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Evaluation of Proximate Composition, Phytochemicals and Selected Mineral Elements Content of Methanol Extract of *Acmella radicans* (Jacquin) Leaves

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ABSTRACT
There is recently, a growing consumption of *Acmella radicans* (white spot flower) as food among many communities in northwestern Nigeria, a development which poses the need to evaluate its nutritive and therapeutic properties. This study, therefore, evaluated the proximate composition, phytochemicals and selected mineral elements contents of methanol extracts of leaves of *A. radicans* plant. The leaves were collected, dried, and extracted using methanol. The resulting extract was subjected to standard procedures for the analyses. The findings indicated that the extract of *A. radicans* had Ash, carbohydrate, crude protein, crude fibre, lipid and moisture contents of 10.28 ± 0.003%, 72.90 ± 0.042%, 3.42 ± 0.013%, 1.32 ± 0.003%, 6.99 ± 0.006% and 9.40 ± 0.039%, respectively. The qualitative phytochemicals analysis showed that the extract contain alkaloids, flavonoids, steroids, glycosides, cardiac glycosides, tannins, saponins, saponin glycosides, balsam and volatile oils. Anthraquinones were not detected. Quantitative phytochemical analysis of the extract revealed the concentrations (in g%) of alkaloids, cardiac glycosides, flavonoids, saponins and tannins as 6.44 ± 0.36, 0.46 ± 0.04, 24.56 ± 0.03, 0.42 ± 0.06 and 0.35 ± 0.20, respectively. The results also revealed the presence of mineral elements, such as sodium, potassium, calcium, phosphorus and magnesium, as 0.002 ±0.003, 0.053 ±0.009, 1280±2.887, 3.94 ± 0.015 and 360± 2.880, respectively. These results suggest that the leaves of *A. radicans* are rich source of macronutrients, bioactive compounds and mineral elements, and could be used as a potential dietary supplement to address protein-energy deficiencies, as well as for nutraceutical and pharmacological applications.

Keywords: *Acmella radicans;* proximate composition; phytochemicals; mineral elements

INTRODUCTION
Plants are considered indispensable for the sustenance of life on earth as they provide oxygen, food, and shelter for many organisms (Taiz & Zeiger, 2010). The plants produce various secondary metabolites that possess biological activities, such as alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds, which are responsible for the medicinal value of the plants, including properties such as antioxidant, anti-inflammatory, antimicrobial, antiviral, and anticancer (Wink, 2013). Therefore, the investigation of under-utilized plants, such as *Acmella radicans* for their nutritional potentials and biological activities is important in the quest for novel natural products with nutritive and therapeutic properties (Chandra & Lata, 2011). Among the plant parts, leaves play a vital role in the growth and development of plants because they serve as storage organs for carbohydrates and other nutrients.

Acmella radicans (Jacq.) R.K. Jansen is known with common names, such as white spot flower, toothache plant or electric daisy, and locally, as "Kabewan Daji" in Hausa language. It is a shrub that belongs to the Asteraceae family. The stem of the radicans is woody, green, and cylindrical, and the plant can grow up to 2 m in height. Previously, it is commonly found in the Southwestern United States and Mexico, but it is now found in many parts of the world, including northern Nigeria (Ahmed *et al.*, 2008). *A. radicans* contains several active compounds, such as flavonoids and phenolics, which have been found to possess various pharmacological properties, and it has therefore, been traditionally used for medicinal applications, such as anti-inflammatory, antimicrobial, analgesic and antiseptic (Alam and Akash, 2023). There is also a growing consumption of *A. radicans* as food among many communities in Northwestern Nigeria, recently. This development poses the need to evaluate its nutritive and therapeutic properties due to paucity of scientific literature on its nutritional composition, phytochemical constituents and biological activities. Thus, the results of this study could contribute to plant conservation efforts, facilitating food, drug and natural product development.

MATERIALS AND METHODS

Sample Collection and Preparation

The fresh leaves of *A. radicans* plant were plucked from the swampy farm in Usmanu Danfodiyo University Sokoto Main Campus and the plant was identified and authenticated in the herbarium section of the Botany unit, Department of Biological Sciences of the University, where a specimen was deposited for record purposes with Voucher Number; UDUH/ANS/478. The plant leaves were separated from its stems and roots, washed with distilled water, transferred to an open bowl, and dried to a constant weight at room temperature. The dry leaves were then pulverized into a powered form and stored in an airtight container.

