Original Research Article

PROXIMATE AND PHYTOCHEMICAL ANALYSIS OF CUCUMBER (CUCUMIS SATIVUS L.CV PIPINO) AND WATERMELON (CITRULLUS LANATUS (THUNB.) MATSUM AND NAKAI CV RED.

ABSTRACT

Nutritional and phytochemical constituents of plants are essential for the survival and general wellbeing of all life forms. These constituents were analyzed from *Cucumis sativus* and *Citrullus lanatus* which are heavily consumed fruits in most households in Nigeria. Both the pulp and rind components of the fruits were analyzed using the standard methods described by Association of Official Analytical Chemist (AOAC) and other scholars. Proximate analysis of the two fruits showed a significant difference in the organic constituents. The percentage ash content, crude protein and total carbohydrate contents were higher in *Cucumis sativus* compared to those of *Citruslanatus*with Mean+SD values of 10.50±0.50, 4.00±1.00 and 19.07±6.27 as against 8.47±0.47, 2.33±0.58 and 4.67±1.32 respectively. The moisture contents, percentage fat and crude fibre content were however higher in amount in *Citrullus lanatus* than those recorded in *Cucumis sativus*. In general, both fruits had highest amount of moisture and lowest fat contents when compared to other proximate constituents. Qualitative results of the various phytochemicals tested in both fruits revealed that Alkaloids were slightly present in the two fruits. Saponins and flavonoids were heavily present in *Cucumis sativus* but slightly present in *Citrullus lanatus*. The results also show that both fruits have glycosides and tannins in smaller quantities while Oxalate was slightly present in *Cucumis sativus* but in much small quantity in *Citrullus lanatus*. Quantitative analysis of the phytochemicals showed that flavonoid was highest in *Cucumis sativus* (7.13%) followed by saponin (6.16%) also in *Cucumis sativus*. Saponin was the only phytochemical found in larger quantity in *Citrullus lanatus* (2.50%). This study revealed that *Cucumis sativus* and *Citrullus lanatus* contains appreciable nutrients and phytochemical constituents that can be used by all living organisms.

KEYWORDS: Proximate analysis, Phytochemical constituents, Cucumber, Life forms, Watermelon.

INTRODUCTION

Plants are essential for the survival and wellbeing of all life forms. Plants and its derivatives provide man with basic necessity of life such as clothing, shelter and food [1]. The significant of fresh fruits and vegetable in our daily nutrients' requirements cannot be overestimated [2]. According to a study by Ngoddy and Ihekoronye[3], fruits and vegetables offered the most rapid

methods of providing adequate supplies of vitamins, minerals and fibre to people. They are also food sources with low energy density which are useful in weight management [4].
Plants have vast array of biologically-active constituents that pose potential danger to man and other animals. With the knowledge on the ability of the compounds to exert toxic and biological response when ingested, there is a called for urgent investigation, with a view to ascertain their activities in the biological systems [5]. Natural substances of plant origin such as alkaloids, tannins, trypsin (protease), saponnins, flavonoids, phenols, gums, terpenoids, polysaccharides, oxalates, phytates, oxalic acid, cyanogenic glycosides, haemagluttinins (lectins), coumarins and gossypol have been proven active with enormous ability to elicit biological responses [6]. Harborne [7] and Okwu [8] refer to these metabolic chemicals as "secondary metabolites." Preventive medicine has been immensely enhanced by the use of these natural plants' antioxidants.

