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Validation of the Chemical Constituents of Crude Leaf Extract of *Datura discolor* with Qualitative Phytochemical Screening and GC-MS Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study sought to investigate into the crude extract of the leaves of *Datura discolor* Bernh. with a view to validate the chemical constituents of the leaves. Extraction was achieved via maceration using methanol and solvent was removed after filtration by evaporation to dryness in the open.

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Preliminary phytochemical screening of the crude extract was qualitatively performed using standard operating procedures used by renowned scholars. Gas chromatography tandem mass spectrometry (GC-MS) was used to further elucidate the secondary metabolites present in the leaves of the plant. Phytochemicals detected are alkaloids, steroids, terpenoids, glycosides, saponins, phenolic compounds, tannins and lipids. Major active secondary metabolites identified via GC-MS are cis-9-Octadecenoic acid (29.59%), hexadecanoic acid (19.72%), octadecanoic acid (7.56%), 1,2-epoxyhexadecane (6.35%), methyl hexadecanoate (6.22%), methyl-11-octadecenoate (5.57%), methyl-15-tetracosenoate (4.16%), and tetradecanoic acid (3.50%). Other compounds which are present in traces with percentage abundance less than 3 % include 3,7,11,15-tetramethyl-2-hexadecen-1-ol; methyl-trans,trans--9,12-octadecadienoate; trans-13-docosenoic acid; 2,6,10,18,22-tetracosahexaene; methyl octadecanoate; dodecyl hexyl oxalate; 2-Methyl-2-(3-methyl-1-vinyl-2-butenyl)oxirane; methyl tetradecanoate; and 2-methyl-3-methylene-2-(4-methyl-3-pentenyl)bicyclo[2.2.1]heptane. These results affirms that *Datura discolor* Bernh, is rich in secondary metabolites which have diverse pharmacological activities with fatty acids and their esters being the most abundant constituents.

Keywords: Datura discolor; phytochemicals; angel's trumpet; thorn-apple; GC-MS analysis.

1. INTRODUCTION

Naturally, humanity is endowed with a plethora of chemical substances occurring in living organisms and these substances are known as natural products. Most of these substances are normally biologically active and are harnessed for one purpose or the other, medicinally and otherwise. The term natural product, therefore, refers to any biologically active organic compound that is manufactured naturally in plants and animals [1]. Natural products are broadly classified into primary metabolites and secondary metabolites.

Primary metabolites are natural products that are associated with intrinsic cellular functions such as nutrient assimilation and energy production, and are essential to the growth, development and survival of the organism that produces them [2,3]. They constitute basic metabolic pathways that ate are required for life, and that is to say that, the organism would die without these metabolites. Examples of primary metabolites include the core building block molecules – nucleotides, amino acids, sugars, fatty acids and vitamins that are required to make the four major macromolecules within the body (nuclei acids, proteins, carbohydrates, and lipids respectively) responsible for sustaining life. Primary metabolites have little or no medicinal properties [2,3]. Secondary metabolites, on the other hand, are natural organic molecules manufactured by living organisms which are not absolutely essential to their survival but typically play extrinsic functions that mainly affect other organisms outside of the producer, and increase the competitiveness of the producer-organism within its own environment [4]. Hence, secondary metabolites are dispensable unlike the primary counterparts. The classes of secondary metabolites are nuclei acids, proteins, carbohydrates, and lipids [5]. Secondary metabolites are commonly known as phytochemicals. They have a diversity of structures and include compounds such as pigments (carotenoids, anthocyanins), alkaloids (e.g. morphine, cordeine, etc.), phenylpropanoids, polyketides, terpenoids, steroids, phenolic compounds, saponins, flavonoids, tannins, lipids, carbohydrates, toxins (e.g. albrin, ricin), lectins (e.g. concanavalin A), drugs (e.g. vinblastine, curcumin) and these are responsible for many biological or pharmacological activities [2,3]. Phytochemicals are found in plant tissues located in the leaves, flowers, roots, vegetables, stem bark, fruits, seeds and nuts, spices, grains and other plant parts [6]. Human beings ingest these bioactive compounds when they consume fruits, seeds, nuts, vegetables and leaves as well as herbal extracts and concoctions of these plant parts.

These plant chemicals have enormous beneficial effects, and when they get into the blood stream, they help to combat different forms of ailments and health conditions [7] such as cancer [4,8–12], tumor [13], diabetes mellitus [14,15], arthritis [16], typhoid, stomach upset, malaria, diarrhoea, yellow fever, pile, body pain, jaundice, measles, fibroid, eczema, low sperm count/impotence, hiccups, dysentery, asthma, ulcer, convulsion, gonorrhoea, syphilis, infertility, obesity, tuberculosis, constipation [15], periodontal disease [17], and all of that.

Plant secondary metabolites (phytochemicals) and their derivatives have high structural

diversity and exhibit various pharmacological activities in humans and animals e.g. antibacterial, antifungal, anti-inflammatory, antimalarial, antiallergic, antihelmintic, hepatoprotective, analgelsic, neuroprotective, immune-modulatory and worm-expelling activities [18–20]. Hence, they play a key role in traditional medicine and modern drug discovery for several ailments [1,21]. Common examples include aspirin, derived from a precursor extracted from the leaves of the willow tree and applied for its health benefits for very many years [22]; paclitaxel (sold as Taxol) which has an anticancer effect, and was originally extracted from the Pacific yew tree [23]; and digoxin which is a heart medicine [24], extracted from the foxglove plant. They are also useful in cosmetics (skin-care products, hair-care products, anti-ageing agents, toiletry preparations) as well as in nutraceuticals (food and nutrition industries) due to their numerous beneficial properties. Terpenoids, for example, are well known natural products for essential oils, with a strong and characteristic pleasant odour which has been harnessed in the making of perfumes [22,25,26].