Sample Extract

Exactly 5g of the powdered sample was weighed and soaked in a beaker containing 100 ml of methanol. The mixture was allowed to stand for 24 hours before being filtered using Whatman no.1 filter paper. The filtrate obtained was used to perform the analyses.

Proximate Analysis of the Extracts

Determination of Moisture Content

This is based on the principle that a known weight of biologically active material is exposed to heat under controlled conditions, this is achieved by placing the sample in an oven at 105ºC for 24 hours. The water from the material evaporates leaving the dry matter, and the difference in weight after heating gives the moisture content of the material (AOAC 1999). The Weight of empty petri-dish was measured and recorded as W_1. 2g of sample was poured in the petri-dish and measured as W_2. It was then placed in the oven at 105ºC for 24 hours after which the petri-dish was reweighed and recorded as W_3 the percentage moisture was calculated as:

$$\% \text{ Moisture } = \frac{W3 - W1}{W2} X\ 100$$

Determination of Ash Content

This is based on the principle that biological material, such as food when heated in muffle furnace at higher temperature of 600ºC have their organic matter burnt off, leaving the inorganic substance in the form of ash and the weight is expressed as percentage (AOAC, 2016). The weight of the empty crucible was measured and recorded as W_1. 2g of the sample was poured into the crucible and measured as W_2.

The crucible was then placed into a drying oven at a temperature of 600°C for 5 hours. It was allowed to cool in a desiccator, reweighed and recorded as W_3. The percentage ash content was calculated as:

$$\% \text{ Ash} = \frac{W3 - W1}{W2} X\ 100$$

Determination of Crude Protein

This process involves the oxidation of protein with Sulphuric acid (H_2SO_4) and the reduction of nutrient to ammonium sulphate, the subsequent addition of excess amount of NaOH in a closed system neutralizes the acid and release ammonium which was diluted into Boric solution and treated against 0.1N HCl to end point (Kjeldahl, 1883). For digestion, 0.4g of the sample was poured into the micro kjeldahl's flask. A catalyst (0.6g Selenium tablet) was added and 10ml of Sulphuric acid was poured. The flask was heated in a fume chamber until digestion was completed (indicated by clear solution or color change). After cooling, volume of the mixture in the flask was made to 50ml with distilled water. The solution (aliquot) of 10ml was poured into a conical flask and 20ml of Boric acid (indicator) was added and placed under the tip of the condenser. After distillation, 10ml of the solution (aliquot) and 20ml of NaOH were measured and poured into Kjeldahl's flask and heated. For titration, 0.01ml of HCl was titrated against the distillate until a colour change. The initial and final burette readings were recorded, and percentage crude protein was calculated as:

$$\% \text{ Nitrogen (\%N)} = \frac{TV\ X\ 0.014\ X\ 0.01\ X\ 50}{0.5\ X\ 10}\ X\ 100$$

Where TV= titer value; NA (normality of acid) = 0.01N; DF (dilution factor) = 0.014
Weight of sample = 0.5g; Volume of solution (aliquot) = 10ml; Volume of mixture = 50ml
% Crude protein = % N x 6.25 (Conversion factor)

Determination of Lipids

The lipids determination was based on the principle that non-polar components are easily extracted into organic solvent, which gives proportions of the true fat present, but does not give the particular fatty acid present (Oyeleke, 1984). Sample of 2g was weighed and recorded as W_2. The weight of empty flask was taken as W_1. The condenser, heater and the flask were arranged, the solvent and sample were poured, and oil was extracted for 6 hours. After cooling, the weight of the flask was measured and recorded as W_3. The measurement of W_3 was repeated in three replicates for variable results. The percentage lipid extracted was calculated as:

$$\% \text{ Lipid} = \frac{W3 - W1}{W2}\ X\ 100$$

Determination of Crude Fiber

This was based on sequential digestion of the sample with dilute acid and alkaline solution. Sample of 2g (W_3) was weighed and poured into a beaker, 20ml of 10 % H_2SO_4 was added, boiled for 30 minutes, filtered and the residue was washed with distilled water. The residue was placed into a beaker and 20ml of 10 % NaOH was added, boiled for 30 minutes and filtered.

