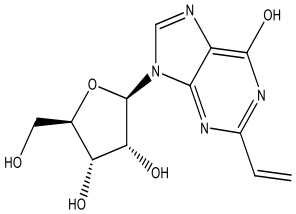
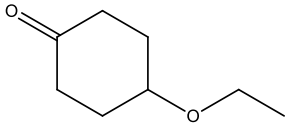
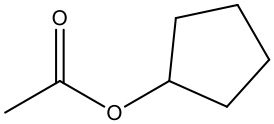
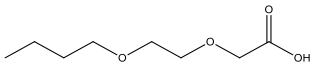
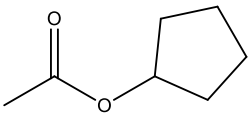
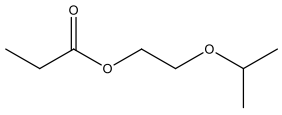
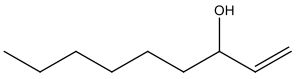
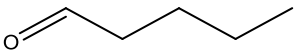
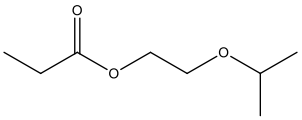
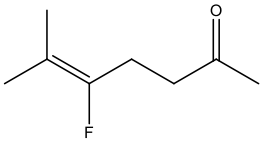
The National Institute of Standards and Technology's database was used for the GC-MS interpretation results (NIST). A comparison was made between the mass spectra of the unknown and known components recorded in the NIST collection.

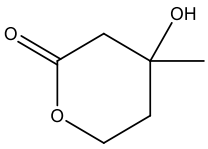
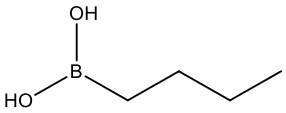
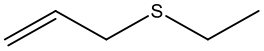
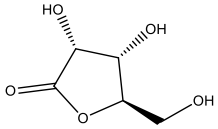
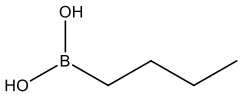
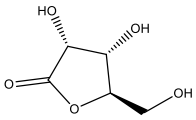
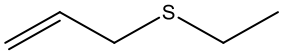
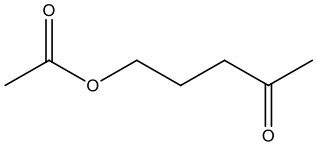
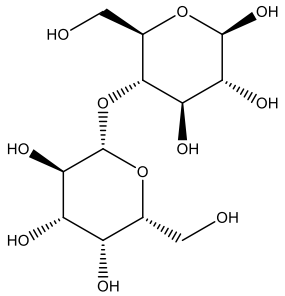
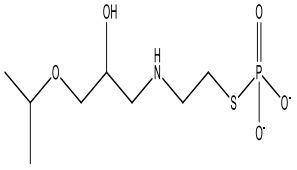
4. Results

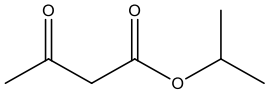
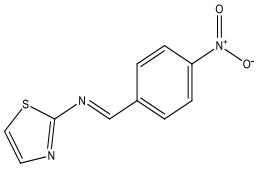
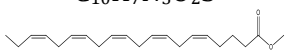
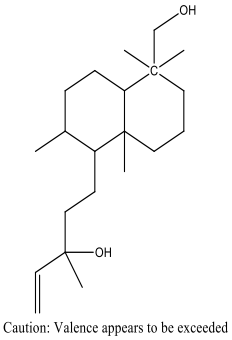
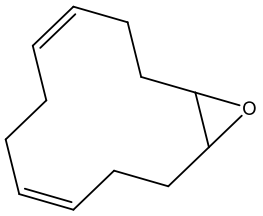
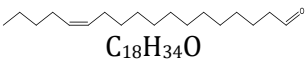
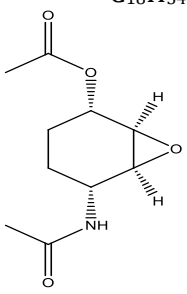
4.1. Components identified from the NIST library are presented in Table 1, and the chromatogram of the gas chromatogram is displayed in Figure 1.

