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ASSESSMENT OF THE PHYTOCHEMICALS AND PROXIMATE COMPOSITION OF THE AQUEOUS EXTRACT OF *Psidium guajava* LEAVES

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ABSTRACT

Guava is a tropical fruit tree with a rich history of traditional use for medicinal purposes. This study aimed to determine the qualitative and quantitative phytochemical and proximate composition of Guava leaves. The Guava leaves samples were obtained from Kumbiya-Kumbiya area of Gombe State, North Eastern Nigeria and brought to Gombe State University. The leaves were rinsed gently with distilled water, shade dried and ground in to a fine powder using laboratory mortar and pestle. They were packed in an air tight container and stored readily for further studies. The aqueous Guava leaves extract was prepared using maceration method. The study involved the use of standard methods for the determination of phytochemical constituents such as alkaloids, flavonoids, saponins, tannins, anthraquinones, steroids and glycosides and proximate composition which are moisture, ash, fat, fibre, protein and carbohydrate content, and were also determined by AOAC (1990). The results of the study revealed that *Psidium guajava* leaves contain significant amounts of crude protein, crude fiber, ash, fat, moisture and carbohydrate which were found to be 2.55 %, 2.43 %, 3.22 %, 0.70 %, 8.52 % and 82.58 % respectively. The phytochemical screening of the leaves revealed the absence of steroids and the presence of flavonoids, alkaloids, tannins, saponins, anthraquinones and glycosides which were found to be 0.49 %, 0.05%, 0.60 µg/ml, 2.78 µg/ml, 0.08 µg/ml and 3.55 µg/ml respectively. The high presence of these phytochemical constituents indicates that *Psidium guajava* leaves possess potential medicinal properties such as antioxidant, anti-inflammatory, and anti-diabetic activities. It was concluded that *Psidium guajava* leaves could be used as a natural source of medicine and nutrition.

Keywords: Phytochemicals, proximate composition, *Psidium guajava,* digestion, nutrients.

INTRODUCTION

Psidium guajava commonly called Guava is a well-known tropical tree, which is mainly grown for fruit. It belongs to phylum magnoliophyta, class magnoliopsida and the family myrtaceae (Dakappa *et al.,* 2013). It is a small tropical tree that grows up to 35feet tall; it is widely grown for its fruits in tropics. It has about 133 genera and more than 3,800 species, it is thought to have originated from Central America and not indigenous to Nigeria. But like Mangoes, Guava was brought into Africa and into Nigeria by slave traders, who often used such exotic fruits as part of their bargaining chip (Dakappa, 2015).

Psidium guajava's leaves and stem bark have been employed in many parts of the world as panacea against fever, diabetes, diarrhea, microbial infection, dysentery, wound, cancer and many other diseases (Nwinyi *et al.,* 2008; Alabi *et al*., 2010).

Guava has been reported to contain many phytochemicals, which are compounds derived from plants that are non-nutritive secondary metabolic compounds occurring in different parts of plants and protective

against disease, hence required by the human body to sustain life (Begum *et al.,* 2010). It is very rich in antioxidants and vitamins and also high in lutein (Joseph and Priyar, 2011).

Proximate analysis also termed "conventional analysis" is a system of analysis of nutrients in food substances in which the gross components (protein, fat, carbohydrate, ash, moisture content etc.) rather than individual nutrients (amino acid, fatty acids, monosaccharides, mineral, etc.) are determined (Prohp *et al.,* 2006).

The growing trend in the use of medicinal plants as natural resources for the development of new drugs, and the increasing evidences on the immense medicinal importance of *Psidium guajava* from various studies have necessitated further research into the bioactive components of *Psidium guajava* plant considering the fact that the plant varies in nutrient content across cultivars as reported by Shiruth *et al*., (2013) and is readily available in the tropics within the reach of the local populace. This current study is aimed to determine the bioactive constituents and nutritional composition of the aqueous extract of *Psidium guajava* leaves to provide scientific information towards validating the various acclaimed uses of this plant.

