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GC-MS analysis and hypoglycemic properties of methanolic leaf extract of *Cnidoscolus aconitifolius* on alloxan-induced diabetic Wistar rats

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Abstract

The GC-MS analysis and hypoglycemic properties of methanolic leaf extract of *Cnidoscolus aconitifolius* were carried out on alloxan-induced diabetic Wistar rats. The GC-MS results revealed the presence of thirty-seven compounds consisting of hydrocarbons, alcohols, phenols, carboxylic acid esters, aldehydes, esters, fatty acids, and nitriles with some of them having known biological properties. The hypoglycemic properties of the methanolic leaf extract of *Cnidoscolus aconitifolius* were accessed by treating groups 3, 4, and 5 diabetic rats with 100, 150, and 200 mg/kg bw respectively of plant extract. Group 1 (negative control group) received only distilled water while group 2 (positive control group) was treated with 10 mg/kg bw of chloropropamide, a known anti-diabetic drug. A significant decrease in blood glucose levels was observed for groups treated with plant extract as compared to groups treated with chloropropamide. From the result obtained, the plant, *Cnidoscolus aconitifolius* could be used by diabetic patients to control blood glucose levels.

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1. Introduction

The wide use of plants to treat various ailments of man has made such plants with these therapeutic efficacies to be called medicinal plants. These plants, provided by nature, are found in different geographical locations of the earth; in terrestrial, aquatic, and arboreal habitats. Different parts of a medicinal plant, such as its leaves, stem, bark, roots, fruits, seeds, and flowers are used traditionally in both disease prevention and healing.

According to the World Health Organization; traditional medicine is the total knowledge, skills, and practices based on theories, beliefs, and experiences indigenous to different cultures, explainable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness [1]. The use of plants in traditional medicine is based on its observable benefits. This type of medication may pose challenges due to difficulty in controlling the dose and quality of

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the product, which can lead to risks and damage to health [2]. Most plants that are widely used as food in Nigeria according to [3] have medicinal values. However, due to paucity of information on the bio-protective properties of these plants, their uses as medicine have languished in obscurity. The health benefits derived by humans in using plants for traditional medicine are attributed to the presence of biologically active and naturally occurring chemical compounds found in plants which are known as phytochemicals. Phytochemicals are known as secondary metabolites and have biological properties such as antimicrobial effect, antioxidant activity, modulation of detoxification enzymes, and stimulation of the immune system, anti-cancer properties, and modulation of hormone metabolism [4]. These chemical compounds are used for protection by plants but recent research has shown that many phytochemicals can also protect humans against diseases [5]. One of such chemical compounds, 4-(4-Phenyl-1,4-dihydronaphthalen-1-yl) pentenoic acid, isolated from the stem bark of *Brachystegia eurycoma* Harmsas reported by Igwe & Echeme [6] could be the reason behind the utilization of the plant in traditional medicine for the treatment of wounds and infections. The secondary metabolite, 2-(4-ethylphenyl)-5-hydroxy-3-methyl-6,7-dihydrofuro-chromen-4-one, isolated from the seeds of *Brachystegia* eurycoma Harms by Igwe & Okwu [7] was reported to have antioxidant activities. Secondary metabolites, because of their medicinal value to humans are processed into drugs such as insulin (dahlias plant), morphine and codeine (poppy plant), quinine (cinchona plant), and digoxin (foxglove plant) [8].

Diabetics, often referred to as diabetes mellitus (DM), is a metabolic disorder that is characterized by high blood glucose (hyperglycemia) or low blood glucose (hypoglycemia), either because insulin production is inadequate or because the body's cells do not respond properly to insulin, or both [9]. Treatment of diabetes mellitus is focused on controlling and lowering blood glucose levels in the vessel to a normal level, 80 mg/dL to 120 mg/dL [10]. Besides modern medication which according to [11] has many side effects that cause serious medical problems, traditional medicines have been used for a long time and play a significant role as alternative medicines. According to WHO, ethno-medicine is still the primary health care of about 75 – 80 % of the world's population especially in developing countries with these medicinal plants [12]. Ethno-medicine is still the main healthcare of patients in developing countries because of cultural acceptability, efficacy, and lesser side effects than modern therapies. The use of transition metal complexes as medicinal compounds which have not only anti-cancer properties but also have been used as anti-inflammatory, anti-infective, and anti-diabetic compounds have been reported [13]. Recent reports have revealed the use of medicinal plants in the management of diabetes worldwide. More than 400 plant species having hyperglycemic activity have been available in the literature [14]. *Cnidoscolus aconitifolius* is one of the plant species its hypoglycemic properties is been assessed in this work.

