GAS CHROMATOGRAPHY-FLAME IONIZATION DETECTION ASSESSMENT OF PHYTOCHEMICAL CONSTITUENTS OF *CNIDOSCOLUS ACONITIFOLIUS* LEAVES EXTRACTS

Commented [RRH1]: Change the title as " DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS IN CNIDOSCOLUS ACONITIFOLIUS LEAVES EXTRACTS BY GC-FID

ABSTRACT

Cnidoscolus aconitifolius, commonly called 'Chaya' and in Southeastern Nigeria 'hospital too far', or 'catholic vegetable' is a medicinal plant from the family Euphorbiaceae that has been used since pre-Columbian times as food and in treating diseases. The aim of this study was to determine the phytochemicals present in the ethanol, methanol, and aqueous extracts of *Cnidoscolus aconitifolius* leaves, which could be accountable for the plant's acclaimed therapeutic properties, using ~~g~~Gas ~~c~~Chromatography-~~f~~Flame ~~i~~Ionization ~~d~~Detection (GC-FID) technique. Cold maceration was used in the extraction process, while ~~gc-fid~~GC-FID was employed for the qualitative and quantitative analysis. The phytochemical screening revealed the presence of lunamarine, cardiac glycoside, anthocyanin, spartien, cyanogenic glycoside, flavonones, steroids, keampferol, epicatechin, flavones, oxalate, resveratol, sapogenin, epihedrine, flavan-3-ol, proanthocyanin, naringin, ribalinidine, naringenin, catechin, tannin, rutin, and phytate. However, the extracts did not all contain the same phytochemicals nor quantity of phytochemicals. While the ethanol extract recorded phytate (18.9224µg/ml) as the highest yield, methanol had spartein (17.2035µg/ml) and the aqueous extracts had epicatechin (9.2402 µg/g). The presence of these pharmacologically active substances, which has been researched to have antimicrobial, anti-diabetic, anti-oxidant, and anti-inflammatory properties amongst others, supports the efficacy of *Cnidoscolus aconitifolius* leaves in treatment of various pathologies and use in ethnopharmacology. It also projects *Cnidoscolus aconitifolius* leaves as a probable raw material for antibiotic formulation.

Keywords: *Cnidoscolus aconitifolius*, GC-FID, Medicinal plants, Phytochemicals, Aqueous extracts, ethanol extracts and methanol extracts

1.0 INTRODUCTION

Since time immemorial, medicinal plants have been used traditionally as therapeutics to various illnesses. Long before recorded history, people employed plants for medical purposes and as early as 3000 BC, descriptions of plant medicines were found in Chinese and Egyptian papyrus literature [1]. Medicinal plants are plants that "possess therapeutic properties or exert beneficial pharmacological effects on and/or in" the body, and are used globally especially in developing countries for treatment of one ailment or the other [2] with greater efficacy and fewer or no significant adverse effects [1]. The WHO estimates that 80% of developing countries populations rely on traditional medicines, mostly plant drugs, for their primary health care needs [3]. The therapeutic properties of medicinal plants can be largely attributed to the wide variety of bioactive compounds, such as saponin, phytate, flavonoids, tannins, and alkaloid, the plants contain [4] which makes them serve as core components of new drugs. For instance, phenolics aid in preventing cardiovascular diseases and cancer [5] while alkaloid is used in the manufacture of antimalarial, analgesic and antihypentensive drugs [6]. With over 500,000 kinds of medicinal plants on earth, there is tremendous hope for the discovery of new pharmacological compounds [1] but there is also the challenge of ascertaining the phytochemicals in these plants responsible for their therapeutic actions. This knowledge would not only aid in drug production but would also create a scientific reference base as a guide to the populace. Hence, this research aimed at analyzing the phytochemicals present in a widely consumed medicinal plant in Southeastern Nigeria - *Cnidoscolus aconitifolius*.

