CODEN (USA): IAJPBB ISSN: 2349-7750
INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.377023

Available online at: http://www.iajps.com *Research Article*

PHARMACOGNOSTIC STUDIES OF THE LEAF OF CNIDOSCOLUS ACONITIFOLIUS

***Ugwoke Christopher Emeka Chukwunonye [1] Anze Sunday Patrick Gaza[1], Chukwube Vincent Obisike[1] Nweze Anthony Ejiofor[2] and Diovu Edith Obioma [1]**

[1] Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, Nigeria.
[2] Department of Plant Science and Biotechnology, University of Nigeria, Nsukka
Corresponding author: DR. UGWOKE CEC.

Received: 29 December 2016 **Accepted:** 10 February 2017 **Published:** 28 February 2017

Abstract:

Purpose: *The work focused on the pharmacognostic standardization of the leaf of Cnidoscolus aconitifolius.* ***Background:*** *C. aconitifolius is a fast growing, drought resistant and heavy rain tolerating perennial shrub, highly nutritive and of wide medicinal applications. Medicinal plants are regarded as safer and cheaper alternative to synthetic drugs but these are prone to adulteration and substitution. This therefore necessitates pharmacognostic standardization of these plants to ensure identity, quality control and purity of the plant drug material.* ***Materials and Methods****: This involved macroscopic and microscopic (quantitative and Qualitative, as well as chemo microscopic) studies, anatomical sectioning with photomicrograps, and physicochemical (analytical) parameters.* ***Results:*** *Leaves were petiolate and palmately compound and 12.73 x 7.53 average size. The leaf was isobilateral with a thicker upper (EP1) and smaller-celled lower epidermis (EP2), a starch sheath around the vascular tissues, a wide parenchymatous pith, anomocytic stomata with 6 – 7 surrounding epidermal cells formed like the wings of a butterfly, scapula-shaped large prisms of calcium oxalate, numerous and tiny starch grains. Cellulose, tannins, calcium carbonate, proteins, lignins, fats and fatty oils were detected. Average palisade ratio was 42.5, stomata number for EP1 and EP2 were 1.5 and 5.75, silmilarly, stomatal index was 9.68 and 29.11. Average vein-islet number and vein terminations were 2 and 5 respectively. Leaf powder exhibited characteristic colours under 254 and 365 nm UV wavelengths. Average moisture content, Ash value, acid-insoluble ash and water soluble ash values were 10.56, 13.5, 02 and 08. Water and alcohol (ethanol) extractives were 10 and 04.73.* ***Conclusion:*** *The features of this plant as shown are important in creating a monograph for the plant especially as no known analysis in this regard has been done.*

Key words: *Cnidoscolus aconitifolius, Standardization, Pulverization, Anatomical sections*

Corresponding Author:
Ugwoke Christopher Emeka Chukwunonye,
Department of Pharmacognosy and Environmental Medicine,
University of Nigeria Nsukka,
Nigeria.

QR code

Please cite this article in press as Ugwoke Christopher Emeka Chukwunonye et al, ***Pharmacognostic Studies of the Leaf of Cnidoscolus Aconitifolius****, Indo Am. J. P. Sci, 2017; 4(02).*

;

INTRODUCTION:

The use of herbs as medicine pre-dates the use of synthetic drugs but the zeal to use herbal drugs seemed to have declined due to availability of numerous synthetic alternatives. However, over time, several reports of adverse reactions due to synthetic drugs forced a return to natural medicines, especially from herbs. Thus herbal is now gaining wider popularity and acceptance. Many varied and numerous claims on therapeutic significance of herbs are also becoming common. These attributes are further strengthened by a belief that all herbal products are safe and effective because they are sourced directly from nature [1]. However, medicinal plants can be poisonous if the wrong plant/plant parts or wrong concentrations are used [2]. Poisoning can also arise from adulteration or substitution of original plant material with adulterants, which could make up the poison in it. Consequently, it has become imperative on researchers to carryout

Fig. 1: a twig of *C. aconitifolius*

pharmacognostic evaluation of various medicinal plants in order to lay down parameters for their standardization. Some medicinal plants with a long history of use both as medicinal and edible plants include *Cnidoscolus aconitifolius* [3], (Fig. 1). It is an ornamental, evergreen, drought resistant [4], perennial shrub with a tolerance for heavy rain [5] belonging to the family Euphorbiaceae, subfamily Crotonoideae and tribe Manihoteae [6]. It is commonly called cabbage star, tree spinach in English [7] (Rehm, 1994), *efo Jerusalem* in Western Nigeria [8], "hospital too far" in Niger Delta of Nigeria [9], or hospital leaf in Middle belt region of Nigeria. Due to its ease of propagation, high productivity, tolerance of poor growth conditions, and resistance to pest and diseases [5] , it is cultivated in domestic gardens and available all through the year [8]. The morphology of the twig is as shown in Fig. 1.

It is believed to have originated as a domesticated leafy green vegetable in the Maya region Guatemala, Belize and Southeast Mexico during the pre-Cambian period [5] . It is commonly found in the tropic and sub-tropical regions worldwide including Africa [10]. It is widely used in the states [8] , and in Nigeria due to high nutritive value [11,5]. However, it has cyanide [12], a poisonous substance, thus the leaves are usually cut into pieces and boiled for at least five minutes[13] to release the volatile hydrogen cyanide thereby making it safe for consumption [12]. *C aconitifolius* has several therapeutic uses, some of which have been scientifically investigated. These medicinal uses are due to the phytochemicals [14,15] found in the plant. These include: alkaloids, tannins, saponins cardiac glycosides, terpenoids [8,16,17], flavonoids [18]. In addition, it is a source of protein, vitamins and minerals [11]. Thus the plant is useful in food preservation and drug formulations [19], against several infectious diseases and for general well-being[17,20-25]. *C. aconitifolius* is reported to be more effective than some commercial antibiotics such as Tetracycline and Cotrimoxazole, and comparative to Gentamycin and Chloramphenicol [8].

