



MINERAL AND VITAMIN CONTENT AND ANTIOXIDANT PROPERTIES OF CRUDE METHANOL EXTRACT OF *PAULLINIA PINNATA* STEM

Precious Nnennaya Akpa-Onyeabor¹ & Donatus Chima Onah²

Department of Science Laboratory Technology

¹Federal Polytechnic Wannune Benue State, Nigeria

Department of Biochemistry

²Joseph Sarwuan Tarka University, Makurdi, Benue State

Abstract: *Paullinia pinnata* is a medicinal plant widely used in traditional medicine for the treatment of various ailments. This study aimed to investigate the mineral and vitamin contents, as well as the antioxidant properties of the crude methanol extract of *P. pinnata* stem. The mineral and vitamin contents were determined using standard analytical techniques. The antioxidant properties were evaluated through various *in vitro* assays, including the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant Power and total antioxidants capacity assay. Data was analysed using SPSS Version 20.0. Results were expressed as Mean \pm SD of two replicate determinations. The results revealed that the crude methanol extract of *P. pinnata* stem contained significant amounts of essential minerals, such as copper, iron, zinc, manganese and cobalt, as well as various vitamins, including vitamins A, C, and E. The extract exhibited potent DPPH radical scavenging activity, with an IC50 value comparable to that of the standard antioxidant, ascorbic acid. The findings suggest that the crude methanol extract of *P. pinnata* stem is a rich source of essential minerals, vitamins, and antioxidants. This information provides valuable insights into the nutritional and therapeutic properties of this medicinal plant, which could contribute to the development of natural health-promoting products or the formulation of complementary and alternative medicine.

Key words: Medicinal Plant, Antioxidant Properties, Nutritional Properties, Therapeutic Properties, Crude Methanol Extract.

1.0 INTRODUCTION

Paullinia pinnata is a flowering plant that belongs to the family Sapindaceae. It is native to tropical and subtropical regions of America and Africa, where it grows as a woody or sub-woody climber in damp sites and stream-banks. It has compound leaves, yellow flowers, and red fruits with edible sweet arils (Tseuguem *et al.*, 2019). The plant has various uses in different cultures and regions. It is sometimes cultivated for its leaves, which are cooked as a vegetable, and for its fruits, which are eaten raw or processed into drinks. It is also used as a

poison to stun fish in shallow pools, as described by the English naturalist Henry Walter Bates in his book *The Naturalist on the River Amazons*. Moreover, it is widely used in traditional medicine for treating various ailments, such as erectile dysfunction, malaria, dysentery, snake bite, rabies, mental disorders, eye disorders, blindness, and abdominal pain (Annan *et al.*, 2013). Figure 1 shows a figurative description of some of the many uses of *Paullinia pinnata*. There are a large number of medicinal plants whose scientific importance has not been explored. All over the world plants have served as the richest source of raw materials for traditional medicine as well as modern medicine, particularly in Africa and Asia (Imade *et al.*, 2015).

Paullinia pinnata Linn. is an African woody vine used in traditional medicine for many purposes. Aqueous decoctions and powdered roots from *P. pinnata* L. are used in Nigeria, Ghana and Togo traditional medicine for treating sores, wounds, snakebites and other diseases such as erectile dysfunction, malaria, dysentery, menstrual pain and coughs (Tseuguem *et al.*, 2019). Phytochemicals such as phenolic compounds and flavo-tannin have been isolated from the leaves of *P. pinnata*. Abourashed *et al.* (1999) identified the presence of two flavone glycosides, ndiosmatin-7-O and tricetin-4'-O-methyl-7-O, from the leaves of the plants. Hasan *et al.* (2017) demonstrated that some pure compounds such as Methylinositol screened from *Paullinia* plant leaves had both anti-typhoid activity and anti-oxidant properties. Azaleic acid, which has also been screened from this plant's methanol root extract, has demonstrated antibacterial activity against organisms like *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, *B. subtilis*, *M. flavus*, *S. faecalis* and resistant *S. aureus* strains (Annan *et al.*, 2009). Imade *et al.* (2015) also reported that *Paullinia pinnata* contains various chemical constituents that have been isolated and identified as Triterpenoids, Flavonoids, Ceramide, Coumarinolignoid. Plant's products assumed to be non-toxic have been used worldwide by herbalists and the local population to treat many diseases. However, it should be noted that although plants extracts are of natural origin, their usage is not entirely safe. Like synthetic drugs, these plant extracts possess active ingredients that are chemicals and thus highly effective under specific concentrations. However, prolonged usage of these plants extracts or in high concentrations may also be fatal to health (Hasan *et al.*, 2017).