Cnidoscolus aconitifolius, with common name 'hospital too far', 'catholic vegetable', Spinach tree or Chaya, is a perennial herb from the family Euphorbiaceae and is recorded to have originated from South–East Mexico during the pre-Cambrian period [2]. The leaves of "*C. aconitifolius* has serrated edges, with a long petiole length, without pubescences, with sagittate base, with the presence of glands and with white flowers" [20]. It is a traditionally used medicinal plant in South-Eastern Nigeria with many claims from local consumers of its blood-replenishing properties. The leaf extract is employed traditionally in the treatment of anaemia, diabetes, cardiovascular diseases [7], and serve as diuretic, laxative, and an antimicrobial agent [2]. The whole plant is used in treating fever, respiratory, kidney and urinary disorders [8] while the juice, and pounded leaves are applied to wounds and refractory ulcers, scabies, eczema and ringworm [9] [7]. Furthermore, *Cnidoscolus aconitifolius* have been reported to possess essential phytochemicals such as anthraquinones, tannins, polyanxanthone c, lignins, phlobatannins, alkaloid, saponin and sesquiterpenes, which have antimicrobial, antidiabetic and anti-mutagenic properties [10]. Most times, the availability of these secondary metabolites depend on the extract and processing techniques employed; and with the paucity of literature on likely phytochemicals, which could be identified using GC-FID technique, this study was aimed at screening the phytochemical constituents of the ethanol, methanol, and aqueous extract of *Cnidoscolus aconitifolius* leaves using GC-FID technique. This research would bridge this gap in knowledge and supply adequate information on the phytochemicals present in this plant for therapeutic purposes and drug synthesis.

2.0 MATERIALS AND METHODS

2.1 Plant Material

Fresh plant leaves of *Cnidoscolus aconitifolius* were harvested from a farm in ifite-awka, Awka South Local Government Area, Anambra state, Nigeria and identified by the Botany department of Nnamdi Azikiwe University, Awka, Anambra state. Nigeria. The herbarium number is NAUH-168[A]. The leaves were then air-dried, pulverized and stored in airtight sterile containers at room temperature for further use.

Commented [RRH2]: Specify separately the names of chemicals/solvents used with make, grade and purity.

2.2 Extracts Preparation for Phytochemical Analysis

Cold maceration method was used in the extraction process. The leaf powder was weighed using a weighing balance. Exactly 350g of it was macerated in 2L of sterile water, ethanol, and methanol separately, shaken vigorously for some minutes and left to stand for 72 hours at 37°C. These afterward, were sieved using muslin cloth and the filtrates concentrated using a rotary evaporator at 40°C except the aqueous extract (Buss and Butler 2010). The percentage crude extract yield was calculated using the formula:

Percentage yield = mass of extract (g) / mass of sample (g) x 100

For the extraction of phytochemicals from the crude ethanol extract, ethanol was used as a solvent. 1g of the ethanol extract was weighed and transferred into a test tube and "15ml of 95% absolute ethanol and 10ml of 50% m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at 60^0C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which were all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000µl of pyridine of which 200µl was transferred to a vial for analysis" [12] [11]. Methanol and water were used as solvents for the phytochemical extraction from crude methanol and aqueous extracts respectively.

2.3 Identification and Quantification ~~U~~using GC-FID

The phytochemical analysis was carried out using a BUCK M910 Gas chromatography ~~fitted~~ with a ~~f~~Flame ~~i~~Ionization ~~d~~Detector ~~according to the method of~~ [13]. ~~The column was a~~ A GC capillary column, RESTEK (make)~~15 meter~~ MXT-1 (15m length x 250µm IDx 0.15µm film thickness) was used for the separation. Helium (5.0 pa.s) was used as ~~the~~ a carrier gas, ~~flowing at~~ with a flow rate of 40 ml/min. The injector temperature was set to 280^{0}C with a splitless injection volume of 2 µL ~~of~~ sample and a linear velocity of 30cm^{-1}. The GC oven temperature was ~~first~~ set to ~~function at~~ 200^{0}C; ~~it was~~ and then raised to 330^{0}C at a rate of 3^{0}C per minute and maintained there for 5 minutes. ~~,~~ ~~t~~The detector ~~operated at a~~ temperature ~~of~~ was set to 320^{0}C.

