RJPHYTO
Research Journal of
Phytochemistry

OPEN ACCESS

Phytochemical and Mineral Content in Flesh of *Psidium guajava* Fruit

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ABSTRACT

Background and Objective: *Psidium guajava* is one of the top-rated tropical fruits, rich in high-profile nutrients, such as minerals and phytochemicals. Guava contains a large number of antioxidants and phytochemicals. This research analyses the phytochemical and mineral content of *Psidium guajava*. **Materials and Methods:** The phytochemical analysis was carried out using standard methods of AOAC while elemental analysis was conducted using agilent FS240AA atomic absorption spectrophotometer according to the method of the American Public Health Association. **Results:** The result shows the various phytochemical constituent concentration of *Psidium guajava*, tannins 11.48±1.45, cardiac glycoside 6.98±3.01, flavonoid 2.49±1.40, anthocyanide 7.345±0.997. The mineral constituent result review potassium 7.028±0.47, sodium 5.49±1.19 and calcium 5.93±0.82. The presence of these essential phytochemicals and minerals in guava confer high nutritional qualities and antioxidant ability, flavonoids in plants are known for their scavenging ability as a result of the presence of hydroxyl groups contained in them. **Conclusion:** The result suggested that the guava fruit if consumed in sufficient amounts could contribute greatly towards meeting the human nutritional requirement for normal growth and adequate protection against disease arising from Reactive Oxygen Species (ROS). From the result, *Psidium guajava* could be recommended for use for nutritional purposes.

KEYWORDS

Guava fruit, improved specie, local specie, mineral element, phytochemicals

INTRODUCTION

Guava (*Psidium guajava)* is one of the top-rated tropical fruits rich in high-profile nutrients. With its unique flavour, taste and health-promoting qualities. *Psidium guajava* and its all parts have an old history of medicinal value[1]. It has a huge content of antimicrobial and antibacterial compounds[2]. Ethanol extracts of the stem have a high anti-diabetic activity[3,4]. Guava contains a large number of antioxidants and phytochemicals including essential oils, polysaccharides, minerals, vitamins, enzymes, triterpenoid acid alkaloids, steroids, glycosides, tannins, flavonoids and saponins[5]. Guava contains a higher content of vitamin C and vitamin A. Guava is also a very good source of pectin which is an important dietary fibre. It has a high content of flavonoids[6], fructose sugar and carotenoids.

Guava (*Psidium guajava*) is a common tropical fruit cultivated in many tropical and subtropical regions. It has been cultivated and is one of the most common fruits in Nigeria[7]. It has become popular because

ISSN: 2151-6081 (Online)
ISSN: 1819-3471 (Print)
https://doi.org/10.17311/rjphyto.2022.88.96

Received: 11 Jan. 2022
Accepted: 28 Jul. 2022
Published: 01 Oct. 2022

of its availability almost throughout the year[8]. The fruit is prominently rich in vitamin C and this fruit provides remarkable antioxidant property[9]. The root, bark, leaf and immature fruits are commonly used in traditional medicine. *Psidium guajava* (common name-guava) is a well-known tropic tree which is abundantly grown for fruit. It belongs to the phylum Magnoliophyta, class Magnoliopsida and Myrtaceae family[10]. Ascorbic acid and citric acid are the major ingredients of guava that play important role in anti-mutagenic activity[11].

The skin of guava fruit contains ascorbic acid in very high amounts, the strong pleasant smell of the fruit is credited to the carbonyl compounds. Guava fruit contains terpene, caryophyllene oxide and p-selinene in large quantities which produce relaxation effects[12].

Essential oil is present in leaves which contain α-pinene, limonene, β-pinene, isopropyl alcohol, menthol, terpenyl acetate, caryophyllene, longicyclene and β-bisabolene. Oleanolic acid is also found in the guava leaves. Leaves have a high content of limonene about 42.1% and caryophyllene about 21.3%. Leaves of guava have a lot of volatile compounds[13].

