CHEMICAL CONSTITUENTS, ANTIBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITIES OF THE AERIAL PARTS OF *Cnidoscolus aconitifolius*

Hamid, Abdulmumeen A.[1*], Oguntoye, Stephen O.[1], Negi, Arvind S.[2], Ajao, Ajibola[1], Owolabi, Nurudeen O.[1].

[1]Department of Chemistry, University of Ilorin, Ilorin, Nigeria
[2]Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India
*Corresponding Author: Tel No: +2347035931646 E-mail: hamidmemo@gmail.com, hamid.aa@unilorin.edu.ng
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ABSTRACT

Preliminary phytochemical investigation of crude n-Hexane, ethyl acetate and methanol extracts of the aerial parts of *Cnidoscolus aconitifolius* revealed the presence of anthraquinones, glycosides, steroids, flavonoids, tannins, saponins and terpenoids. All the crude extracts gave a clear zone of inhibition against the growth of the test bacteria (*Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, *Salmonella typhi, Klebsiellae pneumonae*) and fungi (*Candida albicans, Aspergillus niger, penicillium notatum and Rhizopus stolonifer)* at different concentrations, except ethyl acetate extract which showed no antifungal property on *Rhizopus stolonifer*. Ethyl acetate and methanol extracts exhibited significant antioxidant activities by scavenging DPPH free radicals with IC_{50} of 12.14 and 93.85 μg/ml respectively. GC-MS analysis of n-hexane and methanol extracts showed nine compounds each, while ethyl acetate extracts afforded ten compounds. Phytol is the most abundant constituent in n-hexane, ethyl acetate and methanol extracts with their corresponding percentage of abundance of 41.07%, 35.42% and 35.07%.

Keywords: *Cnidoscolus aconitifolius,* Antioxidant activity, GC-MS analysis, Phytochemicals, Phytol.

INTRODUCTION

Cnidoscolus aconitifolius sometimes called Chaya or spinach tree, belongs to the tribe Manihoteae of the subfamily Crotonoideae of Euphorbiaceae (Webster 1975). It is a fast-growing perennial shrub that produces lots of attractive, large, dark green leaves. It is an evergreen drought-deciduous shrub of up to six meters in height with alternate, palmately lobed leaves, milky sap, and small, white flowers on dichotomously branched cymes. Leaves are large and chartacious or sometimes succulent, up to 32 cm long and 30 cm wide, on petioles up to 28 cm in length. Despite recent work claiming the contrary (Carbajal *et al.,* 1998), the species is monoecious, with separate male and female flowers each exhibiting defunct reproductive organs of the opposite sex. The whole plant has been used in traditional medicine to treat different diseases such as blood inflammation, respiratory disorders, skin disease, fever, bronchitis, asthma, kidney and urinary disorder. Usually cooked leaves are eaten, or teas or infusions are made from the leaves (Berkelaar, 2006). Although many medicinal claims have been made for the plant, traditionally it has been recommended for a number of ailments including diabetes, obesity, kidney stones, hemorrhoids, acne, and eye problems (Diaz-Bolio, 1975). *Cnidoscolus aconitifolius* roots and leaves have been taken as a laxative, diuretic, circulation stimulant, to increase digestion, stimulant for lactation, and to harden the fingernails (Rowe, 1994; Kuti, and Torres, 1996). Oyagbemi *et al.,* (2011) reported the proximate analysis and mineral composition of *C. aconitifolius* leaves. *C. aconitifolius* leaves showed high levels of crude protein, ash, and fiber, in that order, and low fat content with concomitant presence of minerals such as sodium, manganese, magnesium, potassium, calcium, iron, phosphate, and zinc. The presence of different natural products in the plant has initiated our interest to further investigate this plant with a view of determining the antimicrobial and antioxidant activities as well as phytochemical composition of different extracts of the aerial parts of *Cnidoscolus acontifolius*.

MATERIALS AND METHODS

Sample Preparation and Extraction

The aerial parts of *Cnidoscolus aconitifolius* were collected from Amurin, Ondo state, Nigeria in the month of November 2014. The plant was identified and authenticated by Mr. Bolu Ajayi of the Department of Plant Biology, University of

Ilorin, Ilorin, Nigeria where a voucher identification number (UIH001/1145) was assigned to the plant and was deposited at the herbarium.