The ratio between the area and mass of internal standard and the area of the phytochemicals that has been identified was used to determine the concentration of the phytochemicals in ug/g.

Commented [RRH3]: Mention fuel gas flow rates(Zero air, Hydrogen and Make-up gas) used in the method for FID function.

Commented [RRH4]: Specify the name of Internal standard used and its concentration.

Commented [RRH5]: Specify how the concentration of components were quantified (by area normalization or against reference standard area); mention a formula for calculation of % of components from the chromatogram.

~~**2.4 Fixed Setting**~~

~~Usually, for the instrument to function as expected, the operator fixed conclusively the temperatures of the injector and detector and set the gas in such a way that it flowed to the appropriate chambers, which are the column, the inlets, the detectors, and the split ratio. In order to avoid or reduce to the barest minimum the possibility of analyte precipitation, the detectors are usually placed at that of the high end of the oven temperature range. All these were done as required to ensure a correct result.~~

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3.0 RESULTS AND DISCUSSION

RESULTS

3.1 Percentage Yield of the Extract of *Cnidoscolus aconitifolius* Leaves

This (Table 1) revealed that with the same quantity of pulverized leaves, ethanol extract had the highest percentage yield of crude plant extract followed by methanol and aqueous extract.

TABLE 1: Percentage Yield of the Extract of *Cnidoscolus aconitifolius* Leaves

Solvents	Weight of the pulverized leaves (g)	Weight of Extract (g)	Percentage Yield (%)
Ethanol	350	50	14.29
Methanol	350	44	12.57
Aqueous	350	38	10.86

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3.2 Phytochemical screening of *Cnidoscolus aconitifolius*leaves extracts

Gas chromatography-~~f~~Flame ~~i~~Ionization ~~d~~Detection technique screening of *Cnidoscolus aconitifolius* leaves (Fig 1) indicates that the extracts do not contain the same phytochemicals. While Flavan-3-ol was present only in the ethanol extract, proanthocyanin was detected only in the methanol extract and no phytochemical was peculiar to the aqueous extract of *Cnidoscolus aconitifolius* leaves. Naringin, ribalinidine, and naringenin were found only in the ethanol and methanol extracts, catechin and tannin

were present only in the methanol and aqueous extract while rutin and phytate were present only in the ethanol and aqueous extract of the plant leaves. However, lunamarine, cardiac glycoside, anthocyanin, spartien, cyanogenic glycoside, flavonones, steroids, keampferol, epicatechin, flavone, oxalate, resveratol, sapogenin, and epihedrine were found in all three extracts.

The chromatogram (Fig. 2, 3 and 4) shows the quantitative evaluation of the phytochemicals extracted from the plant. In the ethanol extract, phytate (18.9224µg/ml) and naringin (14.1273µg/ml) had the highest concentration while resveratol had the lowest concentration of 3.8888 ppm. For methanol extract, rich amounts of spartein (17.2035µg/ml) and oxalate (10.3147µg/ml) were present while proanthocyanin was there in minute quantity (0.2552ppm). The aqueous extract had high amounts of epicatechin (9.2402 µg/g) and phytate (7.1889 µg/ml) with considerable quantity of spartein (4.4838 µg/ml), and rutin (3.6003 µg/ml) while resveratol (2.5998 ppm) and flavones (2.7896 ppm) were present in minute concentration.

