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GC-MS Analysis of *Cnidoscolus aconitifolius* Leaf Aqueous Extracts

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Abstract: ***Cnidoscolus aconitifolius is a leafy vegetable consumed for its nutritional values and medicinal purposes. The leaves were extracted with water (a polar solvent), then subjected to preliminary phytochemical screening and further GC-MS analysis. The preliminary screening showed the presence of cardiac glycosides, flavonoids, phenols, anthraquinones and triterpenoids while the GC-MS result revealed 42 compounds. The major compounds were Borneol (1.41%), Caryophyllene oxide (2.73%), 1H-cycloprop (e) azulene (2.02%), 4-(1,5-Dimethyl hex-4-enyl) cyclohex-2-enone (4.15%), Farnesol (2.51%), Spiro (4.5) dec-6-en-8-one (6.87%), Longipinane (4.18%) and Benzene (13.37%). This study shows that Cnidoscolus aconitifolius contain many biologically active compounds in various concentrations which could have been responsible for its numerous biological actions.***

Keywords: *Cnidoscolus aconitifolius,* GC-MS, Phytochemical, Biologically active compounds

1. Introduction

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 sub-tropical 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).

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).

The antibacterial, antidiabetic and ameliorative effects of various extracts of *Cnidoscolus aconitifolius* on anemia and osmotic fragility induced by protein energy malnutrition have been reported. (Sarmiento – Franco *et al.,* 2003; Awoyinka *et al.,* 2007; Oladeinde *et al.*, 2007; Oyagbemi *et al.*, 2008). 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).

The aqueous leaf extract has been recommended as a female contraceptive (Yakubu *et al,* 2008). Mordi *et al*, (2003) also reported the use of this leaf as amethystic agent (reducing alcohol absorption).

However, there has been no information to the best of our knowledge, on the bioactive constituents of this plant, which are needed to support its numerous claims of efficacy. As such, this study aims to ascertain the chemical constituents of this plant, so that it will be of benefit to pharmaceutical industries and researchers in the discovery of natural plant therapeutic agents.

2. Materials and Methods

2.1 Plant Material

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

2.2 Preparation of Powder and Extraction

The leaves were air dried and grounded using an electric blender to obtain a fine powder. The powder was further sieved to obtain finer particles. 25g of powdered plant material was separately soaked in 250ml of water (a polar solvent). The solution was allowed to stand for 48 hours with occasional stirring. The mixture was then filtered using whatman number 1 filter paper and the filtrate evaporated in a water bath until dried.

2.3 Qualitative Phytochemical Studies

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

2.4 Cardiac glycosides

2ml of 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.

2.5 Alkaloids

1ml of 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.

2.6 Anthraquinones

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

2.7 Flavonoids

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

2.8 Phenols

200µl of extract 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.

2.9 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.

2.10 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.

2.11 Steroids

1ml of extract 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.

2.12 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.

2.13 Identification of Components

Interpretation of mass spectrum GC-MS 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.

3. Results

GC-MS is one of the advanced technique to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids and esters etc. The GC-MS analysis of *Cnidoscolus aconitifolius* leaves aqueous extract 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. The phytochemicals identified through GC-MS analysis showed many of the biological activities relevant to this study are listed in Table 3. 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:

Table 1: Phytochemical constituents of *Cnidoscolus aconitifolius*

S/N	Cardiac glycosides	Steroids	Triterpenoids	Flavonoids
1	++	-	+	+
2				
3	Anthraquinones	Phenols	Tannins	Alkaloids
4	+	+	-	-

Table 2: List of compounds found in the aqueous extract of *Cnidoscolus aconitifolius*