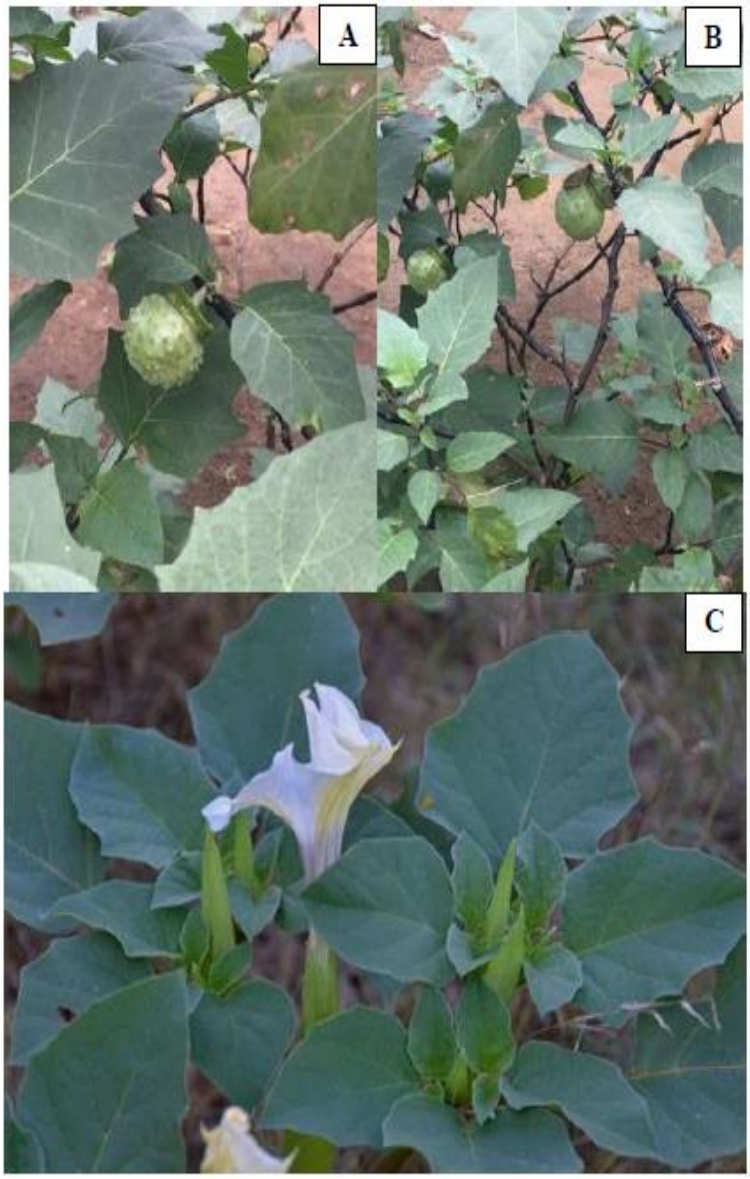


Fig. 1. Photos of *Datura discolor* (A, B) leaves and fruits (C) leaves and trumpet-shaped flowers

The genus *Datura* L. (Solanaceae) is one of the important plant groups known for traditional and modern medicinal uses as a source of tropane alkaloids. Worldwide the genus comprises about 14 species which are native to North America, but vastly diversified in Mexico [27]. *Datura discolor* Bernh., commonly known as angel's trumpet (or desert thorn-apple) is a herbaceous annual shrub native to the Sonoran Desert of western North America and belongs to botanical family, Solanaceae which has more than 90 genera and over 3,000 species distributed around the world. Common plants in this family include genus Solanum (e.g. eggplant, tomato, potato), genus Nicotiana (e.g. tobacco), genus Datura (e.g. angel's trumpet which is *Datura discolor*), as well as genus Capsicum (e.g. Capsicum annuum). *Datura discolor* is an upright or low-lying shrub that can grow to 4.5 feet (1.4 m) tall. It has a light green foliage, with conspicuous purple stripes on its stalks. The leaves are ovate-shaped and can be whole or toothed [28]. It produces an upward-growing tubular trumpet-shaped flowers that are up to 15 cm long, which manifest different colours – white in the bell, and pale to dark violet from the narrow part of the bell to the base. Hence, the specific epithet discolor, meaning "various colors," was appended to its name. *Datura discolor* has large flowers, which make it attractive for garden cultivation. The flowers open for only one night and wilt the following day. Like most other Daturas, its seed capsule is thorny and can be up to 3 inches (76 mm) long and 2.5 inches (64 mm) in diameter. It is a sun-loving plant which does well underneath overhanging leaves that can protect its flowers from damage by rainfall [28]. All parts of *Datura discolor* Bernh. is rich in a mix of alkaloids that are potentially lethal when excess dosage is ingested. Death may occur from careless recreational use of this plant and related plants [29]. *Datura discolor* Bernh. has been in local use by rural dwellers in Southeastern Nigeria who believe that the live plant repels snakes and other kinds of harmful reptiles away from the vicinity of the plant. However, these claims are albeit without any scientific proof at the moment. Coupled with its ornamental disposition, the rural dwellers, therefore, grow *Datura discolor* around residential buildings as a safety measure against snake bites. Again, very little is currently known about *Datura discolor* the phytochemical profile of the plant, the toxicity levels, extraction and isolation of secondary metabolites from the plant, or the names of the bioactive compounds contained in it. This study, therefore, seeks to derive and analyze the crude extract from the leaves of *Datura discolor*, with a view to determining the active chemical constituents.