The residue was washed with distilled water and then washed with Methanol and was scrapped into a crucible and heated at 105°C (drying). The crucible was weighed (W_1) and then placed into a muffle furnace and heated at 550°C. The crucible was reweighed as (W_2). The percentage crude fiber was then calculated as:

$$\% \text{ Crude fiber} = \frac{W3 - W1}{W2} X\ 100$$

The Qualitative Phytochemical Analysis

Test for Alkaloids

Exactly 2ml of 10% HCl was added into the test tube containing 2ml of the extract. 1ml was treated with a few drops of Wagner's reagent and the second 1ml portion was also treated with Mayer's reagent. Turbidity or precipitation with either of these reagents indicates the presence of alkaloids (Harbone, 1973).

Test for Flavonoids

Into 3ml extract, 1ml of 10% NaOH was added, and the development of yellow color indicates the possible presence of flavonoids (El-Oleyi *et al*; 1994, Harbone, 1998).

Test for Saponins

Into 5ml extract, 5ml distilled water was added and the mixture was mixed evenly. The whole tube was filled with a froth that lasts for minutes indicates presence of saponins (Harbone, 1973).

Test for Cardiac Glycosides

Into 0.5ml extract, 2ml of 3.5% Ferric chloride solution was added and allowed to stand for some minutes. 2ml of H_2SO_4 was carefully added along the wall of the test tube to form a layer. A reddish-brown ring at the interface indicates the presence of cardiac glycosides (Levine, 2017; Hui, 2008).

Test for Anthraquinones

Exactly 5ml extract sample was shaken with 10ml Benzene solution, followed by 5ml 10% Ammonia solution. The mixture was shaken and the formation of pink, red, or violet color in the ammoniacal phase indicates the presence of anthraquinones (Smith *et al.*, 2010).

Test for Steroids

Exactly 2ml extract was dissolved in 2ml Chloroform solution, and 2ml of Sulphuric acid was gently added to form a lower layer. A reddish-brown color at the interface indicates the presence of a steroid ring (Harbone, 1973).

Test for Glycosides

The 2.5ml of 50% H_2 SO_4 was mixed with 5ml of the extract in a test tube. The mixture was heated in boiling water for 15 minutes. It was then cooled and neutralized with 3ml of 10% NaOH, and 5ml of Fehling's solution was added into the mixture and boiled again. The formation of brick-red precipitation indicates the presence of glycosides (Harbone, 1973).

Test for Tannins

Into 2ml extract, 2ml of 5% Ferric chloride solution was added. The formation of colored products is confirmed. Condensed tannins give a dark green color while hydrolysable tannins give a blue-black color (Trease and Evans, 1978).

Test for Saponin Glycoside

The 2.5ml extract was treated with 2.5ml Fehling's solutions A and B. The formation of a bluish-green precipitate showed the presence of saponin glycosides (El-Oley *et al.*, 1994).

Test for Balsams

The 2 ml extract was mixed with an equal volume of 2ml 90% Ethanol, and 2 drops of alcoholic Ferric chloride solution were added to the mixture. The formation of dark green color indicates the presence of Balsams (El-Oley *et al*., 1994).

Test for Volatile Oils

The 1ml of the extract was mixed with dilute HCl. The formation of a white precipitate indicates the presence of volatile oils (Evans, 1980).

Quantitative Phytochemical Analysis

Determination of Steroids

The method involves extracting steroid compounds from plant samples and quantifying them using various analytical techniques. Steroids are a class of lipophilic compounds that are widely distributed in plants and have diverse biological activities. A powdered sample (0.1g) was placed inside a 100ml volumetric flask and 10ml of 100% methanol was added, the mixture was allowed to stand for 1 hour, and it was filtered using Whatman's filter paper in a 100ml beaker. Three test tubes were cleaned and labeled as test, standard, and blank. 1 ml of methanol extract was placed initially in the test tube, 1 ml of standard reagent was added into a standard test tube, and 1 ml of distilled water was added into a blank test tube. 2ml of 0.5% $FeCl_3$ was added into all the three test tubes respectively, 2ml of 4N H_2SO_4 was added into all the test tubes, and 0.5ml of potassium hexacyanoferrate was added in each test tube respectively. The solution was mixed and boiled for 20 minutes at 60°C and cooled. The absorbance of both the sample and the standard was measured spectrophotometrically at 780nm (Harbone, 1973). The concentration of steroids was calculated using the formula:

$$\text{Concentration of steroids} = \frac{\text{Absorbance of sample} \times \text{Conc of standard}}{\text{Absorbance of standard}}$$