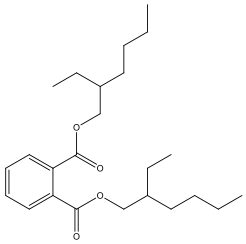
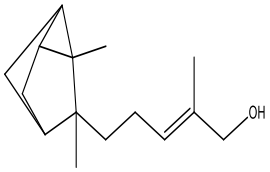
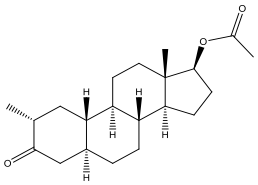
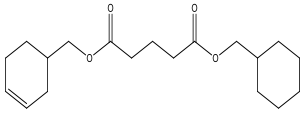
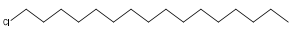
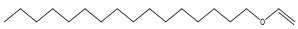
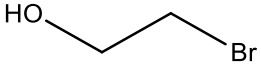
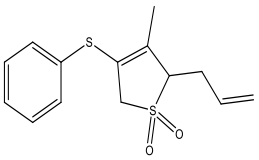
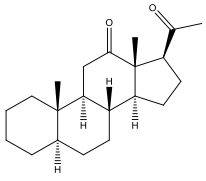
Table 1. Phytochemical constituent in ethyl acetate extract of *Indigofera diphylla*

S/No.	Retention time	Compound Name	Structure/Formula	Molecular weight	% of the Peak Area
1.	6. 2851	Dimethyl Sulfoxide	 C_2H_6OS	78.13	1.0208
2.	7.0574	Methenamine	 $C_6H_{12}N_4$	140.19	4.9173
3.	12.5902	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-	 Caution: Stereochemical terms discarded: rel $C_{15}H_{24}$	204.35	7.4902
4.	14.0342	1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol	 $C_8H_{16}N_2O_7$	252.22	0.0282
5.	14.1929	Cyclopentanol, acetate	 $CH_{12}O_2$	128.17	0.1849

6.	14. 2320	5-Fluoro-6-methyl-5-hepten-2-one	 $C_8H_{13}FO$	144.19	0.0537
7.	14.4161	2-Vinyl-9-[.beta.-d-ribofuranosyl]hypoxanthine	 $C_{12}H_{14}N_4O_5$	294.26	1.0987
8.	14.6655	Cyclohexanone, 4-ethoxy-	 $C_8H_{14}O_2$	142.20	1.7707
9.	14.8232	Cyclopentanol, acetate	 $C_7H_{12}O_2$	128.17	2.2887
10.	14.9579	2-(2-Butoxyethoxy)acetic acid	 $C_8H_{16}O_4$	176.21	2.9349
11.	15.1394	Cyclopentanol, acetate	 $C_7H_{12}O_2$	128.17	2.3037
12.	15.5226	2-Isopropoxyethyl propionate	 $C_8H_{16}O_3$	160.21	17.1205
13.	15.6382	1-Nonen-3-ol	 $C_9H_{18}O$	142.24	5.7045
14.	15.7138	Pentanal	 $C_5H_{10}O$	86.13	3.54
15.	15.8878	2-Isopropoxyethyl propionate	 $C_8H_{16}O_3$	160.21	4.0051
16.	19.1627	5-Fluoro-6-methyl-5-hepten-2-one	 $C_8H_{13}FO$	144.19	0.0936

17.	19.1967	dl-Mevalonic acid lactone	 $C_6H_{11}O_3$	130.14	0.0314
18.	19.3368	1-Butaneboronic acid	 $C_4H_{11}BO_2$	101.94	0.2623
19.	19.3677	3-Ethylthio-1-propene	 $C_5H_{11}S$	102.20	0.0809
20.	19.4287	D-(+)-Ribonic acid .gamma.-lactone	 Caution: Stereochemical terms discarded: + $C_5H_8O_5$	148.11	0.2165
21.	19.4698	1-Butaneboronic acid	 $C_4H_{11}BO_2$	101.94	0.2023
22.	19.5757	D-(+)-Ribonic acid .gamma.-lactone	 Caution: Stereochemical terms discarded: + $C_5H_8O_5$	148.11	0.4213
23.	19.7995	3-Ethylthio-1-propene	 $C_5H_{10}S$	102.20	1.0102
24.	19.8440	2-Pentanone, 5-(acetyloxy)-	 $C_7H_{12}O_3$	144.17	0.1405
25.	19.8691	.beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	 $C_{12}H_{22}O_{11}$	342.40	0.0757
26.	23.8761	S-[2-[2-Hydroxy-3-isopropoxypropylamino]ethyl]thiophosphate	 Caution: A net charge appears to be present $C_8H_{18}NO_5PS^{2-}$	271.27	1.0548

