

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

ABSTRACT

Cnidoscopus 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 *Cnidoscopus aconitifolius* leaves, which could be accountable for the plant's acclaimed therapeutic properties, using gas chromatography-flame ionization detection (GC-FID) technique. Cold maceration was used in the extraction process while 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, saponin, 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 *Cnidoscopus aconitifolius* leaves in treatment of various pathologies and use in ethnopharmacology. It also projects *Cnidoscopus aconitifolius* leaves as a probable raw material for antibiotic formulation.

Keywords: *Cnidoscopus 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 antihypertensive 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 - *Cnidoscopus 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.

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:

$$\text{Percentage yield} = \text{mass of extract (g)} / \text{mass of sample (g)} \times 100$$

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°C 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 Quantification Using GC-FID

The phytochemical analysis was carried out using a BUCK M910 Gas chromatography fitted with a flame ionization detector according to the method of [13]. The column was a RESTEK 15 meter MXT-1 (15m x 250µm x 0.15µm). Helium 5.0 pa.s was used as the carrier gas, flowing at a rate of 40 ml/min. The injector temperature was 280°C with splitless injection of 2 µL of sample and a linear velocity of 30cm⁻¹. The oven was first set to function at 200°C; it was then raised to 330°C at a rate of 3°C per minute and maintained there for 5 minutes, the detector operated at a temperature of 320°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.

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.

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

3.2 Phytochemical screening of *Cnidoscolus aconitifolius* leaves extracts

Gas chromatography-flame ionization 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,

spartein, 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 (18.9224 $\mu\text{g}/\text{ml}$) and naringin (14.1273 $\mu\text{g}/\text{ml}$) had the highest concentration while resveratol had the lowest concentration of 3.8888 ppm. For methanol extract, rich amounts of spartein (17.2035 $\mu\text{g}/\text{ml}$) and oxalate (10.3147 $\mu\text{g}/\text{ml}$) were present while proanthocyanin was there in minute quantity (0.2552ppm). The aqueous extract had high amounts of epicatechin (9.2402 $\mu\text{g}/\text{g}$) and phytate (7.1889 $\mu\text{g}/\text{ml}$) with considerable quantity of spartein (4.4838 $\mu\text{g}/\text{ml}$), and rutin (3.6003 $\mu\text{g}/\text{ml}$) while resveratol (2.5998 ppm) and flavones (2.7896 ppm) were present in minute concentration.

