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GC-MS Determination of Bioactive Constituents of the Methanolic Fractions of *Cnidoscolus aconitifolius*

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Authors' contributions

This work was carried out in collaboration between both authors. Author NKA participated in all operations of this manuscript. Author NKA wrote the first draft of the manuscript. Author OCO designed the study and wrote the protocol performed. Author NKA managed the analyses of the study and managed the literature searches. Author OCO revised the manuscript and author NKA has the final responsible for all information presented. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetes mellitus is a major metabolic disorder affecting a huge population all over the world. *Cnidoscolus* species have been extensively used for the management of diabetes in folkloric medicine. The presence of diverse secondary metabolites has been reported from species of the genus *Cnidoscolus*. However, there has not been much information available on phytochemical components and biological activity in the leaf methanol extract of *Cnidoscolus aconitifolius.*

Objective: This study was designed to extract and identify some bioactive compounds in the leaf methanol fractions of *C. aconitifolius* which may provide insight on its pharmacological properties and its use in traditional medicine.

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Place and Duration of Study: Department of Biochemistry, Michael Okpara University of Agriculture, Umudike and National Cereals Research Institute Zaria, Nigeria between June 2012 and July 2013.
Methodology: Twenty grams of the powdered sample was subjected to column chromatography over silica gel (60-120 mesh) and eluted with 100 ml each of n-hexane, petroleum ether, chloroform, methanol and respectively at the rate of 1ml/min. The eluates were concentrated and labeled as F_I, F_2, F_3 and F_4. The percentage yields of the fractions were 7.55%, 6.00%, 15.5%, and 65.00% (w/w) respectively. n-hexane and petroleum ether did not elute much of the compounds. The active methanol fraction of *C. aconitifolius* extract (F_4) which showed the highest hypoglycaemic effect as identified by Oral Glucose Tolerance Test (OGTT) in rats was taken for Gas Chromatography- Mass Spectrometry (GC-MS) analysis for separation of the bioactive components. GC-MS analysis was performed using a GC-MS (Model: QP2010 PLUS SHIMADZU, JAPAN) comprising a AOC-20i auto-sampler and gas-chromatograph interfaced to a mass spectrometer.
Results: The bioactive organic components of the GC-MS analysis provided peaks of six different phytochemical compounds, with their retention time (RT) and peak area (PA) in addition to minor constituents. The major compounds are dodecanoic acid-1, 2, 3- propanetriyl ester (RT:25.74, PA: 51.18%), cyclotetradecane (RT: 23.39, PA:15.59%), eicosanoic acid (RT:20.61, PA:18.47%); octadecanoic acid (RT: 16.82, PA:1.21%), 4-nitrosophenyl-beta-phenyl propionate (RT: 11.53, PA: 4.38%), benzene acetic acid, phenyl malonic acid and 3-oxo-4-phenylbutyronitrile (RT:10.34, PA: 9.17%). The presence of these compounds in the plant extract may at least be responsible for one of the pharmacological properties of *C. aconitifolius* and thus recommended as plant of phyto-pharmaceutical importance

Keywords: Phytochemials; Bioactive compounds; GC-MS analysis; Cnidoscolus aconitifolius.

1. INTRODUCTION

Use of plants as a source of medicine has been inherited and is an important component of the health care system. Herbal medicines derived from plant extracts are being utilized increasingly to treat a wide variety of clinical diseases, although relatively little is known regarding their modes of action. Studies have shown that commonly consumed medicinal plants are good sources of polyphenols, saponins, flavonoids and phenyl propanoids [1]. These compounds display a vast variety of pharmacological activities such as anti-inflammatory, anticancer, anticarcinogenic, antibacterial, antioxidant, antifungal, antiviral activities etc. In 2002, World Health Organization (WHO) estimated that more than 80% of the world's population depends on traditional herbal medicine for the treatment of different ailments [2].

Diabetes mellitus is one of the most severe and incurable metabolic disorders characterized by increased blood glucose level as a result of an absolute or relative lack of insulin and failure of insulin to act on its targets tissue [3]. According to the World Health Organization (WHO), almost 70% of the diabetic patients use plants as a primary source of antidiabetic agents in order to satisfy their principal health needs [4].

With the increasing demands for herbal medicinal products in healthcare all over the world, medicinal plant extract manufacturers have started using the most appropriate extraction technologies in order to identify and isolate the chemical entities present in them. The purpose of identification of phytochemicals in plants is to attain the therapeutically desired active portion and to eliminate unwanted materials [5]. A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural density called secondary metabolites. A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also for disclosing new sources of economic phyto-compounds for the synthesis of complex chemical substances and for discovering the actual significance of folklorics.