The plant was extracted using standard procedure according to Das *et al.* (2010). The aerial parts of *Cnidoscolus aconitifolius* were air dried and crushed into smaller pieces using mortar and pestle. The plant was weighed and extracted using serial exhaustive extraction method by moving from a non-polar (hexane) solvent to a medium polar solvent (ethyl acetate) and then to a polar solvent (methanol). These solvents were analytical grade of 99.8% purity obtained from Sigma-Aldrich Chemical Company, Germany.

Phytochemical Screening

Preliminary phytochemical screening of the crude extracts was carried out using the methods described by Pranshant *et al.* (2011).

Antimicrobial Assay

Microorganism:

Cultures of six human pathogenic bacteria made up of four gram negative and two gram-positive bacteria were used for the antibacterial assay. These include: *Salmonella typhii, Escherichia coli, Pseudomonas aeruginosa, Klebsiellae pneumonae, Bacillus subtilis* and *Staphylococcus aureus*. Four fungi were also utilized for the antifungal assay. These are: *Candida albicans, Aspergillus niger, Rhizopus stolon* and *Penicillium notatum*. The whole microorganisms used were fresh clinical strains from the Medical Microbiology (University College Hospital, Ibadan) and screened in the Laboratory of Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria.

Media: Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. n-Hexane, ethyl acetate and methanol solvents were also used in solubilizing the extracts and as negative controls in the assays.

Antimicrobial Agents: Gentamycin (10 μg/ml) and Tioconazole (0.7 mg/ml) were included as standard reference drugs in the study

Antibacterial Activity Determination

Agar Diffusion (Ditch) Method: An overnight culture of each organism was prepared by taking two wire loop of the organism from the stock and inoculating each into the sterile nutrient broth of 5 ml, each incubated for 24 h at 37 °C. Each organism (0.1 ml) was taken from overnight culture, and diffused into 9.9 ml of sterile distilled water to obtain 10^{-2} inoculum concentration of the test organism (bacteria). The test organism (0.2 ml of the 10^{-2} inoculum concentration of bacteria) was taken into the prepared sterile nutrient agar cooled to about 45 °C, then poured into sterile Petri dishes and allowed to solidify for about 60 min. Eight (8) wells were made using a sterile cork-borer of 8mm diameter. The graded concentrations (6.25-200 mg/ml) of the extracts were separately loaded into each well accordingly with the dissolved solvents and Gentamycin was used as controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2 h to allow the extract diffuse properly into the nutrient agar. Plates were incubated for 24 h at 37°C (Collins and Lyne, 1970).

Antifungal Activity Determination

Agar Diffusion (Surface Plate) Method:

A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in triplicates and was allowed to solidify. The test organism (0.2 ml of the 10^{-2} inoculum concentration of the fungi) was spread on the surface of the agar using a sterile Petri-dish to cover the surface of the agar. Eight wells were bored using a sterile cork-borer of 8 mm diameter. Respective concentrations of the extracts (6.25-200 mg/ml) were separately loaded into each well with the dissolved solvents and Tioconazole as controls. All plates were left on bench for 2 h to allow the extract diffuse properly into the agar medium. The plates were incubated at 25 °C for 72 h (Collins and Lyne, 1970).

Antioxidant Activity

The ability of the samples to scavenge DPPH free radicals was assessed by the standard method of Sies (1997). The stock solutions of extracts were prepared in methanol to achieve final concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.99 μg/mL. DPPH (2,2-diphenyl-1-hydrazine) is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to

evaluate antioxidant activity. The absorbance was measured in triplicate at varying concentrations and the mean absorbances were determined. Parallel to examining the antioxidant activity of plant extracts, the value for the standard compound (Ascorbic acid) was obtained (Table 3.16) and compared to the values of the antioxidant activity, percentage inhibitions of the serial concentrations of the methanolic DPPH extracts and that of the ascorbic acid standard were determined at different concentrations using the expression below:

$$\% \ inhibition = \left(\frac{Absorbance\ of control - Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100$$

The IC_{50} (half maximal inhibitory concentration) values were estimated from the % inhibition versus concentration of inhibitor plot, using a non-linear regression plot method.

