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GC-MS ANALYSIS OF *CNIDOSCOLUS ACONITIFOLIUS* LEAF ORGANIC EXTRACTS

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ABSTRACT

Cnidoscolus aconitifolius is a potential folklore medicinal plant used for the treatment of many diseases and infections. The leaves were extracted using non-polar solvents (diethyl ether and ethanol). These were subjected to qualitative phytochemical screening using standard procedures. Subsequent analysis of these extracts by GC-MS revealed that it contains the following main compounds: Copaene, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, Caryophyllene, Gamma Elemene, 1,6-Cyclodecadiene, 3-(1,5-dimethyl-4-hexenyl)6-methylene, Cis-alpha-bisabolene, 1H-cycloprop(e) azulen-7-ol, 1H-cycloprop(a)naphthalene and Caryophyllene oxide. In this study these bioactive compounds have been found to possess a wide range of activities, which help in the protection against diseases.

INTRODUCTION

Medicinal plants are increasingly gaining acceptance in our society, probably due to the increasingly inefficacy of many modern drugs used for the control of infections such as typhoid fever and gonorrhea, as well as increase in resistance by several bacteria to various antibiotics, and the increasing cost of prescribed drugs for the maintenance of personal health (levy 1998; Smolinski *et al.,* 2003). Medicinal plants are known to contain substances which could be used for treatment purposes or used to produce drugs (Sofowora, 1999). According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary health care needs (Derwich *et al.,* 2009). One of such medicinal plants is *Cnidoscolus aconitifolius. Cnidoscolus aconitifolius* (Euphorbiaceae family) is a large fast growing leafy perennial shrub, native of Yacutan Peninsula of Mexico in Central America (Ranhotra *et al.,* 1998). It is commonly found in the tropic and subtropical regions worldwide, including Africa, North and South America, India etc. The plant is commonly called Chaya, Iyana-Paja, or tree spinach depending on its regional source. Iyana-paja leaf is commonly eaten as vegetable in soup in Nigeria, where it serves as a good source of protein, vitamins, minerals and antioxidants (Kuti and Konuru, 2004). *Cnidoscolus aconitifolius* shoots and leaves have been taken as laxatives, diuretic and circulatory stimulant, to improve digestion, stimulate lactation and harden the fingernails (Rowe, 1994). Traditionally, *Cnidoscolus aconitifolius* has been recommended for a number of ailments including digestion, obesity, kidney stones, hemorrhoids, eye problems, atherosclerosis, gall stone and high cholesterol (Diaz-Bolio, 1975; Kuti and Toes, 1996, Oyagbemi and Odetola, 2010). This plant has also been used in ethno medicine for the treatment of alcoholism, insomnia, gout, scorpion stings and as a cure for brain and vision impairment (Atuahene *et al.,* 1999). Hence, the aim of this study is to determine the chemical constituents of *Cnidoscolus aconitifolius*so as to ascertain the scientific basis for its medicinal use.

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MATERIALS AND METHODS

Plant Material

Fresh leaf samples of *Cnidoscolus aconitifolius* were collected from a farm in Federal Girls College, Sokoto. Nigeria.

Preparation of Powder and Extraction

The leaves were air dried and grinded using an electric blender to obtain a fine powder. The powder was further sieved to

obtain finer particles. 25g of powdered plant material were separately soaked in 250ml each of absolute ethanol and diethyl ether. The solutions were allowed to stand for 48 hours with occasional stirring. The mixtures were then filtered separately using Whatman number 1 filter paper and the filtrates were evaporated in a water bath until dried.

Qualitative Phytochemical Studies

The phytochemical analysis of *Cnidoscolus aconitifolius* extracts were conducted by using a modified version of Cock and Kalt, 2013.

Cardiac Glycosides

2ml of each extract was treated with 2ml Glacial acetic acid in a test tube and few drops of Ferric chloride solution was added. 1ml of concentrated Sulfuric acid was carefully added. The presence of red/brown ring at the interface or the formation of a green/blue color throughout the solution indicates the presence of cardiac glycosides.

Alkaloids

1ml of each extract was treated with a few drops of an aqueous solution of hydrochloric acid and 500µl of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of deionized water). A reddish brown precipitate indicates the presence of alkaloid.

Anthraquinones

1ml of each extract were treated with few drops of concentrated sulfuric acid and careful addition of 1ml of ammonia. A rose pink color indicates the presence of free anthraquinones.