In view of the reputed efficacies of this plant, and to foster its standardization thereby seeking to enhance its various health and nutritional benefits, this study aimed at evaluating the physicochemical properties and anatomical features of the leaf of *C. aconitifolius* sourced from Nsukka in Enugu State, Nigeria.

MATERIALS AND METHODS:

Collection, identification and preparation of the plant material

Fresh leaves and twigs of *C. aconitifolius* were collected from a garden in the premises of St. John the Cross Junior Seminary, Edem-Nru in Nsukka local Government Area in Enugu State, Nigeria. A sharp knife was used to cut the leaves and allowed to fall into large black polythene bags to avoid contact with the skin. The collection was done at about 11:00 hrs in August, 2015.

Some leaves were stored in a glass jar under refrigeration and used within 24 hrs for the preparation of anatomical sections [26]. Identification and authentication was done by Mr. AO Ozioko of the International Centre for Ethnomedicine and Drug Development InterCEDD, Nsukka, where voucher specimens were deposited with the number INTERCEDD/Euph./082015.

The leaves were dried under shade in an open air and then pulverized using mortar and pestle [27]. The

powder was stored in airtight containers to avoid moisture.

Macroscopy

This was based on organoleptic properties of the leaf viz. size and shape, colour, surfaces, venation, presence or absence of petiole, nature of apex, margin, base, lamina, texture, odour and taste [27].

Microscopy

Quantitative leaf microscopy

Fragments of the outer epidermal membranous layer were cleared in chloral hydrate by boiling until transparent, mounted with glycerin and observed under a compound microscope. The presence or absence of the following was observed and captured: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution) [27,28].

Microscopical examination of anatomical sections

Permanent slide preparation:

The leaf was fixed in Formalin Acetic Alcohol (FAA) for at least 24 hours. Thereafter, the following were carried out:

i. Rinsing of the plant material for 2 hours

ii. Dehydration by fixing the tissue in 30%, 50%, 70%, 95% alcohol each for 2 hours duration, followed by further fixing in an absolute alcohol for two hours. This last step (i.e. in Absolute alcohol) is done twice.

iii. Cleaning or de-alcoholization: as soon as the tissue was removed from absolute alcohol, it was immediately fixed in Chloroform: Alcohol in the ratios 1:3, 1:1, 3:1, and finally in Absolute Chloroform. In each case, the tissue was allowed to stay for 2 hours and the one in Absolute

Chloroform was done twice.

iv. Impregnation of the Paraffin: little paraffin wax chips molten at 60°C were added; a little at a time by infiltration until chloroform no longer evaporates in which case impregnation process was expected to have taken place in molten paraffin wax at 60°C for 24 hours (overnight).

v. Solidification: The impregnated tissue was kept in a watch glass to properly embed the tissue in the paraffin wax. This was kept in this position until the time for sectioning.

vi. Sectioning: The prepared tissue was then mounted on a microtome set at 5 µm and then sectioned.

vii. Preparation of Slides: The sectioned tissue was placed on a clean slide and fixed on the slide using an adhesive (Glycerin and albumin)

viii. Staining Procedure: The sections were de-waxed in two stages using xylene for 5 mins each. They were then treated as follows:

• In absolute alcohol twice for 2 mins in each case, then 95%, 70%, 50% and 30% alcohol for 2 mins in each case and finally in distilled water for 2 mins.

• It was then stained in sarafin for 30 mins and rinsed in tap water for 1 min

• This was followed by de-staining using 70% Acid: Alcohol for 30 seconds.

• The de-stained tissue was then passed through 70%, 50%, and 30% Alcohol for 1 min in each case. Thereafter, it was washed in distilled water for 1 min.

• A counter staining was done using fast green for 1-2 mins, washed in tap water for 1 min and in distilled water for another 1 min.

• It was later passed through 30%, 50%, 70%, 95%, and in absolute alcohol twice for 1 min in each case.

• It was then passed through xylene in 2 stages for 5 mins in each case and finally mounted in D.P.X. which was a mixure of distrene (polystyrene), a pastilizer (tricresyl phosphate) and xylene or Canada balsam. It was then covered with cover slips.

The prepared permanent slides were then ready for viewing for the microscopical characters [30,31]. Photomicrographs were supplemented using an optical microscopic camera.

Temporary slide preparation:

Free hand transverse section (TS) of the leaf through the lamina and the midrib was taken. It was cleared with chloral hydrate, treated (stained) with phloroglucinol and a drop of concentrated hydrochloric acid to stain the lignified elements [27,29]. The TS was then mounted on a glass slide with glycerine and covered with a cover glass. The cover glass was presses lightly and excess fluid was removed from the margin of the cover glass with a strip of filter paper. The slide was then observed under a compound light microscope [26,28]. It was supplemented with photomicrographs.