GC- MS Analysis of the Chemical Constituents

GC-MS was performed with Agilent 19091GC plus automatic sampler system coupled with a quadruple mass spectrometer 433HP-5MS. Compounds were separated in HP5MS column fused with phenyl methyl silox, (length; 30m x 250µm; film thickness 0.25µm). Samples were injected at a temperature of about 250 °C with a split ratio of 10:1 with a flow rate of helium 1ml/min. The extracts of the aerial parts of *Cnidoscolus aconitifolius* were dissolved in the respective solvent (n-hexane, ethyl acetate and methanol) to form solution. The analysis was done using the GC-MS machine which is made up of two major building blocks, the gas chromatography and the mass spectrophotometer. The gas chromatography uses a capillary column which depends on the column dimension as well as the phase properties. The difference in the chemical properties between the different molecules in the mixture will help separate molecules as the sample travels through the length of the column. The molecules take different retention time to be eluted from the gas chromatography. This allows the mass spectrometer downstream to capture, ionize, accelerate, deflect and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule with ionized fragments and detecting these fragments using their mass to charge ratio.

Identification of Compounds

The data obtained from the GC-MS result revealed ten compounds with different mass fragmentation indicating a mixture of ten different compounds in the extraction or ten different chemical constituents present in the extracts. The interpretation of the spectrum and identification of the compounds were based on the molecular structure, molecular mass and the mass spectra which were matched with that of the standard online library of pherobase from which the name, molecular weight and structure of the compounds were established.

RESULTS AND DISCUSSIONS

The phytochemical composition of crude extracts of the aerial parts of *Cnidoscolus aconitifolius* investigated revealed the presence of some bioactive principles. The n-hexane extract revealed the presence of terpenoids, flavonoids, steroids and cardiac glycosides (Table 1), while ethyl acetate extract indicated the presence of anthraquinones, saponins, terpenoids and steroids. Methanol extract of the aerial parts of the plant contains anthraquinones, tannins, flavonoids, glycosides, saponins, terpenoids and steroids. Meanwhile, alkaloids and phenolics were absent in all the three extracts. Compounds rich in flavonoids, terpenoids and glycosides have been reported to possess antibacterial, antifungal, anti-inflammatory and anti-diarrhoeal properties (Prashant *et al.*, 2011). Hence, presence of these compounds in the crude extracts of *Cnidoscolus aconitifolius* exhibits antibacterial and antifungal activities.

Table 1: Phytochemical Screening of various Crude Extracts of the Aerial Parts of *C. aconitifolius*

Chemical constituents	Fractions		
	n-Hexane	Ethyl acetate	Methanol
Alkaloids	-	-	-
Anthraquinones	-	+	+
Glycosides	+	-	+
Steroids	+	+	+
Phenolics	-	-	-
Flavonoids	+	-	+
Tannins	-	-	+
Saponins	-	+	+
Carbohydrates	-	-	-
Terpenoids	+	+	+

Key: + = present. - = absent

The antibacterial activity was determined using the Agar broth cup diffusion – pour plate method for the bacterial and antifungal activity using the surface plate (Ditch) method for the fungi with wells of 8mm in diameter. The result obtained in this study showed the antimicrobial potency of the extracts of *Cnidoscolus aconitifolius* aerial part on the test organisms. The three crude extracts (n-hexane, ethyl acetate and methanol extracts) showed high zone of inhibition on the six test bacteria and four fresh isolates of fungi at concentration of 50 mg/ml, moderate inhibitory concentration at 50 mg/ml and least inhibitory concentration at 12.5 mg/ml. While ethyl acetate extract exhibited no antifungal activity on *Rhizopus stolonifer*. (Table 2-4). n-Hexane extract of the plant exhibited high antibacterial activity on all test organisms at all concentrations, and also high antifungal activity on all the test fungi at concentrations ranging from 12.5 to 200 mg/ml but showed low inhibition against *Aspergillus niger* and *Rhizopus stolonifer* at low concentrations of 6.25 mg/ml (Table 2). Ethyl acetate extract of *Cnidoscolus aconitifolius* aerial parts inhibited the growth of all the bacterial and fungal isolates at high concentrations greater than 50mg/ml (Table 3). Methanol extract of *Cnidoscolus aconitifolius* leaves showed higher inhibition on all the test bacteria. These include: *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa, Klebsiellae pneumonae* than *Bacillus subtilis and Salmonella typhii* at all concentrations. The extract also exhibited antifungal potency on *Candida albicans, Aspergillus niger, Rhizopus stolonifer* and *Penicillum notatum* at concentrations from 50 to 200 mg/ml (Table 4). The zone of inhibition exhibited by the plant extracts gives evidence to the fact that *Cnidoscolus aconitifolius* is an antibacterial and antifungal plant and hence can be used for the treatment of illness caused by pathogenic bacteria and fungi.