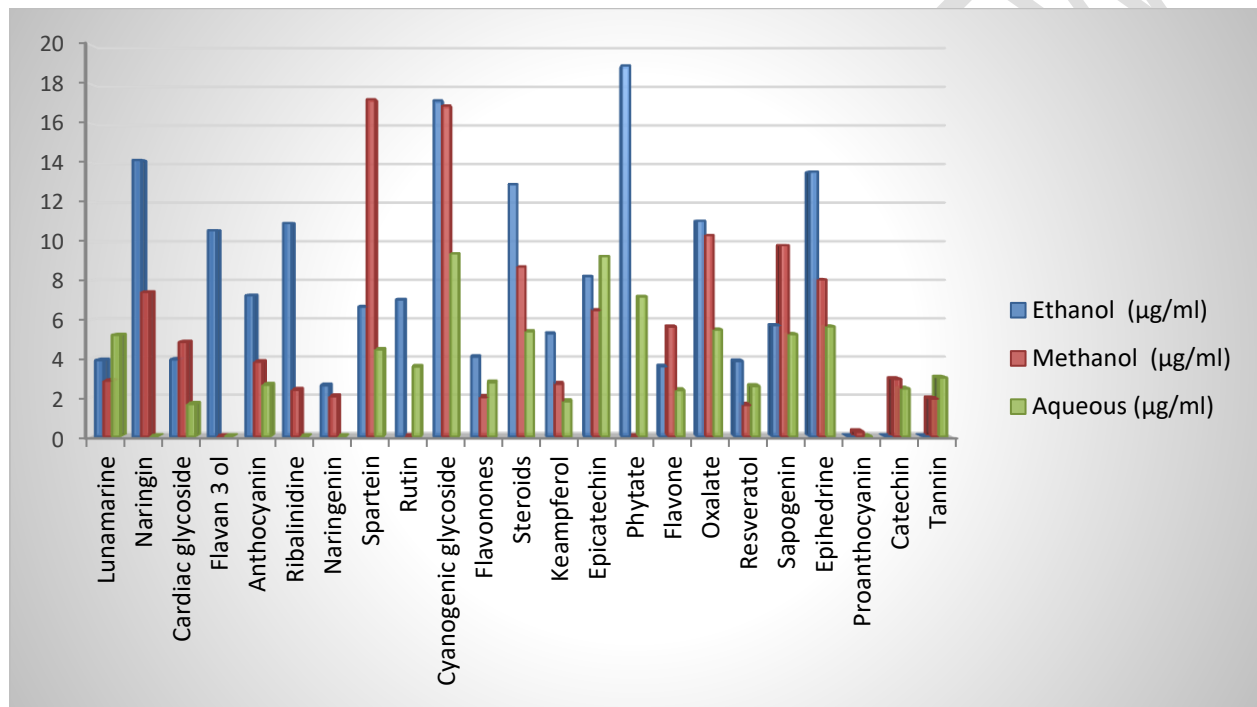
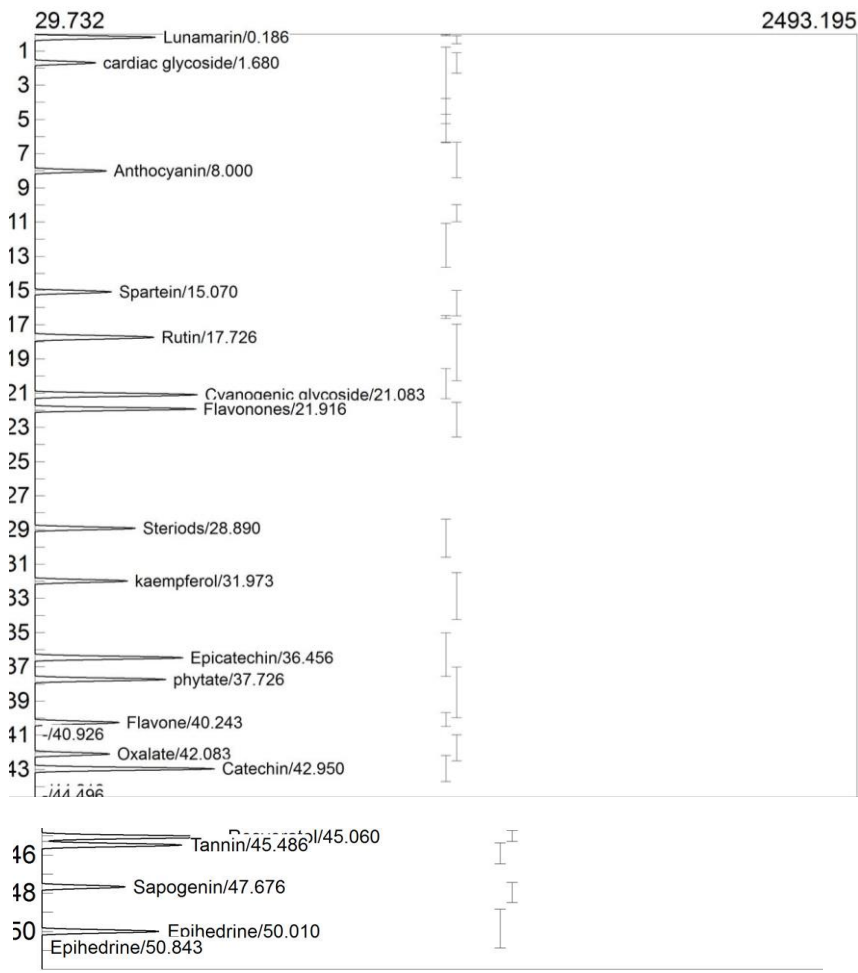


Fig. 1 Phytochemical screening of *Cnidoscopus aconitifolius* leaves

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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
Sparteine	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
Steroids	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

