Phytochemical Analysis of *Cnidoscolus aconitifolius* (Euphorbiaceae) leaf with Spectrometric Techniques.

[*] Omotoso Abayomi E [a], Eseyin O Olorunfemi [b], Suleiman Mikailu [c]

[a] Department of Medicinal and Pharmaceutical Chemistry, University of Port-Harcourt, Port-Harcourt, Nigeria,

[b] Department of Pharmaceutical & Medicinal Chemistry, University of Uyo, Uyo, Nigeria,

[c] Department of Pharmacognosy and Phytotherapy, University of Port-Harcourt, Port-Harcourt, Nigeria.

ABSTRACT

The plant *Cnidoscolus aconitifolius* (Euphorbiaceae) commonly known as '*efo Iyana-Ipaja*' in South-West Nigeria is reported to possess a number of medicinal values. Phytochemical Analysis of the n-hexane, dichloromethane, and ethyl acetate leaf extracts of *Cnidoscolus aconitifolius* were performed by using UV-Vis, FTIR and GC-MS. The crude extracts were scanned in the wavelength ranging from 200-800nm by using UV-spectrophotometer, while FTIR was carried out from 4000 – 400cm^{-1} and their characteristic peaks detected. The GC-MS analysis was carried out using GC-MS Model-QP2010 plus spectrophotometer. The compound detection employed the NIST Ver. 2.0-Year 2005 library. The UV-Vis profile showed different peaks ranging from 400 – 700nm with different absorption respectively. The FTIR spectrum confirmed the presence of alkanes, alkenes, alkynes, alcohols, aldehydes, ketones, carboxylic acids, esters and aromatics. The Phytochemical Analysis of the *Cnidoscolus aconitifolius* leaf extracts revealed 20 phyto-chemotypes with different therapeutic activities, among them, 9-Octadecenoic acid (Z) and its esters, n-Hexadecanoic acid, n-Octadecanoic acid, n-Octacosane, 1,2,3-Propanetriol and its derivatives, and *l*-(+)-Ascorbic acid-2,6-dihexadecanoate, were found to be present in major amount. The presence of some of these phyto-constituents in the plant provides the scientific basis for the therapeutic properties of the plant and thus recommended use as a plant of phyto-pharmaceutical importance.

KEY WORDS: *Cnidoscolus aconitifolius*, Phyto-pharmaceutical, UV-Spectrophotometer, Gas Chromatography-Mass Spectroscopy (GC-MS).

*Correspondence author: abatoseb2001@yahoo.com; abayomi.omotoso@uniport.edu.ng +2348035662535

INTRODUCTION

Cnidoscolus aconitifolius (Euphorbiaceae) is commonly referred to as *'Chaya', 'tree spinach'* in Mexico, '*Efo Iyana Ipaja' or 'Efo Jerusalem'* in Southwest Nigeria and '*Hospital Too Far'* in Niger Delta areas of Nigeria. *Cnidoscolus aconitifolius* is an ornamental, evergreen, drought deciduous shrub up to 6m in height, with alternately arranged palmate lobed leaves, with milky sap and small flowers on dichotomously branched cyme. The leaves are large 32 × 30cm on characteristic succulent petiole. The crop originated as a domesticated leafy green vegetable in Maya region of Guatemala, Belize and south east Mexico during pre-Cambrian period (Ross-Ibarra & Molina 2002; Iwalewa *et al*., 2005). It has continued to be used as food, medicine and ornamental plant till date. Due to its ease of cultivation, potential productivity and substantial nutritional value, the plant has spread all over the world including the tropics (Donkoh *et al.*, 1990*;* Oyagbemi *et al.,* 2003). The shoot and the leaves of *Cnidoscolus aconitifolius* are used as laxative, diuretics, and in circulation aid, lactation stimulants. It has also been recommended for diabetes, obesity, acne, kidney stone and eye problems, ability to strengthen fingernail and darken grey hair, to cure alcoholism, insomnia, gout, scorpion stings, brain and vision improvement (Atuahene *et al*., 1999). Considering the magnitude and potential of drug discovery from medicinal plant in health and world economy, therefore the present study is aimed at determining and comparing the phyto-components present in the non-polar extracts of *Cnidoscolus aconitifolius* using UV-Vis, FTIR and GC-MS analyses by comparison with NIST databases.

MATERIALS AND METHODS

Chemicals

n-Hexane, Dichloromethane, and Ethyl acetate were of analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA).

Plant materials and Extraction

Healthy flowering Cnidoscolus *aconitifolius* leaves was collected from Eleme area of Port-Harcourt, Rivers State, Nigeria, identified and authenticated in Forest Research Institute of Nigeria (FRIN) Ibadan by Mr. Ekundayo A. Adewale, where the voucher specimen was deposited and the Voucher number FHI 109457 was allocated.