2. MATERIALS AND METHODS

2.1 Sample Collection, Preparation and Extraction

Matured leaves of *Datura discolor* were locally harvested from the live plant at Ifakala Community in Mbaitoli L.G.A of Imo State, Nigeria. The plant was first botanically identified by a Plant Scientist. Thereafter, the leaves were sun-dried for 14 days to a constant weight, ground to fine powdered form and preserved. Exactly 775 g of finely ground leaves of *Datura discolor* (DDL) was soaked in 3.1 L of methanol (1:4 parts of sample and solvent) in an airtight container, stirred properly and left to stand for 14 days, with intermittent stirring at 2-day intervals to ensure adequate extraction. Thereafter, the mixture was filtered using a clean piece of muslin cloth [30–32]. The filtrate was collected and the solvent evaporated to dryness by leaving the vessel open under the sun. A slurry greasy solid extract of DDL was finally obtained and the yield was determined.

2.2 Qualitative Tests for Phytochemicals

The crude extract was subjected to the following preliminary qualitative tests.

2.2.1 Detection of alkaloids

(a) Mayer's test: The extract (1 mL) was taken into a test tube and 1 mL of Mayer's reagent (potassium mercuric iodide solution) was added. A cream or white precipitate was formed, indicating the presence of alkaloids [19,20,33].

(b) Hager's test: The extract (2 mL) was taken into a test tube and 1 mL of Hager's reagent was added. Emergence of yellow-coloured precipitate was observed which indicates the presence of alkaloids [33,34].

(c) Dragendorff's test: About 1 mL of the extract solution was measured into a test tube and 1 mL of Dragendorff's reagent (potassium bismuth iodide solution) was added. The solution was shaken and appearance of orange red precipitate was observed which indicates the presence of alkaloids [33,34].

2.2.2 Detection of flavonoids

(a) Alkaline Reagent test: About 2 mL of extract was taken into a test tube and 2-3 drops of 2 % sodium hydroxide solution were added. There was no emergence of an intense (deep) deep yellow colour and this indicates that flavonoids are not present [19,20,33].

(b) Shinoda's test: A 1 mL portion of extract was taken into a test tube and 10 drops of dilute HCl was added, followed by addition of 0.5 g of magnesium turnings. After shaking, there was no emergence of deep pink colour and this indicated the absence of flavonoids [20,33].

2.2.3 Detection of steroids

Salkowski test: The test extract (5 mL) was taken into a test tube and 2 mL of chloroform was added and mixed with it, followed by addition of 3 mL of concentrated H_2SO_4 along the walls of the test tube. After shaking and allowing to stand, a red coloration appeared at the junction of the two liquids which indicates the presence of steroids [19].

2.2.4 Detection of terpenoids

(a) Horizon Test: One milliliter of extract was taken into a test tube and 2 mL of trichloroacetic acid was added. No red precipitate was observed, and this showed that terpenoids were absent [20].

(b) Salkowski test: About 5 ml of extract was taken into a test tube and mixed with 2 mL of chloroform. Then 3 mL of concentrated H_2SO_4 was added carefully to create a layer. No reddish-brown coloration was observed at the interface of the two liquids and this indicates the absence of terpenoids in the extract [19].

2.2.5 Detection of glycosides

Keller-Killiani test: A 2 mL portion of extract was taken into a test tube. A solution of 0.5 mL, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with the extract. Later, 1 mL of concentrated H_2SO_4, was added along the walls of the test tube. The appearance of deep blue colour at the junction of two liquids indicates the presence of cardiac glycosides [20].

2.2.6 Detection of saponins

Frothing test: A 5 mL portion of extract was taken into test tube and a drop of Na_2CO_3 solution was added. After vigorous shaking, it was left to stand for five minutes. Foaming (frothing) having about 2 cm thickness was observed and this indicates that saponins are present [20,33].

2.2.7 Detection of phenol compounds

(a) Ferric chloride test: About 2 mL of extract was taken into a test tube and 2 mL of 5% Ferric chloride solution was added. Formation of blue-black (or deep blue) coloration indicates the presence of phenolic compounds [19,20,35].

(b) Lead acetate test: A 1 mL portion of extract was taken into a test tube and 1 mL of 10% lead tetraacetate solution was added. Formation of red to blue coloration signifies the presence of phenolic compounds [20,33,34].

2.2.8 Detection of tannins

(a) Gelatin test: To 2 mL of 1% gelatin solution, little 10% NaCl solution was added and shaken. The resulting solution was then added to 1 mL of extract in a test tube. Appearance of white precipitate indicates the presence of tannins [33,34].

(b) Ferric chloride test: About 2 mL of extract was taken into a test tube and 5 drops of 10% Ferric chloride solution was added. Formation of blue-black coloration indicates the presence of gallic tannins [19,20,35].