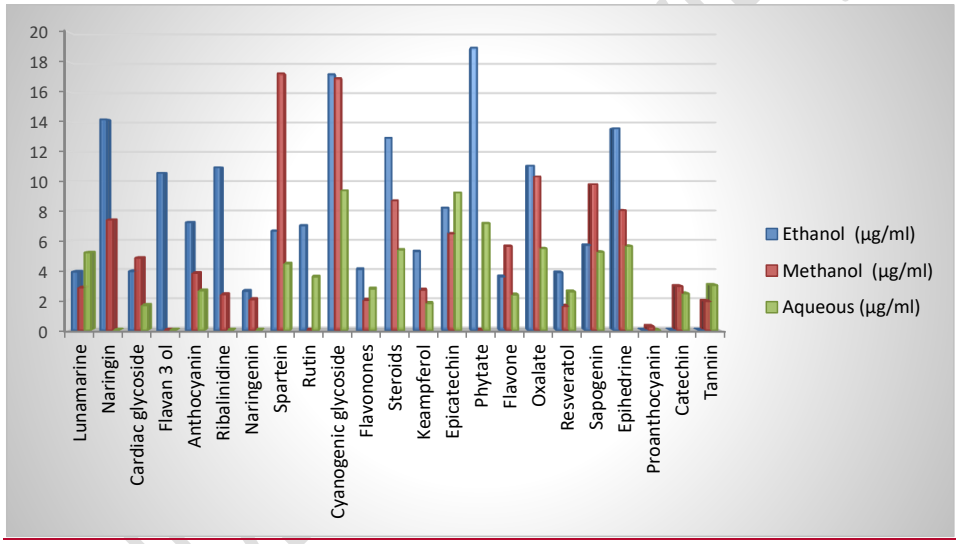
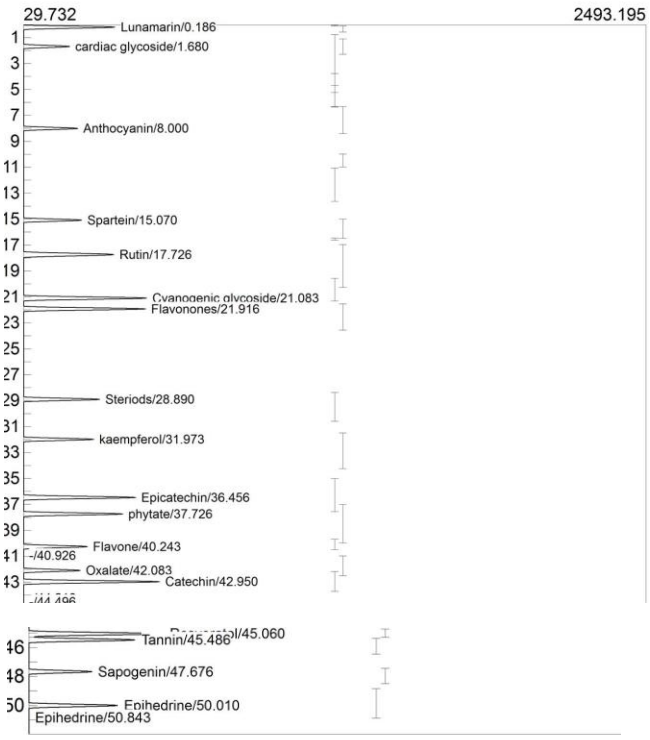