Table 2: Antimicrobial Activity of n-Hexane Extract of the Aerial Parts of *C. aconitifolius*

Isolate	Mean zone of inhibition (mm)							
S.aureus	28	24	20	18	14	10	-	38
E. coli	26	22	18	14	12	10	-	38
B. subtilis	24	20	18	14	12	10	-	40
P. aeruginosa	24	20	18	16	14	10	-	38
K. pneumonia	24	18	16	14	12	10	-	40
S. typhi	24	20	18	14	12	10	-	38
C. ablicans	20	18	16	14	12	10	-	28
A. niger	20	18	14	12	10	-	-	26
P. notatum	20	18	16	14	12	10	-	26
R. stolonifer	18	16	14	12	10	-	-	26
Conc. of extracts(mg/mL)	200	100	50	25	12.5	6.26	-ve	+ve

Key: +ve = Gentamycin10µg/ml (for bacteria), Tioconazole(for fungi); -ve = Solvent of dilution

Table 3: Antimicrobial Activity of Ethyl acetate Extract of the Aerial Parts of *C. aconitifolius*

Isolate	Mean zone of inhibition (mm)							
S.aureus	16	14	12	10	-	-	-	38
E. coli	16	14	12	10	-	-	-	38
B. subtilis	18	14	12	10	-	-	-	40
P. aeruginosa	14	12	10	-	-	-	-	38
K. pneumonia	16	14	10	-	-	-	-	40
S. typhi	14	12	10	-	-	-	-	38
C. ablicans	16	14	12	10	-	-	-	28
A. niger	14	12	10	-	-	-	-	26
P. notatum	12	10	-	-	-	-	-	26
R. stolonifer	-	-	-	-	-	-	-	26
Conc. of extracts(mg/mL)	200	100	50	25	12.5	6.26	-ve	+ve

Key: +ve = Gentamycin10µg/ml (for bacteria), Tioconazole(for fungi); -ve = Solvent of dilution

Table 4: Antimicrobial Activity of Methanol Extract of the Aerial Parts of *C. aconitifolius*

Isolate A	Mean zone of inhibition (mm)							
S.aureus	20	18	14	12	10	-	-	38
E. coli	18	16	14	12	10	-	-	38
B. subtilis	18	14	12	10	-	-	-	40
P. aeruginosa	18	16	14	12	10	-	-	38
K. pneumonia	18	16	14	12	10	-	-	40
S. typhi	16	14	12	10	-	-	-	38
C. ablicans	16	14	12	10	-	-	-	28
A. niger	16	14	12	10	-	-	-	26
P. notatum	14	12	10	-	-	-	-	26
R. stolonifer	14	12	10	-	-	-	-	26
Conc. Of extracts (mg/mL)	200	100	50	25	12.5	6.26	-ve	+ve

Key: +ve = Gentamycin10µg/ml (for bacteria), Tioconazole (for fungi); -ve = Solvent of dilution

n-hexane, ethyl acetate and methanol extracts of *Cnidoscolus aconitifolius* aerial parts exhibited antioxidant activity on DPPH radicals at different concentrations, using ascorbic acid as standard antioxidant. The ethyl acetate extract of the plant showed the highest significant inhibition of DPPH radicals at concentrations in the range of 3.9 to 100 µg/ml, by scavenging the free radicals with IC_{50} of 12.14 µg/ml, the activity was comparable with that of the standard control, ascorbic acid (IC_{50} of 5.67 µg/ml). The methanol extract showed antioxidant activity with IC_{50} of 93.85 µg/ml, while n-hexane extract of the plant revealed low inhibition of DPPH radicals (IC_{50} of 661.4 µg/ml) (Table 5 & 6).

