Asian Journal of Biotechnology and Bioresource Technology

6(2): 45-54, 2020; Article no.AJB2T.57332
ISSN: 2457-0125

Assessment of Proximate, Phytochemical and Selected Mineral Content of *Acanthus montanus* Leaf

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NAA and OTE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NCU and EKCI managed the analyses of the study. Author OVC managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJB2T/2020/v6i230079
Editor(s):
(1) Dr. Md. Shaheed Reza, Bangladesh Agricultural University, Bangladesh.
(2) Dr. Armando Zepeda Bastida, Universidad Autónoma del Estado de Hidalgo, Mexico.
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Reviewers:
(1) Dodo Juliet Dingtsen, University of Jos, Nigeria.
(2) Md. Nasir Ahmed, TechB Nutrigenomics, Bangladesh.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57332

Original Research Article

Received 02 June 2020
Accepted 09 August 2020
Published 19 August 2020

ABSTRACT

Acanthus montanus (Nees) T. Anders belongs to the family Acanthaceae and is one of the most threatened and underutilized species of vegetables in Africa. However the leaves of this plant are part of a consortium infusion (agabada nkwu) given to post-natal mothers within and around Mbaise in Imo State, South Eastern Nigeria, to ensure health and vitality of both nursing mother and child. The proximate, phytochemical and selected mineral contents of the *Acanthus montanus* leaf were investigated to ascertain the various components. The fresh plant sample was

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obtained from Ife, Ezinihitte Mbaise Local Government Area of Imo state, Nigeria. The sample was air-dried at room temperature for 72 hours prior to the various analyses, which were done using standard methods. The results of the phytochemical analysis showed 1.60 % flavonoids, 6.67 % tannins, 5.60 % alkaloids, 6.45 % saponins, 0.26 % phytate, 5.41% oxalate, 0.49 % phenol and 0.019 % Hydrogen Cyanide (HCN). The percentage proximate evaluation for moisture content, carbohydrate, protein, fibre, ash content and fat were: 11.85, 37.86, 17.72, 16.70, 10.56 and 5.31 respectively, while the percentage quantities of selected minerals; iron, magnesium, potassium, calcium, phosphorus, sodium, manganese, zinc and copper were: 0.014, 0.569, 3.152, 0.909, 0.089, 0.202, 0.009, 0.010 and 0.001 respectively. *Acanthus montanus* is reported for its nutritional and medicinal values throughout Africa. The result of this work suggests further exploitation of the parts of *Acanthus montanus* to unveil more of its potential uses for the treatment of diseases.

Keywords: Proximate; phytochemicals; mineral content; Acanthus montanus; leaf.

1. INTRODUCTION

Medicinal plants are plants that can be used in the prevention or treatment of a particular disease or ailment, they are not frequently or indiscriminately consumed as their non-medicinal counterparts, as they may cause harm, since they are "reservoirs" of crude drugs, with unspecified dosage and method of administration [1]. They may occur as wild plant species whereby they grow spontaneously and exist independently of any human activity or as domestic species arising from conscious and careful human activities such as breeding, selection and subsequent management [2]. They are the richest bioresource of drugs of traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs [3]. Sazada, et sl. [4] analysed preliminary phytochemicals in some of the important medicinal and aromatic plants.

Acanthus montanus is an example of a medicinal plant and an endangered species. It is commonly known as False thistle, Leopard's claws or Bear's breech. In Nigeria, it is called àgámobo in Edo, àgámeebu or àgá in Ibo, èdulèè ìmemeìn in Ijaw, and ahòn ékùn in Yoruba. In Sierra Leone, it is called mende kpete-pela.

