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Phytochemical Identification in the Chloroform Fraction of Aqueous-Methanol Extract of *Cnidoscolus aconitifolius* Leaves

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Authors' contributions

This work was carried out by a collaboration of two authors SCI and AN. Author SCI co-designed and carried out the study, managed literature searches, wrote the protocol and the first draft of the manuscript. Author AN co-designed and supervised the work. Both authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aims: This study is designed to identify minor bioactive compounds present in 80% aqueous-methanol chloroform fraction of *Cnidoscolus aconitifolius (CA)* air-dried leaves using proton Nuclear Magnetic Resonance (^{1}H NMR) spectrometry.
Methodology: The air-dried leaves of CA were pulverized and Soxhlet-extracted with aqueous-methanol (1:4, v/v). The dried leaf extract obtained was purified in chloroform and analyzed with ^{1}H NMR- spectrometry using dimethyl sulphoxide *solvent*.
Results: The proton NMR spectra were interpreted to indicate the presence of major phytochemicals like flavonoids, anthraquinones anthranoids), saponins and alkaloids with their minor bioactive compounds. Some of the identified compounds included eupafolin, hispidulin,

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oleonolic acid, β-sitosterol, isoquecetin, kaempferol 3-*O*- gentiobioside, rhamnopyranosyl-(1)-glucopyranosyl-(1)-glucopyranoside, Seneciomine, Retrorsine and Seneciphylline.
Conclusion: The proton NMR-spectrometry characterized and aided the identification of phyto-compounds present in chloroform fraction of *C. aconitifolius* leaf extract. These phytochemicals could be medicinally and toxicologically potent and responsible for the reported effects of the extract. However, bioassay-guided fractionation using HPTLC is still recommended for better extrapolations.

Keywords: Cnidoscolus aconitifolius; leaf extract; ^{1}H NMR; Identification; phytochemical.

1. INTRODUCTION

Recent studies had evaluated the phyto-medicinal, nutritional and electrolyte values of *Cnidoscolus aconitifolius* leaves (CA) consumed in the Niger Delta region of Nigeria [1-4]. These efforts are relevant because World Health Organization (WHO) had specified the need to know the composition of biologically active botanical substances considered for nutritional and medicinal purposes [2]. The phyto-medicinal and nutritive significance of CA could justify its claims of a local name '*ogwu obala'* ("blood tonic") [4,5-12]. Chloroform fraction of crude aqueous-methanol leaf extract of CA had recently been reported to possess antidiabetic properties [13-15]. CA is commonly found in the tropic and sub-tropical regions and is commonly known as *Chaya* or Tree Spinach. It is popular in Mexico and Central America and has been introduced into the United States (mainly South Texas and Florida) for potential use as leafy vegetable and/or as medicinal plant [16,17]. It is commonly eaten as vegetable in soup. Indeed, it has been reported that the level of leaf nutrients are two to threefold greater than any other land-based leafy green vegetables [18-20].

To obtain scientific evidence required to ensure the use of safe, effective and quality products and practices, and to facilitate the understanding of its biological activities, the current study aimed at identifying the major and minor compounds in the chloroform fraction of CA using nuclear magnetic resonance (NMR).

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The plant was identified by its local name in the private residences in Eleme, Port Harcourt, Rivers State, Nigeria. A sample of Fresh plant parts were taken for identification / authentication by a Taxonomist. Fresh leaves of the named *Cnidoscolus aconitifolius* (CA) were collected between March and May, 2012.

2.2 Identification of Plant

Dr. (Mrs.) M. E. Bassey of the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State, Nigeria identified the plant sample as *Cnidoscolius aconitifolius* and it was kept as herbarium I Samuel UUH 026113 (Port Harcourt) in the University of Uyo, Nigeria.

2.3 Extraction Method

The fresh leaves of CA were air dried under room temperature and extraction method was adapted from a previous report [12].

The dried leaves were pulverized with electric grinding machine into minute pieces. Aqueous-methanol (1:4, v/v) extract was obtained using Soxhlet extractor (Model No. 3567, Austria). At the end of the solvent extraction, the extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 45ºC using a rotary evaporator (Gallenkamp UK). The resulting residue called dried leaf extract was transferred to a hot air oven where they were dried to a constant weight at 45ºC. A portion of the residue was used to test for the bioactive constituents of CA. The extracts were stored at 4ºC.

2.4 Phytochemical Screening of the Extract

Phytochemical screening was also carried out at the laboratory of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa Ibom, Nigeria on aqueous-methanol (1:4, v/v) extract of CA dried leaves using reported method [4,11,21].

The residue yield was further fractionated with chloroform solvent.