The bark includes 12-30% of tannin and one source declares that it includes tannin of 27.4%, or polyphenols, resin and crystals of calcium oxalate. Tannin is also present in roots. Leukocyanidin, gallic acid and sterols are also present in roots. Carbohydrates with salts are present in abundance. Tannic acid is also its part. *Psidium guajava* is consumed also as folk medicine in subtropical areas all over the world due to its pharmacologic activities[14]. Guava has a high content of important antioxidants and has a radio-protective ability. Quercetin is considered the most active antioxidant in the guava leaves and is responsible for its spasmolytic activity. This project aimed to investigate the phytochemical and some selected mineral content of improved and local white guava flesh.

MATERIALS AND METHODS

Study area: The study was carried out at Biochemistry Department, Biochemistry Department Research Laboratory, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, from May to June, 2021.

Sample collection: Guava samples were collected from Eke Awka. The samples were identified and authenticated by the curator of the herbarium in the Department of Botany, Nnamdi Azikiwe University.

Sample preparation: Samples were prepared by cutting and separating the seeds from the flesh, then oven-drying them. Drying lasted for one week after which the dried samples were subjected to grinding into fine powder.

Phytochemicals

Oxalate determination by titration method: This determination involves three major steps digestion, oxalate precipitation and permanganate titration

Digestion:

- Weighed 2 g of sample and suspended it in 190 mL of distilled water in a 250 mL volumetric flask
- 10 mL of 6 m HCl was added and the suspension was digested at 100°C for 1 hr
- It was cooled and then made it up to the 250 mL mark before filtration

Oxalate precipitation: Duplicate portions of 125 mL of the filtrate were measured into beakers and four drops of methyl red indicator were added. This is followed by the addition of NH_4OH solution (drop-wise) until the test solution changes from a Salmon pink colour to a faint yellow colour (pH4-4.5). Each portion

was then heated to 90°C, cooled and filtered to remove precipitate containing ferrous ions. The filtrate is again heated to 90°C and 10 mL of 5% $CaCl_2$ solution is added while being stirred constantly. After heating, it was cooled and left overnight at 25°C. The solution was then centrifuged at 2500 rpm for 5 min. The supernatant was decanted and the precipitate was completely dissolved in 10 mL of 20% (v/v) H_2SO_4 solution.

Permanganate titration: At this point, the total filtration resulting from the digestion of 2 g of sample was made up to 300 mL. Then 125 mL of the filtrate was taken and heated. The heated aliquat was titrated against 0.05 M $KMnO_4$ solution to a pink colour that persists for the 30 sec. The calcium oxalate content was calculated using the formula[15].

Calculation:

$$= \frac{T \times (Vme)\ (Df) \times 105\ (mg/100g)}{(ME) \times Mf}$$

where, T is the titre of $KMnO_4$ (mL), Vme was the volume-mass equivalent (i.e., 1 mL of 0.05 m $KMnO_4$ solution was equivalent to 0.00225 g anhydrous oxalic acid), Df was the dilution factor Vt/A (2.4 where, Vt was the total volume of titrating (300 mL) and A was the aliquot used (125 mL), ME was the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of sample used.

Alkaloids determination: Five grams of the sample was weighed into a 250 mL beaker and 200 mL of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs at 25°C. This was filtered with filter paper No. 42 and the filtrate was concentrated using a water bath (Memmert) to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). Then, filter with pre-weighed filter paper. The residue on the filter paper is the alkaloid, which was dried in the oven (precision electro-thermal model BNP 9052 England) at 80°C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed[15,16].

Calculation:

$$\text{Weight of alkaloid (\%)} = \frac{\text{Weight of filter paper with residue-Weight of filter paper}}{\text{Weight of sample analyzed}} \times 100$$

Flavonoids determination: Weighed 10 g of the plant sample and was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight[17].