Determination of Tannins

The method is based on the quantitative consumption of tannins and pseudo-tannins to iodine in an alkaline medium, which is attributed to their phenolic nature. True tannins, in contrast to pseudo tannins, can be removed from the extract by precipitation with gelatin, this can permit the determination of each group of constituents alone. Excess iodine is determined by titration, rendering acidic with sodium thiosulphate standard solution. The powdered sample (100mg) was put into a 100ml conical flask, 50ml of distilled water was added and boiled for 30 minutes in boiling water and filtered using filter paper. Then three test tubes were arranged and labeled as test, standard, and blank. 10ml of the extract was added into a test tube labeled test, 10ml of the standard tannic acid solution was added into a standard test tube and 10ml distilled water was also added into a blank test tube. 10ml of 17% Sodium carbonate was added into all the test tubes, and 2.5ml of Fill-in Dennis reagent was added into all three test tubes, respectively. The sodium was mixed to make the volume of 50ml with distilled water and incubated for 20 minutes at room temperature and the absorbance at 760nm recorded (Trease and Evans, 1978).

$$\text{Tannins} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Determination of Cardiac Glycosides

Cardiac glycosides develop an orange-red color complex with Ballet's reagent (Picric acid in an alkaline medium). The intensity of the color produced is directly proportional to the concentration of cardiac glycosides present in the sample (El-Olemyl *et al.*, 1994). One gram (1g) of the extract was extracted with 10ml of 70% alcohol and the mixture was filtered from the filtrate, 8ml was transferred to a 100ml volumetric flask and the volume was completed to the mark with distilled water. Then 8ml of which was added to 8ml of 12.5% Lead acetate. The mixture was shaken well, the volume completed to 100ml with distilled water, and filtered. 50ml of the filtrate was pipetted into another 100ml volumetric flask and 8ml of 4.7% Disodium hydrogen phosphate solution was added.

The mixture was made-up to the volume with distilled water and mixed. The mixture was filtered twice with filter paper. Then 10ml of Ballet's reagent was added to 10ml of the purified filtrate. A blank sample of 10ml of distilled water was also added to the 10ml Ballet's reagent. The two solutions were allowed to stand for one hour. A blank of 20ml distilled water was used to zero the spectrophotometer. The color was stable for several hours. The percentage of the total glycosides was calculated as digitizing by referring to the given standard curve or by simply using the E1%cm of digitizing which is 170.

$$\% \text{ Glycosides} = \frac{\boldsymbol{A \times 100\ g}\%}{\boldsymbol{17}}$$

Where A is the absorbance of the test sample at 495nm.

Determination of Saponins

Saponins are soluble in water or boiling dilute alcohol precipitated on the addition of Acetone (El-Olemyl *et al*., 1994). A powdered sample (50g) was placed in a 50ml flask containing 300ml of 50% alcohol. The mixture was boiled under reflux for 30 minutes and was immediately filtered through a coarse filter paper. Then 2g of charcoal was added, and the control was boiled and filtered while hot. The extract was cooled. Some saponins may be collected by the precipitate and dissolved in boiling 95% alcohol and filtered while hot to remove any insoluble matter. The filtrate was allowed to cool at room temperature resulting in the precipitation of saponins which was collected by presentation and suspended in about 20ml of alcohol and filtered. The filter paper was immediately transferred to a desiccator containing anhydrous Calcium chloride and the Saponins were left to dry and weighed.

$$\% \text{ Saponins} = \frac{\boldsymbol{W3 - W1}}{\boldsymbol{W2}} \boldsymbol{X\ 100}$$

Determination of Alkaloids

Common among alkaloids salt is soluble in water, while the free basis of alkaloids is soluble in an organic solvent. This fact is made use of in the extraction of free alkaloids with organic solvents. The powdered sample (5g) was weighed into a 250 ml beaker and 200 ml of 20% Acetic acid ethanol was added and covered to stand for 4 hours, this was filtered, and the extract was concentrated using a water bath to 1/4 of the original volume. Concentrated Ammonia solution was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected by filtration using weighed filter paper (Harbone, 1973).

$$\% \text{ Alkaloids} = \frac{\boldsymbol{W3 - W1}}{\boldsymbol{W2}} \boldsymbol{X\ 100}$$

Determination of Flavonoids

The principle of this quantification was based on the precipitation method by Bohme and Kocipal, (1994). The powdered sample (5g) was hydrolyzed by boiling it in 100 ml of the Hydrochloric acid solution for 30 minutes. The hydrolysate was filtered to recover the filtrate. The filtrate was treated with ethyl acetate dropwise until excess.