2.2.9 Detection of free reducing sugars

(a) Benedict's test: Into a test tube, 8-10 drops extract were put and 5 mL of Benedict's reagent was added. After warming for five minutes in a water bath, no dark red precipitate resulted and this indicates the absence of reducing sugars [19,20].

(b) Fehling's Test: The extract (2 mL) was taken inti a test tube and an equal volume of Fehling's (A & B) solution were added in turns. The mixture was heated for five minutes in a water bath. No brick-red (dark red) precipitate appeared and this indicates the absence of reducing sugars [19,20].

2.2.10 Detection of protein

Ninhydrin test: About 1 mL of extract was taken into a test tube and 2 drops of freshly prepared 0.2% Ninhydrin solution added. Purple colour did not appear and this shows the absence of proteins [19,20].

2.2.11 Detection of anthraquinones

One gram (0.5 g) of the DDL powder was placed in a dry test tube and 10 mL of chloroform was added. This was heated in steam bath for 5 minutes, filtered while hot and allowed to cool. An equal volume of 10% ammonia solution was then added to the filtrate and the solution was shaken. The upper aqueous layer showed no bright pink (rose pink) coloration implying the absence of anthraquinones [36].

2.2.12 Detection of lipids

(a) Solubility test: The sample was taken into three different test tubes and labeled as A, B and C. Then, very polar solvent (water), ethanol and non-polar solvent (chloroform) were added in each of the test tubes A, B and C, shaken and the tubes were allowed to stand for 1 minute. Sample dissolved in the non-polar solvent (chloroform) but was partially soluble in ethanol and insoluble in water.

(b) Transparent spot test: A filter paper was taken and a drop of water was applied at one end of it, while a drop of the sample was applied at the other end. Appearance of a translucent spot on the filter paper at the sample area shows that lipids are present.

(c) Sudan IV test: About 1 ml of the sample was taken into a test tube. Then 2 drops of Sudan IV reagent were added. Appearance of red-orange colour in the solution indicates that the sample contains lipids.

2.3 GC-MS Analysis of Extract

Gas chromatography tandem mass spectrometry (GC-MS) was carried out on a GCMS-QP2010 PLUS SHIMADZU instrument at the National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna State, Nigeria. The column used was a Perkin Elmer – 5 capillary column which measured 30 m x 0.25 mm with a film thickness of 0.25 mm, comprising of dimethyl polysiloxane. Helium was used as a carrier gas at a flow rate of 0.5 ml/min. A 1 µl sample injection volume was utilized. The inlet temperature was maintained at 250^0C. The oven temperature was programmed initially at 80^0C for 4 minutes, and was thereafter increased to 200^0C, and finally programmed to 280^0C at a rate of 20^0C ending with 5 minutes, resulting to a total run time of 25 minutes. The MS transfer line was maintained at a temperature of 200^0C and the source temperature was maintained at 180^0C. GC-MS was run using electron-impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectra of the components were compared with the database of spectra of known compounds stored in the GC-MS library [37– 39].

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of Extract

The yield and percentage yield of the methanol extract of *Datura discolor* leaves is shown in Table 1.

3.2 Phytochemical Constituents

The detected phytochemical constituents of the methanol extract of *Datura discolor* leaves are presented in Table 2.

The table reveals that the crude methanol extract of *Datura discolor* leaves is rich in alkaloids, saponins, glycosides and lipids, but also contains traces of steroids, terpenoids and phenolic compounds. These results therefore seem to agree with what has been reported of Solanaceae plants which holds that all parts of plants in the Solanaceae family, Datura species inclusive, are rich in alkaloids.

3.3 GC-MS Results

The GC-MS chromatogram of the compounds detected in the methanol extract of *Datura discolor* leaves is presented in Fig. 2. The chromatogram shows the presence of 17 active phytochemical compounds in the methanol extract of *Datura discolor*. The mass spectra of the 17 identified compounds are presented in Fig. 3.

Table 1. Percentage yield of methanol extract of *Datura discolor* leaves (DDL)

Mass of DDL Powder	Mass of Extract	% Yield
775 g	61.6 g	7.95

Table 2. Detected phytochemical constituents in methanol leaf extract of *Datura discolor*

S/N	Phytochemical	Test	Status
2	Alkaloids	Mayer's test	+++
		Hager's test	++
		Dragendorff's test	+++
	Flavonoids	Shinoda's test	–
		Alkaline reagent test	–
3	Steroids	Salkowski's test	+
4	Terpenoids	Horizon test	++
		Salkowski test	++
5	Glycosides	Keller-Killiani test	++
6	Saponins	Frothing test	+++
7	Phenolic compounds and	Ferric chloride test	+
	Tannins	Lead tetraacetate test	–
8	Tannins	Gelatin test	+
9	Carbohydrate	Benedict's test	–
		Fehling's test	–
10	Proteins	Biuret test	–
11		Ninhydrin test	–
12	Anthraquinones		–
	Lipids	Solubility test	+++
		Transparent spot test	++
		Sudan IV test	+++

Key: (–) = absent; (+) = present in traces; (++) = moderately present; (+++) = abundantly present

The gas chromatogram data are presented in Table 3 showing the peak numbers, retention times (RT) as well as area and height parameters, while the identified secondary metabolites are presented in Table 4 with their molecular formulae, molecular weights (MW), percent abundance (peak area %), IUPAC names and some common names.