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

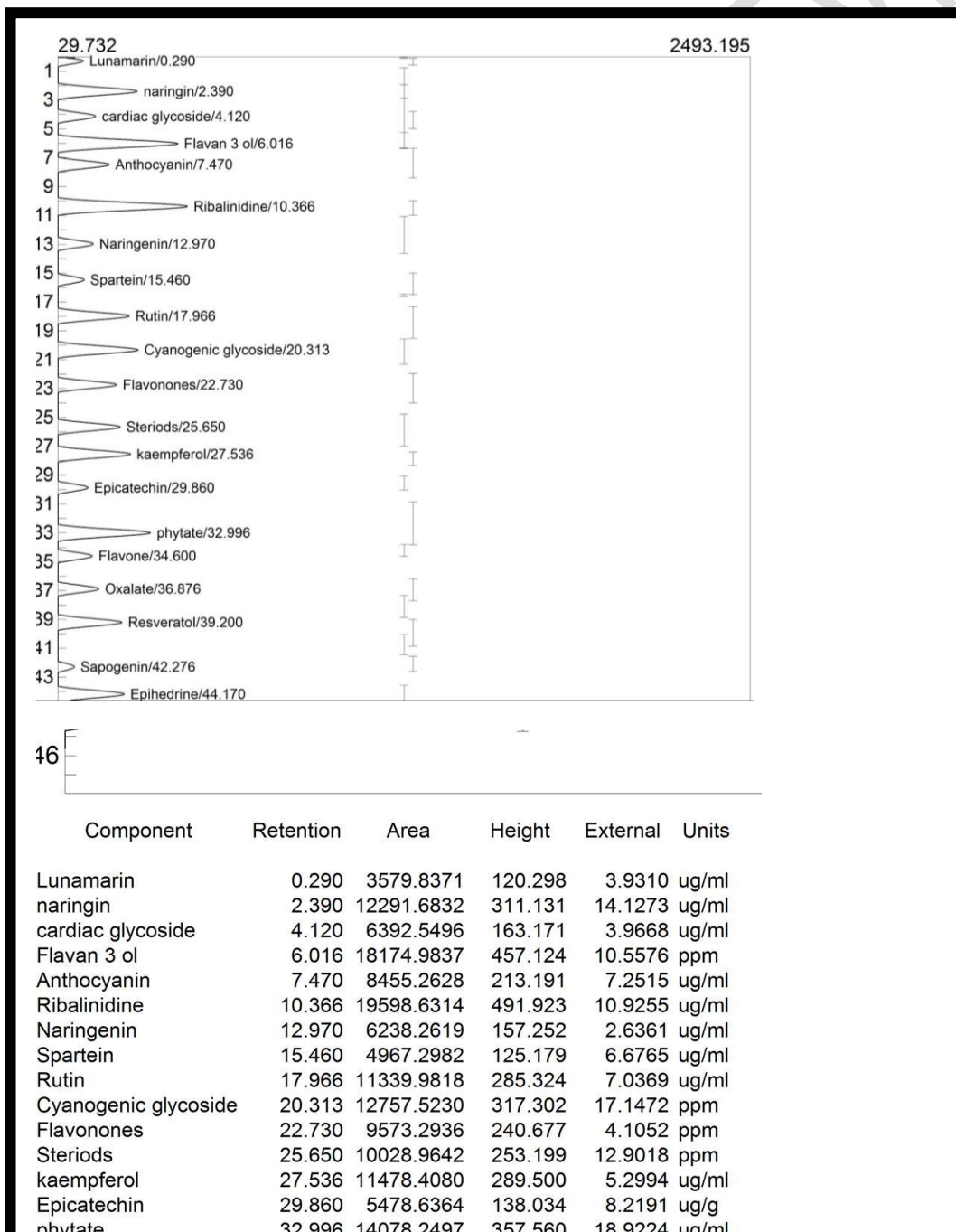


Fig. 3: Chromatogram of the ethanol extract of *Cnidoscolus aconitifolius* leaves