2.5 Nuclear Magnetic Resonance Analysis

The dried chloroform fraction was taken to Department of Chemistry, University of Cape Town for identification of the major and minor phytochemicals.

The Varian Unity 400 (400 MHz) Spectrometer (Varian, Inc., Palo Alto, CA) was used to record spectra of magnetically active nuclei for structure elucidation of molecular structures. John Wiley and Sons Chemical Concepts FTIR spectra library was consulted during interpretation.

The NMR method partially elucidated the phytochemicals contained in the sample. The ^{1}H NMR analysis is the application of NMR spectroscopy with respect to hydrogen nuclei within the molecules of the extract fractionates, in order to determine the structure of its molecules [22]. In samples where natural hydrogen (H) is used, practically all the hydrogen consists of the isotope ^{1}H (hydrogen-1; i.e. having a proton for a nucleus)

The NMR fingerprints was interpreted as shown in the results.

3. RESULTS AND DISCUSSION

The major compounds identified to be present in CA were: Flavonoids, Anthraquinones, Saponins and Alkaloids with minor derivatives are as shown in Table 1.

The role of plants present in the environment has significant importance to health [23,24]. The NMR phytochemical analysis of this medicinal and edible plant leaves is essential in line with WHO requirements [2]. Though Gori and Campbell [25] emphasized that some herbs only have mild or placebo effects, it is necessary to elucidate the phytochemical basis of their safety and effectiveness.

The results of this study confirmed the presence of flavonoids, saponins, anthraquinones and alkaloids as reported recently [3,4]. It went further to characterize and identify the component compounds with the aid of proton NMR spectrometry.

Table 1. Major and minor compounds identified with NMR in the chloroform fraction of hydromethanolic leaf extract of *C. aconitifolius*

S/N	Major compounds*	Minor compounds
1	*Flavonoids*	*Eupafolin, Hispidulin, Oleanolic acid,* β-sitosterol, Campesterol, Isoquercitrin,
2	Anthraquinonine (Anthranoids)	kaempferol 3-*O*- gentiobioside,
		Aloe-emodine 8-*O*-glucopyranoside, rhein 8-*O*- glucopyranoside, torachrysone 8-*O*-glucopyranoside, isorhamnetine 3-*O*-gentiobioside,
3	Saponins	Rhamnopyranosyl-(1)-glucopyranosyl-(1)-glucopyranoside, diosgenyl-glucopyranoside, diosgenyl -rhamnopyranosyl-(1)-glucopyranoside, diosgenyla-rhamnopyranosyl-(1)-rhamnopyranosyl-(1)-glucopyranoside, rhamnopyranosyl-(1)-xylopyranosyl-(1)-glucopyranoside, rhamnopyranosyl-(1)-arabinofuranose
4	Alkaloids	Seneciomine, Retrorsine, Seneciphylline, Riddelline Moncrotaline, Spectabiline, Retronecine, Platyphylline, Hygrophylline, Senkirkine, Pinene, P-Myrcene, Ocimene, Linalool, Rose oxide *(cis),* Lsopulegol, Geraniol, Tetrahydrogeraniol, Linalyl formate, Dihydrocitronellol, Terpineol, 2-Phenylethyl formate.

(Not all compounds were identified because some fractionates appeared clumpsy and require HPTLC. Dimethylsulfoxide (DMSO) NMR solvent was used to dilute the NMR samples.)*

Proton NMR is a powerful tool for the molecular structure characterization of the fractionates obtained from *C. aconitifolius*. The proton NMR spectra are characterized by chemical shifts in the range +14 to -4 ppm and by spin-spin coupling between protons. The integration curve for each proton reflects the abundance of the individual protons. This determined the specific characteristics that phytochemicals are made of.

Phytochemicals have their own unique sets of "fingerprints" based on the chemicals from which they are built. They radiated energy at a specific, unique and therefore identifiable frequency when investigated with the NMR spectrometer.

The minor compounds elucidated in this plant extract could have both medicinal and toxicological implications [26,27,28,29]. For instance, the reported antidiabetic effect of the extract [13] could be due the pharmacodynamic mechanisms of flavonoids like eupafolin, hispidulin, oleonolic acid, β-sitosterol and quercitrins identified by the study.

The study was limited by some clumsy fractionates which could necessitate the use of high powered thin layered chromatography (HPTLC).

4. CONCLUSION

Prior to this study, the medicinal use of *Cnidoscolus aconitifolius* leaves was often attributed to its major phytochemicals like flavonoids and saponins. This present study had partially elucidated the active compounds responsible for its reported medical and toxicological importance. The reported pharmacodynamic properties of *Cnidoscolus aconitifolius* leaves could now be better understood. However, further fractionation with HPTLC and drug development and safety works are recommended on this extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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