MATERIALS AND METHODS

Plant Sample

The guava leaves samples were obtained from Kumbiya-Kumbiya area of Gombe State, North Eastern Nigeria and brought to Gombe State University. The sample was identified at the Department of Botany, Gombe State University.

Reagents preparation

- Tetraoxosulphate (vi) acid (H_2SO_4): (1.5%) and 1.5g of sodium hydroxide (NaOH) were dissolved with distilled water in two different 100ml volumetric flasks and filled to the mark.
- 2% Boric Acid (H_3BO_3): (2g) of Boric acid powder was weighed, transferred into a 100ml volumetric flasks and dissolved using a small quantity of distilled water and then filled to the mark.

Preparation of the Aqueous Extract of Guava Leaves

The leaves sample were rinsed gently with distilled water, shade dried and ground in to a fine powder using laboratory mortar and pestle. The powdered obtained was packed in an air tight container and stored readily for further use. Fifty gram (50g) of the guava leaves powder was weighed using an electric weighing balance and placed in a 1000ml conical flask. 500ml of distilled water was added; the mixture was then placed in a mechanical shaker shaken at 250rpm for 30min. The mixture was kept for 24hr, then placed in a mechanical shaker and shaken again at 250rpm for 30min. The mixture was filtered into a beaker using a Whatmann number one filter paper. The beaker was then placed in an oven until all the water has evaporated leaving only the crude extract.

Qualitative Phytochemical Screening

The qualitative phytochemical screening was carried out to find out the phytochemical constituents in the guava leaves extract. The aqueous leaves extract was subjected to test for the presence of alkaloid, anthraquinone, flavonoid, saponin, tannin, and glycoside using the methods of Ajuru *et al*. (2017) while steroid was determined using the method of Edeoga (2005).

Quantitative Phytochemical Analysis

The quantitative phytochemical screening was carried out for each of the phytochemicals as follows:

Determination of Tannin

0.5g of the leave extract was put into 250ml conical flask; 75ml of distilled water was added. The mixture was boiled for 30 minutes and centrifuged at 2000rpm. The supernatant was collected in a 100ml volumetric flask and was made up to 100ml. Then 1m of the sample extract was transferred into 100ml volumetric flask containing 75ml of distilled water, 0.5ml of Folin-Denis reagent and 1ml of sodium carbonate was added. The absorbance was read at 700nm after 30minutes. Standard tannic acid was prepared using 0.1g/100ml of stock solution and the standard tannic acid graph was plotted.

$$x = \frac{y-c}{m}$$

Where; x = concentration of the tannic acid (µg/ml)

y = absorbance of the sample

c = intercept from the standard tannic acid graph

m = slope from the standard tannic acid graph

Determination of Flavonoid

50ml of 80% aqueous ethanol and 20% distilled water was added to 2.5g of the leaves extract in a 250ml beaker. The mixture was covered and allowed to stand for 24hours at room temperature. The supernatant was discarded and the residue was re-extracted (three times) with the same volume of ethanol. Filter paper was used to filter the mixture and left to evaporate to dryness.

$$\%\text{Flavonoid} = \frac{Weight\ of\ flavonoid}{Weight\ of\ sample} \text{ x } 100$$

Determination of alkaloid

To 5g of the sample in a 250ml beaker, 200ml of 20% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed (Ajuru *et al.*, 2017).

$$\%\text{ Alkaloid} = \frac{Weight\ of\ alkaloid}{Weight\ of\ sample} \text{ x } 100$$

Determination of Saponin

Five gram (5g) of the leaves extract was taken and heated at 55℃ in 100ml of 20% ethanol for 4 hours after which it was filtered. The residue was treated with 100ml of 20% ethanol and was combined with the filtrate, which was concentrated to 40ml by heating in a water bath at 90℃ . The filtrate is treated with 20ml diethyl ether shaken vigorously in a separating funnel, the ether layer was then discarded and the aqueous layer was mixed with 60ml n-butanol and the solution was evaporated to dryness in an oven (George, 2017).

$$\%\text{ Saponin} = \frac{Weight\ of\ saponin}{Weight\ of\ sample} \text{ x } 100$$

Proximate Analysis

The crude fat, moisture content, ash content, crude fibre sample were carried out according to A.O.A.C methods (1990).