The medicinal value of plant lies in the phytochemical (bioactive) constituents of the plant which shows various physiological effects on human body. Therefore, through phytochemical screening one could detect the various important compounds which may be used as the bases of modern drugs for curing various diseases [9]. According to WHO/FAO, [l0], consumption of fruits and vegetables at least 400g daily as well as whole-grains, cereals and legume at least 30g daily is recommended as the optimum diet for everyone. Fruits contain high quantity of water, carbohydrates, vitamins A, B_1, B_2, C, D, E and minerals such as calcium, magnesium, zinc, iron, potassium and organic compounds which are required in small amounts to make the body function properly [11,12,13].Watermelon is a pleasant-tasting fruit and one of the most economically important fruit in the Curcurbitaceae family. The fruit has both nutritional and medicinal values [14]. The juice expressed from the pulp can be made into wine while the seeds are consumed as snacks. Cucucmber on the other hand is also an important vegetable and a member of the cucurbitaceae family. The plant like other members of the family has fruit which contains high water content of about 95%. Its rind has high potassium and magnesium which help to relax nerves and muscle and keeps blood circulating smoothly [15].

With the increasingly importance of the fruits in many households in Nigeria as part of daily diets, the selection of the fruits for this study becomes paramount. This is traceable to the prevalent of cases of diabetics and other related diseases that are caused by the consumption of carbohydrate foods that form the major diets of people in tropical countries like Nigeria. This study was aimed at examining the proximate and phytochemical composition of the two fruits (*Cucumis sativus* and *Citrullus lanatus*).

MATERIALS AND METHODS

Collection and preparation of samples

The fresh fruit samples namely, *Cucumis sativus* and *Citrullus lanatus* were purchased at opened markets in Calabar and taken to the Chemistry Laboratory, Faculty of Physical Sciences; University of Calabar after identification by Plant Biologists. The sampled fruits were washed in

5% hypochloride solution. They were then rinsed several times with distilled water before subjecting them to analysis. The fruit samples were sliced with a cleaned knife to separate the rind (exocarp) from the pulp (mesocarp). The seeds were carefully removed from the pulp. The rind was chopped into tiny cubes while pulp was shredded. Each sample was transferred into separate trays lined with foil, appropriately labeled and dried at room temperature for minimum duration of three weeks. The rinds and pulps of each fruit samples were mixed and grounded into powdered form as described by Martin *et al.*,[16] and stored for subsequent use.

Proximate Composition

Proximate analysis of the powdered samples was conducted using the standard methods described by Association of Official Analytical Chemist (AOAC) [17]. The methods are outlined as follow:

Determination of Moisture Content

Clean aluminum dish was in each case dried at a temperature of 105^{o}C for a period of one hour in an oven after which it was cooled in a desiccator. The empty aluminum dish was then weighed. The dish was reweighed after adding 2g of the sample and was returned to the oven where it was allowed to dry to a constant weight at a temperature of 105^{o}C. The dish was removed, cooled in a desiccator and reweighed.The percent moisture content of the samples was then calculated.

Determination of Ash Content

Crucible container was well cleaned and weighed. Two gram (2g) of the pretreated sample powders were in each case poured into the crucible and reweighed. The crucible containing the samples was then placed in a furnace set at 600 ^{o}C, and was allowed to stay for a period of 3 hours; long enough for the sample to turn to a whitish-grey ash after which it was transferred to a desiccator and allowed to cool. The crucible containing theash was then reweighed. The percent ash content of the samples wasthen calculated.

Determination of Crude Protein Content

Crude protein in the sample was determined by Kjeldahl method. Approximatelsy 1.0 g of the ground samples were taken in digestion flask. Ten milliliter (10ml) of concentrated sulphuric acid (H_2SO_4) and 8 g of digestion mixture ($K_2SO_4CuSO_4$ in the ratio of 8:1) was added. The flask was swirled to mix the contents thoroughly and placed on heater to start digestion till the mixture becomes clear. Complete digestion was achieved for 2 hours 30 minutes. The digest was cooled and transferred to 100 ml volumetric flask and volume was made up to mark by the addition of distilled water. 10 milliters of digests were in each case introduced in the distillation tube and 10 ml of 0.5 N NaOH was gradually added. Distillation was continued for at least 10 min and NH_3 produced was collected as NH_4OH in a conical flask containing 20 ml of 4% boric acid solution with few drops of methyl red indicator. The distillate was titrated with against standard 0.1 N HCl solution till the appearance of pink colour. A blank was also run through following the steps as explained above. Percent crude protein for the two samples was calculated as %N × 6.25* (*Correction factor).