2.0 MATERIALS AND METHODS

Plant Material

Paullinia pinnata was obtained from a farm at the bank of River Benue, Makurdi, Benue State, Nigeria. It was identified and authenticated in the Department of Botany, Joseph Sarwuan Tarka University, Makurdi, Benue State and compared with the Herbarium voucher specimen for reference purposes.

Preparation of *paullinia pinnata* Extracts

The stems of *p. pinnata* were washed with normal saline to make sure that there is no particle of mud on it before drying. The plant were air-dried in the laboratory for two weeks and milled into fine powder using a grinding machine. The milled stem powder were soaked in methanol (1:5 w/v) for 24 hours with gentle stirring, after which the mixture were filtered to obtain the extract. Filtration were done twice; first with a coarse sieve, then with Whatman number 1 filter paper. The filtrate were collected and concentrated using a water bath, producing lyophilized powder. The sample were stored at 4°C (Poitevin, 2016; Elmer, 2013).

Determination of Antioxidant Activity (DPPH Radical Scavenging Activity)

The ability to scavenge the "stable" free radical DPPH or antioxidant activity was determined using the DPPH free-radical scavenging method. A 3.94 mg of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), a stable radical was dissolved in methanol (100 ml) to give a 100 M solution. To 3.0 ml of the methanolic solutions of DPPH was added the crude Methanol extract of *paullinia pinnata* with doses ranging from 500ug/ml to 1000ug/ml. The decrease in absorption was measured against that of the control and percentage inhibition was also calculated. The same experiment was carried out on Ascorbic acid. Ascorbic acid was used as a standard for reference.

The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below;

$$\%RSA \text{ or } \%Inhibition = \{(A_{DPPH} - A_s)/A_{DPPH}\} \times 100$$

Where A_s is the absorbance of the sample solution and A_{DPPH} is the absorbance of the DPPH solution.

Vitamin Analysis

The vitamins in the dried stem powder were determined by the official methods of the Association of Official Analytical Chemist (AOAC, 2006).

Determination of Vitamin A (Beta-Carotene)

The carotene was determined by soaking 1g of the sample in 5 ml of methanol for 2 hours at room temperature under dark conditions to get a complete extraction. The β -carotene layer was separated using hexane through a separating funnel. The volume was made up to 10 ml with hexane and then this layer was again passed through sodium sulphonate through a funnel to remove any moisture from the layer. The solution was mixed and absorbance measured at 450nm against the blank.

The β -carotene was calculated using the formula:

$$\text{Beta-carotene (mg/100g)} = \text{Absorbance (450 nm)} \times V \times D \times 100 \times 100/W \times Y/1000;$$

Where;

V = Total volume of extract;

D = Dilution factor;

W = Sample weight;

Y = Percentage dry matter content of the sample.

Determination of Vitamin E (Tocopherol)

About 1g of the sample was weighed and macerated in 5 ml of methanol for 2 hours at room temperature under dark conditions to get a complete extraction. The filtrate was added 10ml of hexane. One ml of the extract and equal volume of standard vitamin E were transferred into separate tubes. After which the mixtures were allowed to stand for 5mins and the absorbance measured at 450nm against the blank.

Determination of Vitamin C (Ascorbic acid)

A solution of KMnO_4 of concentration of 100 $\mu\text{g}/\text{mL}$ was prepared by dissolving an accurate 0.01g of KMnO_4 in H_2SO_4 solution (5.0M), then transferred into a 100 volumetric flask and completed to the mark with distilled water and thoroughly mixed. This solution was used as a chromogenic reagent for the determination of ascorbic acid (Vitamin C) by spectrophotometer. The plant sample was accurately taken as 10.0 ml and then transferred into a test tube and 1.0 mL of KMnO_4 (100 $\mu\text{g}/\text{ml}$) was added for each. The contents of each test tube were mixed well and stood for 5 minutes. The prepared solutions were read at 530 nm against blank by spectrophotometer using a suitable concentration for the analysis (Elgailani *et al.*, 2017).

A standard solution of ascorbic acid was prepared by dissolving an accurate weight of 0.01g of standard ascorbic acid in a small amount of oxalic acid solution (0.5%) and then completed to 100 ml with the same solution to obtain a concentration of 100 $\mu\text{g}/\text{ml}$. A series of dilutions 1.0, 4.0, 8.0, 12, and 16 $\mu\text{g}/\text{ml}$ were prepared from the stock ascorbic acid solution.