Determination of Protein

The protein content was determined using three different steps as follows:

Digestion

Two (2g) of the powder sample was weighed and transferred into a digestion flask, 0.5g of $CuSO_4$ and 5.0g of sodium sulfate was added as well as 25ml of concentrated H_2SO_4 solution. A significant amount of antibump was added and the digestion flask was placed on a hot plate and it was heated gently until a clear green color was observed.

Distillation

After complete digestion, 100ml of distilled water was added, as well as 10ml of 20% NaOH solution, to the digest. The measuring cylinder used to measure the NaOH solution was rinsed with 50ml distilled water and the content transferred to the digestion flask. Antibump was added and the distillation set-up was connected to the upper chamber of the apparatus (Kjeldahl apparatus). 20ml of 2% H_3BO_3 was transferred into a receiving flask; 3 drops of screened methyl red indicator were added. The receiving flask was placed at the middle chamber of the apparatus and the delivery tube was immersed into the pinkish solution in the receiving flask. The heat knob of the upper chamber was turned on for distillation to begin, and about 20ml of the resulting pale yellow solution was collected for titration.

Titration

After complete distillation, the pale yellow receiving solution was then titrated with 0.05M H_2SO_4 solution until a permanent pink color was observed, which indicated the end point.

$$\% \text{ Nitrogen} = \frac{\text{Titer Value} \times 0.0014}{Weight\ of\ sample} \text{ x } 100$$

Percentage protein (% P), is calculated by multiplying the %N by the Jones factor, F (6.25), corresponding to the protein source, as shown below:

$$\% \text{ Protein} = \%N \times F$$

RESULTS

The result obtained from the qualitative phytochemical analysis is depicted in (Table 1). The results showed the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones and glycosides but steroids was absent.

Table 1: Qualitative phytochemical constituents of *Psidium guajava* leave extract

Parameter	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Anthraquinones	+
Steroids	-
Glycosides	+

Keys: (+) indicates present and (-) indicates absent.

The results for the quantitative phytochemical analysis (Table 2) showed high concentrations of glycosides (3.55 µg/ml) and saponins (2.78 µg/ml), moderate level of flavonoids and tannins with a concentration of 0.49 and 0.60 µg/ml respectively, but low level of alkaloids, and anthraquinones with a concentration of 0.05 and 0.08 µg/ml respectively. This is depicted in Table 2 below.

Table 2: Quantitative phytochemical constituents of *Psidium guajava* leaves extract

Parameter	Concentration
Alkaloids (%)	0.05 ± 0.01
Flavonoids (%)	0.49 ± 0.04
Saponins (µg/ml)	2.78 ± 0.0
Tannins (µg/ml)	0.60 ± 0.04
Anthraquinones (µg/ml)	0.08 ± 0.01
Glycosides (µg/ml)	3.55 ± 0.07

Values are presented as mean ± standard deviation of duplicate measurement.

The results for the proximate composition of Guava leaves (Table 3) showed a high percentage of carbohydrate (82.58 %), followed by 8.52 % moisture content, 3.22 % ash, 2.55 % protein, 2.43 % fiber and a low fat concentration of 0.70 %.

Table 3: Proximate composition of *Psidium guajava* leaves

Parameter	Concentration (%)
Moisture	8.52
Ash	3.22
Fat	0.70
Fibre	2.43
Protein	2.55
Carbohydrate	82.58

DISCUSSION

The aqueous extract of *Psidium guajava* leaves was screened for the presence of phytochemicals such as glycosides, flavonoids, saponins, alkaloids, anthraquinones and tannins on qualitative basis. The extract was found to contain all the tested bioactive components (Table 1). The phytochemicals have been reported to possess various physiological activities (Mohan *et al.,* 2010). For instance, flavonoids which are hydroxylated polyphenolic compounds were found to possess antimicrobial activity in addition to antioxidant properties (Górniak *et al*., 2019). Similarly, saponins and tannins were found to possess inhibitory effect against certain gram positive bacteria that include *Staphylococcus aureus* and *Bacillus cereus* (Biswas *et al.,* 2013).