C. aconitifolius (Miller) of family Euphorbiaceae is commonly referred to as *'Chaya', ' Tree spinach'* in Mexico, *'Efo-Iyanaipaja'* or *'Efo-Jerusalem'* in southwest Nigeria and *'Hospital Too Far'* in eastern part of Nigeria [6]. It is an ornamental evergreen drought deciduous shrub of about 5m tall with 32 cm long and 30 cm wide palmate leaves alternately arranged [7]. The leaves are commonly eaten as vegetable, serve as blood builder [8] and possess most essential amino

acids thus, making the leaves a potential panacea for kwashiorkor and other related protein-deficiency diseases [9]. A wide variety of the folkloric use of this herb in ethno medicine includes treatment for alcoholism, diabetes, kidney stone, insomnia, gout, scorpion stings and as cure for brain and vision improvements [10]. Meanwhile it has been utilized extensively as a major component for the treatment of noninsulin-dependent diabetes mellitus. It has also been reported recently that the use resulted in a satisfactory hypoglycemic effect in diabetic animal models [11]. Basic research involving animal models have shown that this herb attenuates renal dysfunction caused by ethanol toxicity, and also exhibits insulinogenic property in inbred type-2 diabetic mice [12]. It also reported to elicit hepatoprotective activity in rats intoxicated with mega dose of paracetamol [13]. The intrinsic potency of medicinal plants is attributable to the chemical constituents present. Evaluating the biological potency provides a direct assessment of its pharmacological quality.

In order to validate the pharmacological properties of this plant, there is need to identify the chemical components and bioactive principles present. Therefore the present study was aimed at identification of the phytochemical constituents present in the methanol fraction of *C. aconitifolius* leaves using GC-MS analysis.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Fresh leaves of *C. aconitifolius* (CA) were collected with hand in glove from Amaekpu in Ohafia Local Government Area of Abia State, Nigeria, in the morning hours between the month of October and December 2012. Samples were identified and authenticated by Mr. IbeNdukwe, a taxonomist in the Herbarium Section of the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. A voucher specimen was kept at the herbarium in the same Department (Specimen No. FHI-107727).

2.1.1 Preparation of plant material

The leaves of *C. aconitifolius* were sorted, washed thoroughly with distilled water to remove dirt and debris, cut into smaller pieces before it was shade dried for 3 weeks at room temperature (28±3ºC). The dried leaves were pulverized into fine powder using electric blender (CORONA-REF. 121, Landers and Qlink blender, Model No. OBL-15L40). The powdered materials were stored in air tight polyethene bags protected from direct sunlight until required for use.

2.1.2 Plant sample extraction

One hundred grams of the powdered leaves was extracted with 500 ml of 40% ethanol overnight in a stopped bottle and with occasional stirring at room temperature (28±3ºC). The sample was first sieved using muslin cloth and then filtered using Whatman No.1 filter paper. This process was repeated three times. The filtrate was concentrated under reduced pressure at 40ºC for 45 min in a rotary vacuum evaporator, and then lyophilized to get a brown aromatic solid extract (ethanol extract of CA). The yield of the extract was expressed in terms of the percentage of the dry weight of initial plant material used (yield 35.37% w/w). The dry extract obtained was kept in a refrigerator at 4ºC until required for use.

2.1.3 Column Fractionation of Ethanol extract

The dry crude extract was subjected to column chromatography according to standard methods [14,15]. The sample for the column was prepared by adsorbing 20 g of the ethanol extract of *C. aconitifolius* with 60 g of silica gel G (60-120 mesh). The mixture was air dried and carefully layered on top of the packed silica gel in the column (14 cm length) using a glass funnel. The extract in the column was eluted with 100 ml each of petroleum ether, n-hexane, chloroform and methanol respectively at the rate of 1 ml/min. The eluates were concentrated and labeled as F_1, F_2, F_3 and F_4. The percentage yields of the fractions were 7.55%, 6.00%, 15.5%, and 65.00% (w/w) respectively. Petroleum ether and hexane did not elute much of the compounds. The methanol fraction of *C. aconitifolius* leaves which showed the highest hypoglycaemic effect as identified by Oral Glucose Tolerance Test (OGTT) in rats was further analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).

