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Comparative Characterization of the Phytomedicinal Constituents of *Cnidoscolus aconitifolius* Leaf Extracts.

***Iwuji Samuel Chidi[1], Nwafor Arthur[2], Egwurugwu Jude[3], Ejeta Kenneth[1] , Akpan Utibe[1]**
1.Department of Biomedical Technology, School of Health Technology, Federal University of Technology Owerri. PMB 1526 Owerri, Nigeria.
2. Department of Human Physiology, Faculty of Basic Medical Science, University of Port Harcourt, Rivers State, Nigeria.
3. Department of Human Physiology, Faculty of Basic Medical Science, Imo State University Owerri, Imo State, Nigeria.

ABSTRACT

The increasing search and utilization of phytochemicals for medicinal purposes necessitated this comparative isolation and characterization of the medicinal constituents in aqueous, hydro-methanolic (1:4, v/v), hydro-ethanolic (1:4, v/v) leaf extracts of *Cnidoscolus aconitifolius*. The extracts were tested for twelve important medicinal constituents. Alkaloids, tannins, saponins, flavonoids, Salkowski's test were positive though at different strengths in the three extracts. The presence of combined anthraquinons, saponins, free anthraquinones, terpenes and Liberman's test appeared weak (33.3%) in aqueous and hydro-ethanolic (1:4, v/v) extracts though totally absent in hydro-methanolic (1:4, v/v) extract. Phlobatanins and cyanogenetic glycosides were absent in the three extracts. Hydromethanolic (1:4, v/v) extract contained at least 66.7% alkaloids, tannins, flavonoids, Salkowski's test and Kellerkillian's test and these gave cumulative percent – strength of 300.1% compared to aqueous extract that had 233.3% and hydroethanolic (1:4, v/v) extract that had 200%. The results implied that hydromethanolic (1:4, v/v) extraction yields more medicinal constituents than aqueous and hydroethanolic (1:4, v/v) extractions. Further analysis of these isolated phytomedicinal constituents are recommended to understand the physiological and therapeutic implications of *Cnidoscolus aconitifolius* utilizations.

Keywords: *Cnidoscolus aconitifolius*, aqueous, hydro-methanolic, hydro-ethanolic, phytomedicinal constituents

*Corresponding Author Email: iwujisc@yahoo.com
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INTRODUCTION

Plants secondary metabolites which have recently been referred to as phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect.[1,2] consequently, these secondary metabolites could be phytomedicinal.

Antioxidants protect other molecules (*in vivo*) from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the aetiology of many diseases and in food deterioration and spoilage. [3, 4, 5]

The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth. Herbal medicine was practiced by ancient people in Africa, Asia, Europe, and the Americas. [6]

Cnidoscolus aconitifolius (CA), commonly known as Chaya or Tree Spinach, belongs to the family of *Euphorbiaceae*. It is an evergreen, drought deciduous shrubs up to 6 m in height with alternate palmate lobed leaves, milky sap and small flowers on dichotomously branched cymes.[7] It is commonly found in the tropic and sub tropical regions worldwide, including Africa, South of Sahara, North and South America, India, etc. It is commonly eaten as vegetable in soup.[8] The edible parts of the plant provide important nutritional sources for protein, vitamin (A and C), minerals (calcium, iron and phosphorus), niacin, riboflavin and thiamine among populations that cannot afford expensive foods rich in these nutrients.[9] *Cnidoscolus aconitifolius* shoots and leaves have been taken as laxative, diuretic, circulatory stimulant, to improve digestion, stimulate lactation and to harden the fingernails.[10]

In the western part of Nigeria it is called different names such as *efo Iyana Ipaja* and *efo Jerusalem*[11]. Due to the claim that it boosts haemopoiesis, Ibos in eastern Nigeria call *ogwu obala*. Although the plant is mainly cultivated as food it has continued to be an important medicinal plant.

CA is one of the most productive green vegetables.[12] It is a good source of protein, vitamins, calcium, and iron; and is also a rich source of antioxidants.[13] In fact, levels of leaf nutrients are two to threefold greater than any other land-based leafy green vegetable. CA leaves have a possible antidiabetic effect[14,15] antibacterial activities[16, 17] and it also ameliorates anaemia and osmotic fragility induced by protein energy malnutrition.[18]

Traditionally CA leaves are cooked for at least 20 minutes before eating. The aqueous extract

obtained is safely consumed as the cyanide is volatilized as hydrogen cyanide (HCN) during cooking. [12] In this study, the phytomedicinal constituents of CA will be extracted using different solvents: water, hydro-methanol and hydro-ethanol. The results of their comparative characterization will suggest the preferable extraction method / solvent and the expected extent of phytomedicinal activities of these extracts.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh leaf sample of *Cnidoscolus aconitifolius* (CA) was collect from a private residence in Eleme at Port Harcourt, Rivers state Nigeria in 2012. Identification and taxonomical classification were carried out at Department of Botany University of Uyo, Nigeria.