The leaf of *Cnidoscolus aconitifolius* was thoroughly washed with distilled water, shade dried at room temperature and pulverized into coarse powder using manual grinder. The powdered leaf (100g) was taken for sequential extraction using four solvents of increasing polarity viz: n-hexane, dichloromethane, and ethyl acetate by maceration for 72hr each at room temperature (25 ± 2^{O}C). The extracted fractions were concentrated under vacuum and reduced pressure (BUCHI, Rotavapour R-205, BUCHI Labortechnik AG CH-9230, Flamil, Switzerland) at 40^{O}C. The dried extracts were then stored in a refrigerator (-5^{O}C) till further analysis.

UV- VIS and FTIR spectroscopic analysis

The individual dried extract (100mg) was diluted with 500µl of the corresponding solvents and centrifuged at 3000rpm for 10min and thereafter filtered through Whatmann No 4 filter paper using vacuum pump. A further 1:10 dilution of the

centrifuged solution was made with the solvent. The extract thus obtained was 200-800nm using UV-2500PC series Ver. 2.30 spectrophotometer and the characteristic peaks were detected. The diluted extract above was used in carrying out the FTIR analysis using FTIR-8400S Spectrometer equipment; the characteristic peaks were also detected. The peak values of the UV-Vis and FTIR were recorded. The above analyses were carried out in duplicate.

GC-MS analysis

Preparation of extracts for GC-MS analysis

The different dried extracts were re-dissolved in their respective extraction solvents, vortexed and left overnight. The supernatant was filtered with a syringe filter. One microlitre aliquot of the sample solution was injected into the GC-MS equipment.

Instrumentation and Chromatographic conditions

GC-MS analysis was carried out on a GC-MS (Model: QP2010 Plus Shimadzu, Tokyo, Japan) comprising a AOC-20i auto-sampler and gas-chromatograph interphased to a mass spectrometer (GC-MS) instrument equipped with a VF 5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70Ev was used. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.58ml/min, injector and mass transfer line temperature were set at 250^{O}C and 200^{O}C respectively, and an injection volume of 1µl was employed (split ratio 10:1), the oven temperature was programmed from 80^{O}C (isothermal for 2min), with an increase of 9^{O}C/min to 200^{O}C for 4min, 10^{O}C/min to 280^{O}C, ending with a 5min isothermal at 280^{O}C. The MS operating parameters were as follow : ionization energy, 70eV; ion source temperature, 200^{O}C, solvent cut time, 2.5min, relative detector gain mode, scan speed 1666µ/sec; scan range 40-800u, the interface temperature is 250^{O}C. The total running time of GC-MS was 30min. The relative percentage of the extract was expressed as percentage with peak area normalization.

Identification of component

The relative percentage amount of each phyto-component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standard and Technology) Ver. 2.0-Year 2005 library. The compounds biological activity prediction is based on Dr. Duke's phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. Interpretation of GC-MS was conducted using the database of NIST having more than 62,000 patterns. The spectrum of the unknown phyto-components was compared with the spectrum of the known components stored in the NIST library. The name and molecular weight of the phyto-components of the test materials were ascertained.

RESULTS

The qualitative UV-VIS spectrum profile of *the* extract were selected at a wavelength from 200-800nm. The n-Hexane UV-VIS Spectrum showed peaks at 409, 470 and 669nm with the absorption values of 2.216, 0.744 and 0.863 respectively (Table 1; Fig. 1). The UV-VIS profile of dichloromethane extract showed peaks at 412.5, 481.0 and

666nm with the absorption values of 2.748, 0.848 and 0.841 respectively (Table 1; Fig. 2) The UV-VIS profile of ethyl acetate extract showed peaks at 407.5 and 665.5nm with the absorption values of 1.011 and 0.328 respectively (Table 1; Fig 3).

The results of FTIR peak values and functional groups were represented in Table 2. The FTIR spectrum profile was illustrated in Fig. 4, 5 & 6.

Table 1: UV-VIS absorbance of different leaf extracts of *Cnidoscolus aconitifolius.*

n-Hexane		Dichloromethane		Ethyl acetate	
λ_{nm}	Absorbance	λ_{nm}	Absorbance	λ_{nm}	Absorbance
409.00	2.216	412.50	2.748	407.50	1.011
470.50	0.744	481.00	0.848	665.50	0.328
669.00	0.863	666.00	0.841	-	-