Cnidoscolus aconitifolius is one of the Chaya group of plants of the genius *Cnidoscolus*, which is part of the *Euphorbiaceae* [15]. It is a nutritious vegetable, domesticated in pre-Columbian times, mainly used today as food, medicine, a living fence-post, and an ornamental plant by the Mexican and Mesoamerican peoples [16]. The plant is propagated by stem cutting and the about 40 cm long the stem are dried 1 – 4 days before being planted [17]. The plant is known as "*Efo Iyana Ipaja*" or "*Efo Jerusalem*" in the Southwest of Nigeria and "Hospital too far" in the Niger Delta areas of Nigeria [18]. The leaves of *Cnidocolus aconitifolius* according to [19] are used in ethno-medicine to boost blood volume, lower blood cholesterol, and manage and treat diabetes mellitus. This research is aimed at identifying the secondary metabolites present in the leaves of *Cnidoscolus aconitifolius* using GC-MS and also to authentic the claims of diabetic individuals in using the leaves of this plant to reduce blood glucose levels.

2. Materials and methods

2.1. Sample collection and processing

The leaves of *Cnidoscolus aconitifolius* were harvested from the plant at Umuda Isingwu in Umuahia North L.G.A., Abia State, Nigeria. The plant sample was identified in the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaf samples were properly washed, chopped, and air-dried under shade to a constant weight and then ground into powder with an electric blender.

2.2. Extraction of plant materials

The powdered sample was introduced into a big glass container and 100 % of methanol was poured into the container to the brim. The container was thus covered and kept. After two weeks, the sample was filtered, and the filtrate was concentrated for one week by evaporating the solvent to obtain the crude methanol extract of *C. aconitifolius*. The dried crude extracts were weighed to give 106.176 g. It was thus labeled *Cnidoscolus aconitifolius* crude methanol extract (CNACM).

2.3. GC-MS analysis

GC analyses were carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm X 50 m) and the conditions were as follows: temperature 300 ^{0}C, injection temperature 250 ^{0}C, carrier gas nitrogen at a flow rate of 1 mL/min, split ration 1:75. GC-MS analysis was conducted using GCMS-QP 2010 Plus Shimadzu Japan with injector temperature of 230 ^{0}C and carrier gas pressure of 100 Kpa. The length was 30 m with a diameter of 0.25 mm and a flow rate of 50 mL/min. The eluents were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 KV and a sampling rate of 0.2 seconds. The mass spectrum was also equipped with a computer-fed mass spectra data bank. Hermle Z 233 M-Z centrifuge Germany was used. The solvents were all of analytical grade.

Table 1: Compounds present in the GC-MS analysis of methanol leaf extract of *Cnidoscolus aconitifolius*