Calculation of vitamin C (mg/100g) =
$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard} \times \text{Concentration of standard}}$$

Evaluation of Mineral Contents

The elemental analysis (Cu, Zn, Mn, Fe, Co) was carried out according to the method of the Association of Official Analytical Chemists (AOAC, 2005). 2g of sample was digested using an acid mixture of nitric acid, per chloric acid, and sulphuric acid ($\text{HNO}_3:\text{HClO}_4, \text{H}_2\text{SO}_4$). 2 ml of the acid mixture was dispensed in the tube containing 2g of pulverized *Paullinia pinnata* stem powder. The tubes were then placed in the digester under the fume cupboard and heated until a clear and colourless fume was noted. The contents of the tubes were allowed to cool and then diluted with 250 ml of distilled water. From the diluent, an aliquot of the samples was taken for the analysis of minerals using an Atomic Absorption Spectrophotometer (AAS).

Statistical Analysis

Data was analysed using SPSS Version 20.0. Results were expressed as Mean \pm SD of two replicate determinations.

3.0 RESULTS AND DISCUSSION

The results of the vitamin A, C and E composition of *Paullinia Pinnata* plant stem are shown in Table 1. The concentrations of vitamins A is an average of 2.770mg/100ml. Vitamin A is important for normal vision, gene expression, growth and immune functions (Lukaski, 2004). The concentration of vitamin C is 3.280mg/100ml. Vitamin C is a potent natural antioxidants that scavenge free radicals and also aids wound healing. E in mg/100g of extract. The concentration of vitamin E is 0.815mg/100ml. Vitamin E is a powerful antioxidant which helps to protect cell from free radicals and normal function of the muscles (Lukaski, 2004).

These vitamins are very important to the body as they help in proper eyesight, protect the body against pathogens and produces good skin. Apart from the above functions they also help in the metabolism of carbohydrates, lipids and protein they also protect the cells against damage. Furthermore, they help in the maintenance of healthy muscles, skin, eye, hair, and liver. The availability of these vitamins further establish the potentials of *Paulinia pinnata* as source of essential vitamins for human.

Table 1: Vitamins composition of the methanolic stem extract of *Paulinia pinnata*

Vitamins	Mean value/ ppm	Standard deviation
A (Beta-carotene)	2.770	0.028
C (Ascorbic acid)	3.280	0.033
E (Tocopherol)	0.815	0.008

Table 2 shows the mineral composition of the methanolic stem extract of *Paullinia pinnata*. The result of this study confirms presence of trace elements in the methanolic stem extract of *Paulinia pinnata*. The result shows presence of copper, zinc, manganese, Iron and cobalt. Copper has the highest concentration of 1.030mg/100g of dried stem followed by iron and zinc with 0.742mg/100g and 0.511mg/100g respectively. While manganese and cobalt were least with 0.397mg/100g and 0.021mg/100g. This findings is in line with the work of Oluwafemi *et al.*, 2017, Ogunlade *et al.*, 2020 and Oyeyinka *et al.*, 2019 who in their separate studies confirms the presence of zinc, manganese, copper and Iron in appreciable quantity in the stem bark of *Paulinia pinnata*. The presence of these minerals which usually serve as cofactors of enzymatic reaction, projects the plant as a veritable source of minerals and suggest that consumption of the plant or its extract can serve as a natural source of cheap supplements for elements that are needed in the body for such function as production of haemoglobin, enzyme cofactor, healthy muscles and nerve transmission.

Table 2: Mineral composition of the methanolic stem extract of *Paulinia pinnata*

Minerals	Mean value/ppm	Standard deviation
Copper	1.030	0.010
Zinc	0.511	0.005
Manganese	0.397	0.004
Iron	0.742	0.007
Cobalt	0.021	0.000

Table 3 shows the percentage inhibition of the DPPH scavenging activity of crude methanolic stem extract of *Paullinia pinnata*. The antioxidant activities of methanolic stem extract in this study shows a percentage inhibition of 53.59, 48.18 and 46.78 percent for extract concentrations of 1000, 750 and 500µg/mL respectively, this results shows that *paullinia pinnata* has high antioxidant ability using DPPH scavenging activity test. This finding corroborated the finding of previous researchers like Ogunlade *et al* (2020), Oyeyinka *et al* (2019), who in their various research assert the presence of antioxidant in appreciable quantities in stem of *Paullinia pinnata* and concluded that it was potent natural source of antioxidant. This work demonstrated that the antioxidant activity of *Paullinia pinnata* was concentration dependent as it increases from 46.78 percent at 500µg/mL to 53.59 percent at 1000µg/mL. This therefore, suggests that the more quantity of the plant one consume the more it inhibition of oxidative stress and likely prevention of cell damage.