Determination of Crude Fibre

Five grams (5g) of the moisture free samples were accurately weighed, after extracting for about 3 hrs with petroleum ether, using Soxhlet apparatus. The fat free samples were transferred to a 1 litre flask. Two hundred millitres (200ml) of dilute Sulphuric acid (H_2SO_4)was taken in a beaker and bring to boiling. The whole content of the boiling acid was transferred to the flask containing the fat free samples and immediately the flask was connected with a water-cooled reflux condenser and heated to boil within a minute. The flask was rotated frequently, taking care not to allow the sample to stick to the sides of the flask and not to keep the sample out of contact with the acid. Boiling was continued for 30 minutes. The flask was removed and filtered through a fine linin cloth (about 18 threads to the centimeter) held in the funnel and washed with boiling water until the washings were no longer acidic to litmus. Two hundred millitres of Sodium hydroxide (NaOH) solution was boiled under reflux condenser. The residues on the linen cloth were washed into the flask with 200ml of boiling Sodium hydroxide solution and immediately connected the flask with reflux condenser and boil for exactly 30 minutes.

After 30 minutes, the flask was removed immediately and filtered through the filtering cloth. The residue was thoroughly washed with boiling water and transferred to a compact layer of ignited asbestor wash (the residue – thoroughly, first with hot water and then with about 15ml of ethyl alcohol 95 percent by volume). The Gooch crucible was dried with content at 105+2°C in an air oven to constant weights cooled and weighed. The contents of the Gooch crucible were then incinerated in an electric muffle furnace at 600±20°C until all the Carbonaleous matter was burnt. The Gooch crucible containing the ash was cooled in a dessicator and weighed.

Determination of Fat Content

The fat content was determined using Soxhlet apparatus. Two gram (2g) of the sampleswas weighed into a thimble and 200 ml of petroleum ether was measured using measuring cylinder. The solution was poured into round bottom flask and was heated at 45^{o}C for 2 hours at 1hour interval. The collecting flask was removed and cooled in a desiccator for 15 minutes and percentage fat of the sample was determined.

Determination of Carbohydrate

Carbohydrate was determined by difference. This was achieved by subtracting the sum of moisture, ash, protein, crude fat and crude fibre percentage from hundred.

Phytochemical Analysis

The phytochemical analysis for the present of saponins, tannins, alkaloids, oxalates, flavonoids and cyanogenic glycosides in the samples was carried out according to standard methods. Each analysis was done in duplicates. These were as follow:

Determination of Saponins

(a) Qualitative test

This test was done according to a method by Ram and Sinha, [18]. Half gram (0.5g) of extract was taken in a test tube and few drops of 5% Sodium bicarbonate ($NaHCO_3$) solution were added. The mixture was shaken vigorously and kept for 30 minutes. Formation of honey comb like froth showed the presence of Saponins.

(b) Quantitative Analysis

Five grams (5g) of sample was dispensed in 50cm^3 20% ethanol in a beaker. The suspension was heated over a hot water bath for 4 hrs with continuous stirring at about 60°C.The mixture was filtered after 4hrs and the residue was re-extracted with another 20cm^3 of 20% ethanol. The combined extract was concentrated to reduce to 40 cm^3 over water bath at 90°C. The content was transferred into a separatory funnel and 20 cm^3 of diethylether was added and shaken thoroughly. Aqueous layer of the extract was recovered while the other layer was discarded.

The purification process was repeated and 60 cm^3 of n-butanol was added and the extract was washed twice with 10 cm^3 of 50% aqueous Sodium chloride. The remaining extract was evaporated in a water bath and doiled in oven to a constant weight. The Saponin content was then calculated in percentage.