Table 5: The DPPH Antioxidant Activity and % Inhibition of Ascorbic Acid

Concentration (µg/ml)	Absorbance1	Absorbance 2	Absorbance 3	Average ± Standard Deviation	%Inhibition of Ascorbic Acid
1000	0.138	0.138	0.140	0.139±0.0012	89.02
500	0.150	0.150	0.150	0.15±0.000	88.14
250	0.161	0.162	0.160	0.161±0.001	87.26
125	0.180	0.180	0.180	0.180±0.000	85.79
62.5	0.193	0.195	0.194	0.194±0.001	84.26
31.25	0.245	0.245	0.245	0.245±0.000	80.67
15.62	0.311	0.311	0.311	0.311±0.000	75.440
7.81	0.453	0.452	0.454	0.453±0.001	64.18
3.9	0.782	0.781	0.78	0.781±0.001	38.26
1.95	0.991	0.991	0.991	0.991±0.000	21.66

DPPH Absorbance of Control: 1.265

Table 6: DPPH Antioxidant Activity and % Inhibition of Extracts of the n-Hexane Aerial Parts of *Cnidoscolus aconitifolius* (CAAH), Ethyl acetate Arial Parts of *Cnidoscolus aconitifolius* (CAAE) and Methanol Aerial Parts of *Cnidoscolus aconitifolius* (CAAM) with 0.518, 0.333and 0.389 as Absorbance of the Control

ANTIOXIDANT ACTIVITY OF N -HEXANE EXTRACT OF CAA					
Conc.(µg/mL)	Absorbance	Absorbance	Absorbance	Mean absorbance	% Inhibition
1000	0.245	0.242	0.243	0.243±0.0015	53.09
500	0.265	0.262	0.263	0.263±0.0015	49.23
250	0.265	0.264	0.267	0.265±0.0015	48.84
125	0.278	0.278	0.277	0.278±0.0006	46. 33
62.5	0.269	0.271	0.269	0.270±0.0012	47.88
31.25	0.274	0.273	0.274	0.274±0.0006	47.10
15.62	0.278	0.278	0.279	0.278±0.0006	46. 33
7.8	0.283	0.281	0.280	0.281±0.0015	45.75
3.9	0.289	0.289	0.289	0.289±0	44.21
1.95	0.296	0.294	0.292	0.294±0.0016	43.20
ANTIOXIDANT ACTIVITY OF E.A EXTRACT OF CAA					
Conc.(µg/mL)	Absorbance	Absorbance	Absorbance	Mean absorbance	% Inhibition
1000	0.152	0.151	0.154	0.152±0.0015	54. 35
500	0.160	0.161	0.159	0.160±0.0010	52.05
250	0.154	0.154	0.151	0.154±0	53.75
125	0.152	0.149	0.159	0.151±0.0017	54.65
62.5	0.159	0.159	0.159	0.159±0	52.25
31.25	0.159	0.159	0.160	0.159±0.0006	52.25
15.62	0.162	0.162	0.162	0.162±0	51. 35
7.8	0.170	0.173	0.173	0.172±0.0017	48. 35
3.9	0.205	0.206	0.206	0.206±0.0006	38.14
1.95	0.220	0.221	0.221	0.221±0.0006	33.63
ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF *CAA*					
Conc.(µg/mL)	Absorbance	Absorbance	Absorbance	Mean absorbance	% Inhibition
1000	0.174	0.174	0.174	0.174±0	55.16
500	0.146	0.148	0.148	0.147±0.0012	62.21
250	0.165	0.165	0.168	0.166±0.0017	57. 33
125	0.186	0.186	0.184	0.185±0.0012	52.44
62.5	0.205	0.204	0.204	0.204±0.0006	47.56
31.25	0.210	0.208	0.209	0.209±0.0010	46.27
15.62	0.222	0.221	0.221	0.221±0.0010	43.19
7.8	0.227	0.226	0.226	0.226±0.0010	41.90
3.9	0.224	0.224	0.224	0.224±0.0006	42.42
1.95	0.224	0.221	0.222	0.222±0.0015	42.93

In Gas chromatograph-Mass spectroscopy (GC-MS) analysis, nine compounds were detected in the n-hexane extract, ten compounds in the ethyl acetate extract and nine compounds in the methanol extract of *Cnidoscolus aconitifolius* aerial parts.