2.1.4 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was carried out on a GC-MS (Model: QP2010 PLUS Shimadzu, Japan) comprising a AOC-20i auto-sampler and gas-

chromatograph interfaced to a mass spectrometer (GC-MS) The instrument is equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. The temperatures employed were; column oven temperature 80ºC, Injection Temp 250ºC at a pressure of 108.0 kPa, with total flow and column flow of 6.20 ml/min and 1.58 ml/min respectively. The linear velocity was 46.3 cm/sec and a purge flow of 3.0 ml/min. The GC program ion source and interface temperature were 200.00ºC and 250.00ºC respectively with solvent cut time of 2.50 min. The MS program starting time was 3.00min which ended at 30.00 min. with event time of 0.50 sec, scan speed of 1666 µl/sec, scan range 40-800u and an injection volume of 1 µl of the plant extract (split ratio 10:1). The total running time of GC-MS was 30 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

2.1.5 Identification of phytocompounds

Interpretation on the mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The fragmentation pattern spectra of the unknown components were compared with those of known components stored in the NIST library (NIST Ver. 2.0 of 2005). The compound bioactivity prediction is based on Dr. Duke"s Phytochemical and Ethnobotanical Databases (Dr. Duke Database, 2014 http: www.ars-grin.gov/cgi-bin/duke/ethnobot [16]. The relative percentage amount of each phyto-component was calculated by comparing its average peak area to the total area. The name, molecular weight and structure of the components of the test materials were ascertained.

3. RESULTS AND DISCUSSION

Chromatographic purification of the ethanol extract of the leaves of *C. aconitifolius* with different solvents produced four fractions (F_1-F_4) with the methanol fraction producing the highest yield and hypoglycaemic effect as observed by oral glucose tolerance test in rats. This may suggest higher proportion of the plant component were extracted in the methanol fraction. The compounds present in the methanol extract of leaves of *C. aconitifolius* were identified by GC-MS analysis as shown in Fig. 1.

The active principle, area of peak concentration (%), retention time ((RT) molecular weight (MW), and molecular formula (MF) in the methanol extract as identified through the NIST database is listed in Table 1.

The main component of the methanol extract of the leaves was recognized as the major metabolites responsible for its antidiuretic effect. The organic compounds in methanol extract of the leaves were identified through their fragmentation patterns to include 4- dodecanoic acid-1, 2 3- propanetriyl ester (RT: 25.744, PA: 51.18%), cyclotetradecane (RT: 23.39, PA: 15.59%), eicosanoic acid (RT: 20.61, PA: 18.47%) and octadecanoic acid (RT: 16.82, PA: 1.21%). Others are 4-nitrosophenyl-beta-phenyl

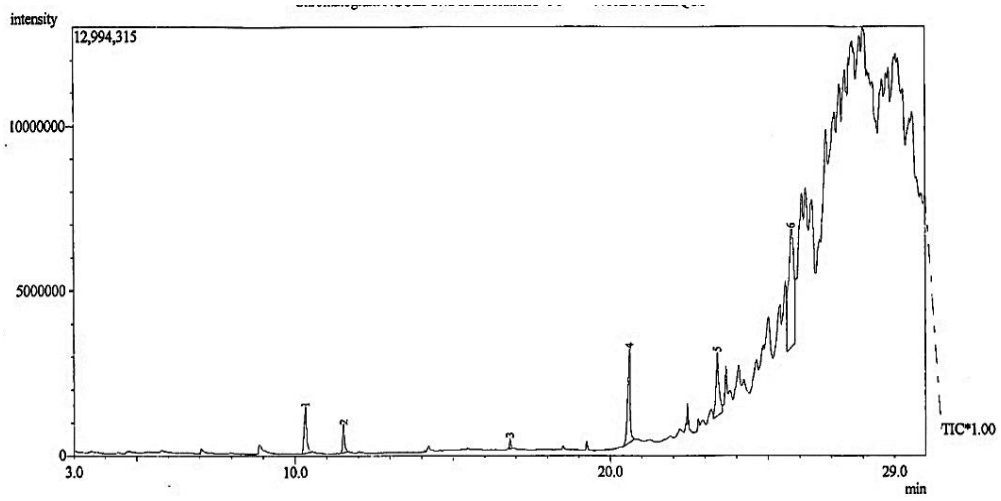

Fig. 1. GC-MS Chromatogram of methanol fraction of *Cnidoscolus aconitifolius* leaves

Table 1. Phyto-components identified in the methanol extract of *Cnidoscolus aconitifolius* by GC-MS analysis