27.	24.1226	Butanoic acid, 3-oxo-, 1-methylethyl ester	 $C_7H_{12}O_3$	144.17	1.4356
28.	24.5692	Dodecanoic acid, 1-methylethyl ester	 $C_{15}H_{30}O_2$	242.40	1.35
29.	27.4460	Hexadecanoic acid, 1,1-dimethylethyl ester	 $C_{20}H_{40}O_2$	312.53	0.7019
30.	28. 2687	2-(4-Nitrobenzylideneamino)thiazole	 $C_{10}H_7N_3O_2S$	233.25	0.948
31.	28.7150	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	 $C_{21}H_{32}O_2$	316.48	4.7441
32.	29.7449	1-Naphthalenepropanol, .alpha.-ethenyldecahydro-5-(hydroxymethyl)-.alpha.,2,5,5,8a-pentamethyl-	 Caution: Valence appears to be exceeded $C_{21}H_{39}O_2$	323.53	0.8326
33.	30.3599	1,2-Epoxy-5,9-cyclododecadiene	 $C_{12}H_{18}O$	178.27	2.8357
34.	30.4419	13-Octadecenal, (Z)-	 $C_{18}H_{34}O$	266.46	1.1294
35.	33. 2188	Cyclohexane, 1R-acetamido-4-cis-acetoxy-2,3-cis-epoxy-	 $C_{10}H_{15}NO_4$	213.23	0.3873

36.	33.5129	Bis(2-ethylhexyl) phthalate	 $C_{24}H_{38}O_4$	390.56	3.7969
37.	36.8123	.alpha.-Santalol	 $C_{15}H_{24}O$	220.35	0.3921
38.	36.8812	Estran-3-one, 17-(acetyloxy)-2-methyl-, (2.alpha.,5.alpha.,17.beta.)-	 $C_{21}H_{23}O_3$	332.48	0.678
39.	36.9893	Glutaric acid, (cyclohex-3-enyl)methyl cyclohexylmethyl ester	 $C_{19}H_{30}O_4$	322.44	0.6729
40.	37.1783	1-Chloroeicosane		316.99	0.8468
41.	37. 2273	Hexadecane, 1-chloro-	 $C_{16}H_{23}Cl$	260.89	1.2399
42.	38.0132	Hexadecane, 1-(ethenyloxy)-	 C_2H_5BrO	268.48	0.1371
43.	38. 2267	Ethanol, 2-bromo-			0.5911
44.	38. 2814	3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	 $C_{14}H_{16}O_2S_2$	280.41	0.4129
45.	38.3725	5.alpha.-Pregnane-12,20-dione	 $C_{21}H_{32}O_2$	316.48	0.1803