Calculation:

$$\text{Flavonoids (\%)} = \frac{(\text{Weight of crucible+residue}) - (\text{Weight of crucible})}{\text{Weight of sample analyzed}} \times 100$$

Determination of saponin: Exactly 5 g of the sample was put into 20% acetic acid in ethanol and allowed to stand in a water bath at 50°C for 24 hrs. This was filtered and the extract was concentrated using a

water bath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage[16].

Calculation:

$$\text{Saponin content (\%)} = \frac{(\text{Weight of filter paper+residue})-(\text{Weight of filter paper})}{\text{Weight of sample analyzed}} \times 100$$

Cardiac glycosides determination: 1 mL of the extract was added 1 mL of 2% solution of 3,5-DNS (Di-nitro Salicylic acid) in methanol and 1mL of 5% aqueous NaOH. It was boiled for 2 min (Until brick-red precipitate was observed) and the boiled sample was filtered. The weight of filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50°C till dryness and the weight of the filter paper with residue was noted[18].

The cardiac glycoside was calculated in percentage.

Calculation:

$$\text{Cardiac glycoside (\%)} = \frac{(\text{Weight of filter paper+residue})-(\text{Weight of filter paper})}{\text{Weight of sample analyzed}} \times 100$$

Tannin determination by folin's dennis titration: The Folin's Dennis titrating method[19] was used. To 20 g of the crushed sample in a conical flask was added 100 mL of petroleum ether and covered for 24 hrs. The sample was then filtered and allowed to stand for 15 min allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100 mL of 10% acetic acid in ethanol for 4 hrs. The sample was then filtered and the filter ate collected.

The 25 mL of NH_4OH was added to the filter ate to precipitate the alkaloids. The alkaloids were heated with an electric hot plate to remove some of the NH_4OH still in the solution. The remaining volume was measured to be 33 mL. The 5 mL of this was taken and 20 mL of ethanol was added to it. It was titrated with 0.1 NaOH using phenolphthalein as an indicator until a pink endpoint was reached. Tannin content was then calculated in percentage ($C_1V_1 = C_2V_2$) molarity.

Calculation data:

C_1 = Concentration of tannic acid
C_2 = Concentration of base
V_1 = Volume of tannic acid
V_2 = Volume of base

Therefore:

$$C_1 = \frac{C_2 V_2}{V_1}$$

$$\text{Tannic acid content (\%)} = \frac{C_1}{\text{Weight of sample analyzed}} \times 100$$

Phytate determination: Phytate contents were determined[20]. The 0.2 g of each of the differently processed corns was weighed into different 250 mL conical flasks. Each sample was soaked in 100 mL of 2% concentrated HCL for 3 hrs, the sample was then filtered. The 50 mL of each filtrate was laced in a 250 mL beaker and 100 mL distilled water was added to each sample. The 10 mL of 0.3% ammonium thiocynate solution was added as the indicator and titrated with standard iron (111) chloride solution which contained 0.00195 g iron per 1 mL.

$$\text{Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19}{\text{Weight of sample}} \times 100$$

Estimation of total phenols

Principle: Phenols react with phosphomolybdic acid in the Folin-Ciocalteu reagent to produce a blue-coloured complex in an alkaline medium, which can be estimated spectrophotometrically at 650 nm[18].

Reagents:

- Ethanol (80%)
- Folin-Ciocalteu reagent (1N)
- Sodium carbonate (20%)
- Standard catechol solution (100 μg mL^{-1} in water)

Procedure: Exactly 0.5 g of the sample was homogenized in 10× volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The sample ion was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipette out and the volume in each tube was made up to 3.0 mL with distilled water. Folin-ciocalteu reagent (0.5 mL) was added and the tubes were placed in a boiling water bath for exactly 1 min. The tubes were cooled and the absorbance was read at 650 nm in a spectrophotometer (Genesis 10-S, USA) against a reagent blank. Standard catechol solutions (0.2-1 mL) corresponding to 2.0-10 μg concentrations were also treated as above.