S/N	Compound	Retention Time (RT)	Concentration %
1	Ethylcyclododecane	INA	INA
2	2-Butyl-1-octanol	5.264	0.53
3	Decane	6.537	0.77
4	2,4-Di-tert-butylphenol	6.841	7.87
5	3-Chloropropionic acid, heptadecyl ester	7.697	1.56
6	5-Octadecene, (E)-	10.035	2.70
7	1,12-Dodecanediol	10.557	0.38
8	Nonadecanoic acid	11.253	0.35
9	7-Hexadecenoic acid, methyl ester(Z)	11.502	0.38
10	1,7-Dichloroheptane	11.828	0.65
11	N-Hexadecanoic acid	12.002	13.42
12	2-Bromopropionic acid, pentadecyl ester	12.232	3.48
13	11-(2-Cyclopenten-1-yl)undecanoic acid	12.476	1.34
14	4-Methyl-1-heptene	13.734	2.91
15	Cis-13-Octadecenoic acid	14.149	18.99
16	Cis-Oleic acid	14.418	2.71
17	2-Bromopropionic acid, pentadecyl ester	14.702	1.25
18	9-Hexadecenoic acid	16.143	1.08
19	9-Octadecene, (E)-	17.426	0.64
20	Cis-11-Hexadecenal	18.636	2.51
21	Bis-(2-ethylhexy)phthalate	19.686	2.66
22	9,12-Octadecadienoic acid	22.924	1.02
23	4,9-Decadienoic acid, 2-nitroethyl ester	22.943	0.26
24	Undec-10-ynoic acid	22.970	0.42
25	Hexadecenoic acid, Z-11-	23.037	1.02
26	2-Methyl-Z,Z-3,13-octadecadienol	23.074	1.33
27	Cyclopentaneundecanoic acid	29.572	1.32
28	7,11-Hexadecadienal	30.376	12.23
29	Docosanoic acid, isobutyl ester	30.603	5.15
30	Octanedinitrile	30.719	1.37
31	2-Methyl-E,E-3,13-octadecadien-1-ol	30.739	3.30
32	Pentadecanoic acid	31.156	0.82
33	Cyclopentaneundecanoic acid	31.208	1.24
34	6-methyl-3-octyne	33.323	0.77
35	17-Octadecynoic acid	33.362	0.69
36	Trans-9-Dodecenol	34.467	0.82
37	Trans,trans-2,8-Decadiene	34.488	0.70

2.4. Experimental animals

The experimental animals used were 30 Wistar albino rats of mixed sex. Wistar rats were used for this study because of their uniform size, docility, and ease of handling. They are important tools in the biomedical investigation of health outcomes in both humans and other animals. The rats were obtained from the animal house in the college of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State. They were 4 – 8 weeks old and weighed an average of 24.0 g.

2.4.1. Experimental design and treatment of diabetic rats

The animals were allowed one week of acclimatization after which diabetes was induced after starving them overnight into group 2 to group 5 with Alloxan at a dose of 60 mg/kg body weight. Before and after inducing diabetes, the blood glucose level of the mice was measured using a single-touch Accuchek glucometer, and animals with blood glucose levels of more than 230 mg/dl after 72 hours of inducing diabetes were classified as diabetic and selected for treatment.

The thirty (30) adult Wistar albino rats were randomly grouped into five groups of 6 animals each. Group 1: served as negative control (non-diabetic) and was normally fed with rat chow and water ad libitum; Group 2: served as positive control group and received 10 mg/kg body weight of Chloropropamide; group 3, 4, 5 served as test groups and received 100 mg/kg, 150 mg/kg and 200 mg/kg body weight of *Cnidoscolus aconitifolius* leaf methanol extract respectively and on daily basis by injection for 28 days.

Table 2: Major compounds present in the GC-MS analysis of methanol leaf extract of *Cnidoscolus aconitifolius.*

S/N	RT	Concentration%	Library	Biological Properties
1	6.841	7.87	2,4-Di-tert-butylphenol	antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal [20].
2	12.002	13.42	n-hexadecanoic acid	anti-inflammatory [21], antibacterial and antifungal [22].
3	12.232	3.48	2-Bromopropionic acid, pentadecyl	....
4	14.149	18.99	Cis-13-octadecenoic acid	....
5.	30.376	12.23	7,11-Hexadecadienal	....
6.	30.603	5.15	Docosanoic acid	....
7	30.739	3.30	2-methyl-E,E-3,13-octadecadien-1-0	....

Table 2 shows 7 major compounds out of the 37 compounds identified to be present in the leaves of *Cnidoscolus aconitifolius*. These 7 compounds are classified as major compounds due to their higher percentage concentration. At the time of this research, the biological properties of only two compounds were known as shown in Table 2.

Table 3: Hypoglycemic effects of the crude methanol extract of the leaf extracts of *Cnidoscolus Aconitifolius* on wistar Albino Rat.