Similarly, 2 mm square pieces of the leaf were placed in a solution of chloral hydrate in a test tube. It was boiled in a water bath until sufficiently transparent. It was then mounted in a mixture of equal parts of glycerine and chloral hydrate solution and examined under a compound light microscope [28].

Microscopical examination of powdered drug

About 5 g of the dried leaves was measured into a test tube containing 10 ml chloral hydrate. The tube was boiled over water bath to clear the powder [26,28]. Various microscopic features of diagnostic significance were then observed under the compound light microscope, with photomicrographs taken.

Chemomicroscopic examination of the powdered leaf

The presence of various chemical substances such as tannins, starch grains, lignin and calcium oxalate crystals were investigated as reported below:

Test for cellulose: The powdered leaf was moistened with N/50 iodine and allowed to stay until nearly dry. Then 80% w/w sulphuric acid was added. A blue colour indicates the presence of cellulose in cell walls

of epidermal cells and collenchymas cells of the cortex[27].

Test for lignin: To a sample of the powdered leaf, a few drops of phluroglucinol were added and allowed to stand for 2 – 3 minutes until almost dry. A drop of concentrated hydrochloric acid was then added. A pink or cherry red color indicates the presence of lignified cells; particularly sclerenchyma cells [30].

Test for tannins: a sample of the powdered leaf was dissolved in a test tube using 70 % methanol. Thereafter, a very dilute solution of ferric chloride was gradually added to the solution of the extract which resulted in blue-black colouration indicating the presence of gallitannins and ellagitannins (hydrolysable tannins). As more ferric chloride was added, the colour changes to olive-green showing that condensed tannins are also present in the powdered leaf sample[27].

Test for starch: To about 5 g of the powdered sample, a few drops of N/50 iodine were added. A deep blue colouration indicates the presence of starch [27,28].

Test for fats and fatty oils: About 1-2 drops of Sudan IV were added to the powdered sample and gently heated over a water bath. The fatty substances in the powdered sample are stained orange-red or brick-red indicating the presence of fats and fatty oils [27].

Test for proteins: To about 3 g of the powdered plant sample was added a few drops of ninhydrin and the mixture was warmed gently over a water bath for about 5 minutes. A pink, blue, violet or yellow colour (depending on the amino acid present) indicates the presence of proteins[27].

Test for calcium oxalate crystals: The powdered sample was first cleared using chloral hydrate solution. A little portion of the cleared sample was used to prepare a temporal slide with glycerine used as the moutant. When viewed under the light microscope, the crystal appears as bright structures of definite shape and size. 8% sulphuric acid was then added to the slide and viewed again. This time, the calcium oxalate crystals disappear. This confirms their presence in the original powdered sample [27]

Test for calcium carbonate: To about 5 g of the powdered leaf sample was added 5 ml of acetic acid in a test tube. The calcium carbonate dissolves with effervescence indicating the presence of calcium carbonate. When 50% sulphuric acid was used, needle-shaped crystals of calcium sulphate gradually separate [27].

Analytical evaluation of the crude drug

Determination of moisture content by the "Loss on Drying" method.

• An empty evaporating dish was heated to a constant weight, allowed to cool and placed in a desicccator.

• Thereafter 3 g of the powdered drug was weighed into the dish; the dish with its content were placed in an oven at 105ºC and allowed to dry to a constant weight. This was achieved by checking the weight at 30 minutes interval after an initial drying for about an hour. Two identical weights read off consecutively confirm a constant weight.

• The total loss in weight (i.e. the weight of the moisture content) was determined by subtracting the final weight of the dish and powder from the initial weight of the dish and the powdered leaf.

• Finally, the percentage of the moisture content was calculated with reference to the initial weight of the powdered leaf [27].

Determination of total ash value

- An empty tarred silica crucible was heated in an oven at 105ºC to a constant weight and the weight was noted. The crucible was allowed to cool and kept in desiccators, for 30 minutes[32].
- 2 g of the powdered plant leaf sample was accurately weighed into the crucible and spread in an even layer.
- The crucible and its content were heated gently until it was moisture free and completely charred.
- The heat was gradually increased until most of the carbon (black residue) was vaporized and then the heating was done strongly at 550^{o} C [32] until the residue was free from carbon (until it turned grey).
- The crucible and its content were then cooled in desiccators, for 30 minutes [32], and the constant weight was accurately noted.
- The heating cooling and weighing was repeated at interval of 30 minutes until a particular weight was obtained, to represent the constant weight.
- The weight of the ash was determined by subtracting the weight of the crucible from the final weight (i.e. weight of the crucible containing residue or ash).
- The ash value of the powdered drug (in percentage) was then calculated with reference to its initial weight[33].

Determination of acid-insoluble ash value:

- The ash obtained from the total-ash-value determination was transferred to a beaker containing 25 ml of 2M hydrochloric acid and boiled for 5 minutes.
- This was filtered through an ash-less filter paper, and rinsed with hot water 3 times until filtrate was neutral and collected at the tip of the coned filter paper.
- The funnel was then dried along with the filter paper in an oven at 105°C [34].
- A clean tarred silica crucible was heated, cooled in desiccators, for 30 minutes[32] and its constant weight was noted.

- The filter paper with the residue earlier dried in an oven was folded and transferred into the original crucible. These were heated gently until the filter paper was completely charred then the heat was applied strongly (to 550^{o} C) for 3 hours to constant weight [32].
- The weight of the residue (the acid insoluble ash) was determined by subtracting the weight of the crucible from the final weight. The acid insoluble ash value (in percentage) was calculated with reference to the initial weight of the powdered drug from the total-ash-value experiment [33].