Gas chromatography hyphenated to mass spectrometry (GC-MS) is aimed at enhancing the analytical power of gas chromatography with mass spectrometry. This allows each compound exiting the gas chromatograph to be analyzed separately. Hence, both the gas chromatogram and the mass spectra of the exited compounds when combined will allow the unambiguous identification of each compound.

Table 3. Gas chromatogram parameters of various secondary metabolites identified in methanol extract of *Datura discolor* leaves

Peak #	R. Time	Peak area	Area % or Conc.	Peak height	Height %	A/H
1	10.550	2390981	0.42	973272	0.97	2.46
2	10.692	9562959	1.67	3881892	3.88	2.46
3	13.550	6888455	1.20	3085329	3.08	2.23
4	14.276	20001876	3.50	2906277	2.90	6.88
5	15.243	36303987	6.35	11017932	11.00	3.29
6	16.836	35558649	6.22	8860595	8.85	4.01
7	18.080	112805514	19.72	10692855	10.68	10.55
8	19.859	13937393	2.44	4390974	4.38	3.17
9	19.963	31851070	5.57	9717267	9.70	3.28
10	20.202	15484217	2.71	4456754	4.45	3.47
11	20.309	11654542	2.04	3787371	3.78	3.08
12	20.860	169300570	29.59	12579204	12.56	13.46
13	21.083	43234954	7.56	7817707	7.81	5.53
14	21.920	11786859	2.06	3758544	3.75	3.14
15	23.802	14501339	2.53	4114210	4.11	3.52
16	24.461	23780237	4.16	6305376	6.30	3.77
17	27.769	13030351	2.28	1802252	1.80	7.23
		572073953	**100.00**	**100147801**	**100.00**	

Table 4. Compounds identified in the methanol extract of *Datura discolor* leaves by GC-MS

Peak #	Area %	Mol. Formula	Mol. Wt.	IUPAC Name of Compound
1	0.42	$C_{15}H_{24}$	204	2-methyl-3-methylene-2-(4-methyl-3-pentenyl) bicyclo [2.2.1] heptane (β-Santalene or norbornane, a sesquiterpene)
2	1.67	$C_{10}H_{16}O$	152	2-Methyl-2-(3-methyl-1-vinyl-2-butenyl) oxirane (Santolina epoxide)
3	1.20	$C_{15}H_{30}O_2$	242	Methyl tetradecanoate
4	3.50	$C_{14}H_{28}O_2$	228	Tetradecanoic acid (Myristic acid)
5	6.35	$C_{16}H_{32}O$	240	1,2-Epoxyhexadecane
6	6.22	$C_{17}H_{34}O_2$	270	Methyl hexadecanoate
7	19.72	$C_{16}H_{32}O_2$	256	Hexadecanoic acid (Palmitic acid)
8	2.44	$C_{19}H_{34}O_2$	294	Methyl-trans, trans--9,12-octadecadienoate
9	5.57	$C_{19}H_{36}O_2$	296	Methyl-11-octadecenoate
10	2.71	$C_{20}H_{46}O$	296	3,7,11,15-tetramethyl-2-hexadecen-1-ol (Phytol, an acyclic diterpene alcohol)
11	2.04	$C_{19}H_{38}O_2$	298	Methyl octadecanoate
12	29.59	$C_{18}H_{34}O_2$	282	Cis-9-Octadecenoic acid (cis-Oleic acid)
13	7.56	$C_{18}H_{36}O_2$	284	Octadecanoic acid (Stearic acid)
14	2.06	$C_{20}H_{38}O_4$	342	Dodecyl hexyl oxalate
15	2.53	$C_{22}H_{42}O_2$	338	Trans-13-Docosenoic acid (Brassidic acid)
16	4.16	$C_{25}H_{48}O_2$	380	Methyl-15-Tetracosenoate
17	2.28	$C_{30}H_{50}$	410	2,6,10,14,18,22-Tetracosahexaene (Squalene, a triterpene)
	100.00			

Table 3 reveals that the chromatogram contains 17 peaks which indicates the presence of the presence of a total of 17 compounds. The 17 compounds (secondary metabolites) detected by the GC-MS instrument with different retention times and their concentration are presented in Table 4. These phytochemical constituents were identified by comparing the m/z values of the unknown peaks in each of the target mass spectra with the m/z values in the spectra of known compounds as obtained from the database stored in the library of the GC-MS instrument. Identification was achieved when a match was obtained in each case, after which the details of the compounds were tabulated (Table 4) showing the molecular formulae and names.

From Table 4, it can be observed that the prevalent compounds in the methanol extract of *Datura discolor* leaves are cis-9-Octadecenoic acid otherwise known as cis-oleic acid (29.59%), hexadecanoic acid otherwise known as palmitic acid (19.72%), octadecanoic acid otherwise known as stearic acid (7.56%), 1,2-epoxyhexadecane (6.35%), methyl hexadecanoate or methyl palmitate (6.22%), methyl-11-octadecenoate or methyl trans-vaccenate (5.57%), methyl-15-tetracosenoate (4.16%), and tetradecanoic acid or myristic acid (3.50%). Other compounds are present in traces with %area less than 3 %.