Preparation of *Cnidoscolus aconitifolius (CA)* leaf extracts

The fresh leaves of CA were air dried and extraction method was adapted from a previous report.[19]The dried leaves were pulverized with electric grinding machine into minute pieces. Hydro-methanolic (1:4, v/v), hydro-ethanolic (1:4, v/v), and aqueous extractions were carried out with Soxhlet extractor (Model No. 3567, Austria). At the end of each respective extraction, the extract is filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 45^0C using a rotary evaporator (Gallenkamp UK).

The resulting residues called dried leaf extracts were transferred to a hot air oven where they were dried to a constant weight at 45^0C. A portion of the residue was used to test for the bioactive constituents of CA. The extracts were stored at 4 ^{0}C.

Phytomedicinal screening of the Extracts

Phytomedicinal screening was carried out at the Laboratory of Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo Akwa Ibom, Nigeria on aqueous, hydro-methanol (1:4, v/v), and hydro-ethanolic (1:4, v/v) extracts of the leaves of CA using reported methods. [20, 21].

Statistical Analysis

All data collected were summarized in percentage (1 dec.).

RESULTS AND DISCUSSION

Table 1shows the phytomedicinal constituents assessed in aqueous, hydromethanolic (1:4, v/v) and hydroethanolic (1:4, v/v) leaf extracts of *Cnidoscolus aconitifolius* (CA) and the absence or percent- strength (%) of each in the different extractions. Of the twelve important medicinal constituents assessed, alkaloids, tannins, saponins, flavonoids, Salkowski's test were present at

different strengths in all the extracts. The presence of combined anthraquinons, saponins, free anthraquinones, terpenes and Liberman's test appeared weak (33.3%) in most of the extracts. However, phlobatanins and cyanogenetic glycosides were absent in all the extracts.

Hydromethanolic (1:4, v/v) extract contained at least 66.7% alkaloids, tannins, flavonoids, Salkowski's test and Kellerkillian's test which gave cumulative percent – strength of 300.1 compared to aqueous extract that had 233.3 and hydroethanolic (1:4, v/v) extract that had 200. This could imply that hydromethanolic (1:4, v/v) extraction yields more medicinal constituents than aqueous and hydroethanolic (1:4, v/v) extractions as indicated earlier.[20]

Table 1: Comparison in Percent-strength (%) of Phytomedicinal Constituents in Leaf Extracts of *Cnidoscolus aconitifolius***.**

S/N	Phytomedicinal constituent Assessed	Aqueous extract (%)	Hydromethanolic (1:4, v/v) extract (%)	Hydroethanolic (1:4, v/v) extract(%)
1	Alkaloids	33.3	100.0	66.7
2	Tannins	100.0	66.7	33.3
3	Saponins	33.3	33.3	33.3
4	Flavonoids	66.7	66.7	66.7
5	Liberman's test	-	33.3	33.3
6	Salkowski's test	33.3	100.0	33.3
7	Kellerkillian's test	-	66.7	-
8	Combined anthraquinons	33.3	-	33.3
9	Free anthraquinones	33.3	-	33.3
10	Phlobatanins	-	-	-
11	Terpenes	33.3	-	33.3
12	Cyanogenetic glycosides	-	-	-

Key: - = absent; 33.3% = weakly present; 66.7% = moderately present; 100% = strongly present

The results agreed with previous study[17] that there are presence of alkaloids, tannins and saponins in water and hydroethanolic leaf extracts of CA. Meanwhile, no previous work had been reported on the phytomedicinal constituents of hydromethanolic extract of CA. Table 1 above confirmed these contents. This work, however, also showed the presence of flavonoids in contrast to the earlier report.[17]

The negligible (33.3%) presence of haemolytic saponins[21] could justify the reported use of CA in treating anaemia[18] and absence of cyanogenetic glycosides in this result showed that even though fresh leaves contain cyanides (CN)[12], the extraction process had removed the poison (CN). The presence of tannins may suggest the reported anti-diarrhoeic and anti-haemorrhaegic potentials of CA.[22]

CONCLUSION

Aqueous, hydromethanolic (1:4, v/v), hydro-ethanolic (1:4, v/v) leaf extracts of *Cnidoscolus*

aconitifolius were found to contain alkaloids, tannins, saponins, flavonoids, Salkowski's test though at different strengths in the three extracts. The presence of combined anthraquinons, saponins, free anthraquinones, terpenes and Liberman's test appeared weak (33.3%) in aqueous and hydro-ethanolic (1:4, v/v) extracts though totally absent in hydromethanolic (1:4, v/v) extract. Phlobatanins and cyanogenetic glycosides were absent in the three extracts. The results implied that hydromethanolic (1:4, v/v) extract yields more medicinal constituents than aqueous and hydroethanolic (1:4, v/v) extracts.

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