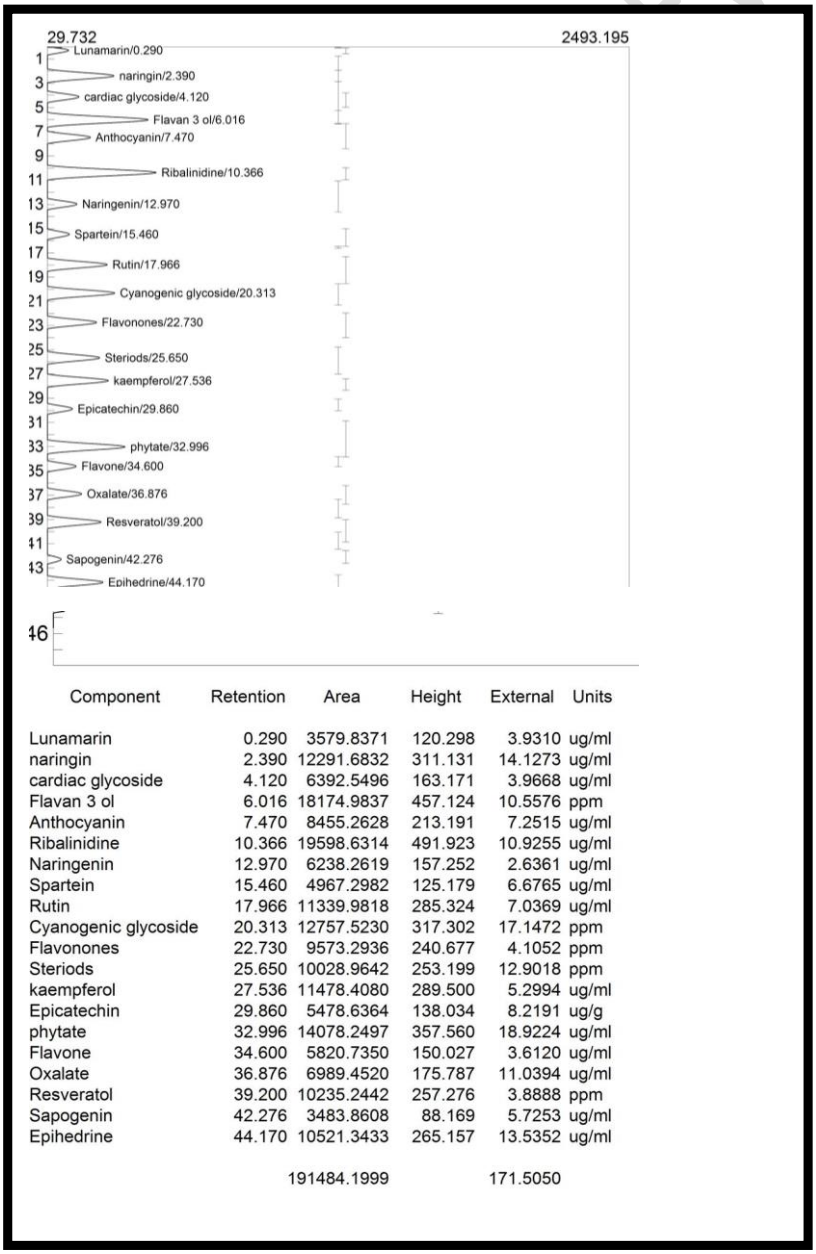