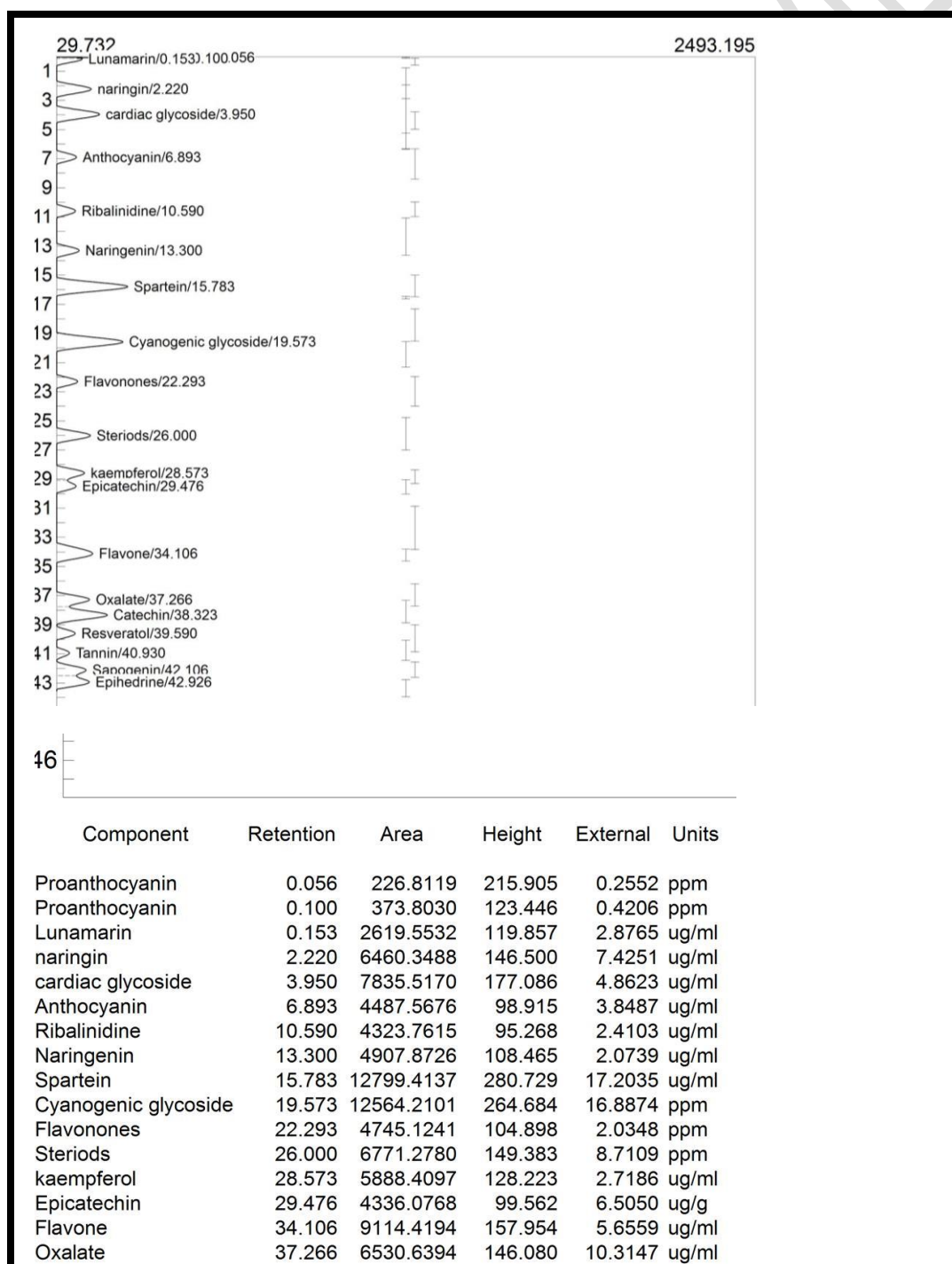


Fig. 4: Chromatogram of the methanol extract of *Cnidoscolus aconitifolius* leaves

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 ephedrine, 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, ephedrine 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, antihemolytic 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 *Cnidoscopus 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 *Cnidoscopus 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

Cnidoscopus 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.