Determination of water soluble ash value:

- The ash obtained from the total-ash-value determination was transferred to a beaker containing 25 ml of water and boiled for 5 minutes.
- This was filtered through an ash-less filter paper, and rinsed with hot water 3 times until filtrate was neutral and collected at the tip of the coned filter paper.
- The funnel was then dried along with the filter paper in an oven at 105°C [34].
- A clean tarred silica crucible was heated, cooled in desiccators, for 30 minutes [32] and its constant weight was noted.
- The filter paper with the residue earlier dried in an oven was folded and transferred into the original crucible. These were heated gently until the filter paper was completely charred then the heat was applied strongly (to 550^{o} C) for 3 hours to constant weight [32].
- The constant weight of the insoluble matter (residue) and the water soluble ash was determined by subtracting the weight of the insoluble matter from that of the ash. The percentage of water soluble ash value was calculated with reference to the initial weight of the air-dried drug from the total-ash-value experiment [33,36].

Determination of extractive values

Alcohol-soluble extractive value:

Five grams of the powdered leaf was accurately weighed into a 250 ml glass-stoppered conical flask. 100 ml of 90 % ethanol was added and the stopper replaced firmly. It was then shaken on a mechanical shaker for 6 hours and then allowed to stand for 18 hours. The mixture was filtered quickly. The weight of a clean tarred flat-bottomed evaporating dish was accurately determined. 25 ml of the filtrate was poured into dish and evaporated to dryness, on a water bath [32]. It was further dried in the oven at 105^{o} C for 1 hour in an oven, cooled in desiccators for 30 minutes and weighed without delay [32]. The weight of the residue was determined from the 25 ml extract by subtracting the weight of the evaporating dish from the final weight. The alcohol-extractive value was calculated with reference to the initial weight of the air-dried powdered drug [28,33].

Water-soluble extractive value:

The procedure of alcohol extractive-value above was repeated but using chloroform-water (0.25% v/v chloroform in distilled pure water). The water-soluble extractive value was the determined as for the alcohol extractive [28,33].

RESULTS:

The transverse section (TS) of the leaf across the mid-rib (Fig. 2a) shows an isobilateral leaf arrangement with a straight, thick double-walled upper epidermis and a lower epidermis of smaller cells. The petiole in TS (Fig. 2b) shows numerous roundish parenchymatous cells of the cortex that are bigger towards mesophyll. A starch sheath was visibly seen to differentiate the vascular tissues. A wide parenchymatous pith is also prominent. The stomata were anomocytic, seen surrounded by similar several epidermal cells formed like the wing of a butterfly. The cells of the epidermis were variously shaped, from irregular to polygonal (Fig. 2c). Other features were a distinct spiral xylem vessel (Fig. 2d), scapula-shaped calcium oxalate prisms (Fig. 2e) and numerous tiny starch grains of irregular shapes (Fig. 2f).

Macroscopic (organoleptic) characters of *C. aconitifolius* as shown in Table 1. Quantitative microscopic leaf characteristics are shown in Table 2. Chemomicroscopic features of the leaf revealed various chemical substances shown in Table 3. Fluorescence analysis revealed characteristic colours exhibited by the leaf powder in various solvents under both short and long wavelength (Table 4). The average physicochemical parameters of coarse leaf powder of *C. aconitifolius* (Table 5).