The concentration of phenols is expressed as mg g^{-1} tissue.

Heamagglutinin determination: Accurately 2 g of each of the samples were added to 20 mL of 0.9% NaCl and suspension shaken vigorously for 1 min. the supernatant was left to stand for 1 hr, the sample was then centrifuged at 2000 rpm for 10 min and the suspension filtered. The supernatants in each were collected and used as crude agglutination extract. Absorbance read at 420 nm[18].

Determination of anthocyanin in the water of life using the gravimetric method of harborne

Principle: Acid hydrolyzed sample when filtered reacts with ethyl-acetate to enable extraction of anthocyanin. Upon addition of amyl alcohol, anthocyanin was extracted and after drying, the percent composition was determined concerning the weight of the original sample gravimetrically[15].

Procedure: The 5.0 g of the powdered sample was boiled in 100 mL of 2MHCl for 30 min. The hydrolysate was filtered using whatman filter paper. The filtrate was transferred into a separation funnel and an equal volume of ethyl-acetate was added, mixed and allowed to separate into two layers. The ethyl-acetate layer was recovered while the aqueous layer was discarded.

The extract was dried over a steam bath. The dry extract was then treated with 50 mL of concentration Amy-alcohol to extract the anthocyanin. After filtration, the alcohol extract was dried. The weight of anthocyanin was determined and expressed as a percentage of the original sample.

Calculation:

$$g\ (\%) = \frac{\text{Weight of anthocyanin}}{\text{Weight of the original sample}} \times 100$$

Determination of steroid content: The 1.0 g of the powdered sample was weighed and mixed with 100 mL of distilled water in a conical flask. The mixture was filtered and the filtrate was eluted with 0.1 N ammonium hydroxide solution. The 2 mL of the eluent was put in a test tube and mixed with 2 mL of chloroform. The 3 mL of ice cold acetic anhydride was added to the mixture in the flask. Two drops of (200 mg dL^{-1}) standard sterol solution were prepared and treated as described for the test as blank. The absorbance of standard and test was measured, zeroing the spectrophotometer with blank at 420 nm[18].

Calculation:

$$(\text{mg}/100\ \text{mL}) = \frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

Methods for the elemental analysis of samples: Elemental analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA[21] (American Public Health Association).

Working principle: Atomic absorption spectrometer's working principle was based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiation interferences. The amount of energy of the characteristic wavelength absorbed in the flame was proportional to the concentration of the element in the sample.

Procedure: The sample was thoroughly mixed by shaking and 100ml of it was transferred into a glass beaker of 250 mL volume, to which 5 mL of concentration nitric acid was added and heated to boil till the volume was reduced to about 15-20 mL, by adding concentration nitric acid in increments of 5 mL till all the residue is completely dissolved. The mixture was cooled, transferred and made up to 100 mL using metal-free distilled water. The sample was aspirated into the oxidizing air-acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed.

Sample digestion:

- Exactly 2 g of the dried sample was weighed into a digestion flask and 20 mL of the acetic acid mixture (650 mL concentration HNO_3, 80 mL perchloric acid and 20 mL concentration H_2SO_4) was added
- The flask was heated until a clear digest is obtained
- The digest was diluted with distilled water to the 100 mL mark

Appropriate dilutions were then made for each element.

Statistical analysis: After the experiment, the data obtained were presented as Mean±SD and were subjected to statistical analysis using the student t-test, to determine the level of significance at $p \leq 0.05$.