REFERENCES

1. Karo MB, Kamelia E, Miko H, Simanjuntak TP and Hatta M. Effects of Herbal Plants on Candidiasis Vulvovaginalis Therapy. American Journal of Laboratory Medicine 2016; 1(3):65-68.
2. Fagbohun ED, Egbebi AO and Lawal OU. Phytochemical screening, proximate analysis and in-vitro antimicrobial activities of methanolic extract of *Cnidoscopus aconitifolius* leaves. International Journal of Pharmaceutical Sciences Review and Research 2012; 13:1.
3. Soetan KO and Aiyelaagbe OO. The need for bioactivity-safety evaluation and conservation of medicinal plants - A review. The Journal of Medicinal Plants Research 2009; 3(5):324-328.
4. Orji OU, Ibiam UA, Aja PM, Ugwu PC, Uraku AJ, Alope C *et al.* Evaluation of the Phytochemical and Nutritional Profiles of *Cnidoscopus aconitifolius* Leaf Collected in Abakaliki South East Nigeria. World Journal of Medical Sciences 2016; 13(3): 213-217.
5. Ozcan T, Akpinar-Bayazit A, Yilmaz-Ersan L and Delikanli B. Phenolics in human health. International Journal of chemical engineering and applications 2014; 5(5):393.
6. Mattijs KJ, Albert K, Herman JW, Wim JQ and Oliver K. Combinatorial biosynthesis of medicinal plant secondary metabolites. Biomolecular Engineering 2006; 23: 265–279.

7. Oladoye SO, Ayodele ET, Abdul-Hammed M and Idowu OT. Characterisation and Identification of Taraxerol and Taraxer-14-en-3-one from *Jatropha tanjorensis* (Ellis and Saroja) Leaves. Pakistan journal of scientific and industrial research Series A: physical Sciences 2015; 58(1):46-50.
8. Berkelaar D. Chaya, ECHO technical note 2006; 1739 Durrance Road, North Myers, FL33917, USA.
9. Kirtikar KR and Basu BD. Indian Medicinal Plants, Oriental Enterprises, Dehradun, India. 2001; 2nd edn, Vols. I-4.
10. Numa S, Rodríguez L, Rodríguez Da and Coy-Barrera E. Susceptibility of *Tetranychus urticae* Koch to an ethanol extract of *Cnidoscopus aconitifolius* leaves under laboratory conditions. Springerplus 2015; 4:338.
11. Buss AD and Butler MS (Eds.). Natural product chemistry for drug discovery. The Royal Society of Chemistry Cambridge 2010. p. 153.
12. Kelly da and Nelson R. Characterization and quantification by gas chromatography of Phytochemicals. Journal of the Brazilian Chemical Society 2014. Vol 25.
13. Bezerra KS and Filho NRA. Characterization and quantification by gas chromatography of free steroids in unsaponifiable matter of vegetable oils. Journal of the Brazilian Chemical Society 2014; 5:238-245.
14. Panghal A, Janghu S, Virkar K, Gat Y, Kumar V and Chhikara N. Potential non-dairy probiotic products—a healthy approach. Food bioscience 2018; 21:80-89.
15. Hamid AA, Oguntoye SO, Negi AS, Ajao A and Owolabi NO. Chemical constituents, antibacterial, antifungal and antioxidant activities of the aerial parts of *Cnidoscopus aconitifolius*. Ife Journal of Science 2016; 18: 2.
16. Oyagbemi AA, Ogunleye AO, Lawal TO and Azeez IO. The effect of *Cnidoscopus aconitifolius* on multi-drug resistant micro-organisms. African Journal of Biotechnology 2011; 10(3):413-415.
17. Adaramoye OA, Aluko A and Oyagbemi AA. *Cnidoscopus aconitifolius* leaf extract protects against hepatic damage induced by chronic ethanol administration in wistar rats. Alcohol Alcohol 2011; 46(4): 451-458.
18. Parekh J and Chanda S. In vitro antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz. Flower (Lythraceae). Brazilian Journal of Microbiology 2007; 38(2):204-207.
19. Sarkar T, Salauddin M, Pati S and Sheikh HI. Application of raw and differently dried pineapple (*Ananas comosus*) pulp on rasgulla (sweetened Casein Ball) to enhance its phenolic profile, shelf life, and in-vitro digestibility characteristics. Journal of Food Processing and Preservation 2021; 45:e15233.
20. Kuri-García A, Chávez-Servín JL and Guzmán-Maldonado SH. Phenolic profile and antioxidant capacity of *Cnidoscopus chayamansa* and *Cnidoscopus aconitifolius*: A review. Journal of Medicinal Plants Research 2017; 11(45):713-727.
21. Fan YM, Xu LZ, Gao J, Wang Y, Tang XH and Zhao XN. Phytochemical and anti-inflammatory studies on *Terminalia catappa*. Fitoterapia 2004; 75:253-260.
22. Shittu SA, Olayiwola OA and Adebayo OR. Nutritional Composition and Phytochemical Constituents of the Leaves of *Cnidoscopus aconitifolius*. American Journal of Food Science and Nutrition Research 2014; 1(2):8-12.
23. Prashant T, Bimlesh K, Mandeep K, Gurpreet K and Harleen K. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Scientia 2011; 1(1):98- 106.
24. Panghal A, Shaji AO, Sajitharan D, Nain K, Garg MK, Chhikara C. *Cnidoscopus aconitifolius*: Nutritional, phytochemical composition and health benefits – A review. Bioactive Compounds in Health and Disease 2021; 4(11):260-286.
25. Westerndarp H. Effect of tannins in animal nutrition. Dtsch. Tierarztl. Wochenschn 2006; 113(7): 264-268.