Flavonoids

1ml of sodium hydroxide solution was added to 3mls of each extract. The formation of intense yellow color which becomes colorless on addition of 1ml dilute hydrochloric acid, indicates the presence of flavonoids.

Phenols

200µl of extracts was added to 2ml of 3% aqueous sodium carbonate, followed by the addition of 200µl Folin's Ciocalteu reagent. The mixture was allowed to stand for 30min at room temperature. The formation of deep blue or black color indicates the presence of phenolic compounds.

Tannins

2ml of extract was treated with 1ml of 1% ferric chloride solution. The mixture was observed for the formation of blue-black or greenish coloration which indicates the presence of tannins.

Triterpenoids

2ml of extract was treated with 1ml of chloroform followed by careful addition of 1ml concentrated sulfuric acid. The formation of reddish brown or purple color indicates the presence of triterpenoids.

Steroids

1ml of extracts was treated with few drops of acetic anhydride and concentrated sulfuric acid. The solution was allowed to stand at room temperature for 5min. The formation of deep blue/green color indicates the presence of steroids.

GC-MS Analysis

The GC-MS analysis was conducted at Central Research Laboratory, University of Lagos, Lagos State. It was injected into a GC model 7890 (Agilent Technologies) coupled to a MS model 5975c (Agilent Technologies). The mobile phase was helium gas with a flow rate of 1ml/min. The injector temperature was 250^0C, the injection volume was 1µl and the oven temperature was initially programmed at 30^0C for 2min. This was then increased by 10^oC per minute to a final temperature of 240^oC for 6 minutes.

Identification of Components

Interpretation of mass spectrum GCMS was conducted using data base of National Institute Standard and Technology (NIST) and Wiley spectra libraries. Spectrum of the unknown component was compared with spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were recorded.

RESULTS

GC-MS is one of the advanced technique to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The GC-MS analysis of *Cnidoscolus aconitifolius* leaves revealed the presence of phytochemical constituents that could contribute the medicinal value of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Table and Fig 2, 3, 4 & 5. The phytochemicals identified through GC-MS analysis showed many of the biological activities relevant to this study are listed in Table 3 & 5. The biological activities listed are based on Dr. Duke's Phytochemical and Ethanobotanical Databases created by Dr. Jim Duke of Agricultural Research Service/USDA.

The preliminary qualitative result is as follows:

DISCUSSION

The more information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS) (Cong *et al.,* 2007). The GC-MS analysis of *Cnidoscolus aconitifolius* leaves revealed the presence of seventeen compounds. The identified compounds possess many biological properties.

Table 1. Phytochemical constituents of *Cnidoscolus aconitifolius*

Compounds	Ethanol Extract	Diethyl Ether Extract
Cardiac glycosides	++	++
Steroids	+	-
Tannins	+	-
Flavonoids	+	+
Anthaquinones	+	+
Triterpenoids	+	+
Phenols	+	+
Alkaloids	+	+

Table 2: Compounds Present in Ethanol Extract of *Cnidoscolus aconitifolius*

S/N	Compound	RT	Area %
1.	1,6-Octadien-3-01	5.287	1.59
2.	3-cyclohexene-1-methanol	6.821	0.18
3.	Alpha Cubebene	9.985	2.25
4.	Copaene	10.048	2.27
5.	Naphthalene	10.271	4.92
6.	IH-Cycloprop (a) naphthalene	10.363	4.94
7.	Cyclohexane, 1-ethenyl-1- methyl-2,4-bis (1-methylethenyl)	10.494	5.18
8.	Caryophyllene	10.780	3.28
9.	Gamma Elemene	11.129	2.78
10.	Alpha Caryophyllene, Aromadendrene	11.341	2.47
11.	1,6-Cyclodecadiene	11.822	5.30
12.	IH –Cycloprop (e) azulene	11.988	2.42
13.	Cyclohexene, 1-Methyl-4,5-methyl-1-methylene-4-hexenyl)-	12.171	2.49
14.	Cyclohexene, 3-(1,5-dimetyl-4-hexenyl)-6-methylene.	12.434	4.33
15.	Cis-Alpha Bisobolene	12.548	0.97
16.	Cadala-1 (10), 3,8-triene,alpha calacorene	12.611	0.56
17.	1,6,10 Dodecatrien-3-01	12.938	2.40
18.	IH-Cycloprop (e) azulen-7-01	13.172	4.06
19.	Caryophyllene oxide	13.206	1.52
20.	Beta Humulene	13.315	0.23
21.	Cis-z-alpha-Bisabolene epoxide	13.458	1.43
22.	(-)- Spathulenol	13.659	1.05
23.	Isoaromadendrene epoxide	14.128	0.50
24.	Longifolenaldehyde	14.196	0.35
25.	Aromadendrene oxide –(2)	14.305	1.03
26.	Alpha Farnesene	14.557	0.90
27.	Beta Elemenene	14.717	0.72
28.	Ledene alcohol	14.935	0.47
29.	3,7-Cyclodecadien-1-one	15.003	0.28
30.	Cycloheptane	15.238	1.08
31.	Spiro (5.5) undec-2-ene	15.570	0.38
32.	Spiro (4.5) dec-6-en-8-one	15.650	0.29
33.	(-)-Neoclovene-(1)	15.982	0.35
34.	Alloaromadendrene oxide –(1)	16.720	0.21
35.	3. alpha, 4. alpha – Epoxymurolan -9(1) en-10-01	16.828	0.07
36.	Cyclononasiloxane	20.130	0.10
37.	Hexasiloxane	23.655	0.21
38.	Cyclononasiloxane	26.539	0.28