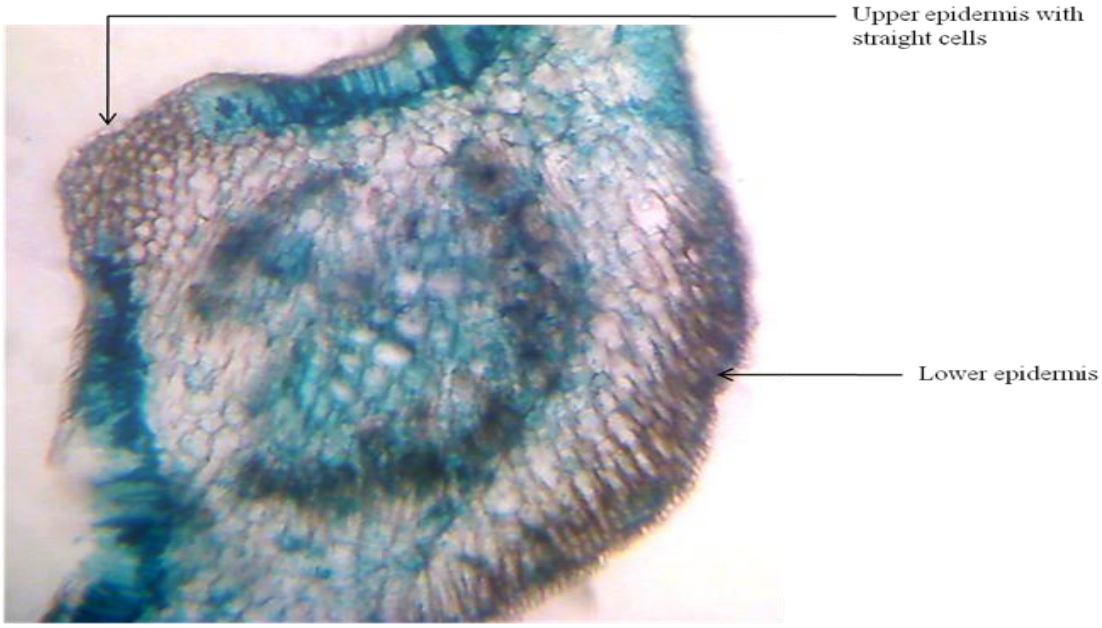

Fig. 2a: TS of *C. aconitifolius* leaf through mid-rib

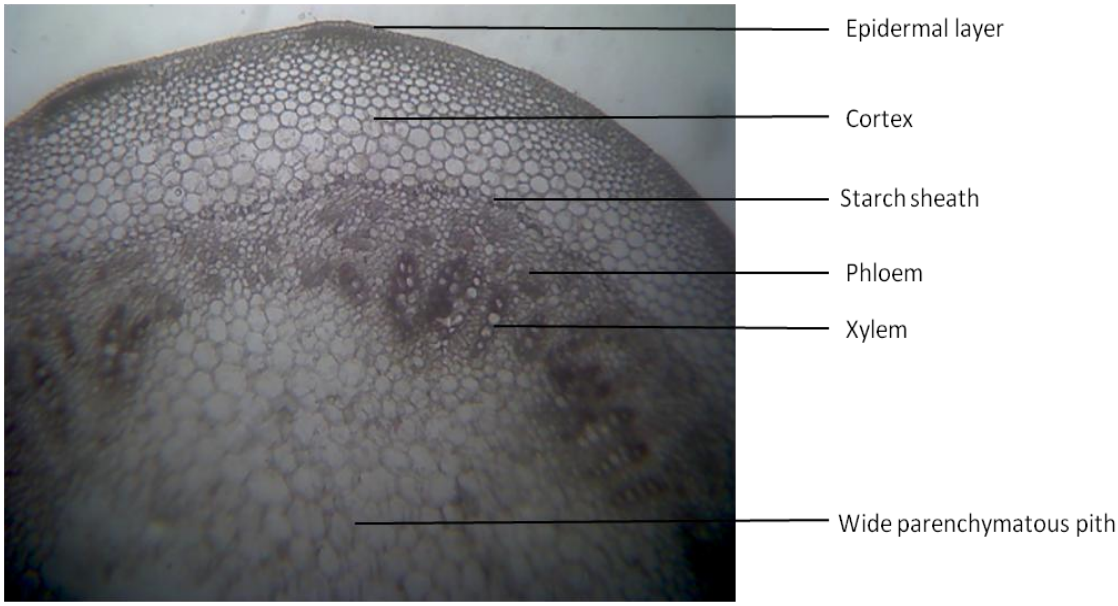

Fig. 2b: Petiole of *Cnidoscolus aconitifolius* in TS

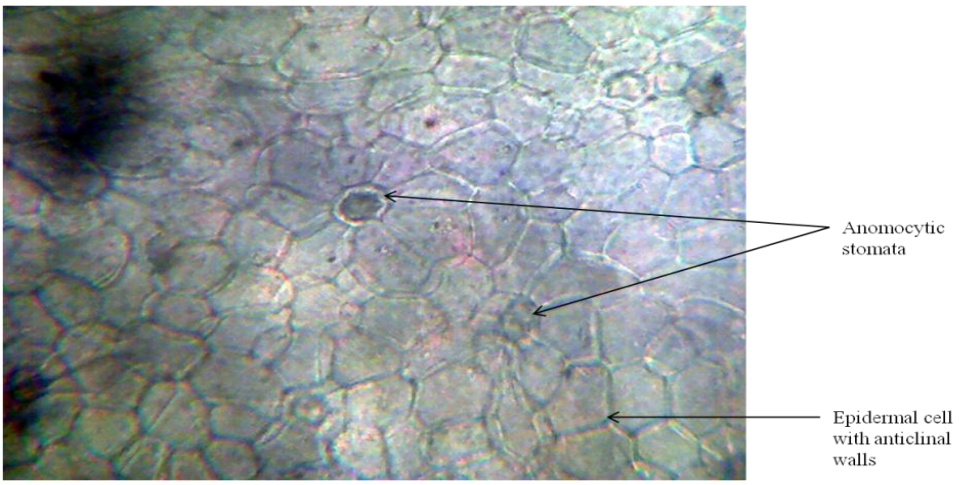

Fig. 2c: Epidermal tissue of *C. aconitifolius* leaf showing anomocytic stomata

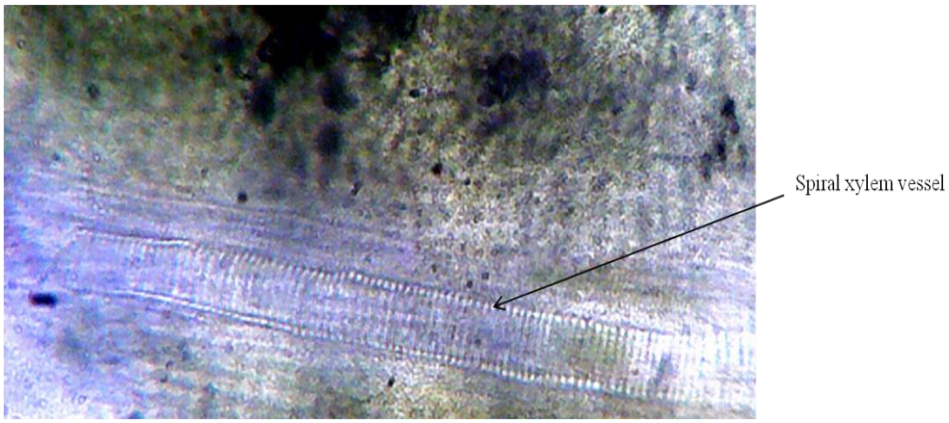

Fig. 2d: spiral xylem vessel of *C. aconitifolius* leaf powder

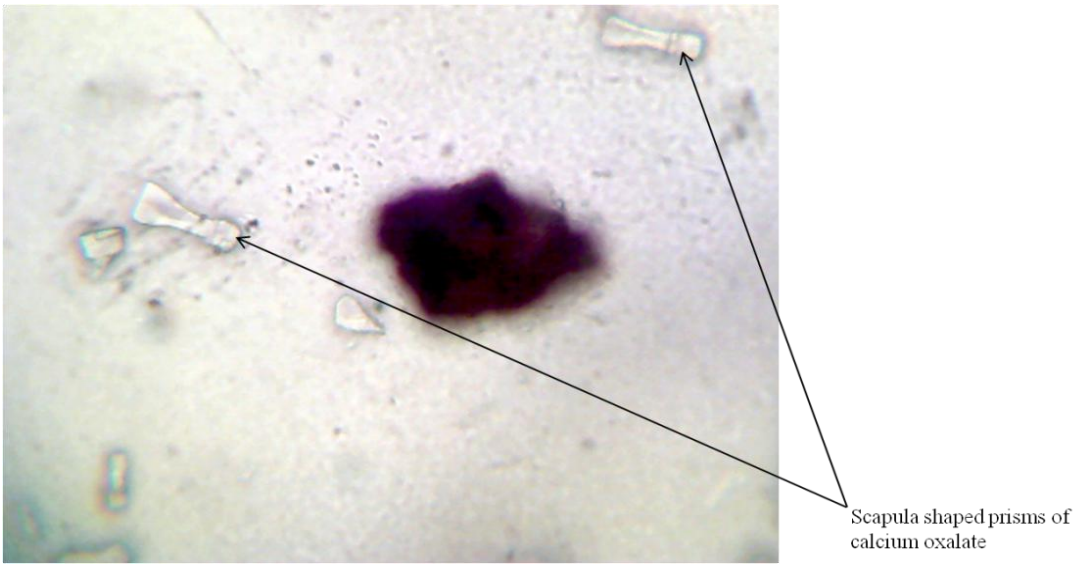

Fig. 2e: Scapula shaped calcium oxalates of *C. aconitifolius* powdered leaf

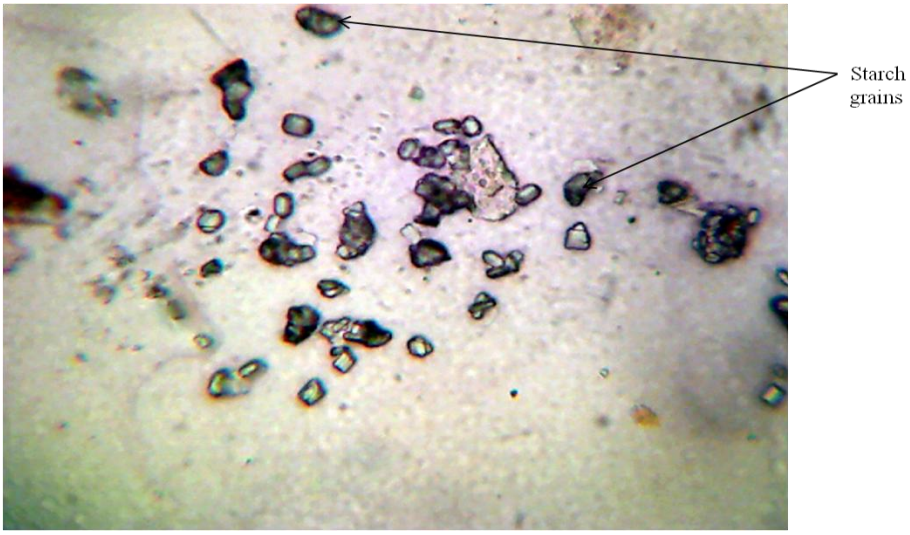

Fig. 2f: Numerous starch grains of *C. aconitifolius* powdered leaf

Table 1: Result of Macroscopical Examinations of *C. aconitifolius*

S/N	Character	Observation
1	Colour	Dark green
2	Odour	Characteristic
3	Taste	Bland
4	Length (average)	12.73 cm
	Width (average)	7.53 cm
5	Shape	Palmate
6	Texture	Smooth
7	Surface	Glaborous
8	Apex	Acuminate
9	Base	Sagitate
10	Margin	Serrated
11	Leaf arrangement	Alternate
12	Venation	Reticulate
13	Presence/absence of petiole	Petiolate
14	Lamina	Intact
15	Presence/absence of stipule	No stipule
16	Leaf type	Compound

Table 2: Result of Quantitative Microscopy of the Leaf of *C. aconitifolius*

Parameter	Mean
Palisade ratio	42.5
Stomata number Upper surface	1.5
Stomata number Lower surface	5.75
Stomatal index Upper surface	9.68
Stomatal index Lower surface	29.11
Vein islet number	2
Veinlet termination number	5