S/N	Compound	RT	Area %
1.	Borneol	6.329	1.41
2.	3-cyclohexene-methanol	6.815	0.77
3.	Copaene	9.962	0.88
4.	Cyclohexene, I-ethenyl-1- methyl-2,4- bis	10.214	0.89
5.	1,3,6 10-Dodecatetraene	10.403	0.39
6.	IH-cycloprop(e)azulene	11.227	1.48
7.	Benzene, 1- (1,5-dimethyl-4- hexenyl-4-methyl	11.656	13.37
8.	Naphthalene	11.845	1.45
9.	Isoledene, IH-Cycloprop(e) azulene	11.885	1.82
10.	Cyclohexene, 1-methyl-4- (5-methyl-1-methylene-4-hexenyl)1, (5) -	12.051	4.02
11.	Cyclohexene, 3-(1,5 – dimethyl-4-hexenyl)-6-methylene-	12.222	4.36
12.	Ethanone	12.411	0.74
13.	Alpha calacorene	12.434	0.40
14.	1,6,10-dode catriene – 3-0L	12.674	1.36
15.	Caryophyllene oxide	12.983	2.73
16.	Beta humulene	13.241	0.28
17.	IH-cycloprop(e) azulene	13.332	2.02
18.	Isocaryophyllene	13.407	0.97
19.	Isoaromadendrene epoxide	13.544	2.34
20.	Tau-cardinol	13.687	0.98
21.	1,4-methanoazulene	13.738	0.38
22.	IH-indene	13.824	1.19
23.	2-Napthalenamine	13.939	2.01
24.	Calarene epoxide	14.133	0.75
25.	4 – (1,5-Dimethy/hex-4-enyl) cyclohex-2- enone	14.334	4.15
26.	Farnesol, acetate	14.408	2.51
27.	1-0-Tolylprop-2-en-I-one	14.562	2.30
28.	1-Oxaspiro (2.5) octane	14.900	0.94
29.	2-Hydroxy -6-methylpyridine-3-carboxylic acid	14.923	0.94
30.	Benzaldehyde	15.049	1.78
31.	Longiverbenone	15.089	1.84
32.	2-Heptanone	15.198	1.00
33.	Bicyclo (4.1.0) heptanes	15.306	0.91
34.	2,6,10-Dodecatrien-1-0L	15.489	2.98
35.	Spiro (4.5) dec-6-en-8-one	15.804	6.87
36.	Alloaromadendrene oxide-1	15.930	0.99
37.	Longipiname	16.439	4.18
38.	Pyridine, 3-butyl-1-oxide	16.668	2.48
39.	Camphorsulfonic acid	17.000	1.86
40.	Bicyclo (31.1.) hept-2-en-4-01	17.143	1.53
41.	39,9-Dimethyldodecahydrocyclo-hepta(d) inden-3-one	17.509	1.06
42.	Ethanone	22.997	0.80

Table 3: Major Constituents of *Cnidoscolus aconitifolius* aqueous leaf extract using GC-MS analysis.

S/N	*RT*	*Area %*	*Libra/ID/C:\Database\NISTO 8L*	*Biological Properties*
1	6.329	1.41	Borneol	Antihypertensive, antioxidant (14).
2	12.222	4.36	Cyclohexene, 3 – (1,5 – dimethyl -4-hexenyl)-6-methylene,	---
3	12.983	2.73	Caryophllene oxide	Antioxidant, Analgesic and anti-inflammatory Antifungal and anti-tumor (15).
4	13.332	2.02	IH-cycloprop (e) azulene	Antifungal (19)
5	13.544	2.34	Isoaromadendrene epoxide	---
6	14.334	4.15	4-(1,5- Dimethylhex – 4 – enyl) cyclohex-2-enone	---
7	14.408	2.51	Farnesol, acetate	Antimicrobial (18)
8	14.562	2.30	1-0-Tolylprop – 2 – en-1-1one	Anti diabetic, antimicrobial (18)
9	15.089	1.84	Longiverbenone	Antibacterial (16), cytotoxic, anti-fungal (19)
10	15.489	2.98	2,6,10-Dodecatrien-1-01	---
11	15.804	6.87	Spiro (4.5) dec-6-en-8-one	---
12	16.439	4.18	Longipinane	Anti-pedant and cytotoxic activity. (17)
13	16.668	2.48	Pyridine, 3-butyl-1-oxide	---
14	17.000	1.86	Camphorsulfonic acid	---
15	11.656	13.37	Benzene, 1-(1,5-dimethyl-4-hexenyl) -4-methyl	Potent antifungal and antioxidant (20).

3.1 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. 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 auqeous extract forty two compounds. The identified compounds possess many biological properties. For instance, Borneol (R/T 6.329) can have Antihypertensive and antioxidant activity studied (Oyagbemi *et al*., 2008). Caryophllene oxide (R/T 12.983) have shown Antioxidant, Analgesic and anti-inflammatory, Antifungal and anti-tumor activity shown (Oyegbemi and Odetola, 2010). IH-cycloprop (e) azulene (R/T 13.332) have shown antifungal activity (Sarmiento-Franco *et al*., 2003). Farnesol, acetate (R/T 14.408) have shown antimicrobial activity (Rowe, 1984). 1-0-Tolylprop – 2 – en-1-1one (R/T 14.562) have shown Anti diabetic and antimicrobial activity (Rowe, 1984). Longiverbenone (15.089) have shown Antibacterial, cytotoxic and anti-fungal activity (Rahman, Anwar, 2008) and Sarmiento-Franco *et al*., 2003). Longipinane (R/T 16.439) have shown Anti-pedant and cytotoxic activity (Ranhotra *et al*., 1998). Benzene, 1-(1,5-dimethyl-4-hexenyl) -4-methyl – (R/T 11.656) have shown Potent antifungal and antioxidant activity (Sialco, 2014). The selected compounds have shown biologically active.

3.2 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 Phyto equivalence 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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