Table 3 Chemical Composition of *Cnidoscolus aconitifolius*Ethanolic Extract Using GCMS

S/N	RT	AREA %	Library	Biological Properties
1.	9.985	2.25	Alpha cubebene	Antifungal, (21)
2.	10.048	2.29	Copaene	Cytotoxic and cytogenetic effect (22). Antioxidant, antimicrobial and larvicidal effect (12)
3.	10.494	5.18	Cyclohexane, 1-ethenyl-1-methyl 2, 4-bis(1-methylethenyl)	---
4.	10.780	3.28	Caryophyllene	Antioxidant and antimicrobial (12) anesthetic activity (13)
5.	11.129	2.78	Gamma Elemene	Antifungal, antioxidant and biocidal activity.
6.	11.822	5.30	1,6-cyclodecadiene	Antibacterial (23)
7.	12.434	4.33	Cyclohexene, 3-(1,5-dimethyl -4-hexenyl)-6-methylene.	---
8.	13.172	4.06	IH-Cycloprop(e) azulen-7-01	Antifungal, insecticidal, larvicidal activity, antimicrobial (24)
9.	10.363	4.94	IH-cyclopropa (a) naphtalene	Antioxidant (17)
10.	11.988	2.42	IH Cycloprop(e) azulene	Antifungal (25)

Table 4. Chemical Composition of Diethyl Ether Extract of *Cnidoscolus aconitifolius*

S/N	Compound	RT	Area %
1.	1,3-Cyclohexadiene	9.344	1.01
2.	Alpha Cubebene	9.544	0.65
3.	Copaene	10.008	6.67
4.	IH-Cycloprop (e) azulene	10.523	0.84
5.	Caryophyllene	10.769	10.88
6.	Gamma Elemene	10.935	5.90
7.	4,7, -methanoazulene	11.043	2.20
8.	Alpha caryophyllene	11.238	6.48
9.	IH –Benzocycloheptene	11.358	2.00
10.	1,6-Cyclodecadiene	11.633	8.47
11.	Naphthalene	11.724	3.71
12.	IH-Cyclopropa (a) naphthalene	11.833	4.94
13.	Cyclohexene, 1-methyl-4 (5-methyl-1-methylene-4-hexenyl)-	12.022	8.40
14.	Bicyclo (4.4.0) dec-1-ene	12.085	0.58
15.	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene	12.188	4.15
16.	Bicyclo (3.H) hept-3-ene	12.239	0.30
17.	Cis alpha Bisabolene	12.359	0.61
18.	Cyclohexane methanol	12.485	0.34
19.	1,6,10-Dodecatrein-3-0L	12.686	2.12
20.	IH – Cycloprop (e) azulen -7-01	12.926	2.65
21.	Caryophyllene oxide	12.972	1.94
22.	3-cyclohexen-1-carboxaldehyde	13.264	0.75
23.	1,7,7, Trimethyl-2-vinylbicyclo(2.2.1)hept-2-ene	13.487	0.47
24.	Naphthalene	13.624	0.87
25.	Isoaromadendrene epoxide	13.756	0.13
26.	Silane	13.870	0.23
27.	4-(1,5-Dimethylhex-4-enyl) Cyclohex-2-enone	14.185	0.27
28.	2-Naphthalenecarboxylic acid	14.448	0.43
29.	Benzene, 1,1, -(1,2-cyclobutanediyl) bis-	14.883	0.29
30.	Cyclononasiloxane	15.678	0.28
31.	(-)-Neoclovene –(1)	15.890	0.17
32.	Hexadecanoic acid	16.725	0.63
33.	Cyclodecasiloxane	17.298	0.27
34.	9,12-Octadecadienoic acid	18.413	0.16
35.	9,12,15 –Octadecatrienoic acid	18.488	0.44
36.	Octadecanoic acid	18.717	0.26
37.	Hexasiloxane	21.629	0.49
38.	1,2 –Propanediol	22.024	0.93
39.	IH-Indole	23.317	0.56
40.	Cyclononasiloxane	23.643	0.72
41.	Squalene	29.634	0.64

