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COMPARATIVE ANALYSIS OF NUTRIENTS AND PHYTOCHEMICAL PROPERTIES OF MATURED AND YOUNG LEAVES OF *Manihot esculenta*, *Arachis hypogeal*, AND *Parkia biglobosa*

Sogoi B. P.*[1], Ogidi O. I.[2], Addy P. S.[1]

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[1]*Department of Biochemistry, Bayelsa Medical University, Imgbi, Bayelsa State, Nigeria*
[2]*Department of Biochemistry, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria*
Corresponding author: Peresogoi@gmail.com; Tel: +2348039529426

ABSTRACT
The nutritive and phytochemical constituents of three plant leaves were investigated. The plants include: Matured *Manihot esculenta*, six weeks old *Manihot esculenta*, matured *Arachis hypogeal*, two weeks old *Arachis hypogeal*, matured *Parkia biglobosa* and two weeks old *Parkia biglobosa*. The nutrients and phytochemicals determined quantitatively were: soluble protein, soluble sugar, total protein, nitric oxide radical scavenging activity, saponin, alkaloid, and tannin. The results of the quantitative analysis of these plants show that all the vegetable plant leaves contained all the parameters analyzed in varying proportion. For the quantitative analysis, matured *M. esculenta* had the highest amount of total protein (36.69% FW) and saponin (0.21g/g FW), six weeks old *M. esculenta* had the highest amount of total soluble protein (0.26g/g FW), total soluble sugar (0.12g/g FW) and nitric oxide radical scavenging activity (2.25% FW), matured *P. biglobosa* had the highest amount of alkaloid (0.04g/100g FW). Two weeks old *A. hypogeal*, matured *A. hypogeal* and matured *P. biglobosa* had same amount of soluble protein (0.18g/g FW), matured *M. esculenta*, two weeks old *A. hypogeal* and two weeks old *P. biglobosa* had same amount of alkaloid (0.02g/100g FW), matured *M. esculenta*, two weeks old *A. hypogeal* and two weeks old *P. biglobosa* had same amount of tannin (0.04g/g FW) and matured *A. hypogeal* and matured *P. biglobosa* had same amount of tannin (0.05g/g FW). The significance of these results showed that there are lots of phytochemicals and nutrients present in *M. esculenta*, *A. hypogeal* and *P. biglobosa* leaves which can be utilized as potential vegetables in traditional Nigeria soups.

Keywords: *Vegetable; Phytochemicals; Nutrients; Mature; Young; Leaves*

INTRODUCTION

In Nigeria, especially in the Western and Southern regions of the country, many kinds of soups are made with various vegetables like pumpkin (*Cucurbita maxima*) leaves, waterleaf (*Talinum fruticosum*), bitterleaf (*Vernonia amygdalina*) which are quite rich nutritionally (Tonukari et al., 2013). A number of studies have proved that vegetables contain some nutrients such as sugars, amino acids and vitamins, which have long been recognized for their health benefits to humans (Donoghue et al., 2006; Ovuru et al., 2023). As technology and research techniques are improving, other substances in vegetables that were previously ignored are getting the spotlight. Vegetables are rich sources of bioactive compounds such as flavonoids, carotenoids, anthocyanins, vitamins and other polyphenolics (Lee et al., 2005; Adesina et al., 2013). Such compounds play roles in disease prevention/reduce disease risk factors through antioxidant activity (Enerijiofi & Isola, 2019). Researchers have identified hundreds of compounds in vegetable crops with functional qualities and they continue to make new discoveries surrounding the complex benefits of phytochemicals such as lycopene in tomatoes, curcumin in turmeric, gingerol in ginger, organosulphur compounds in allium species, omega-3 fatty acids in cucurbitaceous vegetable seeds and so on (Ram et al., 2000).

Cassava leaves are largely consumed in Africa and are among the top three African indigenous vegetables rich in nutrients (Umuhozariho et al., 2013). In Nigeria, especially in the Northern part of the country, cassava leaves are used in the preparation of soup. Cassava leaves soup is widely consumed in Central Africa. In Central Africa, cassava leaves soup is called Saka Saka or Pondu (Immaculate, 2021).