Therefore, the various medicinal properties of the *Psidium guajava* leaves and stem bark extracts reported by some researchers (Nwinyi *et al.,* 2008; Alabi *et al*., 2010) could be as result of the presence of these phytochemicals.

Extract of *Psidium guajava* leaves in ethyl acetate has been shown to stop germ infection and thymus production. It can act as anti-viral agent. It can enhance mRNA expression. Guava can alter the heme oxygenase-1 protein's work. And due to this reason, it can be used as anti-inflammatory agent for skin disease. Extract of guava in ethanol has been shown to inhibit lipopolysaccharide from manufacturing nitric oxide, which is an important free radical in the system. It suppresses the expression of estradiol (E2). In this way it works as an anti-inflammatory agent (Jeong *et al*., 2014).

Benzophenone and flavonoids are important compounds found in guava. These compounds are responsible for the inhibition of histamine and nitric acid production (Matsuzak *et al*., 2010)

Psidium guajava leaves extract also show anti-nociceptive activity, thus lowering pains. It happens by acetic acid production.

Phenol is another important compound found to be present in the *Psidium guajava* leaves extract in this research, and it has previously been reported to be involved in anti-allergic and anti-inflammatory activity (Denny *et al*., 2013). A dose of guava extract has been reported to be effective in reversing liver damage inflammation and serum production (Roy *et al*., 2006).

The quantitative phytochemical analysis of the powdered leaves of *Psidium guajava* showed high concentrations of saponins and glycosides, moderate concentrations of flavonoids and tannins, with low concentration of alkaloids and anthraquinone (Table 2). The results further showed the absence of steroids and varying amounts of

other bioactive components. This agrees with the studies conducted by Olayemi, (2011) who also found that not all phytochemicals are present in all plant parts in large amount, and that these differences maybe as a result of different extraction methods used. The presence of Saponins in *Psidium guajava* leaves is of great importance as they have been shown to possess some beneficial (cholesterol lowering) properties (Bamishaiye *et al*., 2011). The *Psidium guajava* leaves also contained alkaloids in low level (0.05ug/ml) which are nitrogen-containing naturally occurring compounds, commonly found to possess antimicrobial properties due to their ability to intercalate with DNA (Olayemi, 2011).

Alkaloids have a wide range of pharmacological activities (including antimalarial and antibacterial). Saponins are thought to have antimicrobial, anti-inflammatory, anti-oxidant and immune-stimulating properties. In addition, ubiquitous flavonoid, which is reported to contain high antioxidant properties (Fleuriet and Macheix, 2003) were detected in the sample. The presence of saponins and flavonoids suggest that the samples may be beneficial in the reduction of cancer risk and heart diseases (Feskanich *et al*., 2000). Tannins are known to exert antioxidant properties and herbivory roles (to prevent entrance of pathogens) respectively (Arima and Danno, 2002; Das, 2011) and were present in the sample studied.

The results of the proximate analysis of the Guava leaves extract showed a high percentage of carbohydrate (82.58 %), followed by 8.52 % moisture content, 3.22 % ash, 2.55 % protein, 2.43 % fiber and a low fat concentration of 0.70 %. This indicates that Guava leaves extract is an important source of nutrients in the body, thus containing both organic and inorganic compounds that promote health and general wellbeing as previously mentioned by Shabbir *et al*., (2020). Polysaccharides were found to be present in the Guava leaves extract studied and they have been shown to perform various physicochemical, biological, and pharmacological properties, such as antioxidant, anti-inflammatory, antidiabetic, immunomodulatory, and antitumor activities (Luo *et al*., 2019).