The precipitated flavonoids were recovered by filtration using a weighed filter paper after drying in an oven at 100°C for 30 minutes, it was then cooled and reweighed. The difference in the weights gave the weighed flavonoids which were expressed as percentage of the weighed sample analyzed (Boham and Kocipai, 1994).

$$\% \text{ Flavonoids} = \frac{W3 - W1}{W2} X\ 100$$

Determination of Selected Minerals' Composition

Sodium and Potassium were determined using Flame photometer, according to the method described by Oladele and Aina, 2007. Phosphorus using Spectrophotometer, while Calcium and Magnesium were determined using Atomic Absorption Spectrophotometer (AAS). Dry digestion method was employed where 2g of the sample was taken into a crucible (which has been dried and weighed) and placed in a muffle furnace at a temperature of 600^0C for 3 hours. This crucible was cooled in a desiccator and weighed, 5ml of 10% HCl, was added to the crucible to dissolve the ash residue.

The Flame photometer was calibrated by aspiring distilled water as blank and 1000mg/l of Sodium standard was used to set the machine to 100 scale reading. The sample was aspirated at 589 nm and the reading recorded. For Potassium, 0.5ml of the aliquot was taken and diluted with 20ml of distilled water, the sample was aspired at 768 nm and the reading recorded. Standard curves were prepared for Sodium and Potassium, from the curves the concentration Na^+ and K^+ in the sample were obtained by extrapolation. All dilutions were considered in making calculations.

Na (mg*l*) = reading value × D.F.; K (mg*l*) = Reading value × D.F.
Where: D.F. = Dilution factor

Determination of Phosphorus (Spectrophotometry)

This method is based on the principle that, in an acid Molybdate solution containing orthophosphate ions, phophomolybdate complex is formed, which can be reduced by ascorbic acid or other reducing agents (example $SnCl_2$) in the presence of potassium antimony tartrate to form a blue coloured heteropolymolybdic complex. Two ml of the diluted sample was taken in to 50ml volumetric flak, 2ml of phosphorus extracting solution and 2ml of Ammonium molybdate were added into the sample and mixed. Distilled water was added to make it 50ml, after addition of 1ml of dilute stannous chloride. The colour intensity was measured at 660nm wavelength using a spectrophotometer. The concentration of phosphorus was obtained on extrapolation from the standard curve, and this was calculated as:

Phosphorus (mg/kg) =Absorbance × DF_a × DF_b × CF
Where: DF_a = Absolute dilution factor; DF_b = Dilution factor
CF= Conversion factor.

Determination of Calcium and Magnesium (AAS)

This method uses EDTA which forms a complex with Calcium and Magnesium ions. A blue dye Eriochrome Black T (ErioT) is used as the indicator. This blue dye forms a complex with the Calcium and Magnesium ions, changing colour from blue to pink in the process. The dye-metal ion complex is less stable than the EDTA-metal ion complex.

Into 2ml of the sample, 2ml of phosphorus extracting solution and 2ml of Ammonium molybdate were added and mixed well. Distilled water was added to make volume up to 50ml, 1ml of diluted Stannous chloride was added and absorbance measured at 660 nm wavelength using Spectrophotometer. This was calculated as:

$$\frac{A\left(\frac{mg}{l}\right) \times Dilution\ Vol.(1)}{Mass\ of\ Sample}$$

Statistical Analysis

All data were subjected to statistical analyses. The values were expressed as Mean ± Standard deviation using the statistical software; Instat 3 version (San Diego, USA). $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

The proximate composition of *A. radicans* leaves extract is presented in Table1. The result reveals that the sample has 9.40% moisture content. Higher moisture content is associated with a rise in microbial actions during storage (Ahmed *et al*. 2008), therefore the leaves should properly be dried in a low temperature environment before storing. The moisture content can also vary depending on the plant's growing conditions and processing methods. A study conducted in India reported a moisture content of 86.9% for *Acmella calva*, a related species. Another study conducted in Nigeria by Nwachukwu and
Ohaeri, 2016 reported a moisture content of 89.03% for *Acmella uliginosa*. These values are higher than the moisture content obtained in this study, which confirmed the drying of this sample before analysis.