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The leaves of cassava are utilized as vegetables and the tuberous roots can be processed into over one hundred food types. Certain cassava varieties are mainly grown for industrial use (Fyhri, 2010). In the rural areas, the roots and young leaves are utilized as a vegetable. Cassava "mash", fufu, is widely consumed by pounding and sieving cassava to make flour which is then put into hot water and other cassava produce.

Groundnut (*Arachis hypogeal*) belongs to the pea and beans family and is a legume. Groundnut is rich in oil and protein, and has a high energy value (Nautiyal et al., 2002). It can be eaten raw, roasted or cooked and the flour is an ingredient in many foods. Groundnut can be used in the preparation of groundnut soup with warm garri. Groundnut soup is mostly cooked by Northerners and some parts of Southern Nigeria (Nautiyal et al., 2002). Groundnut is important in vegetarian diets because of the protein it contains. It provides 13 different vitamins, especially A, the B group, C and E, along with 26 essential trace minerals, including calcium, iron, zinc and boron, and dietary fibre (Nautiyal et al., 2002). Groundnut is a rich source of fat ranging from 36 to 54% (Asibu, 2008). Groundnuts and groundnut butter are energy-rich and nutritious foods, providing a valuable supply of a wide range of vitamins, minerals and dietary fibre (Jennette, 2003).

Beans (*Parkia biglobosa*) are a tropical legume plant and have been used as a staple in the diet, and the health benefits derived from them have been well recognized. Beans are a strong, plant-based source of protein, fibre and iron that offer many health benefits (Kathy et al., 2023). Beans are nutrient-dense in that the amount of nutrients provided per calorie is particularly high. They are packed with protein, carbohydrates, vitamins, minerals, and are low in fat. They are rich in lignans, which may play a role in preventing osteoporosis, heart disease, and certain cancers (Maria, 2008). Vegetables, including legumes/beans are nutrient-dense, low in kilojoules, and are a good source of minerals and vitamins (such as magnesium, vitamin C and folate), dietary fibre and a range of phytochemicals including carotenoids (Darrell et al., 2008).

This study was aimed to determine the nutritional and phytochemical components of mature and young leaves of cassava (*M. esculenta*), groundnut (*A. hypogeal*) and beans (*P. biglobosa*) so as to ascertain the possibility of utilizing them as vegetables in traditional Nigerian soups.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves of plants screened in this investigation were collected from a multiple farming farmland that grows cassava, beans, groundnut, and garden egg located at Ekrejeta, Abraka, Delta State, Nigeria with an agroecological zone within the coordinates 5.7894º N and 6.1023º E.

Samples Extraction

The method used for the extraction of the samples was that of Marinova (2005). Samples weighing 0.5g respectively were ground using a mortar and pestle. All samples were homogenized with 50ml distilled water and transferred to test tubes. Then, the mixtures were centrifuged for 10 minutes at 4,000 rpm. The supernatants were collected and used for the determinations.

Assay for Soluble Protein Content

The protein content of each sample was determined by means of the Biuret Method as described by Gornal et al. (2000) with some modifications. 1.5ml of the biuret reagent was added to 0.5ml of each of the three different formulations in their respective test tubes and thoroughly mixed. Thereafter, the test tubes were left to stand for 30 minutes at 37ºC and then the absorbance of the samples was determined in the spectrophotometer at 540nm wavelength against reagent blank.

Estimation of Soluble Sugar (Phenol Sulphuric Acid Reagent method)

Five milliliters each of the plant leaf extract was homogenized with 10ml of 80% ethanol. Then each homogenate was centrifuged at 2000rpm for 15 minutes. The supernatants were collected separately. To 1ml of alcoholic extract, 1ml of 5% phenol solution was added and mixed. Five milliliters of 96% sulphuric acid was also added. This was done in triplicate. Each tube was gently agitated during the addition of the acid and then allowed to stand in a water bath at 25°C for 20 minutes. The optical density of the characteristic yellow-orange colour thus developed was measured at 490nm in a spectrophotometer. Simultaneously, a standard curve

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was prepared by using a known concentration of glucose. The amount of sugar was expressed as mg/g (Dubois et al., 2002).