Phytochemical screening of the root extract of *Acanthus montanus* yielded an abundance of alkaloids and carbohydrates with traces of saponins, glycosides and terpernoids [5]. GC-MC analysis of ethanolic extract of the leaves of *Acanthus montanus* yielded nine (9) compounds: 2,6-bis (1,1-dimethylethyl) -4-methyl phenol in 13.68%, allyl (2-tetrahydrofuryl methoxy) dimethysilane in 3.86%, sulfurous acid cyclohexylmethyl hexyl ester in 5.67%, alpha-methyl 4-methylmannoside in 8.41%, hexadecanonoic acid methyl ester in 16.12%, 11-octadecanoic acid methyl ester in 19.03%, docosane in 5.85%, N,N-dimethylvaleramide in 18.62% and 2,6,10,15-tetramethyl heptadecane in 8.76% [6]. Study of the alcohol extract of aerial parts of *Acanthus montanus* yielded nine (9) compounds: B-sitosterol-3-B-D-glucoside, palmitic acid, linaroside, homoplantegenin, 5,7,3'-trihydroxy-6,4'-dimethosy flavone-7-0-glucoside, shikimic acid, protochatecuic acid, blepharin and acetosode [7].

Adeyemi, et al. [8] investigated the analgesic effect of the methanolic leaf extract of *Acanthus montanus* in rats and mice. Their results showed dose dependent increase in pain threshold. The study indicated that the analgesic effect of the methanolic extract of *Acanthus montanus* is both centrally and peripherally mediated. The aqueous root extract showed moderate antimicrobial activity on *Staphylococcus aureus,* the usual pathogen in boils. It inhibited 57% topical acute edema in mouse ear induced by xylene and suppressed the development of rat paw enema in a non-dose-related manner. Growing vegetables like *Acanthus montanus* (amongst other edible plants) is an important income source for vegetable growing countries. These vegetables not only have nutritional benefits, but also meet up with personal and social needs such as curing diseases. They are rich in phytochemicals that are important for human health [9].

In Africa, where our daily diet is dominated by starchy staples, African indigenous leafy vegetables are considered the most readily available sources of important vitamins and minerals. These vegetables are important commodities for poor households because they are more easily affordable than other food items [9].

Image. 1. *Acanthus montanus* plant

Although *Acanthus montanus* has been reported to be used for the treatment of some diseases, studies still show that it is one of the most threatened and underutilized species of vegetables in Africa [10]. The probable cause of this is its highly perishable nature in addition to the little or no knowledge people have of the phytochemicals, minerals and vitamins present in these vegetables, hence the negligence of them. Local vegetables whose prices are very affordable can play important and beneficial roles in providing food, nutrition and also in securing the health of the impoverished populace [11]. In South-eastern Nigeria, particularly in Mbaise, Imo state. *Acanthus montanus* is boiled with other herbs and used as a post natal remedy, specifically for "colon cleansing and wellbeing". Igwe and Eleazu [12] investigated the effect of processing on the biochemical contents of raw, sun-dried and boiled samples of *A. montanus* leaf, it is therefore worthwhile to explore the properties of a room-temperature air-dried sample of this wonder plant.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The leaf of *Acanthus montanus* used in this study was collected from Ofeama Ife community in Ezinihitte Mbaise Local Government Area of Imo state. The sample was confirmed as *Acanthus montanus* by Mrs Passion Sam of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia state, Nigeria. The sample was taken to the Crop Science Laboratory in the Federal University of Technology, Owerri (FUTO) and air-dried at room temperature for 72hours, before analyses.

2.2 Quantitative Analysis of Phyto-chemicals

The determination Of Alkaloids, Flavonoids and Oxalate was carried out using the method of [13], which involves the gravimetric method of alkaline precipitation.