Table 3: Result of Chemo microscopy of *C. aconitifolius* leaf powder

Chemicals	Reagents	Observations	Inference
Cellulose	N/50 Iodine + 80 % Sulphuric acid	Blue-black colour	Present
Tannins	70 % Methanol + dil. Ferric chloride	Blue-black colour	Present
Calcium carbonate	Acetic acid + 50 % Sulphuric acid	Effervescence +needle-shaped crystals separated	Present
Fats and fatty oils	Sudan IV + heat	Orange-red or brick red substances	Present
Proteins	Few drops of Ninhydrin + gentle warming for 5 min	Yellow colour	Present
Lignin	Few drops of phloroglucinol + stand for 2 – 3 min + drop of con. Hydrochloric acid	Pink or cherry red colour	Present
Starch	Few drops of N/50 Iodine	Deep blue to pinkish colour	Present
Calcium oxalate			Present

Table 4: Fluorescence analysis of *C. aconitifolius* leaf powder

Detection reagents	UV wavelength / Colour exhibited	
	254 nm	365 nm
Petroleum ether	Brick red	Dark green
Methanol	Brick red	Brick red
50 % Hydrochloric acid	Dark green	Dark brown
50 % Sulphuric acid	Dark green	Dark brown
Ammonia	Blue-green	Dark green
Ethyl acetate	Bright red	Dark red

Table 5: Result of Quantitative (Analytical) Evaluation of *C. aconitifolius*

S/N	Parameters	Leaf powder
1	Moisture content (LOD) (% w/w)	10.56 ±1.02
Ash Values		
2	Total ash (% w/w)	13.50 ±0.29
3	Acid-insoluble ash (% w/w)	02.00 ±0.29
4	Water soluble ash (% w/w)	08.00 ±0.03
Extractive values		
5	Water soluble extractive value (% w/w)	10.00 ±0.26
6	Alcohol soluble extractive value (% w/w)	04.73 ±0.33

DISCUSSION:

The macro and microscopic features of *C. aconitifolius* (Table 1 and Fig. 2) serves a as guide to its correct identification. The formation of the epidermal cells surrounding the stomata and the scapula-shaped prisms of calcium oxalate are some peculiar features of this plant as shown (Fig. 2c and e). Physicochemical evaluation of crude drugs is indispensable for standardization of herbals (WHO, 1996a and b). Ash values and solvent residues gives an idea of characteristic physiological and non-physiological ash and reveals contamination with siliceous matter [33] thereby useful in proving identity and purity of plant materials [37] The moisture content of the plant (Table 5)falls within acceptable range(WHO, 1996a and b) [38,39]. Low moisture protects the plant material from microbial degradation [33]. Solvent extractive values gives an indication of the weight of chemical components extractable in a particular solvent[33]and thus a tool to verify claims of composition of a particular herbal preparation. The result for fluorescence analysis adds to the profile of the plant thereby providing more characteristics for a thorough assessment of the plant for correct identity of the powdered form, which could be difficult to analyze in other perspective.

CONCLUSION:

This study has specified salient pharmacognostic parameters that are imperative for correct identification and quality assurance. The findings of this research would therefore be instrumental in creating a monograph for *C. aconitifolius*.

REFERENCES:

1.Said O, Khalil K, Fulder S, Azaizeh H. Ethnobotanical survey of medicinal herbs of the Middle Eastern region. Journal of Ethnopharmacology 2004; 83:251-6.

2.Fronhe D. Plant intoxicants – Is there a risk from herbal preparation? Journal of Phytotherapy 1999; 20:201-2.

3.Nebel S, Heinrich M. Ta Chòrta: A comparative ethnobotanic linguistic study of wild food plants in a Graecanic area in Calabria Southern Italy. Economic Botany 2009; 63(1): 78 – 92.

4.Kuti JO, Torres ES. Potential nutritional and health benefits of tree spinach. In: Janick J. (Ed.), Progress in new crops. ASHS Press, Arlington, VA.; 1996. 516 – 520.

5.Ross IJ, Molina CA. The ethnobotany of *Chaya* (*Cnidoscolus aconitifolius* ssp *aconitifolius* Breckon): A nutritious Maya vegetable. Economic Botany 2002; 56: 350 – 365.

6.Brako L, Zarucchi JL. Catalogue of the flowering plants and gymnosperms of Peru. Monogr. Syst. Bot. Missouri Botanical Garden 45. 1993 (L Peru).

7.Rehm S. Multilingual dictionary of agronomic plants. 1994.

8.Adeniran OI, Olajide OO, Igwemmar NC, Orishadipe AT. Phytochemical constituents, antimicrobial and antioxidant potentials of tree spinach (*Cnidoscolus aconitifolius*(Miller) I.M. Johnston). Journal of Medicinal Plants Research May 2013; 7(19): 1317 – 1322.

9.Mordi J, Uzuegbu U, Nwagwa E, Okunima A. Effect of the dry aqueous leaf extract of *Cnidoscolus aconitifolius* on blood alcohol clearance in rabbits. Journal of Natural Science Research 2013; 3: 245 – 251.

10.Otitolaiye CA, Asokan C. GC-MS analysis of *Cnidoscolus aconitifolius* leaf aqueous extracts. International Journal of Science and Research 2016; 5(8): 2319 – 7064.

11.Kuti JO, Konuru HB. Antioxidant capacity and phenolic content in leaf extracts of tree spinach (*Cnidoscolus* spp). Journal of Agriculture and Food Chemistry 2004; 14: 117 – 121.

12.Akachukwu D, Okafor PN, Ibegbulem CO. Phytochemical content of *Cnidoscolus aconitifolius* and toxicological effect of its aqueous leaf extract in Wistar rats. Journal of Investigational Biochemistry 2014; 1(1): 29.

