

COMPARISON OF THE ANTIOXIDANT ACTIVITY, PHYTOCHEMICAL AND NUTRITIONAL CONTENTS OF TWO ANTIHYPERTENSIVE ETHNOMEDICINAL PLANTS

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ABSTRACT

The fruits of *Xylopia aethiopica* (Dunal) A. Rich and leaves of *Persea americana* (Mill.) are ethnomedicinally used in combination (1:10) as a remedy for the treatment of hypertension in southwest Nigeria. This study compared the antiradical activity, phytochemical and proximate components of the two plants, with a view to providing scientific information on the therapeutic properties and potential of the plants. The antioxidant activity of the samples was against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The phytochemical and proximate analyses were carried out using standard laboratory techniques. The plants showed varied antioxidant activity. *X. aethiopica* had 39.10 mg GAE/g polyphenol content and gave 67.43% inhibition against DPPH⁺ whereas *P. americana* with 24.3 mg GAE/g polyphenol content had 48.3% inhibition against DPPH⁺. It was observed that there was direct relationship between polyphenol content and antioxidant activity of the plants. There were also significant variations in the chemical components of the plants. Quantitative phytochemical screening showed that alkaloids (1483.33 mg/100g), proanthocyanidins (16.17mg GAE/g), tannins (968.33 mg/100g) and anthraquinones (58.3 mg/100g) were higher in *X. aethiopica* than in *P. americana*. On the other hand, *P. americana* contained more flavonoids (777.67 mg/100g), cardiac glycosides (41.67 mg/100g) and saponins (610 mg/100g) than *X. aethiopica*. *X. aethiopica* was richer in ash (4.47%), fats (22.43%), crude fibre (12.47%), moisture content (10.23%) and energy (2716.30Kcal) than *P. americana*. However, *P. americana* had higher contents of crude protein (21.57%) and carbohydrates (50.73%). The observed antioxidant activity and chemical components of the two plants might be responsible for their therapeutic use as antihypertensive remedy.

Keywords: *Xylopia aethiopica*, *Persea americana*, Antioxidant activity, Phytochemical components, nutritional contents

INTRODUCTION

X. aethiopica, commonly called Ethiopian/African pepper is locally known as “Eeru-alamo” among Yoruba people of southwest Nigeria (Figure. 1). It is an evergreen, aromatic tree, of the Annonaceae family that can grow up to 20m high. It is a native to the lowland rainforest and moist fringe forests in the savanna zones of Africa (Orwa *et al.* 2009; Burkill, 1985). It is an important spice in the preparation of several delicacies, and also used traditionally in the management of several diseases in Nigeria (Mann, 2011).

P. americana known as Avocado is called “Ubeyinbo” (Igbo) or “Pia” (Yoruba) in Nigeria (Figure. 2). It belongs to family Lauraceae. *P. americana* is a medium to large tree, 9-20m in height. The avocado is an evergreen tree with canopy ranges from low, dense, and symmetrical to upright and asymmetrical (Anon, 1986). Leaves and seed cotyledon are used in the management of hypertension and other diseases in Nigeria (Gill,

1992; Lawal *et al.*, 2009).

A decoction of the fruits of *X. aethiopica* and leaves of *P. americana* is used in combination (1:10) as antihypertensive remedy in southwest Nigeria. In addition, *X. aethiopica* and *P. americana* have been reported to possess an array of bioactivities and pharmacological effects such as antimicrobial, anti-inflammatory, hypolipidaemia and hepatoreparative/hepatoprotective effects (Erhirhie and Moke, 2014; Yasir *et al.*, 2010). The hypotensive effect of *X. aethiopica* fruit and *P. americana* leaf as a recipe has been reported by Gbadamosi *et al.* (2016).