λ_{nm} = Wavelengths of maximum absorption

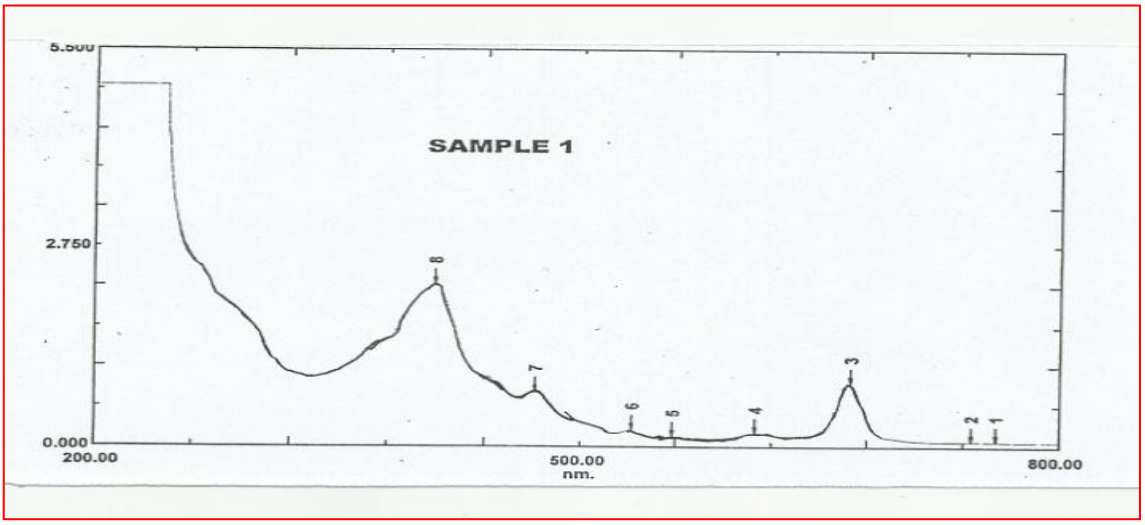

Fig. 1: UV-VIS Spectrum of n-Hexane extracts of *Cnidoscolus aconitifolius* (sample 1).

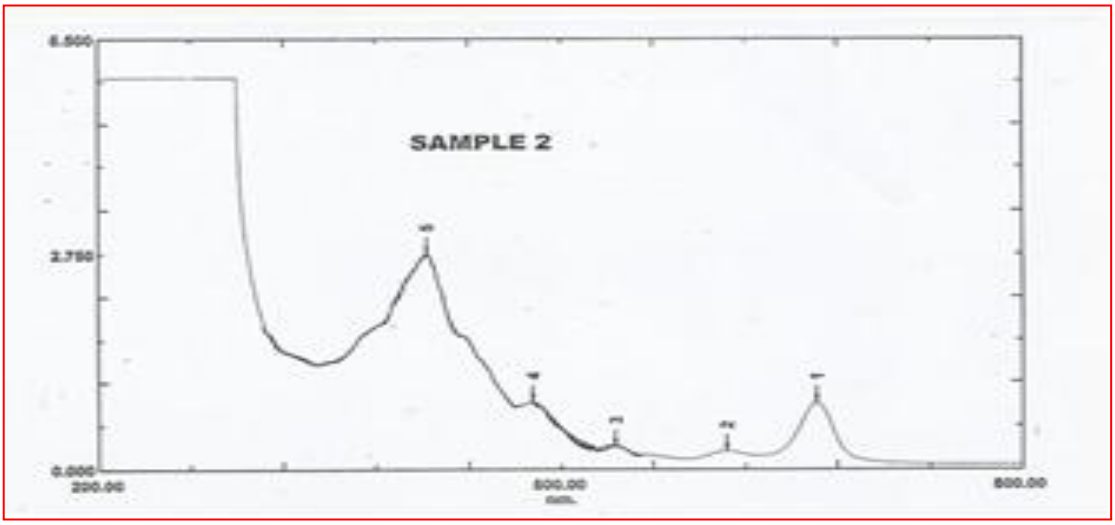

Fig. 2: UV-VIS Spectrum of Dichloromethane extracts of *Cnidoscolus aconitifolius* (sample 2).

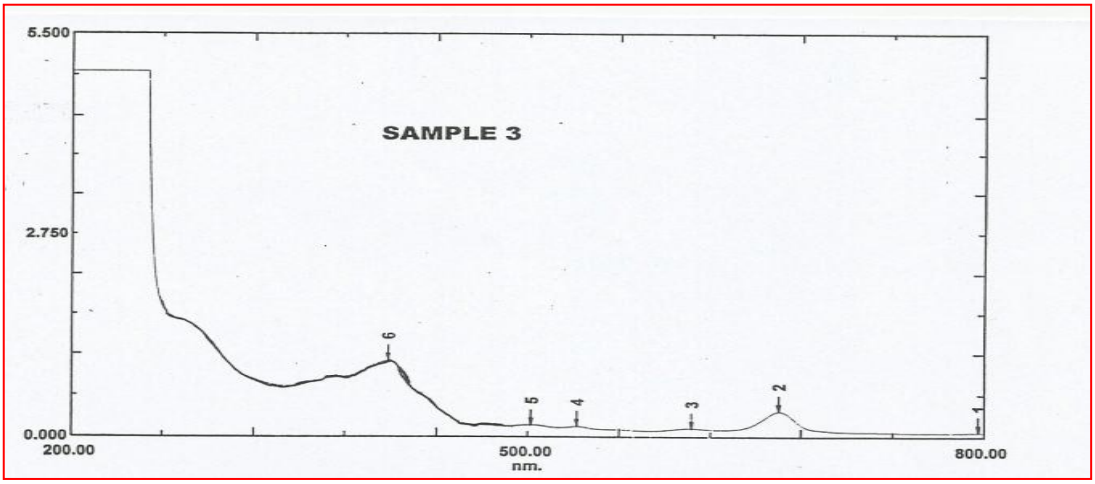

Fig. 3: UV-VIS Spectrum of Ethyl acetate extracts of *Cnidoscolus aconitifolius* (sample 3).