2.2.1 Alkaloids

Alkaloids was determined by adding 100cm3 of 20% v/v ethanolic acid solution to 2 grams of the plant sample powder placed in a 250cm3 Erlenmeyer flask. The flask was covered and allowed to stand for three hours with periodic stirring, filteration and then re-extraction etc. The percentage alkaloid was calculated thus:

$$\%\ Alkaloids = \frac{w3 - w2}{w1} X100$$

Where:

W3 = weight of filter and alkaloids after drying
W2 = weight of filter paper alone
W1 = weight of sample
100 = scaling factor to convert to percentage

2.2.2 Flavonoids

Flavonoids was determined by adding 100cm3 of 80% v/v methanol solution to 2g of the powdered sample in a 250cm3 Erlenmeyer flask, with

periodic stirring for 3 hours. The mixture was filtered using Whatman filter paper No. 42 and the residue was re-extracted with a fresh 100cm3 of 80% v/v methanol solution. The percentage flavonoid was calculated thus:

$$\%\ Flavonoids = \frac{w3 - w2}{w1} X100$$

Where,

w3 = weight of beaker and flavonoid.
w2 = weight of beaker alone
w1 = weight of sample
100 = scaling factor to convert to percentage

2.2.3 Oxalate

The determination of Oxalate was carried out, by adding 20cm^3 of 0.3M Hydrochloric acid solution to 5g of the powdered sample in a 100cm^3 Erlenmeyer flask. The contents of the flask was allowed to stand for 1hour, with periodic stirring and then filtered. The filterate was saved. The process was repeated twice, the filterates were combined and finally made up to 100.0cm3 with distilled water. To 20cm^3 of the filterate; 5drops of Phenolphthalein indicator was added. 5.0M ammonium hydroxide was added in drops till the reaction mixture turned alkaline. Glacial ethanoic acid was added in drops till the pink coloration disappeared. A few more drops were added to make the mixture acidic. 5.0cm3 of 5% calcium chloride solution was added to the solution and mixture was allowed to stand for 3 hours. Centrifugation was done at 300 r.p.m for 15 minutes and the residue was washed 3 times with hot water, using Centrifugation technique. The residue was dissolved in 4.0cm3 of 3.0M Tetraoxosulphate (VI) acid solution. The resulting solution was titrated with freshly prepared 0.01M Potassium permanganate solution till permanent pink coloration that lasted for 30 seconds was obtained. A blank titration was carried out using the same volume of 3.0M Tetraoxosulphate (VI) acid as that used in dissolving the oxalate residue. Thus the percentage oxalate was calculated thus:

MaVa = MbVb

Xg/l = Molarity x Molar mass

$$\%\ Oxalate = \frac{X}{1} x \frac{20a}{1000} x \frac{100a}{20b} x \frac{100b}{5}$$

Where,

X = Weight of oxalate obtained by multiplying molar mass and molarity of oxalate
20a = Volume of extract aliquot taken for analysis
1000 = Reference volume for molar concentration
100a = Total volume of extract
20b = Volume of extract aliquot taken for analysis
100b = Scaling factor to convert to percentage
5 = weight of sample taken for analysis

2.2.4 Phytate, saponin, hydrogen cyanide, phenol and tannin

The Phytate Content determination was carried out using the Colorimetric method as described by [14]. The saponin content was determined according to the method described by [15]. The Hydrogen Cyanide (HCN) content was determined by the simple Picrate spectrophotometric method of [16]. The total Phenol was determined using the Folin-Ciocalteu spectrophotometer [17]. While the tannin content was determined using the Folin-Dennis spectrophotometric method by [18].

2.3 Proximate Analysis

The Proximate evaluations were carried out as described by the method of [19].

2.3.1 Moisture

The moisture content was determined using the desiccator and dry oven method; whereby a container was washed, oven-dried and weighed. 2g of the sample was dried at 105°C, the sample and container were reweighed, oven-dried again and reweighed, until a consistent result is obtained. The percentage moisture content was calculated thus:

$$\%\text{Moisture content} = \frac{w1 - w2}{w1 - w} x100\%$$

Where:

W1 = mass of sample + container before drying
W2 = mass of sample + container after drying
W = mass of container

2.3.2 Ash

This was determined by washing, drying and cooling a crucible in a desiccator. The crucible was weighed. 2g of the sample was weighed into the crucible. The crucible with content was placed in a muffle furnace. The temperature was regulated to 575 +_ 25°C until it carbonised.