Peak no	Retention (RT)(s)	Name of compound	Molecular formula (MF)	Molecular weight (MW-g/mol)	Peak area (%)
1	1249	Phenylmalonic acid	$C_9H_8O_4$	180	9.17
	1641	Benzene acetic acid	$C_8H_8O_2$	136	9.17
	1473	3-Oxo-4-phenylbutyronitrile	$C_{10}H_9NO$	159	9.17
	718	Spiro (2, 4) hepta-4, 6-diene	C_7H_8	92	9.17
2	000	4 Nitrosophenyl-β-phenyl propionate	$C_{15}H_{13}NO_3$	255	4.38
	1349	Benzene propanoic acid	$C_9H_{10}O_2$	150	4.38
	1394	3-Phenyl-Propionic acid	$C_{12}H_{16}O_2$	192	4.38
3	1769	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.21
	2167	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.21
	1968	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.21
4	2366	Eicosanoic acid	$C_{20}H_{40}O_2$	312	18.47
	1869	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	18.47
5	1679	Cyclo-tetradecane	$C_{14}H_{28}$	196	5.59
	1818	5-Octadecene	$C_{18}H_{36}$	252	5.59
	1620	7-Hexadecene	$C_{16}H_{32}$	224	5.59
	2017	5-Eicosene	$C_{20}H_{40}$	280	15.59
6	4336	Do-decanoicacid-1, 2, 3- propane-triyl ester	$C_{39}H_{74}O_6$	638	51.18
	3218	Do-decanoicacid, 1-hydroxy methyl-1, 2-diyl ester	$C_{27}H_{52}O_5$	456	51.18
	1570	Do-decanoicacid, ethenyl ester	$C_{14}H_{26}O_2$	226	51.18

propionate (RT: 11.53, PA: 4.38%), Benzene acetic acid, phenylmalonic acid and 3-oxo-4-phenylbutyronitrile (RT: 10.34, PA: 9.17). dodecanoic acid-1, 2, 3- propanetriyl ester have high retention time (25.74 min) and molecular weight (638), while benzene acetic acid is of low molecular weight (136) and retention time (10.34 min). A similar trend of the presence of fatty acids in extracts of dichloromethane and hexane of *C. aconitifolius* has been reported in studies on the comparative analysis of the nature of the solvent of extraction and chemical composition of the leaves [17,18]. The mass spectrum of the individual components are shown in Fig. 2. (a-h).