The characterizing constituents of the n-hexane extract from the aerial parts of *Cnidoscolus aconitifolius* revealed the presence of nine compounds which are 2,6,6-Trimethylbicyclo[3.1.1]heptanes, Hexahydrofarnesylacetone, Octadec-9-yne, 5-Nonadecen-1-ol, Methyl hexadecanoate, Hexadecanoic acid, Methyl linoleate, (Z,Z,Z)-9,12,15-Octadecatrienoic acid methyl ester and also Phytol (% abundance of 41.07%) as shown in Table 7. The GC-MS analysis of ethyl acetate extract of the plant showed the presence of ten compounds which are Benzoic acid, Methyl 3-Hydroxyhexadecanoate, 2-Phenylacetic acid, Triacetin, 3-(2-Methoxyethyl)nonan-1-ol, (Z)-Tricos-14-en-1-yl formate, Octadec-9-yne, Methyl palmitate, Phytol and Phytol acetate with % abundance of 2.01, 2.41, 1.79, 1.26, 15.93, 3.68, 6.48, 2.18, 35.24 and 29.02 respectively (Table 8).

The GC-MS analysis of methanol extract of *Cnidoscolus aconitifolius* aerial parts showed the presence of nine compounds which are 2-((5-Aminopentyl)amino)-2-oxoacetic acid (% abundance: 1.26), Methyl-1-ethylazetidine-2-carboxylate (% abundance: 7.95), Diethyl phthalate (% abundance: 2.16), (1S,2R,5S)-2,6,6-Trimethylbicyclo[3.1.1]heptanes (% abundance: 2.31), 2-Hexyldodecyl butyrate (% abundance: 4.58), Methyl palmitate (% abundance: 19.20), Methyl linoleate (% abundance: 3.68), Trienoic acid methyl ester (% abundance: 23.79) and Phytol (% abundance: 35.07) (Table 9). Phytol which has molecular formula $C_{20}H_{40}O$ and molecular mass of 296.53 g/mol is the most abundant constituent in n-hexane, ethyl acetate and methanol extracts of the plant with their corresponding percentage of abundance of 41.07%, 35.42% and 35.07%.

Table 7: GC-MS Analysis of n-Hexane Extract of the Aerial Parts of *Cnidoscolus aconitifolius*

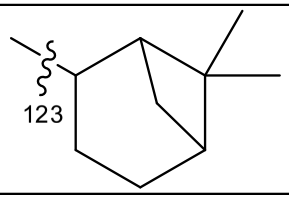
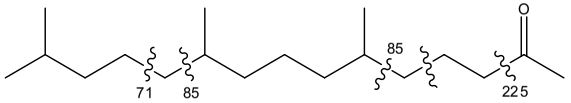
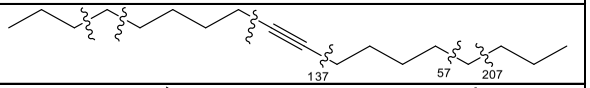
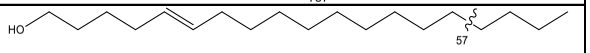
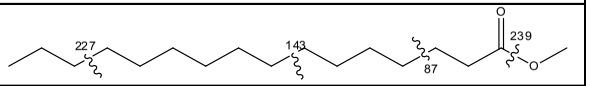
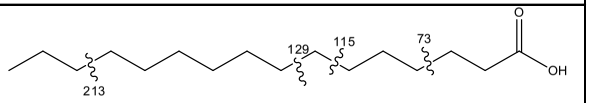
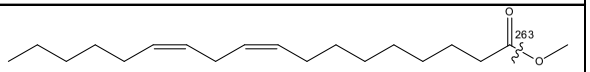
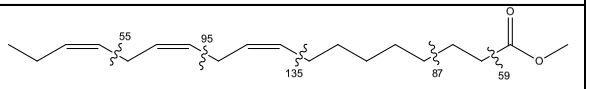
S/N	Compound name	Formula	Peak area %	Molecular Weight (g/mol)	Mass spectral fragments	Retention time (min)	Structure
1	2,6,6-Trimethylbicyclo[3.1.1]heptane	$C_{10}H_{18}$	6.05	138.25	123, 109, 95, 82, 68,57	24.722	
2	Hexahydrofarnesylacetone	$C_{18}H_{36}O$	5.04	268.48	165, 137, 123, 109, 95, 85, 71, 58	24.891	
3	Octadec-9-yne	$C_{18}H_{34}$	1.31	250.46	207, 137, 123, 109, 95, 81, 68, 57	25.348	
4	5-Nonadecen-1-ol	$C_{19}H_{38}O$	1.90	282.50	137, 123, 109, 95, 81, 68, 57	25.798	
5	Methyl hexadecanoate	$C_{17}H_{34}O_2$	3.66	270.45	239, 227, 143, 129, 87, 74, 55	26.899	
6	Hexadecanoic acid	$C_{16}H_{32}O_2$	27.52	256.42	213, 181, 171, 157,129, 115, 97, 83, 73, 60, 57	28.388	
7	Methyl linoleate	$C_{19}H_{34}O_2$	1.40	294.48	263, 150, 123, 109, 95, 81, 67, 55	30.890	
8	(Z,Z,Z)-9,12,15-Octadecatrienoic acid methyl ester	$C_{19}H_{32}O_2$	12.05	292.46	149,135, 121, 108, 95, 87, 79, 67, 59, 55	31.034	
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$	41.07	296.53	137, 123, 109, 95, 81, 68, 55,	31.434	