Calcination was done until black particles were no more. The furnace was turned off and allowed to cool, the crucible with content was then placed in a desiccator and weighed. The percentage Ash content was determined thus:

$$\%\ Ash\ content = \frac{(W3 - W1}{(W2 - W1} x \frac{100)}{1)}$$

Where:

W1 = mass of crucible
W2 = mass of crucible + sample before ignition
W3 = mass of crucible + ash after ignition
W2 - W1 = mass of sample taken for ignition

2.3.3 Crude fibre

Crude fibre was determined by placing 2g of the sample in a hot 200ml of 1.25M H_2SO_4 and boiled for 30minutes. It was filtered through a buckner funnel equipped with muslin cloth and held firm with an elastic band. This was followed by subsequent washing with boiling water, alcohol and then 3 times petroleum ether. The residue was drained, oven-dried to a constant mass, cooled and reweighed, and the percentage crude fibre was calculated as a loss in incineration mass; thus:

$$\%\ Crude\ Fibre = \frac{M3 - M4}{M2 - M2} x \frac{100}{1}$$

Where,

M1 = mass of crucible
M2 = mass of sample + crucible
M3 = mass of crucible + residue after drying
M4 = mass of crucible + ash after incineration

2.3.4 Crude fat

This was determined using the Soxhlet extraction method, after which the percentage crude fat was calculated thus:

$$\%\ Crude\ Fat = \frac{M2 - M1}{M3} x \frac{100}{1}$$

Where,

M1 = mass of the flask
M2 = mass of flask + fat
M3 = mass of sample

2.3.5 Crude protein

This was determined by transferring 0.2g of the sample into a Kjedahl flask containing boiling chips and a mixture of copper and sodium sulphate; added to increase the boiling temperature. 20ml conc. H_2SO_4 was added to assist oxidation and the mixture was heated until clear. It was cooled and transferred to a 100ml flask. A blank was prepared using same procedure. 2ml of the digest was pipetted and transferred to the distillation flask. 10ml of 2% boric acid was measured out into a receiver (small beaker) and two drops of methyl red indicator was added. It was ensured that the tip of the recovery tube extended below the surface of the boric acid solution. 35ml of 40% NaOH was added to the distillation flask and the plug was replaced. The mixture was distilled until 30ml of the distillate was collected. The same procedure was carried out for the blank experiment and titrated against standard 0.1N HCl. The percentage crude protein was calculated thus:

$$\%\ Crude\ Protein = \frac{(T - B) x\ NHCl\ x\ 6.25\ x\ Vol.Made\ x\ 0.00014}{Aliquot\ x\ mass\ of\ substance\ used} x \frac{100}{1}$$

Where:

T = titre value of sample
B = blank titre value
NHCl = normality of HCl used
Aliquot = sample aliquot taken
The volume it was made up to = 100cm3

2.3.6 Total carbohydrates

This was determined 'by difference' calculation, thus:

% Total carbohydrates = 100% - % (crude protein + ash + crude fat + moisture).

2.4 Selected Mineral Analysis

The sodium and potassium contents were determined using the Flame Photometry Method. The calcium and magnesium contents were determined by the complexiometric method using EDTA. The Iron content was determined using the Thiocyanate method. Copper, Manganese and Zinc contents were determined by using the Atomic Absorption Spectrophotometer Analysis, while the phosphorus content was determined by Spectrophotometry

3. RESULTS AND DISCUSSION

Figs. 1, 2 and 3 show the results for the percentage composition of phytochemical,

proximate and selected minerals of leaf of *Acanthus montanus.* This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as flavonoids, alkaloids and tannins were present in the sample. The result of the phytochemical analysis shows that the plant is rich in alkaloids, flavonoids, tannins, oxalate and saponin. Tannins, Saponins, Alkaloids and Oxalate were found to have higher quantities as they were 6.67%, 6.45%, 5.60% and 5.41% respectively while Flavonoids, Phenol, Phytate and Hydrogen Cyanide were in lower quantities as they were1.60%, 0.49%, 0.26% and 0.019% respectively.