Determination of Tannins

(a) Qualitative Test: *Ferric Chloride Test.*

Five millitres (5ml) of aqueous extracts were stirred with 10ml of distilled water, filtered and 1% $FeCl_3$ addedto the filtrate. Blackish blue precipitate indicated the presence of hydrolysis of tannins (Callic) while blackish green precipitate indicated the presence of tannins (Cothecol).

(b) Quantitative Analysis

The analysis was done using the method by Amadi *et al.*,[19]. Half gram (0.5g) of the sample was weighed into plastic bottle and 50 cm^3 of water was added, shaken for 1hr in a shaker. It was then filtered and 5 cm^3 of the extract was measured to a test tube and mixed with 3cm^3 of 0.1NHCl and 3 drops ferrocyanide. It was allowed to stay for 10min, and then measured in the Uv- Spectrophotometer at 605nm. Blank too was determined.

Determination of Alkaloids

(a) Qualitative test

Thiswas doneusing the method described by Sofowora, [20] as shown below:

Preparation of Reagents

I. Mayer's reagent

1.357g of $HgCl_2$ and 5g of potassium iodide (KI_2) were dissolved in 100ml of water to give the working solution.

II. Dragendorff's reagent

1.7g Bismuth sub-nitrate was dissolved in 20ml of Glacial acetic acid. 50% solution of potassium iodide in 100ml water was added to the mixture to give the working solution.

Method

Three milliliters (3ml) of the aqueous extracts was put in a test tube and treated with 3ml of 1% HCl keep for 10 minutes in a water bath. Few drops of Mayer's and Dragendorff reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

(b) Quantitative Analysis

Quantitative determination of alkaloids was according to the methodology by Harborne, [7].Five grams (5g) of sample was weighed into a 250cm^3 beaker. 100 cm^3 of 10% acetic acid in ethanol

(1 to 1 ratio) was added to each powdered sample and allowed to stand for 4 hours. The extracted sample was filtered after four hours. It was then concentrated using water bath to a quantity of the original volume. Ammonia solution was added to the concentrated sample (extract) drop wise until the precipitate was allowed to settle, then was filtered and washed with dilute ammonium hydroxide.

The residue left was taken as the crude alkaloid. It was then dried in an oven and weighed.

Determination of Glycosides

(a) Qualitative test

Five grams (5g)of samples were in each case dissolved in 2ml of pyridine. 5 drops of 2% Sodium nitropouside and 3 drops of 20% Sodium hydroxide were added. Production of deep red colour which fades to brownish yellow is a positive test for cardenolide glycone portion of glycoside.

(b) Quantitative Analysis

This was done using the method by Amadi *et al.*,[19] with slight modifications. Five grams (5g) of each powdered sample was weighed into a 500ml round bottom flask. 200ml of distilled water was added to stand for 2 hours before it was distilled into a 2.5% Sodium hydroxide. 100ml of the distillate was collected. 25 aliquot was taken with additional 8ml of 6N NH_4OH and 2ml of 5% KI and titrated with 0.02N AgNO3 solution until a permanent turbidity indicating the end point. The experiment was in each case repeated two other times.

Determination of Oxalates

(a) **Qualitative test**

Exactly 0.5g of each powdered sample was extracted with dilute HCL. To 5ml of the extract, 1ml of 5N ammonium hydroxide was added and then made acidic with acetic acid in the presence of phenolthlene (2 to 3 drops). One millimeter of 5% $CaCl_2$ was added to the mixture and allowed to stand for 3hours. The white precipitate formed was an indication of the presence of oxalates.