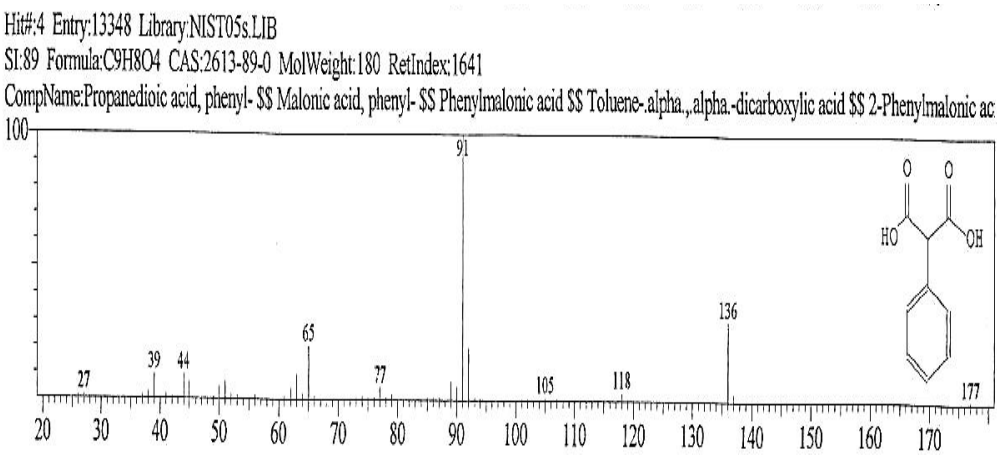

Fig. 2a. Phenylmalonic acid

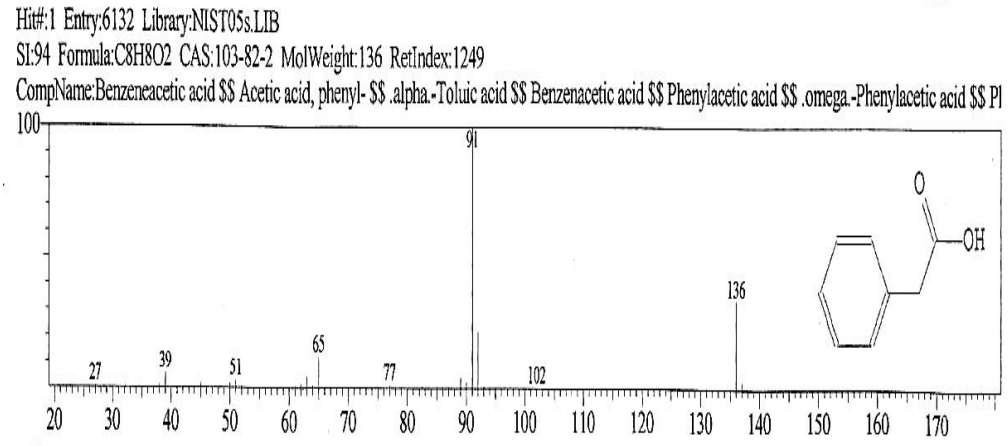

Fig. 2b. Benzene acetic acid

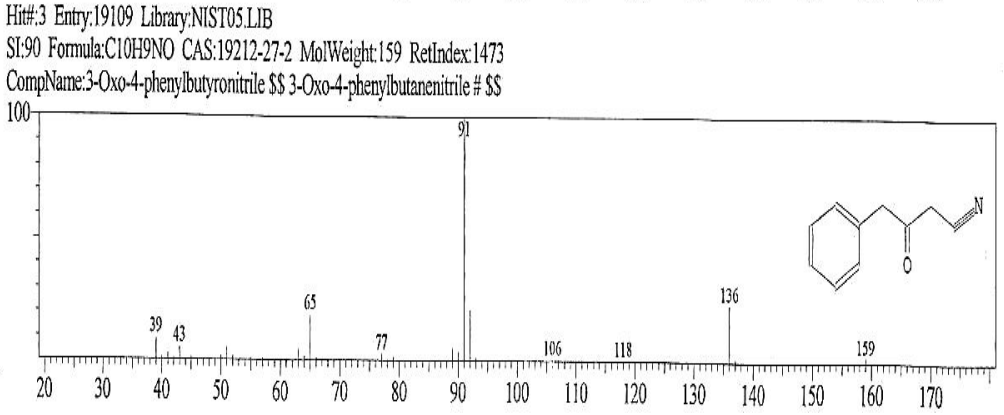

Fig. 2c. 3-Oxo-4-phenylbutyronitrile

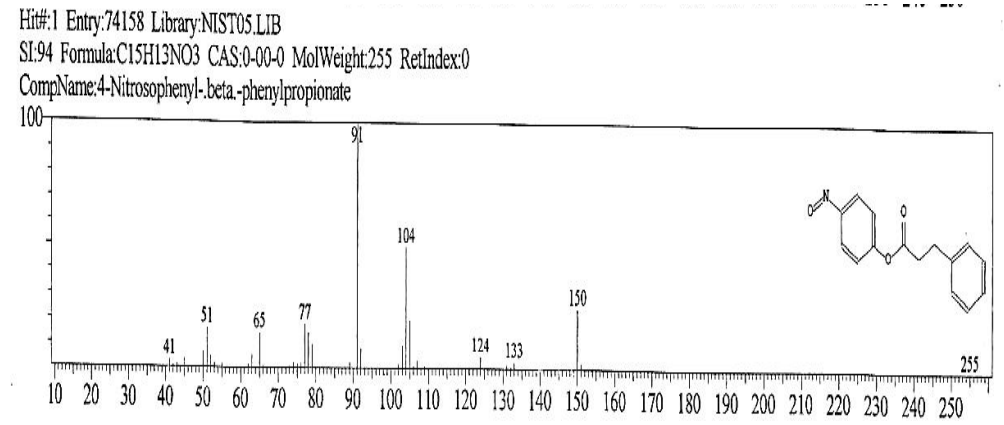

Fig. 2d. 4 Nitrosophenyl-β-phenyl propionate

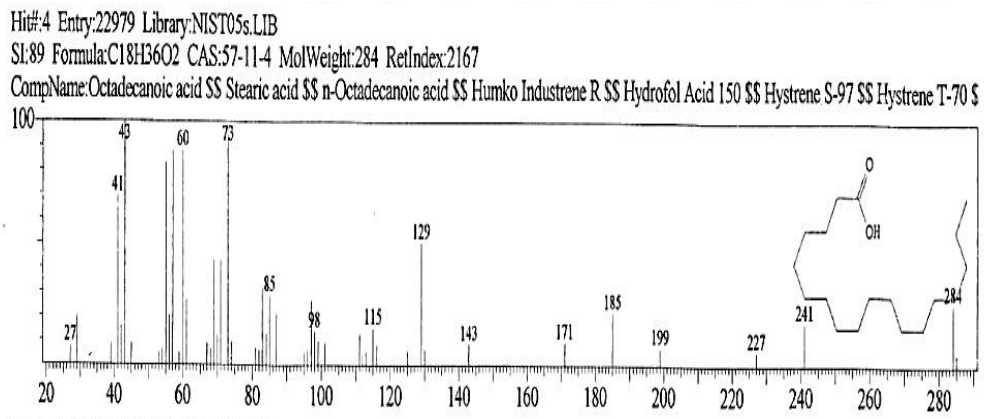

Fig. 2e. Octadecanoic acid

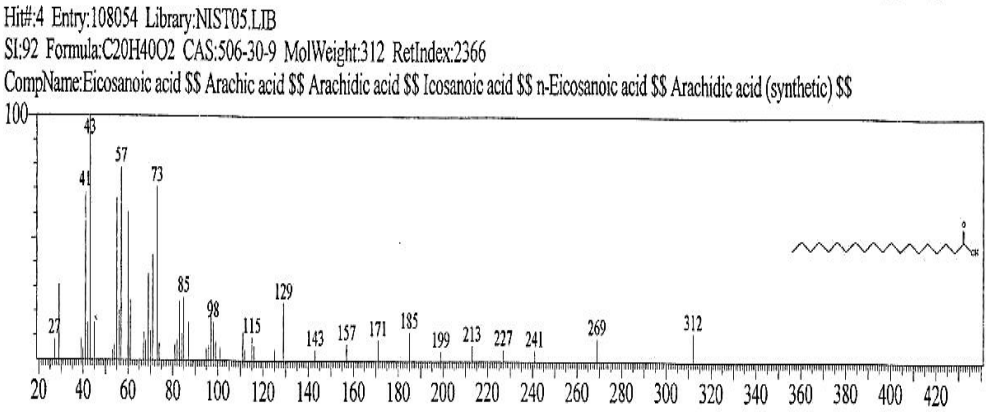

Fig. 2f. Eicosanoic acid

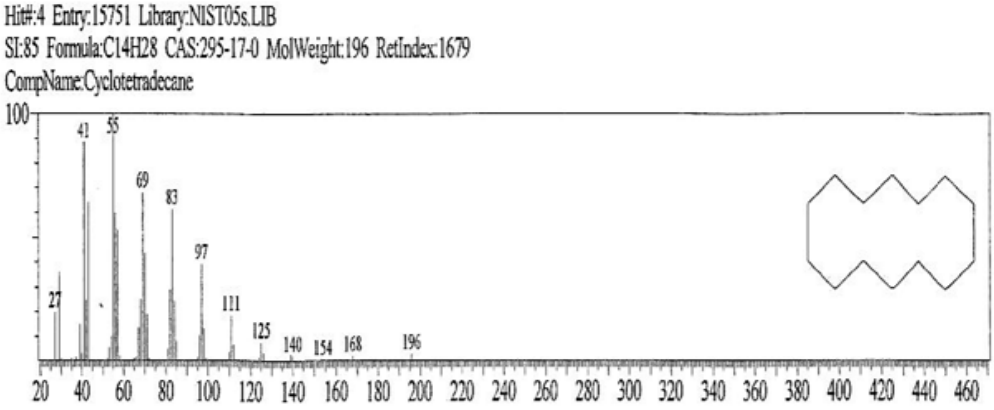