Fig. 1 Phytochemical screening of *Cnidoscolus aconitifolius* leaves



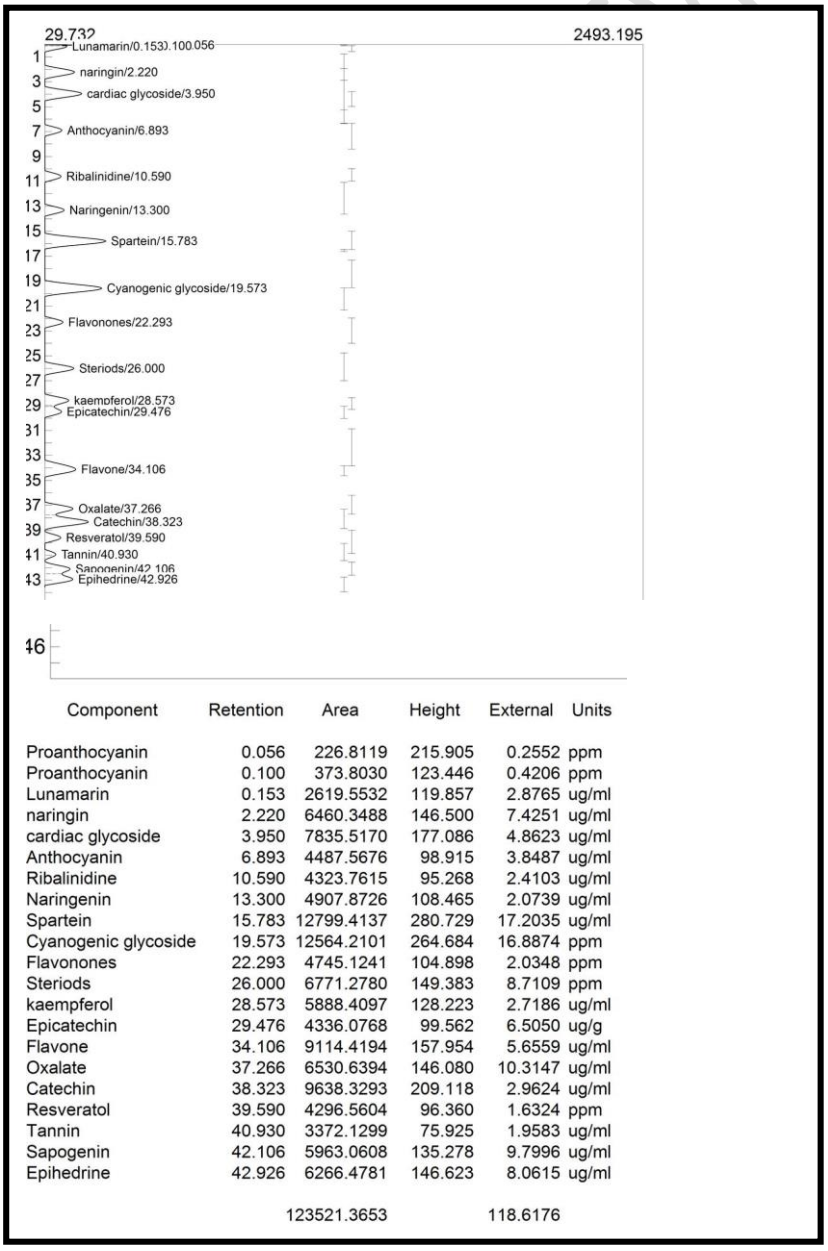
Component	Retention	Area	Height	External	Units
Lunamarin	0.186	4760.4178	392.551	5.2274	ug/ml
cardiac glycoside	1.680	2707.9788	211.396	1.6804	ug/ml
Anthocyanin	8.000	3111.2627	244.086	2.6683	ug/ml
Spartein	15.070	3335.9490	258.741	4.4838	ug/ml
Rutin	17.726	5801.8128	384.892	3.6003	ug/ml
Cyanogenic glycoside	21.083	6981.8836	515.125	9.3843	ppm
Flavonones	21.916	6505.2996	509.978	2.7896	ppm
Steriods	28.890	4207.2472	330.404	5.4124	ppm
kaempferol	31.973	3937.0518	305.767	1.8177	ug/ml
Epicatechin	36.456	6159.2316	469.868	9.2402	ug/g
phytate	37.726	5348.5161	418.085	7.1889	ug/ml
Flavone	40.243	3853.2248	276.485	2.3911	ug/ml
Oxalate	42.083	3483.0057	250.664	5.5012	ug/ml
Catechin	42.950	8047.9474	565.256	2.4736	ug/ml
Resveratol	45.060	6842.5236	505.467	2.5997	ppm
Tannin	45.486	5229.3426	404.177	3.0368	ug/ml
Sapogenin	47.676	3204.8954	251.636	5.2669	ug/ml
Epihedrine	50.010	4403.3232	344.646	5.6647	ug/ml
Epihedrine	50.843	31.1666	0.745	0.0401	ug/ml
		87952.0803		80.4671	

Fig. 2: Chromatogram of the aqueous extract of *Cnidoscolus aconitifolius* leaves



Component	Retention	Area	Height	External	Units
Lunamarin	0.290	3579.8371	120.298	3.9310	ug/ml
naringin	2.390	12291.6832	311.131	14.1273	ug/ml
cardiac glycoside	4.120	6392.5496	163.171	3.9668	ug/ml
Flavan 3 ol	6.016	18174.9837	457.124	10.5576	ppm
Anthocyanin	7.470	8455.2628	213.191	7.2515	ug/ml
Ribalinidine	10.366	19598.6314	491.923	10.9255	ug/ml
Naringenin	12.970	6238.2619	157.252	2.6361	ug/ml
Spartein	15.460	4967.2982	125.179	6.6765	ug/ml
Rutin	17.966	11339.9818	285.324	7.0369	ug/ml
Cyanogenic glycoside	20.313	12757.5230	317.302	17.1472	ppm
Flavonones	22.730	9573.2936	240.677	4.1052	ppm
Steriods	25.650	10028.9642	253.199	12.9018	ppm
kaempferol	27.536	11478.4080	289.500	5.2994	ug/ml
Epicatechin	29.860	5478.6364	138.034	8.2191	ug/g
phytate	32.996	14078.2497	357.560	18.9224	ug/ml
Flavone	34.600	5820.7350	150.027	3.6120	ug/ml
Oxalate	36.876	6989.4520	175.787	11.0394	ug/ml
Resveratol	39.200	10235.2442	257.276	3.8888	ppm
Sapogenin	42.276	3483.8608	88.169	5.7253	ug/ml
Epihedrine	44.170	10521.3433	265.157	13.5352	ug/ml
		191484.1999		171.5050	