Table 1: Proximate Composition of *Acmella radicans* leaves extract

S/No	Parametre	Concentration (%)
1.	Ash	10.28 ± 0.003
2.	Carbohydrate	72.90 ± 0.042
3.	Crude Protein	3.42 ± 0.013
4.	Fibre	1.33 ± 0.003
5.	Lipids	6.99 ± 0.006
6.	Moisture	9.40 ± 0.039

Values are expressed as mean ± SD of three replicates.

Table 2: Qualitative phytochemical screening of *Acmella radicans* leaves extract.

S/No	Phytochemical	Detection
1..	Alkaloid	++
2.	Anthraquinones	ND
3.	Balsams	+
4.	Cardiac glycosides	+
5.	Flavonoids	+++
6.	Glycosides	+
7.	Saponins	+
8,	Saponin glycosides	+
9.	Steroids	++
10.	Tannins	+
11.	Volatile Oils	+

Key: +: present; ++: moderate amount; ND: not detected.

Table 3: Quantitative phytochemical screening of *Acmella radicans* leaves extract.

S/No	Phytochemical	Concentration (g%)
1..	Alkaloids	6.44 ± 0.36
2.	Cardiac glycosides	0.46 ± 0.04
3.	Flavonoids	24.56 ± 0.03
4.	Saponins	0.42 ± 0.06
5.	Steroids	6.01 ± 0.05

Values are expressed as Mean ± SD of three replicates.

Table 4: Selected Mineral Elements Composition of *A. radicans* leaves extract.

S/No	Phytochemical	Concentration (mg/100g)
1.	Sodium	0.002 ± 0.003
2.	Potassium	0.053 ± 0.009
3.	Calcium	1280 ± 2.887
4.	Phosphorus	3.94 ± 0.015
5.	Magnesium	360 ± 2.887

Values are expressed as Mean ± SD of three replicates.

The relatively high moisture content suggests that their plant samples are fresh and has not been subjected to excessive water loss. The moisture content values are important indicators of the plant's freshness and purity (Ahmed *et al*. 2008). These proximate values for *A. radicans* leaves can be compared with those of other plants to assess its nutritional and dietary value. For example, compared to other leafy vegetables, *A. radicans* leaves have relatively low levels of crude protein and fiber but is high in carbohydrates.

The ash content of the leaves shows that they have a good amount of inorganic matter which is an index of the amount of the mineral elements present in the vegetables (Nicholas *et al.* 2017). The ash content of *A. radicans* leaves was similar to 10.04% reported by Nwachukwu & Ohaeri 2016) for *Acmella uliginosa* in Nigeria, a related species of *Acmella radicans*. Another study conducted by Oliveira *et al.,* (2014) reported an ash content of 7.64% for *Acmella oleracea*. The ash content provides information about the presence of minerals and other inorganic compounds in the plant. The result obtained in this study indicate that the leaves of *A. radicans* could supplement the body with some of the required macro elements. The relatively low crude fiber content of *A. radicans* leaves suggests that it may not be an optimal source of fiber for individuals looking to increase their dietary fiber intake. It is believed that fiber content reduces the level of cholesterol in human blood and decreased the likelihood of different cancers, dietary fiber consists of non-stomach polysaccharide such as cellulose, resistant starch, resistant dextrin, etc (Alam and Akash, 2023). Women should try to eat at least 21 to 25 grams of fiber per day, while men should aim for 30 to 35 grams per day, fiber also uses to regulate the body use of sugars, helping to keep hunger and blood sugar in check (Alam and Akash, 2023).

The lipid content of *A. radicans* obtained shows that the leaves extract contains less amount of lipid when compared with the amount of oil present in other oil seeds like groundnut, which may also vary depending on the plant's growing conditions and processing methods. A study conducted in Brazil by Oliveira *et al.,* (2014) reported a lipid content of 10.3% for *Acmella oleracea*, another related species. Another study conducted in India by Shinde *et al.,* (2016) reported a lipid content of 14.33% for *Acmella calva*.