Groups	Treatment	Fasting blood glucose level (mg/dl) (%)		
		Before alloxan induction	After alloxan induction	After drug treatment
Group 1 (negative control)	Distilled water	82.54±2.05	-	-
Group 2 (positive control)	10 mg/kg bw CPA	73.23±2.51	246.37±12.02	206.28±12.33
Group 3 (test groups)	100 mg/kg of leaf extract	82.12±3.01	237.82±9.17	216.13±8.16
Group 4 (test groups)	150 mg/kg of leaf extract	68.61±2.04	247.12±9.33	206.14±11.83
Group 5 (test groups)	200 mg/kg of leaf extract	85.47±2.81	247.38±16.73	182.02±9.16

Results are expressed in Mean±SEM; n=5; * = significant at p level < 0.05 compared with glucose level before alloxan induction.
KEY= CPA – Chloropropamide

After the extract administration, animals were anesthetized with ether after which blood was collected for laboratory determination of blood glucose level.

3. Results and discussion

3.1. GC-MS analysis result of the leaf Cnidoscolus aconitifolius

Table 1 is the 37 compounds identified to be present in the leaves of *Cnidoscolus aconitifolius* using GS-MS showing the retention time and percentage concentration of these compounds. The retention time is the time from the injection of the sample to the time of compound elution while the percentage concentration refers to the quantity of each secondary metabolic compound present in leaves of *Cnidoscolus aconitifolius*.

3.2. Result of hypoglycemic test

Group 1 served as the negative control and was not induced with diabetes. They were properly fed with rat chow and distilled water throughout the experiment. This was to monitor the overall effect of the environment in which the work is been done on the blood glucose level of these animals when non-diabetic.

There was a statistically significant ($P < 0.05$) increase in the fasting blood glucose level of animals after the induction of alloxan as shown in Table 2.

A significant decrease in blood glucose level was observed in group -2 which received 10 mg/kg Chloropropamide. While groups -3, 4, and 5 received 100, 150, and 200 mg/kg bw respectively of leaf extract after induction of diabetes and a significant ($P<0.05$) reduction in blood glucose level was observed for each group after treatment with methanolic leaf extract of *Cnidoscolus aconitifolius*, especially when a high dose of the leaf extract was administered. A dose-dependent reduction in blood glucose level was found to show a maximum reduction at a dose of 200 mg/kg from 247.38±16.73 mg/dl to 182.02±9.16 mg/dl.

A high dose of the methanolic leaf extract administered revealed an improved therapeutic effect compared to the anti-diabetic drug (Chloropropamide) used.

A dose dependent reduction of blood glucose level in streptozotocin induced diabetic mice has been reported when treated with the ethanolic leaf extract of *Cnidoscolus aconitifolius*. The hypoglycemic strength exhibited by this extract may be due to the presence of tannis, saponins [23] and terpenoids [24]. The hypoglycemic activity of *Cnidoscolus aconitifolius* as recorded in this work could be due to the presence of the the terpenoid, Lupeol, extracted from the leaves of this plant [24] which has the ability to decrease blood glucose level and inhibit α-glucosidase [25]. The ant-diabetic activity of the saponin; Tigogenin and Timosaponin A is known [26], just as the anti-diabetic property of the tannins; Procyandin and Epigallocatechin, isolated from the leaves of *Cnidoscolus aconitifolius* are known [27]. The blood glucose level of moderately and severely diabetic mice where lowered by 25.6% and 43.7% respectively when treated with the leaf extract of *Cnidoscolus aconitifolius* [28].

4. Conclusion

The result obtained has shown that the methanolic leaf extract of *Cnidoscolus aconitifolius* possesses anti-diabetic properties. At present, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of orthodox therapy and α-glucosidase inhibitors in addition to insulin. However, due to unwanted side effects, the efficacies of these compounds are contestable thus fueling the potential for alternative therapy in the treatment of diabetes [29]. It is on this ground that plants such as *Cnidoscolus aconitifolius* are been investigated as an alternative therapy for diabetes. The result also justifies the wide use of the leaves of *Cnidoscolus aconitifolius* in ethno-medicine to control blood glucose levels. More research work aimed towards extracting the compounds responsible for the anti-diabetic activity of the leaves of *Cnidoscolus aconitifolius* is hereby recommended as this will help pharmaceutical industries to develop more potent anti-diabetic drugs.

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