(b) Quantitative Analysis

Oxalates quantitative determination was carried out using the method reported by Ejikeme *et al.*,[21] and Munro and Bassir[22]. Exactly 20 cm^3 of 0.3 M HCl in each powdered sample (2.50 g) was extracted two (2) times by warming at a temperature of 50°C for 1 hour with constant stirring using a magnetic stirrer. For oxalate estimation, 1.0 cm^3 of 5 M ammonium hydroxide was added to 5.0 cm^3 of extract to ensure alkalinity. Addition of 2 drops of phenolphthalein indicator, 3 drops of glacial acetic acid, and 1.0 cm^3 of 5% calcium chloride to make the mixture acidic before standing for 3 hours. This was followed by centrifugation at 3000 rpm for 15 minutes. After discarding the supernatant, the precipitate was washed three times using hot water by mixing thoroughly each time centrifugation. Then, to each tube, 2.0 cm^3 of 3M tetraoxosulphate (VI) acid was added and the precipitate dissolved by warming in a water bath at 70°C. Freshly prepared 0.01 M potassium permanganate ($KMnO_4$) was titrated against the content of each tube at room temperature until the first pink colour appears throughout the solution. The solution was allowed to stand until it returned colourless, after which it was

warmed on an electric hot plate at 70°C for 3 minutes, and retitrated again until a pink colour appears and persists for at least 30 seconds. Titration reactions of oxalates in each sample was calculated and recorded.

Determination of Flavonoids

(a) Qualitative Test

The test was carried out using a method by Sofowara[23] and Harborne [7]. Each sample (0.30 g) weighed into a beaker was extracted with 30 cm^3 of distilled water for 2 hours and filtered with Whatman filter paper number 3. To 10 cm^3 of the aqueous filtrate of each powdered extract was added 5 cm^3 of 1.0 M dilute ammonia solution followed by the addition of 5 cm^3 of concentrated tetraoxosulphate (VI) acid. Appearance of yellow colouration which disappeared on standing shows the presence of flavonoids.**(b) Quantitative Analysis**

Thiswas done according to the method reported by Ejikeme *et al.*,[21] and Boham and Kocipai[24]. Five grams of each sample was weighed into beakers and extracted with 50cm^3 of 80% methanol at room temperature for 1hour. The solutions were filtered through Whatman filter paper 3. Each sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath and oven. The contents in the crucibles were cooled in a desiccator and weighed until constant weights were obtained. The results were then recorded.

RESULTS

Proximate Composition

The results of proximate analysis of the powdered cucumber and watermelon showed

Table 1: Proximate Analysis of powdered Cucumber (*Cucumis sativus*)

Proximate	MC	ASH	FAT	CF	CP	TC
R1	60	11	2	8	4	26
R2	65	10	1.2	7	3	13.8
R3	63	10.5	2.4	7.1	5	17.4
Mean +SD	62.67±2.52[a]	10.50±0.50	1.87±0.61	7.37±0.55	4.00±1.00	19.07±6.27

NB: Superscript presents significant Analysis of Variance ($p < 0.05$) for all the proximate parameters replicates

MC = Moisture content, **Ash** = Ash Content, **Fat** = Percentage fat or lipid content, **CF** = Crude fibre content, **CP** = Crude protein, **TC** = Total carbohydrate, **R**= Sample reading

a significant difference in the organic constituents (Tables 1 and 2).All proximate parameters recorded from *Cucumis sativus* and *Citrullus lanatus* differed significantly. The percentage ash content, crude protein and total carbohydrate contents were higher in *Cucumis sativus* compared to those of *Citruluslanatus*. Proximate compositions such as moisture contents, percentage fat and crude fibre content were however higher in amount in *Citrullus lanatus* than those recorded in *Cucumis sativus*. Generally, both fruits showed the highest amount of moisture and lowest fat contents when compared to other proximate constituents.