Several of the identified compounds have been reported to have medicinally important bioactivities. Among the identified secondary metabolites, myristic acid, squalene, palmitic acid and phytol have been found to exhibit antioxidant property, 5-alpha-reductase inhibitory effect, as well as antifibrinolytic, hemolytic and antimicrobial activities [37,40,41]. Squalene (a triterpenoid) is a phenolic compound. It has been reported that terpenes occur in the latex and resins of some plants and perform some physiological functions to defend the plants against certain disease-causing pathogens [40,42]. In addition, squalene has also been reported to exhibit anticancer, chemopreventive, gastropreventive and hepatoprotective effects. It also possesses pesticide, anti-tumor and sunscreen properties [40].

β-santalene is a sesquiterpene, a bridged carbobicyclic compound and a polycyclic olefin which plays a role as a plant metabolite. It is also used in cosmetic products for perfuming, as part of perfume oils and/or flavours. Phytol is an acyclic diterpene alcohol which is a constituent of chlorophyll and used as a precursor in making synthetic forms of vitamin E and vitamin K_1. Phytol has also been reported to find use in both cosmetic and non-cosmetic products [43]. Medicinally, phytol exhibits antinociceptive and antioxidant activities [44] as well as anti-inflammatory and anti- allergic properties [45],

immunostimulant, and antimicrobial activities [46].

Datura discolor leaves have also shown the presence of esters as listed in Table 4 and which includes dodecylhexyl oxalate, methyl-15-tetracosenoate, methyl-trans, trans-9,12-octadecadienoate, methyl hexadecanoate and methyl tetradecanoate. Esters are important organic compounds with increasing number of commercial applications such as in making fragrances, cosmetics, detergents, flavors as well as pharmaceuticals. The epoxide (2-tetradecyloxirane) identified in the leaves of *Datura discolor* is a white solid with a characteristic waxy consistency. It is useful in organic synthesis, as diluent for UV-curable coatings, acid scavengers, stabilizer, deodorizer, and intermediates for agriculture, pharmaceuticals, personal care, surfactants, and lubefuel additives [47].

Stearic acid (octadecanoic acid), a soft waxy solid, is one of the useful types of saturated fatty acids which has been reported to exhibit some neuroprotective effect against oxidative stress [48,49], immunosuppressive action on T cells [50], hepatoprotective effect [51] and anti-inflammatory potential [51]. It has a bifunctional character with a polar head group that can be attached to cations and a non-polar hydrocarbon chain that confers solubility in organic solvents makes it useful as a surfactant and softening agent. No critical pharmacological effect or potential has been recorded of brassidic acid (trans-13-Docosenoic acid), a monounsaturated very long-chain fatty acid. Cis-oleic acid (cis-9-Octadecenoic acid) is the most common fatty acid in nature and classified as a monounsaturated omega-9 fatty acid [52]. It is used as a component in many human foods. Its sodium salt is a good emulsifying agent [53].

Due to the presence of above identified compounds in the methanol leaf extract of the plant, *Datura discolor* may be harnessed in various pharmaceutical and chemical applications.

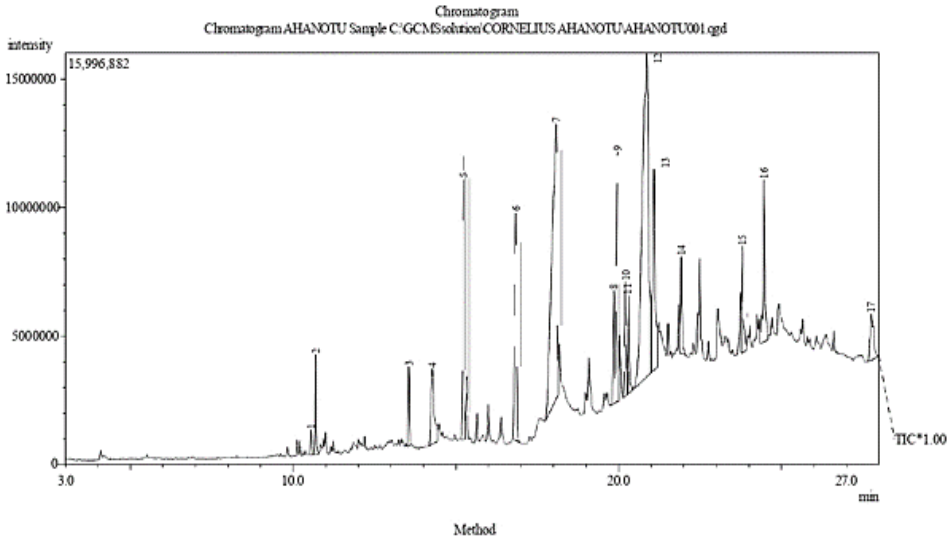
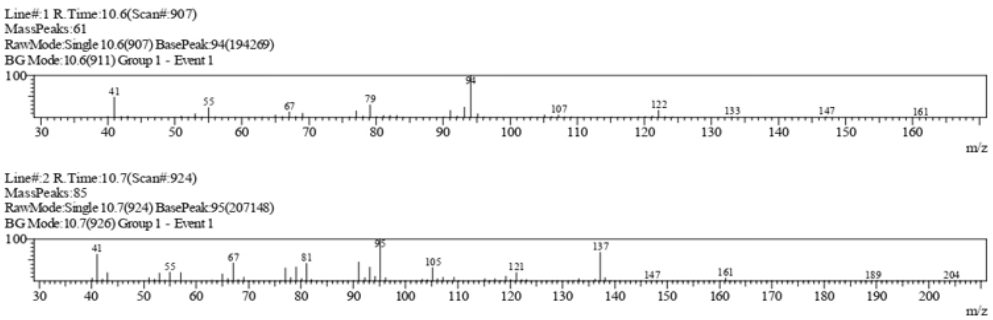
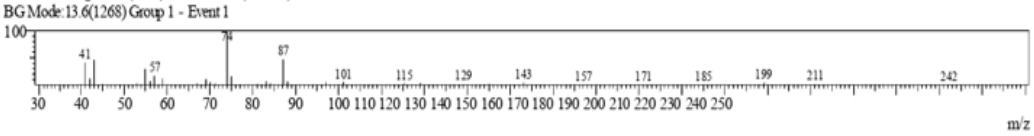