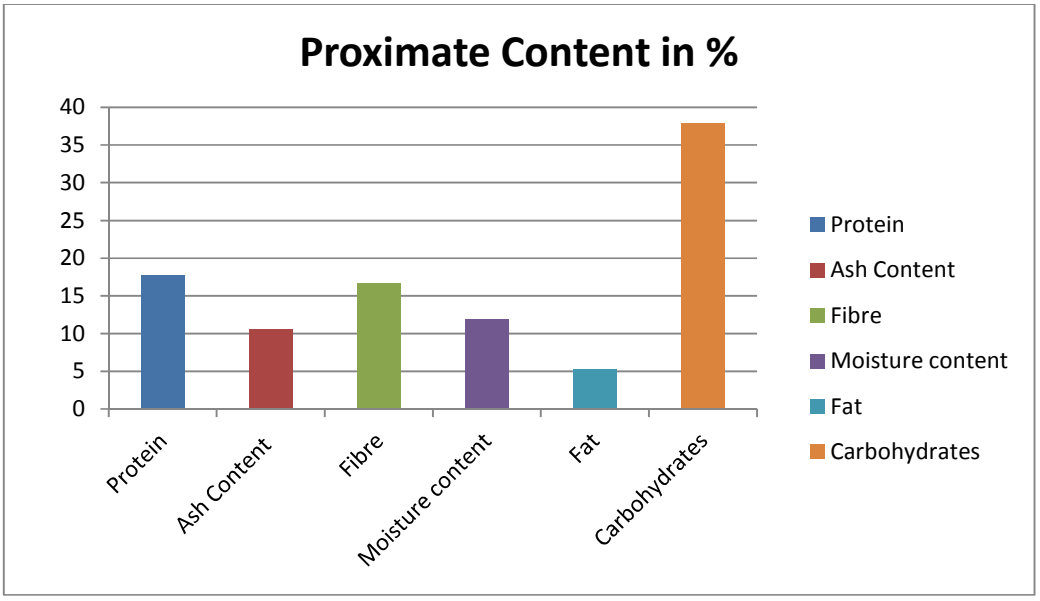

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Fig. 1. Proximate content of the leaf of *Acanthus montanus*