Proximate	MC	ASH	FAT	CF	CP	TC
R1	70	9	2.1	10	3	5.9
R2	75	8.1	2.82	8.81	2	3.27
R3	72	8.3	2.56	9.35	2	4.85
Mean +SD	72.33±2.52[a]	8.47±0.47	2.49±0.36	9.39±0.60	2.33±0.58	4.67±1.32

NB: Superscript presents significant Analysis of Variance ($p < 0.05$) for all the proximate parameters replicates

MC = Moisture content, **Ash** = Ash Content, **Fat** = Percentage fat or lipid content, **CF** = Crude fibre content, **CP** = Crude protein, **TC** = Total carbohydrate, **R**= Sample reading

Phytochemical Analysis

The two fruits (*Cucumis sativus* and *Citrullus lanatus*) were analyzed for the presence of phytochemicals. They were tested qualitatively and quantitatively.

Qualitative Tests

Table 3 showsqualitative results of the various phytochemical components tested in both fruits.

Fruit Sample	Alkaloids	Saponins	Glycosides	Tannins	Oxalates	Flavonoids
Cucumber	++	+++	+	+	++	+++
Watermelon	++	++	+	+	+	++

Heavily present: +++; slightly present: ++; present: +

Alkaloids were slightly present in the two. Whereas there was heavy presence of saponins and flavonoids in *Cucumis sativus*, the two phytochemicals turned to be slightly present in *Citrullus lanatus*. The results also show that both fruits have glycosides and tannins in smaller quantities (present). Oxalate was slightly present in *Cucumis sativus* but in much small quantity in *Citrullus lanatus*.

Quantitative Analysis

Figure 1 shows quantitative analysis of the various phytochemicals tested. Of the examined phytochemicals, flavonoid was highest in *Cucumis sativus* (7.13%) followed by saponin (6.16) also in *Cucumis sativus*.

Saponin was the only phytochemical found in larger quantity in *Citrullus lanatus* (2.50%). The least phytochemical was tannin (0.42 mg/100g) in *Citrullus lanatus*. Student t-test comparism of the various phytochemicals showed a non-significant ($p > 0.05$) value.

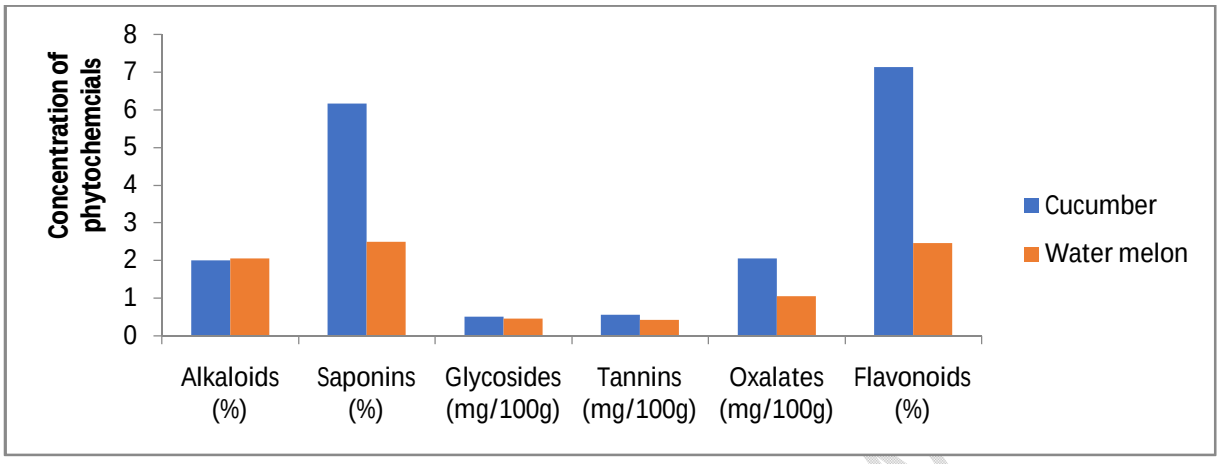

Fig 1: Quantitative analysis of the photochemical components of Cucumber and Watermelon fruits