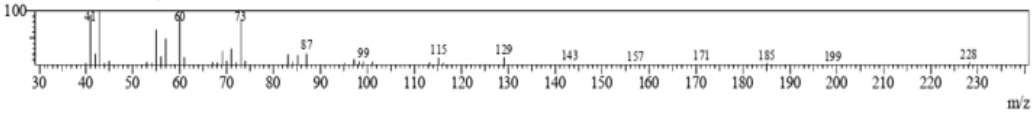
Fig. 2. Gas chromatogram of the *Datura discolor* leaves extract



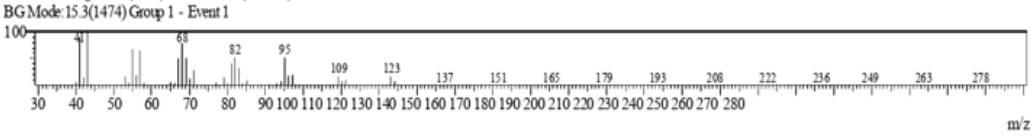
Line#:3 R.Time:13.6(Scan#:1267)
MassPeaks:98
RawMode:Single 13.6(1267) BasePeak:74(102483)
BG Mode:13.6(1268) Group 1 - Event 1



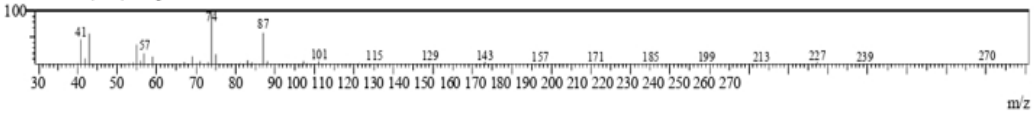
Line#:4 R.Time:14.3(Scan#:1354)
MassPeaks:86
RawMode:Single 14.3(1354) BasePeak:43(79560)
BG Mode:14.3(1357) Group 1 - Event 1



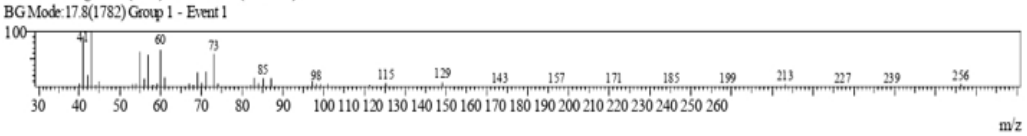
Line#:5 R.Time:15.2(Scan#:1469)
MassPeaks:88
RawMode:Single 15.2(1469) BasePeak:43(834452)
BG Mode:15.3(1474) Group 1 - Event 1



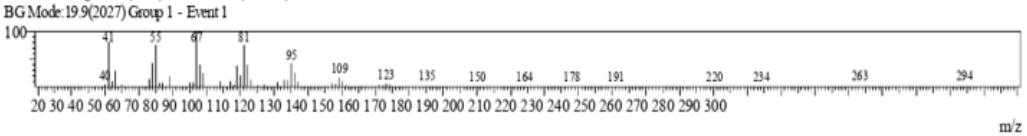
Line#:6 R.Time:16.8(Scan#:1661)
MassPeaks:108
RawMode:Single 16.8(1661) BasePeak:74(1561893)
BG Mode:16.9(1666) Group 1 - Event 1



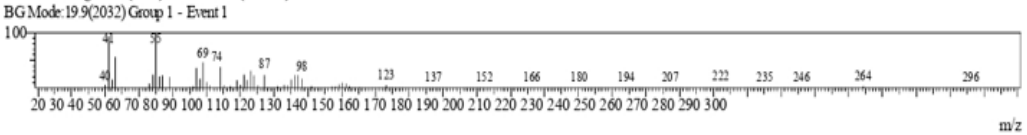
Line#:7 R.Time:18.1(Scan#:1811)
MassPeaks:124
RawMode:Single 18.1(1811) BasePeak:43(1227573)
BG Mode:17.8(1782) Group 1 - Event 1



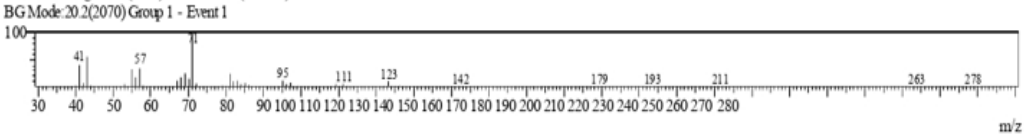
Line#:8 R.Time:19.9(Scan#:2024)
MassPeaks:129
RawMode:Single 19.9(2024) BasePeak:67(242759)
BG Mode:19.9(2027) Group 1 - Event 1