Fig. 2g. Cyclo-tetradecane

The peaks retention time ranged from 10.33 - 25.00 while the peak percentage area ranged from 9.17–15.80. The compounds prediction and biological activities is based on Dr Duke' Phytochemical and ethno botanical databases as tabulated in Table 2.

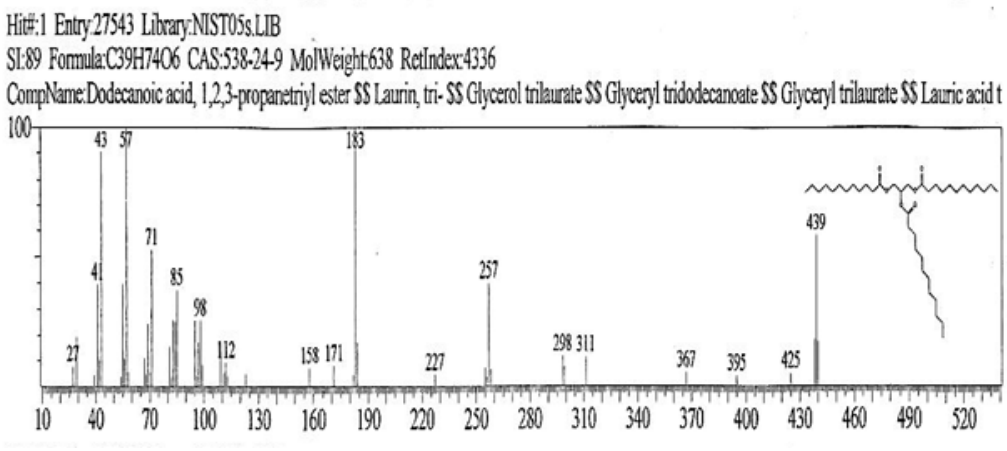

Fig. 2h. Do-decanoicacid-1,2,3- propane-triyl ester

Table 2. Biological activities of some active principles present in methanol fraction of *Cnidoscolus aconitifolius* [20]