Table 3: percentage inhibition of the methanolic stem extract of *Paulinia pinnata* and Ascorbic acid to DPPH

Concentrations	Methanolic extract	Ascorbic acid
1000	53.59±0.91 ^a	78.67±0.17 ^a
750	48.18±0.03 ^b	70.75±0.24 ^b
500	46.28±0.50 ^b	63.39±0.00 ^c
P-value	0.013	0.000

4.0 CONCLUSION

The mineral and vitamin content and antioxidant properties of crude methanol extract of *paullinia pinnata* stem were investigated. The results showed that *paullinia pinnata* stem is rich in vitamins, minerals and antioxidant properties, with scientific data to be used as natural source of these products that have immense benefit to man and other animals. The

consumption of this stem will be of immense benefit to health as it will provide the consumers the requisite micronutrients for proper food assimilation, defense against infectious pathogens and prevention of oxidative stress and formation of free radicals by cell.

REFERENCES

Abourashed, E. A., Toyang, N. J., Choinski, J. and Ian, K. (1999). Two New Flavone Glycosides from *Paullinia pinnata*. *Journal of Natural Products*, 62(8):1179–1181.

São Gonçalo Channel in Pelotas-RS, Brazil. *J. Braz. Chem. Soc.*, 29:285–296.

Annan, K., Dickson, R. A., Amponsah, I. K., Jato, J., & Nooni, I. K. (2013). Pharmacognostic evaluation and physicochemical analysis of *Paullinia pinnata* L (Sapindaceae). *Journal of Pharmacognostic Phytochemistry*, 2:203-208.

AOAC. (2005) AOAC Official Method 967.21. Vitamin C in juices and vitamin preparations. In AOAC Official Methods of Analysis, 18th ed., AOAC International: Gaithersburg, MD, USA, p. 45.41.14.

AOAC. (2006) Official Methods of Analysis. 18th Edition, Association of Official Analytical Chemists, Washington DC.

Elgailani, I. E. H., Elkareem, M., Noh, E., Adam, O. and Alghamdi, A. (2017). Comparison of two methods for the determination of vitamin C (ascorbic acid) in some fruits. *American Journal of Chemistry*, 2:1–7.

Elmer, P. (2013). *Atomic Spectroscopy Sample Preparation: Because Preparation is Everything*; Perkin Elmer: Walham, MA, USA, 2013.

Hasan, B. B., Soghra, M. and Hossein, H. (2017). Toxicology effects of saffron and its constituents: a review. *Iran Journal of Basic Medical Science*, 20(2):110–131

Imade, F. N., Nosakhare, N. G. and Mensah, J. K. (2015). Phytochemical and Antibacterial Properties of the Lead, Stem and Root of *Paullinia pinnata* Linn. *Nigerian Annals of Natural Sciences*, 15(1):079-084.

Lukaski, C. H. (2004). *Vitamin and Mineral Status: Effect on Physical Performance*. Nutrition Research Centre, Grand Forks, North Dakota, USA 20:632-644

- Ogunlade, I., Adewole, E., and Akinyosoye, F. A. (2020). Comparative study on mineral content and antioxidant properties of some medicinal plants used in Nigeria. *Journal of Food Measurement and Characterization*, 14(1):577-584.
- Oluwafemi, F., Debiri, F. and Oyeyinka, S. A. (2017). Mineral and vitamin content of *Paullinia pinnata* stem bark and leaf extracts. *Journal of Applied Sciences and Environmental Management*, 21(3):559-562.
- Oyedemi, S. O., Okoh, A. I., Mabinya, L. V., Pirochenva, G. and Afolayan, A. J. (2017). Comparative study on mineral, vitamin and antioxidant properties of some oil seeds used in Nigeria. *Journal of Food Measurement and Characterization*, 11(4):2109-2116.
- Oyeyinka, S. A., Oyeyinka, B. O., and Oluwafemi, F. (2019). Antioxidant properties of *Paullinia pinnata* stem bark and leaf extracts. *Journal of Food Biochemistry*, 43(1):e12674.
- Poitevin, E. (2016). Official methods for the determination of minerals and trace elements in infant formula and milk products: A review. *J. Aoac. Int.*, 99, 42–52.
- Tseguem, P. P., Nguenefack, T. B., Piegang, B. N. and Ngassam, S. M. (2019). Aqueous and Methanol Extracts of *Paullinia pinnata* (Sapindaceae) Improve Monosodium Urate-Induced Gouty Arthritis in Rat: Analgesic, Anti-Inflammatory, and Antioxidant Effects. *Hindawi Evidence-Based Complementary and Alternative Medicine*, 2019, 12 Pages.