Fig. 3: Chromatogram of the ethanol extract of *Cnidoscolus aconitifolius* leaves



Component	Retention	Area	Height	External	Units
Proanthocyanin	0.056	226.8119	215.905	0.2552	ppm
Proanthocyanin	0.100	373.8030	123.446	0.4206	ppm
Lunamarin	0.153	2619.5532	119.857	2.8765	ug/ml
naringin	2.220	6460.3488	146.500	7.4251	ug/ml
cardiac glycoside	3.950	7835.5170	177.086	4.8623	ug/ml
Anthocyanin	6.893	4487.5676	98.915	3.8487	ug/ml
Ribalinidine	10.590	4323.7615	95.268	2.4103	ug/ml
Naringenin	13.300	4907.8726	108.465	2.0739	ug/ml
Spartein	15.783	12799.4137	280.729	17.2035	ug/ml
Cyanogenic glycoside	19.573	12564.2101	264.684	16.8874	ppm
Flavonones	22.293	4745.1241	104.898	2.0348	ppm
Steriods	26.000	6771.2780	149.383	8.7109	ppm
kaempferol	28.573	5888.4097	128.223	2.7186	ug/ml
Epicatechin	29.476	4336.0768	99.562	6.5050	ug/g
Flavone	34.106	9114.4194	157.954	5.6559	ug/ml
Oxalate	37.266	6530.6394	146.080	10.3147	ug/ml
Catechin	38.323	9638.3293	209.118	2.9624	ug/ml
Resveratol	39.590	4296.5604	96.360	1.6324	ppm
Tannin	40.930	3372.1299	75.925	1.9583	ug/ml
Sapogenin	42.106	5963.0608	135.278	9.7996	ug/ml
Epihedrine	42.926	6266.4781	146.623	8.0615	ug/ml
		123521.3653		118.6176	

Fig. 4: Chromatogram of the methanol extract of *Cnidoscolus aconitifolius* leaves

DISCUSSION

Phytochemicals are secondary metabolites also known as bioactive constituents found in plant, which are responsible for several health advantages when consumed over time in a prescribed dosage [14]. The phytochemicals obtained in this study aligned with the findings of [2] [15] [16] [17] but anthraquinones, and phlobatannins, which they obtained, were absent in this study. The use of GC-FID revealed phytochemicals such as oxalate, specific forms of alkaloid (lunamarine, ephedrine, spartein, ribalinidine), and flavonoid (naringenin, anthocyanin, proanthocyanin, kaempferol, catechin) which before now has not been documented as bioactive constituents of *Cnidoscolus aconitifolius* leaves extract. Rich concentrations of epihedrine, ribalinidine, epicatechin, phytate, naringin, flavan-3-ol, sapogenin, spartein, and steroids were recorded in this work. The presence of these phytochemicals justifies the ability of the plant to treat various ailments.

Alkaloids are the most abundant phytochemicals in plants; the high quantities of alkaloids (spartein, epihedrine and ribalinidine) obtained in this research infer the ability of *C. aconitifolius* leaves extracts to play anti-inflammatory and hepatoprotective roles. Alkaloids also manage hypertension and AIDS related intestinal infections, diverse degenerative diseases [18], diabetes mellitus, and cancer [19].