26. Anands TP and Edwards JKP. Antimicrobial activity in the tissue extract of five species of cowries *Cypraea spp.* Mollusca: Gastropoda and an ascidian *Didemnum Psammathodes* Tunicata: Didemnidae. *Indian Journal of marine sciences* 2002; 31(3):239-242.
27. Vergara-Jimenez M, Almatrafi MM and Fernandez ML. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. *Antioxidants* 2017; 6: 91.
28. Dulger B, Ergul CC and Gucin F. Antimicrobial activity of the macrofungus *Lepista nuda*. *Fitoterapia* 2002; 73:695-697.
29. Scalbert A. Antimicrobial properties of tannins. *Phytochemistry* 1991; 30:3875-3882.
30. Hameed IH, Hussein HJ, Kareem MA and Hamad NS. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS), *Journal of Pharmacognosy Phytotherapy* 2015; 7:107–125.
31. Awoyinka OA, Balogun IO and Ogunnowo AA. Phytochemical screening and in vitro bioactivity of *Cnidoscolus aconitifolius* (Euphorbiaceae). *Journal of Medicinal Plant Research* 2007; 1:63-65.
32. Hodek P, Trefil P and Stilborova M. Flavonoids – potent and versatile biologically active compounds interacting with cytochrome P450. *Chemico-Biological Interactions* 2002; 139(1):1-21.
33. Menaga D, Mahalingam PU, Rajakumar S and Ayyasamy PM. Evaluation of Phytochemical Characteristics and Antimicrobial Activity of *Pleurotus Florida* Mushroom. *Asian Journal of Pharmaceutical and Clinical Research* 2012; 5(4): 102-106.
34. Egwim EC, Ellen RC and Egwuiche RU. Proximate composition, phytochemical screening and antioxidant activity of ten selected edible mushrooms. *American Journal of Food and Nutrition* 2011; 1(2):89-94.
35. Schneider G and Wolfling J. Synthetic cardenolides and related compounds. *Current Organic Compounds* 2004; 8:14.
36. Janporn S, Ho CT, Chavasit V, Pan MH, Chittrakorn S, Ruttarattanamongkol K, and Weerawatanakorn M. Physicochemical properties of *Terminalia catappa* seed oil as a novel dietary lipid source. *Journal of Food and Drug Analysis* 2015; 23:201 – 209.
37. Noonan SC and Savage GP. Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition* 1999; 8(1): 64-74
38. Onasanwo SA, Oyagbemi AA and Saba AB. Anti-inflammatory and analgesic properties of the ethanolic extract of *Cnidoscolus aconitifolius* in rats and mice. *Journal of Basic Clinical Physiology and Pharmacology* 2011; 22(1-2):37-41.
39. Achi NK, Ohaeri OC, Ijeh II and Eleazu C. Modulation of the lipid profile and insulin levels of streptozotocin induced diabetic rats by ethanol extract of *Cnidoscolus aconitifolius* leaves and some fractions: Effect on the oral glucose tolerance of normoglycemic rats. *Biomedicine and Pharmacotherapy* 2017; 86:562-569.
40. Azeez OI, Oyagbemi AA, Oyeyemi MO and Odetola AA. Ameliorative effects of *Cnidoscolus aconitifolius* on alloxan toxicity in Wistar rats. *African Health Sciences* 2010; 10(3):283-291.