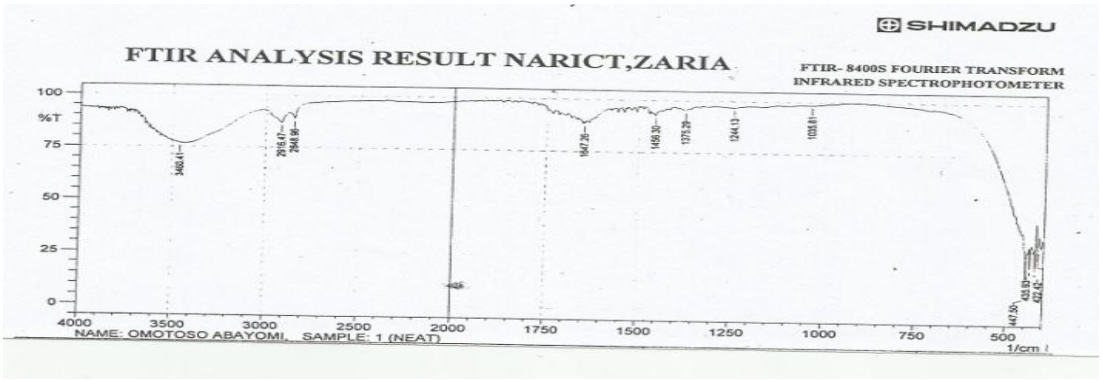

Fig. 4: FTIR Spectrum of n-Hexane extracts of *Cnidoscolus aconitifolius*.

Fig. 5: FTIR Spectrum of Dichloromethane extract of *Cnidoscolus aconitifolius*.

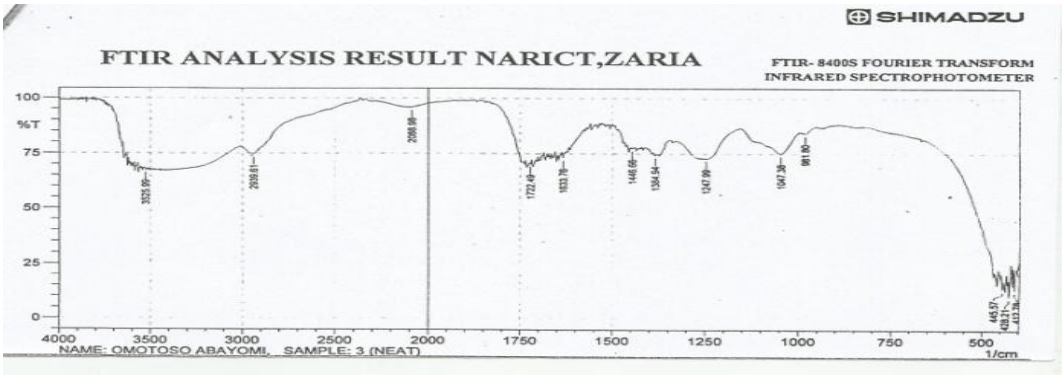

Fig. 6: FTIR Spectrum of Ethyl acetate extract of *Cnidoscolus aconitifolius*.

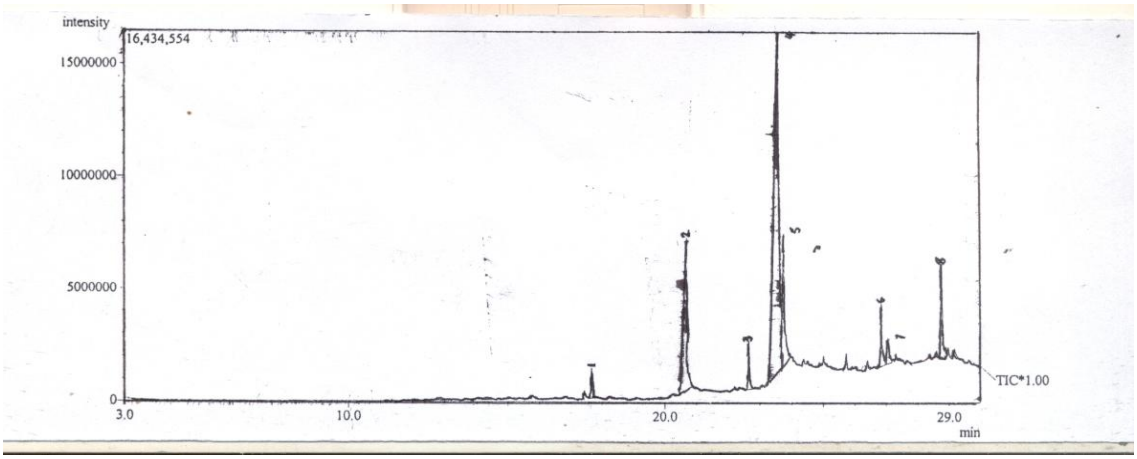

Fig. 8: Gas Chromatograph of n-Hexane extracts of *Cnidoscolus aconitifolius*.