RESULTS

The results were expressed as Mean±Standard deviation with tannins having the highest concentration (11.48±1.45), followed by anthocyanide content (7.345±0.997) and then cardiac glycosides (6.98±3.01)

Table 1: Phytochemical analysis of guava fruit (*Psidium guajava*)

Parameters	Result
Flavonoid (mg/100g)	2.49±1.40
Alkaloids	3.74±0.48
Tannins	11.48±1.45
Phytate	2.75±1.76
Cardiac glycosides	6.98±3.01
Heamagglutinin	3.34±1.64
Cyanogenic glycosides	5.643±1.22
Oxalate	0.05±0.005
Steroids (mg/100 mL)	1.07±0.381
Phenols (mg g^{-1})	1.01±0.299
Anthocyanide	7.345±0.997

Table 2: Mineral compositions of *Psidium guajava* fruit

Parameters	Result (ppm)
Calcium	5.93±0.82
Aluminum	0.0±0.01
Tin	0.00±0.00
Arsenic	0.38±0.48
Copper	0.06±0.08
Manganese	0.06±0.08
Nickel	0.37±0.02
Chromium	0.003±0.004
Iron	1.07±0.57
Cobalt	0.00±0.00
Zinc	0.24±0.17
Lead	0.00±0.00
Cadmium	0.50±0.21
Magnesium	3.18±1.09
Sodium	5.49±1.19
Potassium	7.028±0.47
Vanadium	0.00±0.00
Selenium	1.67±0.37

and cyanogenic glycosides (5.643±1.22) relatively similar. The oxalate content of *Psidium guajava* has the lowest concentration (0.05±0.005) as shown in Table 1. Also, mild concentrations of the phenols and flavonoids were found in the fruit of *Psidium guajava*.

The results were expressed as Mean±Standard deviation. *Psidium guajava* is rich in calcium (5.93±0.82), sodium (5.49±1.19) and potassium (7.028±0.47) with a maximum value recorded to be (5.93±0.82). However, tin, cobalt, lead and vanadium were absent as given in Table 2.

DISCUSSION

The result shows a high level of concentration for many important phytochemicals. Tannins (11.48±1.45), cardiac glycosides (6.98±3.01), cyanogenic glycosides (5.643±1.22) and anthocyanide (7.345±0.997). Hypoglycaemic potentials in the *Psidium guajava* are exhibited by the presence of tannins[22]. Cardiac glycosides, cyanogenic glycosides and anthocyanide, these compounds have since shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties and as blood glucose reducing agents as mentioned in previous study too[23].

Flavonoids (2.49±1.40), are a class of plant phenolic, which contain hydroxyl groups and are responsible for the radical scavenging and chelating properties. *Psidium guajava,* possesses possible strong antioxidant activity, owing to the presence of flavonoids (and at right intake leads high concentration, high flavonoid intakes lead a decrease in LDL oxidation[24], which are known antioxidants. Flavonoids in plants are known for their scavenging ability as a result of the presence of hydroxyl groups contained in them investigated in other study[25].

The phenol contents of the sample as shown in the Table 1 is (1.01±0.299 mg/100 g). Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers, it was reasonable to determine their total amount in the fruit extracts of *Psidium guajava*[26]. Thus the plant could serve as a very good source of phenols and antioxidants confirmed to be bio-available.

Minerals such as calcium (5.93±0.82), sodium (5.49±1.19) and potassium (7.028±0.47) are essential minerals for growth and development and were high in value. This infers that *Psidium guajava* could be used as a good source of calcium, sodium and potassium for deficient people. However, more research would be ideal to determine their bio-availability on direct consumption.

CONCLUSION

Psidium guajava fruit is high in tannins, glycoside and anthocyanide and can serve as a good source of these nutrients. Because of its medicinal and nutritional value, it can be used as a source of antioxidants and food supplements to complement and improve the nutrient content of other food items, which may be low in essential minerals. It can also serve as a food supplement. However, the bio-availability of these as well as the anti-nutritional factors level of the plant needs to be investigated to establish its suitability or otherwise as a food supplement.

SIGNIFICANCE STATEMENT

This study discovered the bioactive components and essential minerals that can be beneficial for medicinal and nutritional purposes. This study will help the researchers to uncover the critical areas of food supplementation that many researchers were not able to explore. Thus a new theory on neutraceuticals may be arrived at.

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