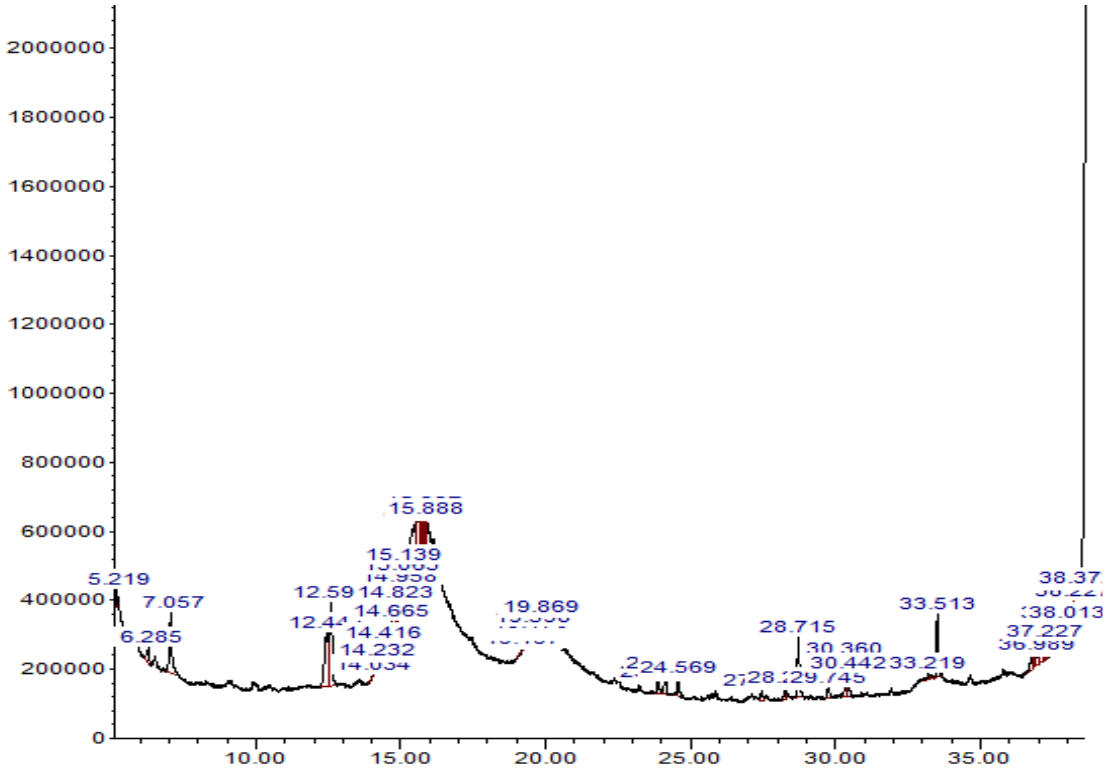


Figure 1. Chromatogram of the phytochemical constituent in ethyl acetate of the root of *Indigofera diphylla.*

4.2. Phytochemical Results

Phytochemical Tested	Result
Alkaloid	-
Glycoside	+
Flavonoids	-
Saponins	+
Taninns	+

5. Discussion

According to a report, varieties of plants belonging to the genus Indigofera have demonstrated that they are prospective sources of chemicals with pharmacological effects. *Indigofera diphylla* root bark extract was used to performed GC-MS analysis, and it revealed 45 compounds, among which 2-isopropoxyethyl propionate had the highest peak area (17.12%) and 1-nitro-2-acetamido-1,2-dideoxy-d-mannitol had the lowest peak area (0.028%). The major components identified from the GC-MS analysis include, Methenamine has been reported to be used as a urinary antiseptic agent for several century ago [10]. 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol was mentioned to have anti-insect activity [11]. 5-Fluoro-6-methyl-5-hepten-2-one is use as defensive glands of nymphalid butterfly Agraulis vanillae [12]. 2-(4-Nitrobenzylideneamino)thiazole and its derivatives have many different biological effects, such as antioxidant, analgesic, and antimicrobial activities that include antibacterial, antifungal, antimalarial, anticancer, antiallergic, antihypertensive, anti-inflammatory, and antipsychotic properties [13]. Hexadecanoic acid, 1,1-dimethylethyl ester can act an anti-inflammatory. According to Aparna *et al*. (2012), Because of its strong entropy-driven

binding to the enzyme as demonstrated by ITC analysis, high active site binding affinity as demonstrated by the formation of binary complex crystals with PLA2 in a 1:1 molar solution, and its binding to the enzyme's active site in the X-ray structure, n-hexadecanoic acid and its derivatives may have anti-inflammatory effects [14].

6. Conclusion

The study examines the phytochemical components in an ethyl acetate root extract of *Indigofera diphylla* collected from the Samaru region of the Zaria Local Government in Kaduna State, Nigeria. The phytochemical include glycoside, saponins, and tannins. There were found to be forty-five (45) chemicals, of which prior studies had reported on their bioactivity or industrial use. According to data from the GC-MS, *Indigofera diphylla* may be a reliable source of pharmaceuticals.

List of Abbreviations

NIST: National Institute of Standard and Technology
GC-MS: Gas Chromatography- Mass Spectrometry
ITC: Isothermal Titration Calorimetry
PLA2: Phospholipase A2

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