Determination of Total Protein

The method used for the determination of total protein was that of Lowry et al. (2002). Five milliliters of the sample was mixed with 10ml of concentrated H_2SO_4 in a digestion flask. Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) was added to catalyze the reaction and then heated under a fume cupboard until a clear solution was obtained. The clear solution was diluted to 100ml in a volumetric flask, filtered, and used for the analysis. Ten milliliters of the diluted solution was mixed with an equal volume of 45% NaOH solution in a Kjeldahl distillation apparatus. The mixture was added into 10ml of 4% basic acid containing three drops of mixed indicator (bromocresol green and methyl red). A total of 50ml distillate was collected and titrated against 0.02N EDTA from green to a deep red endpoint. A reagent blank was also digested, distilled, and titrated. The nitrogen content, hence the protein content, was calculated thus:

$$\%\text{Protein} = \%N_2 \times 6.25$$

$$\%N_2 = \frac{(T - B) \times N \times 1.4 \times V_t}{W \times V_a}$$

Where:

- W = weight of sample analyzed (5ml)
- N = normality of titrant (0.002N H_2SO_4)
- V_t = total digest volume (100ml)
- V_a = volume of digest analyzed (10ml)
- T = sample titre value
- B = blank titre value

Nitric Oxide Radical Scavenging Assay

The scavenging effect on nitric oxide (NO) radical was measured according to the method of Marcocci et al. (2000). One milliliter of the leaf extract was added in the test tube to 1ml of sodium nitroprusside solution (25ml) and the tubes incubated at 37ºC for 2 hours. An aliquot (1ml) of the incubation solution was removed and diluted with 0.6ml of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570nm against distilled water as blank with catechin (500g) used as standard. The results were expressed as percentage radical scavenging activity (RSA):

$$\%\text{RSA} = \frac{\text{Abs. of Control} - \text{Abs. of sample}}{\text{Abs. of Control}} \times 100$$

Quantitative Determination of Saponin

The method used for the determination of saponin was that of Peny et al. (2005). Five grams of the leaf samples were pulverized and placed in a conical flask and 50ml of 20% aqueous ethanol added. The samples were stirred at a constant temperature of 55ºC for 12 hours. Thereafter, the samples were filtered with Whatman No. 1 filter paper and the residue re-extracted with another 100ml of 2% ethanol. The extracts were combined and reduced to 40ml by heating in a water bath at 55ºC. The purification process was repeated two more times. Six grams of NaCl were added to adjust the pH of the solution to 4.5 (confirmed with a pH meter). The solution was shaken with 30ml portion of n-butanol, extracted and washed twice with 10ml of aqueous NaCl. They were dried to constant weight, and the saponin extracted was shaken and expressed as a percentage:

$$\%\text{Saponin} = \frac{\text{Weight of Residue}}{\text{Weight of Sample}} \times 100$$

Alkaloid Determination

Alkaloid content was determined gravimetrically (Sreexidya and Mehrotra 2003). One gram of leaf was dispersed in 10ml of 10% acetic acid in methanol. The suspension was shaken and allowed to stand for 4 hours before it was filtered. The filtrate was evaporated to a quarter of its original volume before concentrated ammonium hydroxide (30%) was added dropwise to precipitate the alkaloids. A pre-weighed filter paper was used to filter the precipitate and it was then washed with ammonium hydroxide solution (1%). The filter paper with alkaloids precipitate was dried at 60ºC until a constant weight was obtained. The content of alkaloid was determined by the difference in the filter paper and expressed as g/100g DW.

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Determination of Tannin

Tannins were determined using the method of Dawra et al. (1998). Zero point two grams of each sample was weighed into a beaker. Each was soaked with a solvent mixture (80ml of acetone and 20ml of glacial acid) for 5 hours to extract tannin. The filtrates were removed and samples were filtered through a double filter paper to obtain the filtrate. Catechin was used as a standard solution. The absorbances of the standard solution as well as that of the filtrates were read at 750nm on a spectrophotometer.

$$\text{Conc. of Samples} = \frac{A_{\text{Sample}} \times \text{Conc. of std.}}{A_{\text{Std.}}}$$

RESULTS

The results obtained from the quantitative analyses of soluble protein, soluble sugar, total protein, nitric oxide radical scavenging assay, saponin, alkaloid, and tannin from the three investigated vegetable leaves are presented in the figures below.