Phyto-components	Nature of compound	Activities (16)
Tetradecanoic acid	Fatty acid	Antioxidant, cancer preventive, nematocide, hypercholesterolemic, lubricant.
Do-decanoic acid,-1,2,3–propane -triyl ester	Fatty acid	Hypercholesterolemic, antiarthritic, nematocide, hepatoprtective.
Octadecanoic acid	Fatty acid	b5-α reductase inhibitor, Cosmetic, Flavour, Hypocholesterolemic, Lubricant, Perfumery, Propecic and Suppository.
Eicosanoic acid	Fatty acid	Antinflamtory, anti- therogenic.
9-Octadecenoic acid	Fatty acid	Anti-inflammatory, Anti-alopecic, Anemiagenic, 5-α reductase inhibitor, α-reductase inhibitor lubricant, Antitumour, Choleretic, Dermatitigenic, Immunostimulant, Anti-leucotriene-D4, Antiandrogenic, Lipoxygenase inhibitor, Allergenic, Flavour, Hypocholesterolemic, Insectifuge, Irritant, Percutaneo-stimulant, Perfumery and Propecic
n-Hexadecanoic acid	Fatty acid	Anti-alopecic, Anti-androgenic, Antioxidant, Haemolytic, Hypercholesterolemic, Lubricant, Nematicide, Pesticide, Propecic, Flavour 5-α reductase inhibitor.
Dodecanoic acid -ethyl ester	Fatty acid ester	Anti-inflammatory, Hypercholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistamine, Anti-eczemic, Anti-acne, 5-Alpha reductase inhibitor, Antiandrogenic, Anti-arthritic, Anti-coronary.
Hexadecanoic acid methyl ester 5-Octadecene	Fatty acid ester Olefins	Antioxidant, Hypercholesterolemic, Lubricant, Nematicide, Pesticide, Hemolytic 5-Alpha reductase inhibitor, Flavour, Antiandrogenic

In the present study, six major compounds have been identified from the ethanol extract of the leaves of *C. aconitifolius* by Gas Chromatography-Mass Spectrometry analysis. Authentication of medicinal plants at genetic and chemical level is a critical step in the use of these botanical materials. Gas Chromatography-Mass Spectrometry (GC-MS) is a valuable tool for reliable identification of phytocompounds [19].

The study on the active principle of methanol fraction of *C. aconitifolius* revealed that the plant contains a wide range of phytochemicals which may contribute to its therapeutical value. The GC-MS analysis showed a fragmentation pattern characteristic of the presence of fatty acids such as octadecanoic acid, tetradecanoic acid, and do-decanoic acid. These phytochemicals are known to have antimicrobial activity, antioxidant, hypercholesterolemic, cancer preventive and hepatoprotective activity [20,21]. Previous studies have demonstrated the presence of similar compounds in methanol fractions of C. *aconitifolius* with 9-octadecanoic acid as the prevailing compound [17].

In addition, the methanol fraction of *C. aconitifolius* showed the presence of phenolic compounds, saturated and unsaturated fatty acids including eicosanoic acid, a component of membranes and precursor of a group of hormones like prostaglandins, thromboxanes and prostacyclines which are important in regulation of diverse physiological processes [22]. The eicosanoids, have been reported to possess anti-inflammatory properties [23]. The bioactivities of these compounds may depend on the lipophilic properties of their functional groups. We report the presence of some important components of methanol extracts of *C. aconitifolius* resolved by GC-MS analysis. Many of the metabolites have been found to possess interesting biological activities and find applications such as pharmaceuticals, insecticides, dyes, flavors and fragrances [20].

4. CONCLUSION

From the present study, GC-MS analysis of the methanol fraction of *C. aconitifolius extracts* afforded active compounds which were easily distinguishable fatty acids that contribute to the therapeutic potential of the plant. This may justify its use as herbal therapy for the treatment of various diseases by traditional medical practitioners. It also suggests that further investigation on these phytochemicals will pave the way for the venture of cost effective drug with less side effect.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

This study will provide additional insights on the use of traditional herbal medicine and to being able to develop new cure for some ailments like diabetes as one of the leading diseases causing numerous deaths worldwide.