Proteins are found to be present in reasonable amount in this present study (Table 3) and they have been reported to play major roles in growth and maintenance, enzyme regulation, cell signaling, and also as biocatalysts (Alberts *et al*., 2002).

Guava leaves extract is thought to contain many important vitamins and minerals and this can be accounted for by the reasonably high ash content (3.22%) in this study. This therefore makes it a highly suitable choice for human nutrition and also as an important component of human diet to prevent micronutrient deficiency (Adrian *et al*., 2015). Ascorbic acid and citric acid are major ingredients in Guava fruit, and these have been found to play important role as anti-mutagenic agents (Grover and Bala, 1993).

Therefore, from the results obtained in the present study we are left with no choice but to agree with Thomas *et al.* (2017) who reported that guava leaves can be utilized as a novel and sustainable dietary source as they are a rich in proteins, carbohydrates, and dietary fibers.

CONCLUSION

The aqueous extract of guava leaves studied showed the presence of phytochemicals such as glycosides, flavonoids, saponins, alkaloids, anthraquinones and tannins and the quantitative phytochemical screening showed that most of these phytochemicals are present in reasonable quantities. The proximate analysis of the *Psidium guajava* leaves extract

showed high percentage carbohydrate content, moderate level of moisture, crude fiber, protein, ash, and low fat content. It is concluded that the aqueous extract of Guava leaves could be highly medicinal considering the presence of these phytochemicals and that it is highly nutritious, hence could be used as an important dietary source.

REFERENCES

Adrian, J.A.L., Arancon, N.Q., Mathews, B.W., Carpenter, J.R. (2015). Mineral composition and soil plant relationships for common guava (*Psidium guajava* L.) and yellow strawberry guava (Psidium cattleianum var. Lucidum) tree parts and fruits. Commun. Soil Sci. *Plant Anal.* 46;1960–1979.

Ajuru, MG., Williams, L.F., Ajuru, G. (2017). Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta Region of Nigeria. *J. Food Nutr. Sci.* 5(5): 198-205.

Alabi, A., Luky O.O., Kate, E.I. (2010). Phytochemical, proximate and metal content analysis of leaves of *Psidium guajava*. *International Journal of Health Research*. 3(4): 217.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P. (2002). *Protein Function. In Molecular Biology of the Cell*, 4th ed.; Garland Science: New York, NY, USA.

AOAC (1990). *Official Methods of Analysis*, 15th ed. Washington, DC: Association of Official Analytical Chemists Arlington, VA, USA. 429-434.

Arima, H. and Danno, G. (2002). Isolation of antimicrobial compounds from guava (*Psidium guajava* L.) and their structural elucidation. *Biosci Biotechnol Biochem.* 66:1727–30.

Bamishaiye, E. I., Olayemi, F. F., Awagu, E. F., & Bamshaiye, O. M. (2011). Proximate and phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. *Advance Journal of Food Science and Technology*, *3*(4), 233-237.

Begum, S., Hassan, S.I., Ali, S.N., Siddiqui, B.S. (2010). Chemical constituents from the leaves of *Psidium guajava*. *National Product Research*. 18(2): 135- 140.

Biswas, B., Rogers, K., McLaughlin, F., Daniels, D., Yadav, A. (2013). Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. *Int J Microbiol* .2013:746165–746467.

Dakappa, S.S., Adhikari, R., Timilsina, S.S., Sajjekhan, S. (2013). A review on the medicinal plant *Psidium guajava* Linn. (Myrtaceae). *J Drug Deliv Ther*. 3(2):162–8.

Das, A.J. (2011). Review on nutritional, medicinal and pharmacological properties of *Centella asiatica* (Indian pennywort). *J Biol Act Prod from Nat*.;1(4): 216–28.