Assay for Soluble Protein Content

The results of the quantitative analysis revealed that soluble protein was present in all the vegetable plant leaves investigated; six weeks old cassava leaves have the highest mean value (0.24g/g FW) compared to that of the matured cassava leaves (0.19g/g FW) (Figure 1). Two weeks old groundnut leaves and matured groundnut leaves have the same mean value (0.18g/g FW) while two weeks old beans leaves have a lower mean value compared to that of the matured beans leaves (Figure 1). This revealed that six weeks old cassava leaves have the highest soluble protein content.

Estimation of Soluble Sugar

From the results, the soluble sugar of the six weeks old cassava leaves is slightly higher than the matured cassava leaves. The mature groundnut leaves have a slight higher mean value (0.07g/g FW) than the two weeks old groundnut leaves (0.06g/g FW) while the two weeks old beans leaves (0.04g/g FW) have a higher mean value than the matured beans leaves (0.02g/g FW). This showed that six weeks old cassava leaves have the highest soluble sugar content (Figure 2).

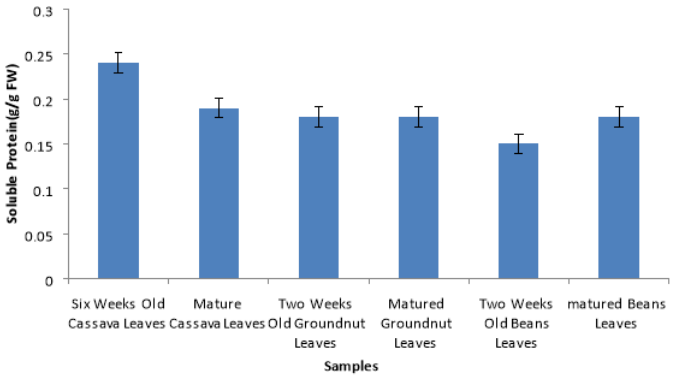

Figure 1: Soluble Protein Content of Cassava, Groundnut, and Beans Leaves

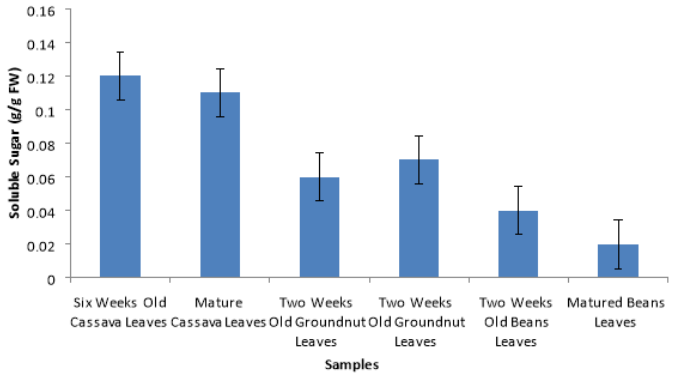

Figure 2: Soluble Sugar Content of Cassava, Groundnut, and Beans Leaves

Determination of Total Protein

The total protein content of the matured cassava leaves have a higher mean value (36.69%) compared to that of the six weeks old cassava leaves (34.86%). The two weeks old groundnut leaves have a higher total protein value (17.96%) than the matured groundnut leaves (15.96%) while the matured beans leaves have a higher mean value (3.82%) than the two weeks old beans leaves (2.82%). This showed cassava leaves have the highest total protein content (Figure 3).

Nitric Oxide Radical Scavenging Assay

As seen, nitric oxide radical scavenging activity of the six weeks old cassava leaves (2.25%) is higher than the matured cassava leaves (2.18%). Although the two weeks old groundnut leaves (2.00%) are slightly higher than the matured groundnut leaves (1.91%), the matured beans leaves have a higher mean value

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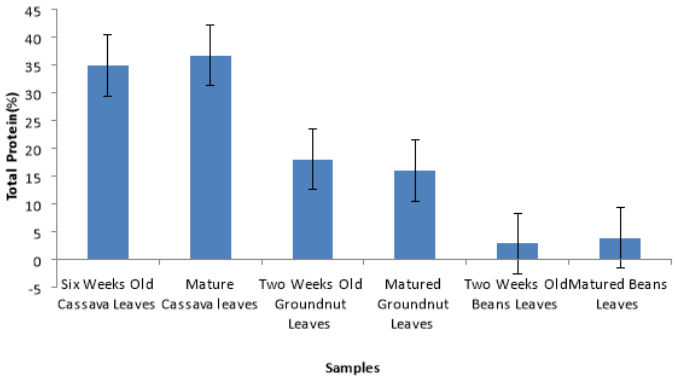

Figure 3: Total Protein Content of Cassava, Groundnut, and Beans Leaves

(2.13%) than the two weeks old beans leaves (1.98%) (Figure 4). This revealed that the six weeks old cassava leaves have the highest nitric oxide radical scavenging assay.