Table 2: FTIR peak values and functional groups of different extracts of *Cnidoscolus aconitifolius.*

n-Hexane		Dichloromethane		Ethyl acetate	
Peak Values ʎ (nm)	Functional group	Peak values ʎ (nm)	Functional group	Peak Values ʎ (nm)	Functional group
3460.41	**O-H stretch, H-bonded**	3419.90	**O-H stretch, H-bonded**	3525.99	O-H stretch, H-bonded
2916.47	**CH_2 stretch asymmetric**	2920.32	**CH_2 stretch asymmetric**	2939.61	CH_2 stretch asymmetric
2848.96	**CH_2 stretch symmetric**	2850.88	**CH_2 stretch symmetric**	2088.98	CΞC stretch
1647.26	**C=C stretching non-conjugated**	1705.13	**C=O stretch**	1722.49	C=O
1456.30	**CH_2 deformation**	1464.02	**CH_2 deformation**	1633.76	N-H deformation
1375.29	**CH_3-C- deformation**	1375.29	**CH_3-C- deformation**	1446.66	C-H deformation
1244.13	**C-N stretch**	1165.04	**C-H wag (-CH_2X)**	1384.94	C-H deformation
1035.81	**C-N stretch**	979.87	**=C-H bend**	1247.99	C-N Aliphatic amines
447.50		443.64		1047.38	C-N Aliphatic amines
435.93		430.14		981.80	=C-H bend
422.42		420.50		445.57	
414.71		403.41		428.21	
--		--		412.78	
--		--		405.06	

Gas Chromatograms of n-hexane, dichloromethane and ethyl acetate extracts are shown in Fig. 8, 9 and 10 respectively. GC-MS analysis of the phyto-components present in different non-polar solvent extracts (n-hexane, dichloromethane, and ethyl acetate) of *Cnidoscolus aconitifolius* clearly showed the presence of twenty phyto-components (Fig. 11). The active phyto-components with their respective retention time (RT), molecular formula, molecular weight (MW), and relative percentages (peak area %) is presented in Tables 3, 4 and 5.

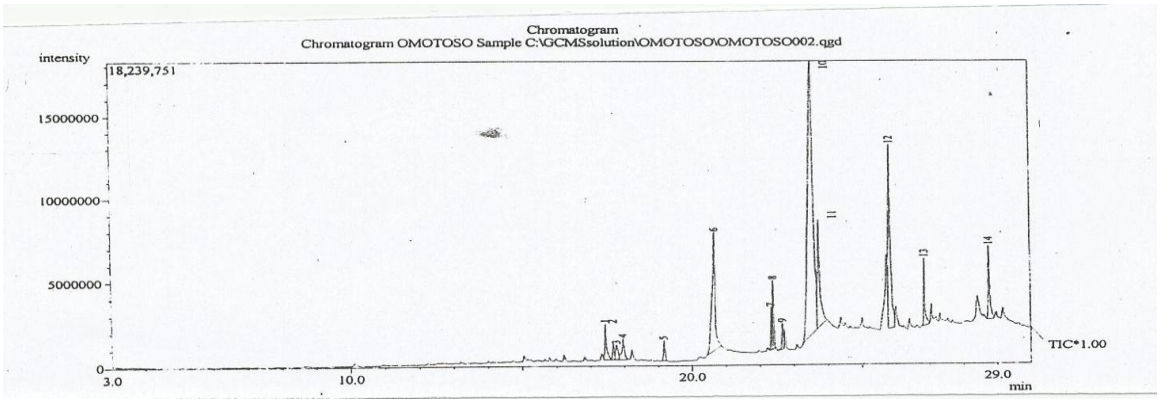

Fig. 9: Gas Chromatograph of Dichloromethane extracts of *Cnidoscolus aconitifolius*.

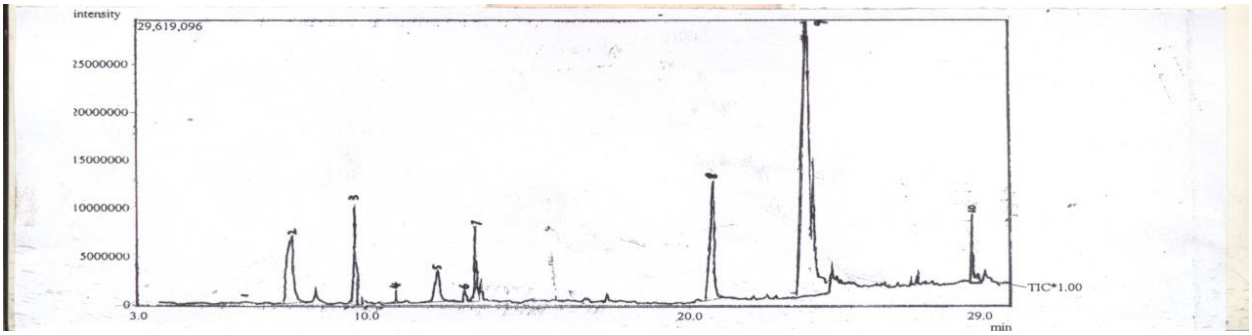

Fig. 10: Gas Chromatograph of Ethyl acetate extracts of *Cnidoscolus aconitifolius*

Table 3: Phyto-components identified in the n-Hexane extract of *Cnidoscolus aconitifolius* by GC-MS.