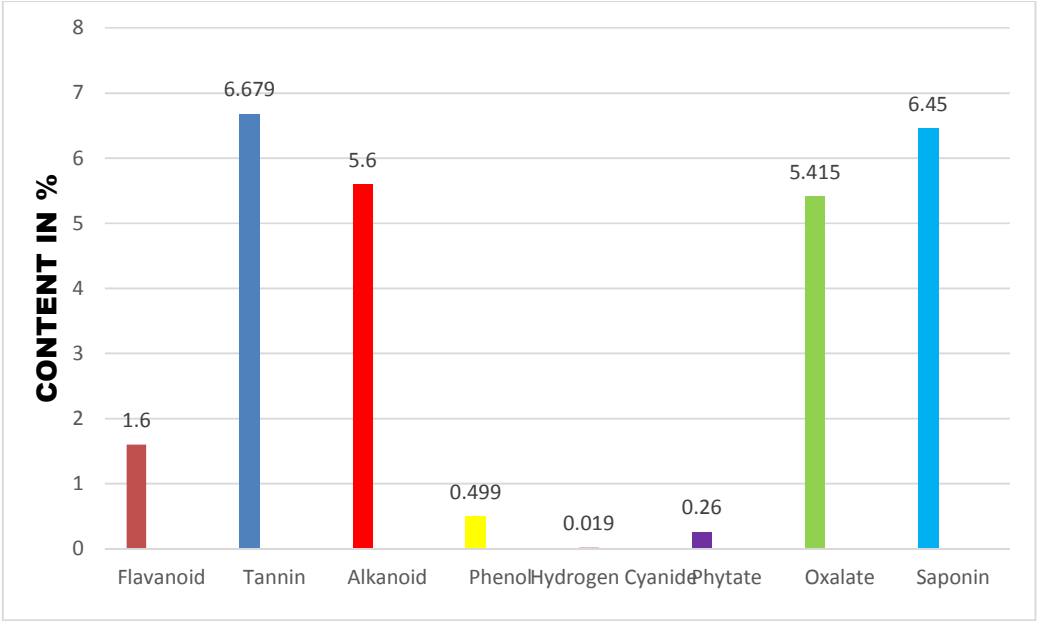

Fig. 2. Percentage phytochemical content of the leaf of *Acanthus montanus*

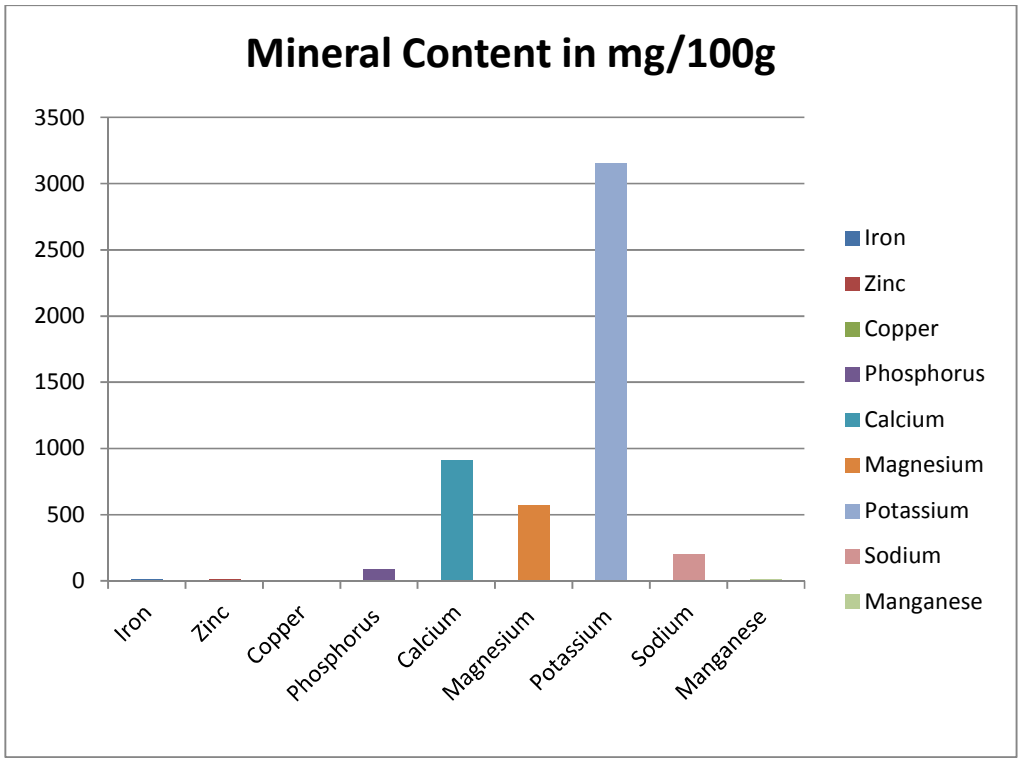

Fig. 3. Percentage mineral content of the leaf of *Acanthus montanus*

In the proximate analysis, Carbohydrate was found to be high, with a value of 37.86%. The leaf was also found to have moderate quantities of protein, fibre, moisture and ash which were 17.72%, 16.70%, 11.85% and 10.56% respectively. The fat content was found to be low (5.31%) compared to other parameters.

The study also revealed the presence of some minerals such as iron, zinc, copper, phosphorus, calcium, magnesium, potassium, sodium and manganese. Most of them occurred in minute quantities, however, potassium was found to occur in a relatively high quantity as it was 3152.48 mg/100 g. Other minerals, namely: Calcium, Magnesium, Sodium, Phosphorus, Iron, Zinc, Manganese and Copper were 909.94 mg/100 g, 569.82 mg/100 g, 202.69 mg/100 g, 89.30 mg/100 g, 14.96 mg/100 g, 10.39 mg/100 g, 9. 69 mg/100 g and 1.02 mg/100 g respectively.