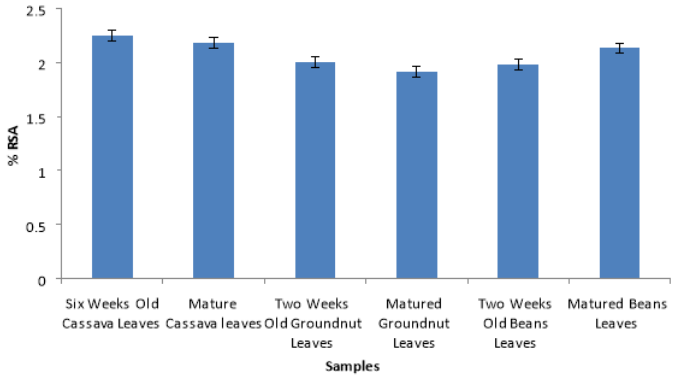

Figure 4: Nitric Oxide Radical Scavenging Assay of Cassava, Groundnut, and Beans Leaves

Quantitative Determination of Saponin

The saponin content of the matured cassava leaves have a higher mean value (0.21g/g FW) compared to that of the six weeks old cassava leaves (0.15g/g FW). The matured groundnut leaves have a higher saponin value (0.14g/g FW) than the two weeks old groundnut leaves (0.11g/g FW) while the matured beans leaves have a higher mean value (0.18g/g FW) than the two weeks old beans leaves (0.16g/g FW) (Figure 5). This showed that the matured cassava leaves have the highest saponin content among all the leaves investigated.

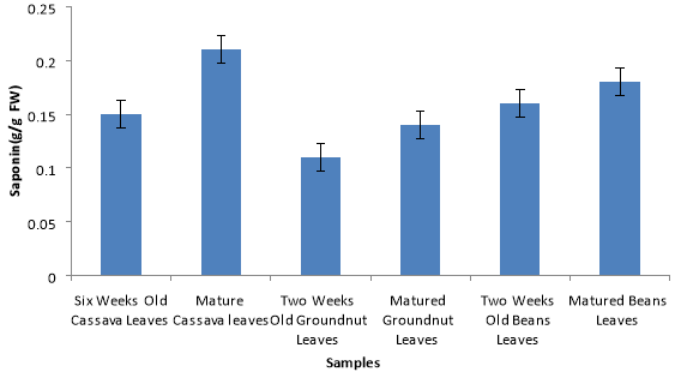

Figure 5: Saponin Content of Cassava, Groundnut, and Beans Leaves

Alkaloid Determination

The alkaloid content of the six weeks old cassava leaves is higher than the matured cassava leaves. The two weeks old groundnut leaves have a higher mean value (0.02g/100g) than the matured groundnut leaves (0.01g/100g) while the matured beans leaves have a higher mean value (0.04g/100g) than the two weeks old beans leaves (0.02g/100g) (Figure 6). This showed that the matured beans leaves have the highest alkaloid content. Alkaloids act on a diversity of metabolic systems in humans and other animals; they almost uniformly invoke a bitter taste (Rhoades et al., 2000).