S/N	RT (min)	Name of Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	17.66	6,10,14-trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	268	1.30
2	20.69	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	17.54
3	22.66	3,7,11,15-Tetramethyl-[R-[R*,R*,R*(E)]]-2-Hexadecen-1-ol	$C_{20}H_{40}O$	296	2.55
4	23.57	9-Octadecenoic acid (z)	$C_{18}H_{34}O_2$	282	56.77
5	23.76	n-Octadecanoic acid	$C_{18}H_{36}O_2$	284	11.12
6	26.84	1,2,3-Propanetriyl ester (E,E,E)-9-Octadecenoic acid	$C_{57}H_{104}O_6$	884	9.21
7	27.05	1-(Hydroxymethyl)-1,2-ethanediyl ester-Hexadecanoic acid	$C_{35}H_{68}O_5$	568	1.51

Table 4: Phyto-components identified in the Dichloromethane extract of *Cnidoscolus aconitifolius* by GC- MS-

S/N	RT (min)	Name of Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	17.44	9—Eicosyne	$C_{20}H_{38}$	278	2.20
2	17.67	6,10,14-trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	268	0.79
3	17.77	3,3,6-trimethyl-4,5-heptadiene-2-ol	$C_{10}H_{18}O$	154	1.13
4	17.96	2-(2-Hydropropyl)-1,4-benzenediol	$C_9H_{12}O_3$	168	2.06
5	19.17	Methyl-14-methylpentadecanoate	$C_{17}H_{34}O_2$	270	1.05
6	20.69	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.82
7	22.34	9,12-Octadecanoic acid (z, z)-methyl ester	$C_{19}H_{36}O_2$	294	1.80
8	22.40	9-Octadecenoic acid (z)-methyl ester	$C_{19}H_{34}O_2$	296	2.43
9	22.67	3,7,11,15-Tetramethyl-[R,-[R*,R*-(E)]]-2-Hexadecen-1-ol	$C_{20}H_{40}O$	296	1.16
10	23.57	9-Octadecenoic acid (z)	$C_{18}H_{34}O_2$	282	43.13
11	23.76	n-Octadecanoic acid	$C_{18}H_{36}O_2$	284	8.36
12	25.85	n-Octacosane	$C_{28}H_{58}$	394	13.59
13	26.85	2,3-dihydroxypropyl ester-9-Octadecenoic acid (z)	$C_{21}H_{40}O_4$	356	3.29
14	28.78	1,2,3-Propanetriyl ester (E,E,E)-9-Octadecenoic acid	$C_{57}H_{104}O_6$	884	4.19

Table 5: Phyto-components identified in the Ethyl acetate extract of *Cnidoscolus aconitifolius* by GC-MS.

S/N	RT (min)	Name of Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	6.23	1,2,3-Propanetriol	$C_3H_8O_3$	92	0.64
2	7.77	1,2,3-Propanetriol monoacetate	$C_5H_{10}O_4$	134	11.18
3	9.68	1,2,3-Propanetriol diacetate	$C_7H_{12}O_5$	176	5.05
4	10.90	1,2,3-Propanetriol triacetate	$C_9H_{14}O_6$	218	0.43
5	12.20	1,2,3-Propanetriol monoacetate	$C_5H_{10}O_4$	134	3.64
6	13.02	1,2,3-Propanetriol triacetate	$C_9H_{14}O_6$	218	0.95
7	13.38	1,2,3-Propanetriol monoacetate	$C_5H_{10}O_4$	134	4.50
8	20.76	*l*-(+)-Ascorbic acid-2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	12.53
9	23.68	9-Octadecenoic acid (z)	$C_{18}H_{34}O_2$	282	57.22
10	28.79	1,2,3-Propanetriyl ester (E,E,E)-9-Octadecenoic acid	$C_{57}H_{104}O_6$	884	3.86

Table 6: Biological activities of some active principles present in different extracts of *Cnidoscolus aconitifolius.*

Phyto-components	**Nature of Compound**	**Biological Activities****
n-Octadecanoic acid	Stearic acid	Flavourant
9-Octadecenoic acid (z)	Oleic acid	Anti-inflammatory, Anti-alopecic, Haemolytic and 5-Alpha reductase inhibitor, lubricant, Antitumour Immunostimulant, Antiandrogenic, Antibacterial, Antifungal, Lipoxygenase inhibitor, Diuretic
n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypercholesterolemic, Lubricant, Nematicide, Pesticide, Hemolytic and 5-Alpha reductase inhibitor, Flavour, Antiandrogenic
3,7,11,15-Tetramethyl-[R,-[R*,R*-(E)]]-2-Hexadecen-1-ol	Acyclic di-terpene alcohol	Antimicrobial, Anticancer, Anti-inflammatory Hypercholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic
9,12-Octadecanoic acid (z, z)-methyl ester	Linoleic acid ester	Anti-inflammatory, Hypercholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistamine, Antieczemic, Anti-acne, 5-α reductase inhibitor, Antiandrogenic, Anti-arthritic, Anti-coronary.
***l*-(+)-Ascorbic acid-2,6-dihexadecanoate**	Vitamin C derivative	Antioxidant, Anti-inflammatory, Anti-nociceptive, Antibacterial, Sperm quality enhancer
Gamma-Aminobutyric lactam	Pyrrolidone	Anticonvulsant, Solvent and Excipient in Veterinary Pharmaceutical products
6,10,14-trimethyl-2-Pentadecanone	Ketone	Arthropod repellant
Hexadecanoic acid methyl ester	Palmitic acid ester	Antioxidant, Hypercholesterolemic, Lubricant, Nematicide, Pesticide, Hemolytic, 5-Alpha reductase inhibitor, Flavour, Antiandrogenic