The presence of phenolic compounds (Flavonoid, phenol, and tannin) in *C. aconitifolius* concurred with the findings of [20] making the plant a suitable raw material in drug production. The various forms of the phenolic compounds: phytate, epicatechin, naringin and flavan-3-ol were also present in rich quantities. Phenolic compounds protect the plant from pathogens. Their presence in *C. aconitifolius* indicates the plant's antioxidants, anti-inflammatory and anticancer abilities [21]. "Phytate has been linked to the prevention of kidney stone, dental decay, and calcification of blood vessels" [22]. The antioxidant nature of flavonoids and phenol can also be utilized in the food industry in the prevention of food spoilage [24].

Flavonoids and glycosides have been researched to be major antioxidants. They exhibit a broad range of biological properties, amongst which are antifungal, antibacterial, cytostatic, antidiarrhoeal, analgesic, and anti-inflammatory [23] [15]. "The antimicrobial attributes of these bioactive constituents have been associated with their abilities to inhibit cell wall formation in fungi, intercalate with DNA, and inactivate microbial adhesions and enzyme" [2]. Flavonoids can also shrink cancer cells and lessen the likelihood of developing neurodegenerative and cardiovascular diseases [24].

"Plants containing tannins are used to treat non-specific diarrhea and inflammation of the mouth" [25] [26], tannins facilitates healing of wounds and is effective in treating inflamed or ulcerated tissues in the body [18]. Tannins have also been reported to possess antioxidant properties and carry out antimicrobial abilities at low concentrations [27] via proteolytic enzymes, cell membrane lysis, microbial adhesions, and inhibition of protein synthesis pathways [28]. According to [22], "Tannins can be toxic to filamentous fungi, yeast, and bacteria" and cause physiological responses in animals when consumed [29]. Aside the biological properties of these bioactive compounds, they also aid in wading off pathogens from the plants hence serving as part of their defense mechanism [30].

The presence of sapogenin (a form of Saponin) is in agreement with the work of [4] [31]. Sapogenin has an innate capacity to eliminate microbes [24] [2], aids in the treatment of hypertension, hyperglycaemia and hypercholesterolaemia, acts as a prophylactic against cancer [32] and possess anti-diabetic, antihaemolytic and anti-inflammatory abilities [33]. Saponin stimulates Ca^{2+} influx, which strengthens the contraction of cardiac muscles in the body [34] [35]. "Its presence in *Cnidoscolus aconitifolius* leaves makes the plant a potential antibiotic" [4].

Steroids were also found in this study in high concentrations. Steroid is consistently utilized in the food, cosmetics, and herbal medicine industries due to their biological attributes; they are also effective antimicrobial and insecticidal agents [36]. Fortunately, the toxic, hydrocyanic glycoside found in the plant can be easily neutralized by soaking and cooking the leaves [2] likewise oxalate, which causes a decrease in calcium and iron absorption in the body [37].

From the three extracts- ethanol, methanol and aqueous- used, the concentrations of these important secondary metabolites were highest in the ethanol extract and least in aqueous extract. Ethanol extract was rich in alkaloids (epihedrine), phenolic compounds (ribalinidine, epicatechin, naringin, flavan-3-ol) and steroids (phytate and steroids), key phytochemicals responsible for the medicinal properties of the plant. This infers the anti-cancer, anti-inflammatory [38], anti-diabetic [39], antioxidant [40] and antimicrobial abilities [2] of the ethanol extract of *C. aconitifolius* leaves. Methanol extract had appreciable quantities of sapogenin and the alkaloid-spartien, hence it could be applied as an antimicrobial agent. Aqueous extract had low concentrations of these vital phytochemicals, cyanogenic glycoside and oxalate inclusive, thus it is less toxic for consumption.

With this array of phytochemicals found in *Cnidoscolus aconitifolius*, it is evident that it is an embodiment of solution to various ailments and if properly harnessed would be instrumental in ethnopharmacology.

4.0 CONCLUSION

Cnidoscolus aconitifolius leaves contain numerous phytochemicals which are responsible for its therapeutic abilities. Knowledge of the bioactive substances in the various extracts used in this study would guide in the proper utilization of this medicinal plant both for consumption purposes and for synthesis of antibiotics.

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