Table 5. Major Compound Present in *Cnidoscolus aconitifolius* Diethyl Ether extract using GC-MS

S/N	RT	AREA %	Library	Biological Properties
1.	10.008	6.67	Copaene	Cytotoxic and cytogenetic effect (22). Antioxidant, antimicrobial and larvicidal effect (12)
2.	10.294	9.23	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-	---
3.	10.769	10.88	Caryophyllene	Antioxidant and antimicrobial (12). Anesthetic activity (13)
4.	10.935	5.90	Gamma-Elemene	Antifungal, antioxidant biocidal agent (14)
5.	11.358	2.00	Beta-Humulene	Antimicrobial and antioxidant (15) anti inflammatory (16)
6.	11.633	8.47	1,6-cyclodecadiene	---
7.	11.833	4.94	IH-Cycloprop (a) naphthalene	Antioxidant (17)
8.	12.022	8.40	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) cis-alpa-Bisabolene	Anticonvulsant (18)
9.	12.972	1.94	Caryophyllene oxide	Analgesic and anti-inflammatory (19) gastro protective (20)
10.	12.926	2.65	IH-Cyloprop(e) azulen-7-ol	---

For instance, the qualitative analysis shown phytochemical constituents of *Cnidoscolus aconitifolius* (Haznagy-Radnal *et al.,* 2007).The GC-MS analysis of *Cnidoscolus aconitifolius*leaves revealed the presence of Ethanol extract thirty eight compounds.

The identified compounds possess many biological properties. For instance, Alpha cubebene (R/T 9.985) can have antifungal activity (Sialco, 2014). Copaene (R/T 10.048) possesses cytotoxic and cytogenetic effect (Siddiqui, *et al.,* 2013) and Antioxidant, antimicrobial activity and larvicidal effect,

Caryophyllene (R/T 10.780) having Antioxidant and antimicrobial activity shown by (Levy, 1998) and anesthetic activity shown by (Lin, *et al.,* 2011). Gamma Elemene (R/T 11.129) has shown Antifungal, antioxidant and biocidal activity by (Madhumitha, *et al.,* 2011). 1,6-cyclodecadiene (R/T 11.822) has shown antibacterial activity (Smolinski, *et al.,* 2003).IH-Cycloprop(e) azulen-7-01 (R/T 13.172) has shown Antifungal, insecticidal, larvicidal, antimicrobial activity studied by (Sofowora, 1999). IH-cyclopropa (a) naphthalene (RT/10.363) was identified as antioxidant shown by (Oyegbemi and Odetola, 2010). H Cycloprop (e) azulene (RT?11.988) has shown as antifungal activity studied by (Turkez, *et al.,* 2014). The GC-MS analysis of *Cnidoscolus aconitifolius* leaves revealed the presence of Diethyl ether forty one compounds. The similar compounds were identified having biological activity which was shown in Ethanolic extract.

Conclusion

The source of the many plants (herbs and spices) can be often identified from the peak pattern of chromatograms obtained directly from the headspace analysis. Similarly, unique qualitative and quantitative patterns from a GC analysis will often help identify the source of the many alcoholic beverages. The technique of fingerprint could really identify the false herbal products. The construction of the chromatographic fingerprints aims to evaluating the quality of Herbal Medicines **(**Yi-Zeng *et al*., 2004**)**. The fundamental reason of quality control of herbal medicines is based on concept of the Phytoequivalence of herbs, and then to use of this conception to identify real herbal medicine and the false one, and further to do quality control analysis. Therefore, GC-MS method is a direct and fast analytical approach for the identification of terpenoids and steroids and only few grams of plant material is required. The importance of study is due to biological activity of some of the compounds. The present study, which reveals the presence of the components in *Cnidoscolus aconitifolius* suggest that contribution of these compounds on the pharmacological activity should be evaluated.

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