DISCUSSION

Plants in the Curbitaceae family have been generally known to have high amount of water in their fruits [25]. The high moisture contents in both fruits are responsible for rapid deterioration when infested by microorganisms. According to the findings of this research, *Citrullus lanatus* will be prone to faster deterioration given its higher moisture content as compared to *Cucumis sativus*. However, Olayinka and Etejere, [25] reported significantly higher moisture content in *Cucumis sativus* than in *Citrullus lanatus*. Fruit difference indicated *Cucumis sativus* contained higher amount of ash content and total carbohydrate than *Citrullus lanatus*.Samples with high percentage of ash and total carbohydrate contents are expected to yield much quantity of pectin - which is another form of carbohydrate. The fat content was generally low in both fruits as compared to all other proximate composition. The result agreed with the observation made by Ngoddy and Ihekoronye[3] and the recent findings of Fila et al.,[26] that fruits contain high percentage of water averaging 85% but have not been a very good source of fat. The crude protein values in *Cucumis sativus* were relatively higher than those in *Citrullus lanatus*. Olayinka and Etejere[25] reported lower values of protein in both fruits.

Chemical compounds produced as a result of metabolic reaction during plant growth are known as phytochemicals [27]. Harbone, [7] and Okwu, [8] refer to such metabolic chemicals as "secondary metabolites" which include flavonoids, tannins, alkaloids, coumarins, terpenes, terpenoids, phenols, gums, polysaccharides, and glycosides. In this research, most of these phytochemical constituents were determined from the two fruits *Cucumis sativus* and *Citrullus lanatus*. Tannins which were expressly present in both fruits are one of the major active ingredients found in plant-based medicines [28]. They serve as caustics of cationic dyes (tannin dyes) used in the dyestuff industry as well as in the production of inks (iron gallate ink). Other uses of tannin are for wine, fruit juice, and beer clarification in food industry [29], antioxidant in beverages, and coagulant in rubber production. Glycosides play numerous important roles in living organisms. Many plants store chemicals are in the form of inactive glycosides which are

mostly used as medications. Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion, and progression of tumors [30]; reduction of coronary heart disease has been reported to be associated with intake of flavonoid [31]. Findings from this research have shown that both fruits contain appreciable quantity of flavonoid, with *Cucumis sativus* having the highest amount. Other biological functions of flavonoid include protection against platelet aggregation, free radicals, hepatotoxins, inflammation, and allergies [32]. Saponins were found in appreciable quantities in both fruits. They protect against microbial attack in plants and are also useful in treating yeast and fungal information [27]. Oxalates are found in almost all plants, but some plants contain very high amounts while others have very little. Findings from this research show that oxalate was slightly present in cucumber but in much small quantity in watermelon. Once consumed, oxalate can bind to minerals to form compounds, including calcium oxalate and iron oxalate. This mostly occurs in the colon, but can also take place in the kidneys and other parts of the urinary tract. Many fruits that contain oxalates are delicious and provide many health benefits. They are vital in regulation of bulk-free calcium levels in tissues and organs, and in light regulation during photosynthesis [33]. According to Sodipoet al.,[34], most phytochemicals serve as natural antibiotics, which assist the body in fighting microbial inversion and infections. Alkaloids, for instance, consist of chemical compounds that contain mostly basic nitrogen atoms which occur naturally, mainly, in plants but may be produced by bacteria, fungi, and animals. In this research, appreciable quantities of alkaloid were obtained in both *Cucumis sativus* and *Citrullus lanatus* (Fig 1). Alkaloids have a wide range of pharmacological activities including antimalaria (e.g., quinine), anticancer (e.g., homoharringtonine), antibacterial (e.g., chelerythrine), and antihyperglycemic activities (e.g., piperine).

CONCLUSION

The findings from this study have shown that the two fruits (*Cucumis sativus* and *Citrullus lanatus*) are good sources of vital nutrients and phytochemicals essential for the survival and wellbeing of many live forms.

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