REFERENCES

1. Loizzo MR, Tundis R, Conforti F, Saab AM, Statti GA, Menichini F. Composition and alpha-amylase inhibitory effect of essential oils from *Cedrus libani*. Fitoteraptera. 2007;78:323-326.
2. World Health Organization **(**WHO). World Health Organization Report, GENEVA, WHO/EDM/TRM. 2002;21,19.
3. Valiathan MS. Healing plants. Curr. Sci. 1998;75:1122-1127.
4. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. Diabetes Care. 1989;12:553-564.
5. Waldesch FG, Konigswinter BS, Blasius H. Herbal medicinal products, Medpharm, Stuttgart, Germany CRS Press, London. 2003;48-54.
6. Oyagbemi AA, Odetola AA, Azeez OI. Ameliorative effect of *Cnidoscolus aconitifolius* on anaemia and osmotic fragility induced by protein energy malnutrition. J. Biotechnol. 2003;7(11):1721-1726.
7. Brekon GJ. Studies on *Cnidoscolius aconitifolius* Euphorbiaceae Brittonia. 1979;*31:125-147.* Kuti JO, Torres ES. Potential nutritional and health benefits of spinach *In:*Jarick, J. Progress in new crops. ASHS Press, Arlington, VA 516-5201996.
8. Oyagbemi AA, Odetola AA. Hepatoprotective and nephroprotective effects of *Cnidoscolus aconitifolius* in protein energy malnutrition induced liver and kidney damage. Phcog Res. 2013;5:260-264

9. Atuahene CC, Poku-Prempeh B, Twun G. The nutritive values of chaya leaf meal (*Cnidoscolus aconitifolius*) studies with broilers chickens. Anim. Feed Sci. Technol. 1999;77:163-172.
10. Adaramoye, OA, Aluko A. Methanolic extract of *Cnidoscolus aconitifolius* attenuates renal dysfunction induced by chronic ethanol administration in Wistar rats. Alcohol. 2011;46:4-9.
11. Oladeinde FO, Kinyua AM, Laditan AA, et al. Effect of *Cnidoscolus aconitifolius* leaf extract on the blood glucose and insulin levels of inbred type 2 diabetic mice. Cell Mol Biol. 2007;53:34-41.
12. Oyagbemi AA, Odetola AA. Hepatoprotective effects of ethanolic extract of *Cnidoscolus aconitifolius* on paracetamol-induced hepatic damage in rats. Pak J Biol Sci. 2010;13:164-169.
13. Chakrabarti R, Vikramadithyan RK, Mullangi R, Sharma VM, Jagadheshan H, Rao YN, et al. Antidiabetic and hypolipidemic activity of *Helicteres isora* in animal models. J. Ethnopharmacol. 2007;81:343-349.
14. Selvamangai G, Bhaskar A. GC-MS Analysis of phytocomponents in the methanlic extract of *Eupatorium triplinerve.* Asian Pacific Journal of Tropical Biomedicine. 2012;S1329-S1343.
15. Duke"s Phytochemical and Ethnobotanical Database; 2014. Available: http://www.ars-grin.gov/cgi-bin/duke/ethnobot/ Assessed 16th Jun. 2014
16. Omotosho AE, Ezealisiji K, Mkparu KI. Chemometric profiling of methanolic extract of *Cnidiscolus aconitifolius* (Euphorbiaceae) using UV-VIS, FTIR and GC-MS techniques. Peak J. Med Plant Res. 2014;2(1):6-12.
17. Omotoso AE, Eseyin OO, Suleiman M. Phytochemical Analysis of *Cnidoscolus aconitifolius* (Euphorbiaceae) leaf with Spectrometric Techniques. Nig J. Pharm. Appl. Sc. Res. 2014;3(1):38-49.
18. Sampath KS, Rama KN. Pharmacognostic and Phytochemical investigation of *Naringi crenulata* (Roxb.) Niicols. Journal of Life. 2011;31(1):17-21.
19. McGraw LJ, Jager AK, Van Staden. Isolation of antibacterial fatty acids from *Schottia brachypetala*. Fitoteraptera. 2002;73:431-433.
20. Siedel V, Taylor PW. *In vitro* activity of extracts and constituents of *Pelagonium* against rapidly growing mycobacteria. Int. J. Anti Microb Agents. 2004;23:613-619.
21. Ueda H, Yamazaki C, Yamazaki M. Luteolin as an antinflammatory and anti-allergic constituents of *Perrila frutescens.* Biol. Pharm. Bull. 2002;25:1197-1202.
22. Asif M. Health effect of Omega-3,6,9 fatty acids: *Perilla frutescens* is a good example of plant oil. Orient. Pharm. Exp. Med. 2011;11:51-59.

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