The findings of this work agree with the work of [20] who reported that *Acanthus montanus* leaf is rich in alkaloids, saponins and tannins but with low phytate and flavonoids, however they reported high phenol content which does not agree with our findings here. It also agrees with Okoli, et al. [21] who reported that alkaloid is highly present in the leaf of *Acanthus montanus* but does not agree that saponin occurred in trace amounts. The presence of these phytochemicals in *Acanthus montanus* leaf makes it a potential source of useful drugs as they have many health benefits. Bonfilius [22] reported that alkaloids have health benefits such as: anti-malarial, painkillers, anti-tumour agents, stimulants and depressants. Alkaloids are the most efficient therapeutically significant plant substances. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents because of their analgesic, antisplasmodic, and antimicrobial properties [23].Yildrim and Kuflu [24] reported that tannins and saponins have anti carcinogenic properties because they can inhibit cancer cells under certain conditions. Also, clinical studies have suggested that saponins decrease blood lipids, lower cancer risks and lower blood glucose response; hence a high saponin diet can inhibit platelet aggregation and can act as an antidote for lead poisoning [25]. Hydrogen cyanide is very poisonous as it inhibits cell respiration; even in minute quantities. Codex Alimantarius Commission established the maximum permissible limit for HCN to be within the range of 2mg/kg – 50mg/kg. Necessary

conversion of the percentage HCN content in our test sample yields 0.19mg per gram of sample, which is still less than the lower permissible limit. In addition, studies have shown that, cooking cyanogenic plants thoroughly in boiling water (as is the case with the conventional preparation and consumption of our test plant), can effectively reduce their toxicity level.

In the proximate analysis, the carbohydrate value was found to be relatively high, which agrees with the findings of [24] but did not agree with the low quantities of protein, fibre and ash, which were moderate in this study. The fat content was found to be low compared to other parameters. The moisture content of the leaf was also moderate, probably due to the air-drying pretreatment of the sample. These values correlate with the previous reports of [10].

The results of the mineral content evaluation also revealed the presence of minerals in the leaf extract of *Acanthus montanus.* Some minerals such as iron, zinc, copper, phosphorus, calcium, magnesium, potassium, sodium and manganese were investigated. Most of them occurred in minute quantities, however, potassium was found to be in a relatively high quantity. The American Heart Association, estimated that increasing potassium intake would decrease the incidence of hypertension by 17% and would increase life expectancy by 5.1 years. A dietary intake of >3500 mg/d of potassium is recommended for the primary prevention of hypertension [26]. The considerable amount of potassium in the leaf of *Acanthus montanus* suggests that it might possess these properties, if consumed in the right amount [27]. However, the percentage mineral contents; with respect to the quantities analyzed, are not up to their recommended daily intake as recommended by [28].

4. CONCLUSION

Medicinal plants are a rich source of secondary metabolites, such as: alkaloids, tannins, saponins, oxalates, flavonoids etc. Medicinal plants play a vital role in preventing various diseases. The phytochemical, proximate and mineral analysis of medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. The test plant screened for phytochemical, proximate and mineral constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. This plant may therefore be exploited as an alternative potential future agent to synthetic preservatives in the pharmaceutical and food industries. The leaf extracts were shown to possess significant amount of phytochemicals such as saponins, tannins, flavonoids, oxalate and alkaloids and minerals like potassium, sodium, calcium and iron. The proximate study of the leaf extract also showed it possesses significant amount of carbohydrates, protein and fibre. These findings have been reported to promote homeostatic balance in patients and are relatively less toxic than synthetic drugs [27]. It can be concluded that the result of this work suggests further exploitation of the parts of *Acanthus montanus* to unveil more of its potential uses for the treatment of diseases which validate the use of undiluted extracts of this species in ethnomedicine and could provide a lead in the treatments of other diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://www.the healthbeat.com
19th May 2020.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57332