Line#:9 R.Time:20.0(Scan#:2037)
MassPeaks:154
RawMode:Single 20.0(2037) BasePeak:55(713845)
BG Mode:19.9(2032) Group 1 - Event 1



Line#:10 R.Time:20.2(Scan#:2065)
MassPeaks:86
RawMode:Single 20.2(2065) BasePeak:71(593766)
BG Mode:20.2(2070) Group 1 - Event 1



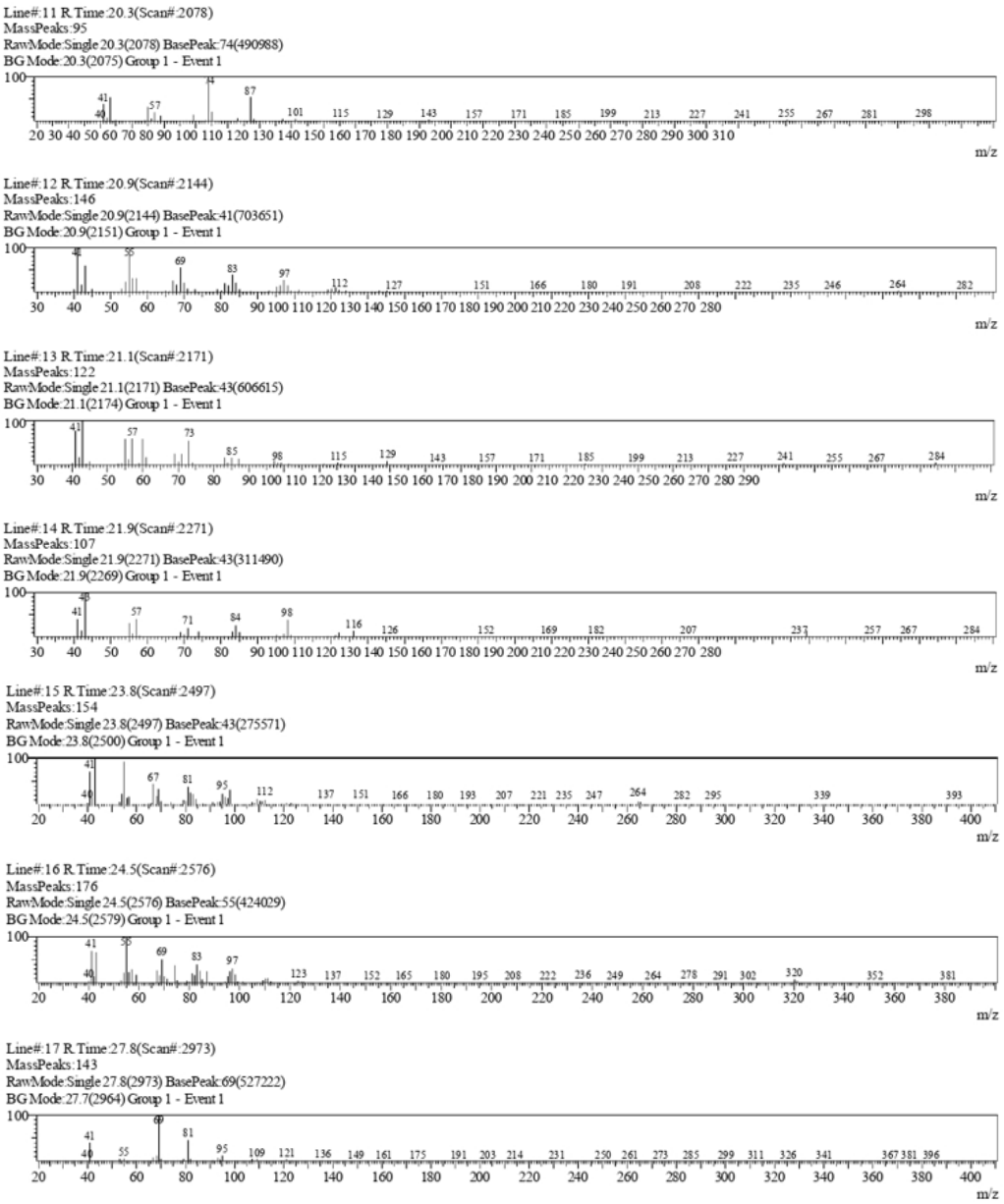


Fig. 3. Mass spectra of the 17 identified compounds indicated by the GC peaks

4. CONCLUSIONS

From the results, the following inferences can be drawn;

1. *Datura discolor* Bernh crude leaf extract is rich in alkaloids, saponins, glycosides and lipids, but also contains traces of phenolic compounds and steroids.
2. Most of the 17 active compounds identified with GC-MS technique are fatty acids and their esters.

5. RECOMMENDATIONS FOR FURTHER RESEARCH

1. Since local populations in the Southeastern region of Nigeria claim *Datura discolor* has snake-repelling tendencies albeit without scientific evidence to support these claims, further research via collaboration with herpetologists and research experts in biochemistry and pharmacology is recommended to investigate the leaf extract to ascertain their biochemical and

pharmacological activities as well as their toxicity levels.

2. Further research is also recommended to isolate and characterize the active compounds in the leaves, stem, flowers and fruits of this plant for structure elucidation as this would serve as literature reference materials in the future.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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