Table 8: GC-MS Analysis of Ethyl acetate Extract of the Aerial Parts of *Cnidoscolus aconitifolius*

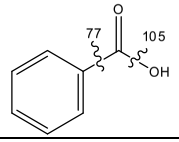
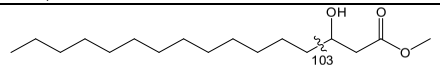
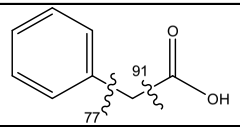
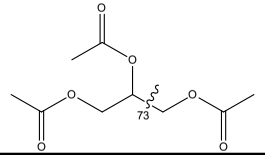
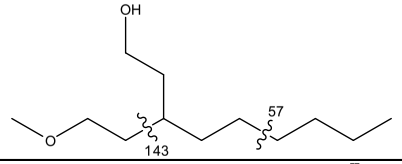
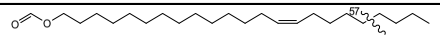
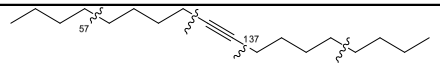
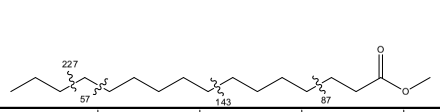
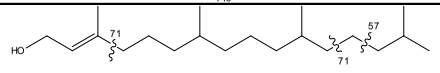
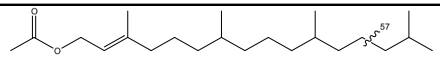
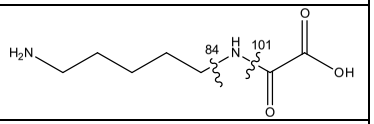
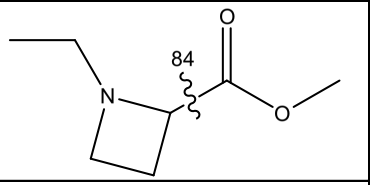
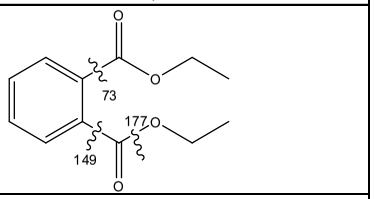
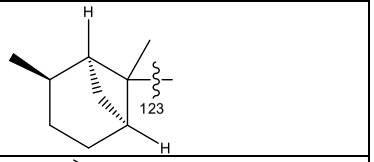
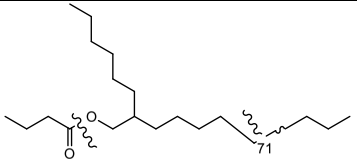
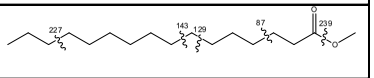
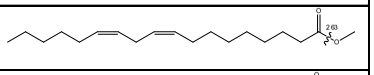
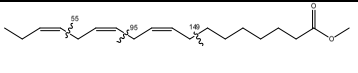
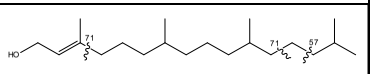
S/N	Compound name	Formula	Peak area %	Molecular Weight (g/mol)	Mass spectral fragments	Retention time (min)	Structure
1	Benzoic acid	$C_7H_6O_2$	2.01	122.12	105, 99, 77, 51	6.175	
2	methyl-3-hydroxyhexadecanoate	$C_{17}H_{34}O_3$	2.41	286.45	103, 7467,61	7.977	
3	2-Phenylacetic acid	$C_8H_8O_2$	1.79	136.15	113, 97, 91, 65	8.552	
4	Triacetin	$C_9H_{14}O_6$	1.26	218.20	145, 116, 103, 86, 7367, 57	10.967	
5	3-(2-Methoxyethyl)nonan-1-ol	$C_{12}H_{26}O_2$	15.93	202.33	144, 137, 123, 109,95, 82,68,57	24.760	
6	(Z)-Tricos-14-en-1-yl formate	$C_{24}H_{46}O_2$	3.68	366.62	278, 135, 123, 109, 95, 81, 68, 57	25.385	
7	Octadec-9-yne	$C_{18}H_{34}$	6.48	250.46	137, 123, 109, 95, 81, 68, 57	25.830	
8	Methyl palmitate	$C_{17}H_{34}O_2$	2.18	270.45	227, 185, 143, 129, 97, 87, 74, 55	26.943	
9	Phytol	$C_{20}H_{40}O$	35.24	296.53	151, 137, 123, 109, 95, 81, 71, 57	31.422	
10	Phytol acetate	$C_{22}H_{42}O_2$	29.02	338.57	278, 137, 123, 95, 81, 68, 57	33.743	