**Source: Dr .Duke's phytochemical and Ethnobotanical databases [Online database].

DISCUSSION

The UV-Vis Spectroscopic method is a useful method for the identification of unsaturated bonds present in plant phyto-constituents. Thus, this method is useful in differentiation of conjugated and non-conjugated system. Different compounds have their characteristic wavelength of maximum absorption, for example the peak values of 280nm and 330nm indicates phenolic compound and its derivatives, peak values of 420 nm and 470 nm indicates the

presence of carotenoids and its derivatives while the peak value of 663nm indicates the presence of chlorophyll and its derivatives in the plant (Zavoi *et al*., 2011). The UV-Vis spectra scanning of different extracts of this plant indicated the presence of phenolic compounds and carotenoids which are useful plant based antioxidants without losing the focus on the plant elaboration of chlorophyll all through the extracts, which may partially explain why the plant is used as ornamental plant. The FTIR confirmed the presence of alkanes, alkenes, alkynes, alcohol, aldehyde, ketone, ester, carboxylic acid and aromatics. Hence, the crude extracts subjected to UV-VIS and FTIR analysis help in the identification of phyto-components present in *Cnidoscolus aconitifolius.*

From the GC-MS results, it was observed that 9-Octadecenoic acid (z) (Synonym: Oleic acid) occurs in highest amount all through [n-Hexane (56.77%), dichloromethane (45.13%), and ethyl acetate (57.22%)] the extracts, while its derivative 1, 2, 3-Propanetriyl ester (E, E, E)-9-Octadecenoic acid is also elaborated in all the extracts but in minor quantity (Tables 3, 4 and 5). The derivatives of the oleic acid observed might have resulted from the adverse conditions the crude extract was exposed to in the gas chromatography column. The major phyto-Component: 9-Octadecenoic acid (z) was reported to possessed an anti-inflammatory, anti-alopecic, Haemolytic 5-α reductase inhibitor, lubricant, antitumor, Immunostimulant, diuretic, Antiandrogenic, antibacterial, antifungal, and Lipoxygenase inhibitor activities (Dr.Duke; Ogunlesi *et al.*, 2010). Other extracted phyto-components possessing Antiandrogenic and Haemolytic 5-α reductase inhibitor activity include palmitic acid and its ester as well as Linoleic acid ester. A well-known acyclic diterpene alcohol (3, 7, 11, 15-Tetramethyl-[R-[R*-R*-(E)]]-2-hexadecen-1-ol) which is present in minor quantity [n-Hexane (2.6%) and dichloromethane (1.2%)] in the extracts has been previously isolated and identified from Jute leaves. It is present as the ester side chain in the molecule of chlorophyll (Harbone, 1998). This diterpene has potent anti-inflammatory, Hypercholesterolemic, antimicrobial, Nematicide, Antiandrogenic and anticancer activity. (Rajasekaran *et al*., 2012; Dr. Duke's)

The ***l***-(+)-Ascorbic acid-2,6-dihexadecanoate (Vitamin C derivative) comprising of 12.5% in the ethyl acetate extract only, has been reported to have an antioxidant, anti-inflammatory, anti-nociceptive, antibacterial (*Staph. aureus* and *E. coli*) and sperm quality enhancing properties (Ogunlesi *et al*., 2010).

There is a significant difference between the number of phyto-components extracted by dichloromethane (fourteen) when compared with that of n-Hexane (seven) and ethyl acetate (ten) which may be due to its extractive ability.Chlorophyll is pigmentation present in plants generally and it is extracted by the non-polar solvents used in this study. Chlorophyll is responsible for the absorption of light energy during the process of photosynthesis. Although chlorophyll can have a negative effect on the oxidative stability of the fatty acids and their ester derivatives which occur as majority of the phyto-components identified in this study, as this can produce the photo-oxidation of the fatty acids or their derivatives if it is exposed to light. (Eyres et al., 2001) Thus, it is imperative that in order to increase the oxidative stability of the concerned fatty acids for long duration, exposure to light

should be avoided, using dark bottles, as well as exposure to oxygen in the air, via the use of nitrogen in the storage.

Though, the leaf extracts of the plant obtained by polar solvent extraction have been investigated for their volatile and semi-volatile constituents in considerable detail (Omotoso *et al.,* 2014), non-polar extracts were not studied till date. Thus, the primary aim of this study is to compare both volatile and semi-volatile phyto-components present in the non-polar extractives of this herb. The above findings will bring to fore another direction in pharmacological and therapeutic investigations with the leaf extracts obtained from non-polar solvent extraction such as n-Hexane, Dichloromethane and Ethyl acetate.