13.Dawn B. Chaya. 17391 Durrance Road, North Fort Myers, FL 33917, USA. 2006.

14.Dufresne C.J. and Farnsworth E.R (2001). A Review of the Latest Findings on Health Promoting Properties in Tea. *J. Nutr. Biochem*. **12**: 404 – 421.

15.Okoko T (2009). In vitro Antioxidant and Free Radical Scavenging Activities of *Garcinia kola* Seeds. *Food Chem. Toxicol*. **47**: 2620 – 2623.

16.Awoyinka AO, Balogun JO, Ogunowo AA. Phytochemical screening and *in vitro* bioactivity of *Cnidoscolus aconitifolius* (Euphorbiaceae). Journal of Medicinal Plant Research 2007; 19(130): 63 – 65.

17.Yakubu MT, Akanji MA, Oladiji AT, Olatinwo AO, Adesokan AA, Oyenike M, Yakubu RW, Owoyele BV, Sumonu TO, Ajao SM. Effect of *Cnidoscolus aconitifolius* leaf extract on reproductive hormones of female rats. Iranian Journal of Reproductive Medicine 2008; 6: 149 – 155.

18.Peixoto Sobrinho TJS, Castro VTNA, Saraiva AM, Almeida DM, Tavares EA, Pisciottano MNC, Amorim ELC. Phytochemical screening and antibacterial activity of four *Cnidoscolus* species (Euphorbiaceae) against standard strains and clinical isolates. Journal of Medicinal Plants Research 2012; 6(21): 3742 – 3748.

19.Loliger J. The use of antioxidants in food. In: Auroma Ol, Halliwell B (Eds.), Free radicals and food additives. Taylor and Francis, London. 1991. 121.

20.Rowe L. plant guards secret of good health. Valley Morning Star, Sept. 4, 1994. A12.

21.Mordi JC, Akanji MA. Phytochemical screening of the dried leaf extract of *Cnidoscolus aconitifolius* and associated changes in liver enzymes induced by its administration in Wistar rats. Current Research Journal of Biological Sciences 2012; 4(2): 153 – 158.

22.Oladeinde FO, Kinyua AM, Laditan AA, Michelin R, Bryant JL, Denaro. Effect of *Cnidoscolus aconitifolius* leaf extract on the blood glucose and insulin levels of inbred type 2 diabetic mice. Cellular Molecular Biology (Noisy-le-grand). 53: 34 – 41.

23.Oyagbemi AA, Odetola AA, Azeez OI. Ameliorative effects of *Cnidoscolus aconitifolius* on anaemia and osmotic fragility induced by protein energy malnutrition. African Journal of Biotechnology 2008; 11: 1721 – 1726.

24.Araújo TAS, Alencar NL, Amorim ELC, Albuquerque UP. A new approach to study medicinal plants with tannins and flavonoids contents from the local knowledge. Journal of Ethnopharmacology 2008; 120: 72 – 80.

25.Oyagbemi AA, Odetola AA. Hepatoprotective effects of ethanolic extract of *Cnidoscolus aconitifolius* on paracetamol-induced hepatic damage in rats. Pakistan Journal of Biological Science 2010; 13: 164 – 169.

26.Birdi TJ, Brijesh S, Daswani PG (2008) Approaches towards the Preclinical Testing and Standardization of Medicinal Plants.
27.Evans WC (2005) Trease and Evans pharmacognosy. (Edn15th) Rajkamal Electric press, Delhi, India, pp. 516 - 536.
28.Khandelwal KR. Practical pharmacognosy. Nirali Prakashan, twelfth Edition 2004.
29.Rachh PR, Dharamsi A. Pharmacognostic studies of *Moringa oleifera* leaflet. Pharma Science Monitor: an International Journal of Pharmaceutical Sciences 2012; 3(3): 125 – 126.
30.African Pharmacopoeia Determination of Extractives (1986) Scientific Publication, No 3, Lagos, Nigeria, 78.
31.Donald A (1940) J Plant Micro Technique. McGraw-Hill Book Company New York and London, 15-64, 126-154, 182-203.
32.Vinotha S, Ira T, Sri Ranjani S. Phyto, physicochemical standardization of medicinal plant *Enicostemma littorale* Blume. IOSR Journal of Pharmacy 2013; 3(2): 52 – 58.
33.Mukherjee P.K (2002). Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. 1st Edition. Business Horizon Publications, New Delhi, India. Pp. 185 – 186, 193, 541 – 542.
34.Sani A, Agunu A, Danmalam HU, Ibrahim H. Pharmacognostic studies of the stem bark of *Detarium microcarpum*-Guill. and Perr. (Fabaceae). Natural Products Chemistry and Research 2014; S1:004.
36.Kadam VB, Momin RK, Wadikar MS, Tambe SS. Determination of water soluble ash values of some medicinal plants of genus *Sesbania*. Journal of Pharmaceutical and Biological Research 2013; 1(1): 1 – 4.
37.Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines – a review. International Journal of Biodiversity and Conservation 2012; 4(3): 101 – 112.
38.WHO (1996a). Quality Assurance of Pharmaceuticals: A Compendum of Guidelines and Related Materials, Good Manufacturing Practices and Inspection. World Health Organization, Geneva. 2.
39.WHO (1996b). Guidelines for the Assessment of Herbal Medicines. WHO Technical Report Series, World Health Organization, Geneva. 863.