Denny, C., Melo, P. S., Franchin, M., Massarioli, A. P., Bergamaschi, K. B., de Alencar, S. M., & Rosalen, P. L. (2013). Guava pomace: a new source of anti-inflammatory and analgesic bioactives. *BMC complementary and alternative medicine*, *13*, 1-7.

Edeoga H. O., Okwu D. E., and Mbaebie B.O. (2005). Phytochemical Constituents of some Nigerian Medicinal Plants. *Afr. J. Biotechnol*. 4:685-688.

Feskanich, D., Ziegler, R.G., Michaud, D.S., Giovannucci, E.L., Speizer, F.E., Willett, W.C., Colditz, G.A. (2000). Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women.

J Natl Cancer Inst. 92:1812–23.

Fleuriet, A., & Macheix, J. J. (2003). Phenolic acids in fruits and vegetables. *Oxidative stress and disease*, *9*, 1-42.

Górniak, I., Bartoszewski, R., Króliczewski, J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochem Rev* 18(1):241–272.

Grover, I. S., & Bala, S. (1993). Studies on antimutagenic activity of Terminalia bellerica (myroblan) in Salmonella typhimurium. *Proc Natl Acad Sci India (Section B)*, *63*, 425-432.

Jeong, S., Cho, S.K., Ahn, K.S., Lee, J.H., Yang, D.C., Kim, J. (2014). Anti-inflammatory effects of an ethanolic extract of guava (Psidium guajava L.) leaves in vitro and in vivo. *J Med Food*. 17(6):678–85.

Joseph, B. and Priya, R.M. (2011). Phytochemical and biopharmaceutical aspects of Psidium guajava (L.) essential oil: a review. *Res J Med Plant.* 5(4):432–42.

Luo, Y., Peng, B., Wei, W., Tian, X., & Wu, Z. (2019). Antioxidant and anti-diabetic activities of polysaccharides from guava leaves. *Molecules*, *24*(7), 1343.

Matsuzaki, K., Ishii, R., Kobiyama, K. (2010). New benzophenone and quercetin galloyl glycosides from Psidium guajava L. *J Nat Med.* 64:252–6.

Mohan, C. V. R., Kasthuri, N., Rajendra, W. (2010). Phytochemical and pharmacological potential of *Psidium guajava*. *International Journal of Pharmaceutical Sciences Review and Research,* 1(1): 12-18.

Nwinyi, O.C., Chinedu, N.S., Ajani, O.O. (2008). Evaluation of antiacterial activity of *Psidium guajava*. *Journal of Medicinal Plant Research*. 2(8): 189-192.

Olayemi, F.F. (2011) Proximate and phytochemical composition of *Moringa oleifera*. *Advance Journal of Food Science and Technology,* 3(4): 233-237.

Prohp, T.P., Ihimire, I.G., Madusha, A.O., Okpala, H.O., Erevor, J.O., Oyinbo, C.A. (2006). Some antinutritional and mineral contents of extra-cotyledonous deposit of pride of Barbados (*Caealpina pulcherima*). *Pakistan Journal of Nutrition*. 5: 114-116.

Roy, C.K., Kamath, J.V., Asad, M. (2006). Hepatoprotective activity of *Psidium guajava* Linn. Leaf extract. *Indian J Exp Biol*. 44(4):305–11.

Shabbir, H., Kausar, T., Noreen, S., Hussain, A., Huang, Q., Gani, A., Su, S., Nawaz, A. (2020). Invivo screening and antidiabetic potential of polyphenol extracts from guava pulp, seeds and leaves. *Animals*, 10, 1714.

Shiruth, D., Adhikari, S.S., Timisma D., and Sajekhan, S. (2013). A review on the medicinal plants, *Psidium guajava*. *Journal of Drug Delivery and Therapeutics*. 3(2): 162-168.

Thomas, L.A.T., Anitha, T., Lasyaja, A.B., Suganya, M., Gayathri, P., Chithra, S. (2017). Biochemical and mineral analysis of the undervalued leaves-*Psidium guajava* L. *Int. J. Adv. Sci. Res*. 2; 16-21.