CONCLUSION

This type of comparative phytochemical analysis study will be useful in differentiating the species from adulterants and other subspecies thus will become the fingerprint for this plant. Subsequently, it may become biochemical marker which is of immense benefit in research and manufacturing settings. The spectra analysis will help the manufacturer of herbal products in quality control and standardization of their herbal formulation. The presence of many phyto-components in *Cnidoscolus aconitifolius* lend credence to its use by both urban and rural dwellers both as food and medicine thereby holding promising future in the production of both nutraceuticals and pharmaceuticals. It would be worthwhile to further isolate the characterized phyto-compounds, determine the specific therapeutic activity (an ongoing work in our laboratory) and also understand the synergistic effect of the different phyto-compounds in the plant.

ACKNOWLEDGEMENTS

The authors thank Mr. Ekundayo AA for plant identification, Mr. Ibrahim of NARICT, Zaria, Nigeria for UV-VIS, FTIR and GC-MS experiments, and Prof. CO Usifoh of Dept. of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin (Nigeria), for his input in spectra interpretation.

REFERENCES

Atuahene CC, Poku-Prempeh B, Twun G (1999). The nutritive values of chaya leaf meal (*Cnidoscolus aconitifolius*) studies with broilers chickens. Anim. Feed Sci. Technol., 77:163-172.

Donkoh A, Kese AG, Atuahene CC (1990). Chemical composition of chaya leaf meal (*Cnidoscolus aconitifolius*) and availability of its amino acids to chicks. Anim. Feed Sci. Technol., 30: 155-162.

Dr. Duke's Phytochemical and Ethnobotanical Database. http://www.ars-gov/duke/ Assessed Thursday 16th Jan. 2014. 22:24pm.

Eyres L, Sherpa N, Hendricks G (2001). Avocado oil: new edible oil from Australasia. Lipid Technol. 13(4): 84-88.

Harbone JB (1998). Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman Hall, New York. Pp. 205

Iwalewa EO, Adewunmi CO, Omisore WO, Adebanjo OA (2005). Pro-antioxidant effect of vegetables in South-West Nigeria. J Med. Food 8, pp 531-534.

Komal KJ, Deri-Prasad AG (2011). Identification and comparison of Biomolecules in medicinal plants of *Tephrosia tinctoria* and *Atylosia albican* by using FTIR. Romanian J Biophys. 21 (1):63-71.

Ogunlesi M, Okiei W, Osibote EA (2010). Analysis of Essential oil from the leaves of *Sesamum radiatum*, a potential medication for male infertility factor by gas chromatography-mass Spectrometry. African Journal of Biotechnology. 9 (7): 1060-1067.

Omotoso AE, Ezealisiji K, Mkparu KI (2014). Chemometric Profiling of Leaf Extracts of *Cnidoscolus aconitifolius* (Euphorbiaceae) with UV-Vis, FTIR and Gas Chromatography-Mass Spectrometric (GC-MS) Techniques. Peak Journal of Medicinal Plant Research. 2(1): 6-12.

Oyagbemi AA, Odetola AA, Azeez OI (2003). Ameliorative effect of *Cnidoscolus aconitifolius* on anaemia and osmotic fragility induced by protein energy malnutrition. J Biotech. 7 (11):1721-1726.

Roessner U, Leudermann A, Brust D, Fiehn O, Lmke T et al., (2001). Metabolic Profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. Plant Cell 13:11-29.

Peter J Houghton, Amala Raman (1998) Laboratory Handbook for the Fractionation of Natural Extracts, London Chapman & Hall Ltd. Pp 22-52.

Rajasekaran M, Archana R, Raviprasadha R (2012). GC/MS Analysis and Identification of phytochemicals present in the leaves of *Beloperone plumbagini* folia (Jacq.) Nees. Intro. J Bio-Engineering and Technology. 3 (2) : 1134-1138.

Ross-Ibarra J, Molina-Cruz A (2002). The Ethnobotany of chaya (*Cnidoscolus aconitifolius*): A nutritious Maya vegetable. J. Ethnobotany. 56: 350-364.

Shellie RA, Poynter SD, Li J, Gatherole JL, Whttock SP et al. (2009). Varietal characterization of hop (*Humulus lupulus* L) by GC-MS analysis of hop cone extracts. J Sep Sci. 32: 3720-3725.

Sofowora A (1993). Medicinal Plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria pp 118-128.

Trease GE, Evans WC (2009). Phytochemistry. In: Textbook of Pharmacognosy (16^{th} ed.) Saunders Elsevier pp 133-148.

Zavoi S, Fetea F, Ranga F, Pop MR, Baciu A, Solacin C (2011). Comparative Fingerprint and Extraction yield of medicinal Herb Phenolic with Hepatoprotective potential, as Determined by UV-Vis and FT-MIR Spectroscopy. Not Bot Horti Agrobo 39 (2): 82-89.