Table 9: GC-MS Analysis of Methanol Extract of the Aerial Parts of *Cnidoscolus aconitifolius*

S/N	Compound name	Formula	Peak area %	Molecular Weight (g/mol)	Mass spectral fragments	Retention time (min)	Structure
1	2-((5-Aminopentyl)amino)-2-oxoacetic acid	$C_7H_{14}N_2O_3$	1.26	174.20	171, 156, 101, 84, 69, 56	6.250	
2	Methyl-1-ethylazetidine-2-carboxylate	$C_7H_{13}NO_2$	7.95	143.18	124, 84, 56	12.009	
3	Diethyl phthalate	$C_{12}H_{14}O_4$	2.16	222.24	177, 149, 121, 105, 73, 57	18.292	
4	(1S,2R,5S)-2,6,6-Trimethylbicyclo[3.1.1]heptane	$C_9H_8NaO_4$	2.31	217.154	189, 151, 123, 109, 95, 82, 68, 57	24.741	
5	2-Hexyldodecyl butyrate	$C_{22}H_{44}O_2$	4.58	340.58	280, 250, 226, 165, 137, 124, 109, 95, 71, 58	24.935	
6	Methyl palmitate	$C_{17}H_{34}O_2$	19.20	270.45	239, 227, 143, 129, 87, 74	26.962	
7	Methyl linoleate	$C_{19}H_{34}O_2$	3.68	294.48	263, 189, 164, 123109, 95, 81, 67, 55	30.927	
8	Trienoic acid methyl ester	$C_{19}H_{32}O_2$	23.79	292.46	264, 236, 189, 163, 149, 121, 108, 95, 79, 67, 55	31.071	
9	Phytol	$C_{20}H_{40}O$	35.07	296.53	137,123,111,95,81,71,57	31.422	

CONCLUSION

The phytochemical screening of crude extracts revealed the presence of anthraquinones, glycosides, steroids, flavonoids, tannins, saponins and terpenoids. The findings of this research showed that the aerial parts of *C. aconitifolius* exhibit antibacterial and antifungal activities with wide zone of inhibition against pathogenic fresh isolates of test organisms which supported the previous study by Leandra *et al*., 2014. Ethyl acetate extract of the plant exhibits the DPPH free radicals scavenging activity with IC_{50} of 12.14 µg/ml, the activity was comparable with that of the standard control, ascorbic acid (IC_{50} of 5.67 µg/ml). The GC-MS of all the three extracts revealed the principal compound, phytol and other bioactive compounds.

The observed antimicrobial and antioxidant efficacies of this medicinal plant can be attributed to the presence of the abundant bioactive compounds in synergy with all other compounds in the plant. It can be concluded that extracts from the aerial parts of *Cnidoscolus aconitifolius* have antimicrobial and antioxidant activities, which support the ethnomedicinal uses of the plant for the treatment of back pain, kidney pain, kidney stones, biliousness, jaundice, boils, warts, pimples or other skin conditions, gum disease and toothache (Obi and Onuoha, 2000).

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