These values are higher than the lipid content reported in this study, which could be due to differences in growing conditions, processing methods, or analytical techniques (Nwachukwu, 2016). Lipids are any of the various organic compounds that are insoluble in water but soluble in organic solvents. Lipid helps in supporting cells and aid in essentials functions such as in protecting nerve cells (Nicholas *et al.* 2017). The crude protein obtained was 3.42%, protein is an essential component of the diet needed for survival of humans and animals, which function basically in nutrition by supplying adequate amount of required amino acids (Pandey *et el.*, 2019),

The high carbohydrate content obtained (72.90 %) suggest that consumption of the leaves could provide the body with the needed fuel and energy for daily activities and exercises (kumar *et al.* 2020) Adequate carbohydrate is also needed for optimum function of the brain, heart, nervous, digestive and immune system, while the deficiency of carbohydrate causes depletion of the body tissues (Han, 1998). These results indicate that *Acmella radicans* is a good source of carbohydrates and has moderate levels of lipids, and can therefore, be useful for individuals looking to incorporate *A. radicans* leaves into their diet for its nutritional benefits.

The qualitative phytochemical result (Table 2) showed the presence of alkaloids, flavonoids, steroids, glycosides, cardiac glycosides, tannins, saponins, saponin glycosides, balsam and volatile oils, while the quantitative result (Table 3) showed alkaloids (6.44), cardiac glycosides (0.46), flavonoids (24.56), saponins (0.42) and tannins (0.35). From the results, the concentration of flavonoids is higher than the rest of the parameters followed by alkaloids and steroids, while the concentration of tannins, saponins, and cardiac glycosides was found to be lower than all the phytochemicals' parameters estimated. These results lend credence to the fact that *A. radicans* leaves have been traditionally used to treat various ailments and suggest that the plant has the potential to be used for therapeutic purposes in the treatment of various diseases. Devi *et al.*, (2013) reported that *A. radicans* exhibits antimalarial, analgesic, anti-inflammatory, antioxidant, and anticancer properties.

The study also reveals the presence of some important mineral elements that play a vital role in maintaining homeostasis (Table 4). The most abundant here is calcium (1280 mg/100ml) and magnesium (360 mg/100ml). Calcium is very essential in blood clotting, muscle contraction and the activity of certain enzymes and metabolic processes and its deficiency can lead to osteoporosis (Owolabi *et al.,* 2015). Magnesium is an actual component for several enzymes and activator for the phosphate transferring enzyme myokinase, and a constituent of bones, teeth and as enzyme cofactor (Rahman *et al.*, 2018). Potassium is essential in the maintenance of cellular water balance, pH regulation in the body and it is also associated with protein and carbohydrate metabolism (Sarojini and Anita, 2013). The sodium obtained was very small, suggesting that *A. radicans* leaves are not good source of sodium, which is an essential nutrient involved in the maintenance of normal homeostasis and in the regulation of fluid and electrolyte balance, as well as high blood pressure (Rahman *et al.*, 2016).

CONCLUSION

From the results of this study, it can be concluded that *A. radicans* leaves contains appreciable amounts of macronutrients, bioactive compounds and some mineral elements and may serve as a potential dietary supplement to address protein-energy deficiencies, as well as for the development of nutraceuticals novel therapeutic agents.

REFERENCE

Ahmed, Z.U., Begum, Z.N.T., Hassan, M.A., Khondker, M., Kabir, S.M.H., Ahmed, M., Ahmed, A.T.A., Rahman, A.K.T. and Haque, E.U. (eds.). (2008). *Encyclopedia of Flora and Fauna of Bangladesh*. Vol.6. Asiatic Society of Bangladesh, Dhaka. pp. 340-341.

Alam, M.M. and Akash, S.R. (2023). In vitro pharmacological activities of methanol extract of *Acmella oleracea* leaves: A variety grown in Dhaka, Bangladesh. *Indian J Microbiol Res* 2023;10(1):43-49.

AOAC (1999). Methods of Analysis of Association of Officials of Analytical Chemists. 16th Ed. Washington D.C. 1: 600 – 792.

Association of Official Analytical Chemists (AOAC). (2016). Official methods of analysis of AOAC International (20th ed.). AOAC International.

Boham, BA., Kocipai-Abyazan, R., (1974). Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium. *Pacific Science*. 48: 458-463.

Chandra, S., & Lata, H. (2011). Variations in secondary metabolites of medicinal plants collected from different natural habitats. *Journal of Herbs, Spices & Medicinal Plants*, 17(4), 341-359.

Devi, R. C., Gupta, G., Deb, L., & Dash, S. K. (2013). *Spilanthes acmella L.*: A Review on its Medicinal Properties. *Journal of Chemical and Pharmaceutical Research*. 5(3), 71-79.