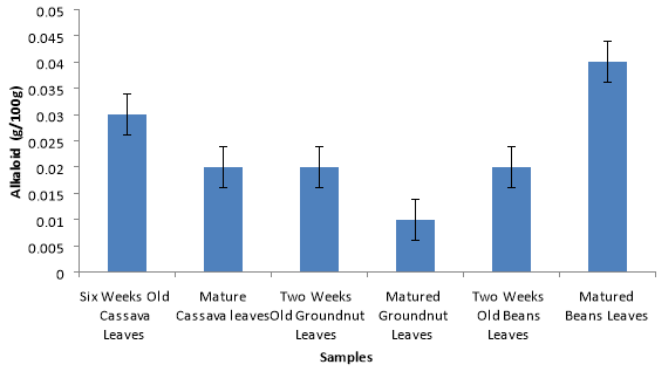

Figure 6: Alkaloid Content of Cassava, Groundnut, and Beans Leaves

Determination of Tannin

The tannin content of the matured cassava leaves (0.04g/g FW) is higher than the six weeks old cassava leaves (0.02g/g FW). Matured groundnut leaves have a higher amount of tannin (0.05g/g FW) compared to

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that of the two weeks old groundnut leaves (0.04g/g FW), while that of the matured beans leaves have a high tannin content (0.05g/g FW) compared to the two weeks old beans leaves (0.04g/g FW) (Figure 7). This revealed that matured beans leaves have the highest tannin content.

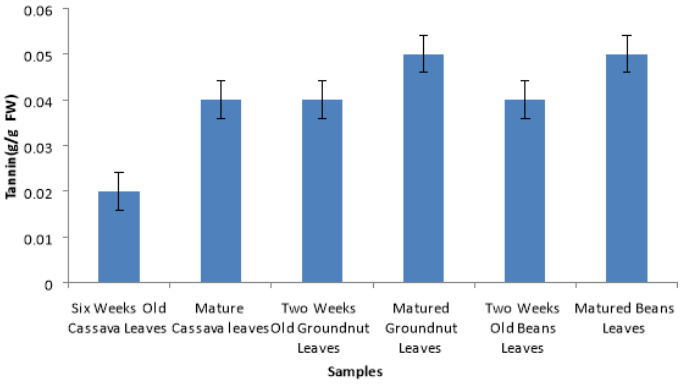

Figure 7: Tannin Content of Cassava, Groundnut, and Beans Leaves

Discussion

The soluble protein, soluble sugar, and nitric oxide radical scavenging concentrations are higher in the young cassava leaves followed by the matured cassava leaves. A study by Ravindran and Ravindran (2010) on cassava leaves during different stages of maturity reported a decrease in solubility in protein and carbohydrate contents from young to mature leaves respectively. Hence their high nutritive value may probably account for their use in catalyzing metabolic reactions while polysaccharides are long carbohydrate molecules of monosaccharide units joined together by glycosidic bonds and serve as a source of energy. The high soluble sugar content of the six weeks old cassava leaves could therefore be used as a source of energy. In humans, nitric oxide radical is an important cellular signaling molecule involved in many physiological and pathological processes (Moncada et al., 2004). This implies that six weeks old cassava leaves is a powerful vasodilator with a short half-life of a few seconds in the blood when used.

For the groundnut leaves, soluble protein, soluble sugar, and total protein concentrations have high nutritional value compared to that of beans leaves and serve as a great source of protein (Shalini et al., 2015). This is an indication that these vegetables have a good amount of nutrients.

The saponin and tannin content of the matured cassava leaves have higher mean values compared to that of the young cassava leaves. Alkaloid of the young cassava leaves is higher than the matured cassava leaves. According to research, cassava leaves contain a high concentration of phytochemical components like saponin, alkaloid, and tannins (Babalola and Ahmed, 2005).

The matured groundnut and beans leaves have higher saponin, alkaloid, and tannin concentrations than the young leaves, except for young groundnut leaves which have a slightly higher mean value than the matured groundnut leaves. This work is in agreement with that of Chelangat and Mukono (2023).

This showed that the bioactive non-nutrient plant compounds in vegetable plant foods have complementary and overlapping mechanisms of action, including modulation of detoxification enzymes, scavenging of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects (Dragsted et al., 2000). The results of the quantitative analysis of these plants showed that all of the plant leaves contained varying proportions according to the nutritive and phytochemical properties in them.

Conclusion

This study showed the determination of the nutritional and phytochemical components of mature and immature leaves of cassava (*M. esculenta*), groundnut (*A. hypogeal*), and beans (*P. biglobosa*) so as to ascertain the possibility of utilizing them as vegetables in traditional Nigerian soups and incorporating them into other dishes. Though the analysed components of the vegetables are not enough to satisfy the recommended dietary allowances (RDAs), they can be used as sources of additional organic nutrients in our daily traditional meals.

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