El-Oleyi, A. M., Barbour, E. K., Al Mariri, A., & Al-Qudah, M. A. (1994). Phytochemical analysis and antibacterial activity of some medicinal plants in Jordan. *Pharmaceutical Biology*. 32(2), 109-114.

Evans, W. H. (1980). A biochemical dissection of the functional polarity of the plasma membrane of the hepatocyte. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *604*(1), 27-64.

Hahn, T. (1998). Classification of phytochemicals: A challenge for nutrition research and public health. *Nutrition Reviews*. 56(3), 84-96. doi 10.1111/j.1753-4887. 1998.tb01652. x.

Harborne, J.B. (1973). Alkaloids. In Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis (2nd ed., pp. 120-124). Chapman and Hall Ltd.

Hui, Y.H. (2008). Handbook of food science, technology, and engineering: 4-volume set. CRC Press.

Kjeldahl, J. (1883). New Method for the Determination of Nitrogen. *Chem. News*. 48 (1240) : 101 – 102.

Kumar, V., Roy, B. K., & Verma, A. K. (2020). Glycosides: classification, properties, and their pharmaceutical applications. *International Journal of Pharmaceutical Sciences and Research*. 11(6), 2556-2568.

Levine, K.E. (2017). Techniques for phytochemical analysis. In M. DeClerck (Ed.), Handbook of Analytical Techniques in Agriculture: Volume 2: Separation and Characterization of Agricultural Products (pp. 149-176). CRC Press.

Nichols, M. G., Ferguson, B. S., & Tracy, C. M. (2017). Cardiac glycosides: New roles for old drugs. Clinical therapeutics. 39(9). 1847-1854.

Nwachukwu, I. N. and Ohaeri, O. C. (2016). Phytochemical and proximate analyses of Acmella uliginosa (Sw.) Cass. *International Journal of Advanced Research in Chemical Science*, 3(11). 14-20.

Oladele, A.K. and Aina, J.O. (2007). Chemical composition and functional properties of flour produced from two varieties of tiger nut (*Cyperus esculentus*). *Afr J Biotechnol* 6: 2473–6.

Oliveira, L. P., Almeida, F. F. C., Santos, M. V. F., Ribeiro, L. P. and Vasconcelos, M. A. S. (2014). Chemical composition and fatty acid profile of shoots and leaves of *Spilanthes oleracea L*. *Journal of Food Science and Technology*. 51(5). 983-987.

Owolabi, M. S., Lawal, O. A., Oguntoye, S. O. and Agbaje, E. O. (2015). Analgesic and anti-inflammatory activities of *Acmella radicans* extract in mice. *Journal of Ethnopharmacology*. 172, 170-173.

Oyeleke, A.O. (1984). Outline of Food Analysis. Department of Biochemistry. Ahmadu Bello University Zaria, Nigeria.

Pandey, K. B., Rizvi, S. I., & Plant, V. (2019). Flavonoids as nutraceuticals: A review. *Tropical Journal of Pharmaceutical Research*. 18(11). 2319-2327.

Rahman, M. A., Uddin, S. B., Wilcock, C. C. and Wood, J. R. (2018). Ethnobotanical and phytochemical review of genus *Acmella* with potential medicinal uses. *Journal of Ethnopharmacology*. 215, 211-236.

Sarojini, V., & Anitha, R. (2013). Pharmacological properties of Spilanthes acmella–A review. *International Journal of Pharmaceutical Sciences and Research.* 4(7). 2389-2396.

Shinde, S. D., Jadhav, V. M. and Yadav, S. R. (2016). Acmella calva DC. (*Asteraceae*): A new record for the flora of Maharashtra, India. *Journal of Threatened Taxa*. 8(8). 9169-9172.

Smith, T., Kaleb, J., Muhammad, C., & Johnson, R. (2010). Phytochemical analysis of medicinal plants. *Journal of Natural Products*. 73(2). 204-215.

Taiz, L., & Zeiger, E. (2010). Plant physiology. Sinauer Associates, Incorporated.

Trease, G. E., & Evans, W. C. (1978). Pharmacognosy. Bailliere Tindall, London.

Wink, M. (2013). Functions of plant secondary metabolites and their exploitation in biotechnology. *Annual Plant Reviews*. 39, 1-3.