Oxidative stress as caused by generation of free radical has been implicated in the pathogenesis of many degenerative diseases such as hypertension, diabetes, strokes, cancer, and neurodegenerative diseases (Uttara *et al.*, 2009). In order to alleviate this menace of oxidative stress, the body naturally synthesizes antioxidants and enzymes with

antioxidant activities such as superoxide dismutase (SOD), catalase (CAT), Glutathione (GSH) and Nitric oxide (NO) whose primary role is to deactivate reactive oxygen species (ROS) (Bharti and Srivastava, 2009). Chemically synthesized antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) are often added to food as preservative to prevent lipid peroxidation (Venkatesh and Sood, 2011). The DPPH assay is reportedly one of the most widely and effective methods for screening antioxidant activities of samples. It allows testing of both lipophilic and hydrophilic compounds (Koleva *et al.*, 2002). In a DPPH radical scavenging assay, BHA has antioxidant ability of about 64% whereas BHT has about 49% (Katalinic *et al.*, 2004). Kahl and Kappus (1993) reported toxic effect of chemically synthesized antioxidants such as BHT and BHA. Their toxic effects include impairment of blood clotting and tumour promoting effect. Specifically, BHA causes tumour in the fore stomach while BHT causes tumour in lungs and liver (Karl and Kappus, 1993). On the other hand, plants polyphenols might serve as antioxidant to 'mop up' these free reactive oxygen species (ROS) with no side effects (Pandy and Rizvi, 2009). Antioxidants of plant origin could have great importance in prevention and treatment of several diseases associated with oxidative stress (Krishnaiah and Sarbaty, 2007).

The presence of phytochemicals in plants lends scientific credence to their ethnomedicinal uses in the treatment of several degenerative disorders. Alkaloids have been reported to have wide range of biological activities such as emetic, diuretic, antidepressant, antihypertensive, antimicrobial and anti-inflammatory among other (Abulude, 2007). Flavonoids are known to be bactericidal, pesticidal and fungicidal in nature (El astal *et al.*, 2005). According to Dharmanda (2003), tannins in plants are known to be astringents, which help in wound healing. Usman and Usuji (2007) reported that they are used for treating intestinal disorders such as diarrhea and dysentery.

Anderson *et al.* (2009) reported that individuals with high intakes of dietary fiber have significant lower risk for developing coronary heart diseases, stroke, hypertension, diabetes, obesity and certain

gastrointestinal diseases. Also, increasing fiber intake lowers blood pressure and serum cholesterol levels. Increased fibre content in the body of organisms could reduce incidence of diseases like diabetes, high blood pressure, piles, digestive disorders (SACN, 2008). Apart from insulating and conserving body temperature in organisms, fatty acid components such as lauric acid in *P. americana*, have been reported to improve health (Fite, 2000). The impact of proteins in body system cannot be over looked. They repair and replace worn out cells, form structural and globular materials that hold the body, form blood proteins, and boost the immune system (Olusanya, 2008). Hofman *et al.* (2002) reported that ash content is generally recognized as measure of quality for the assessment of functional properties of foods. Ash constituents of the investigated samples could be related to their mineral contents and these minerals, which are mostly in forms of chemical compounds, play numerous functions towards the improvement of health in the body of organisms (Onwuka, 2005). Carbohydrates are related to energy generation (Olusanya, 2008).

In view of the reported ethnomedicinal values of the two test plants, this study investigates antioxidant activity, phytochemical and proximate composition of *X. aethiopica* and *P. americana* to provide an empirical basis or justification for their use as antihypertensive ethnomedicinal plants.

MATERIALS AND METHODS

Collection and Identification of Plants

Dried fruits of *Xylopia aethiopica* (UIH-22458) and leaves of *Persea americana* (UIH-22459) were collected from University of Ibadan campus. Voucher specimens were deposited at the University of Ibadan Herbarium (UIH) for reference purpose.

Preparation of Plant Materials

The plants were cut into smaller pieces or macerated and air-dried for two weeks at room temperature (25-30 °C). The dried plants were then ground into powder and stored in air-tight glass containers prior to their use in experiments.

Reagents

Ethanol, sodium acetate, vanillin, methanol,

potassium ferrocyanide, standard tannic acid, acetic acid, ammonium hydroxide, diethyl ether, benzene, n-butanol, standard anthraquinone, 2,2-bipyridine, ferric ammonium sulphate, ferric chloride, concentrated tetraoxosulphate VI acid, glacial acetic acid, acetone, sodium chloride, ethanol, ammonia, 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution, zinc dust.

In Vitro Antioxidant Assay

The method of Brand-Williams *et al.* (1995) was used to determine antioxidant activity via DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay. DPPH (20 mg/dm³) was prepared in 80% methanol. 0.2 mg of extract of sample was added to 2.8 ml of DPPH solution. The mixture was incubated for 20 min in dark room at room temperature (25-30 °C) and the absorbance was read at 517 nm using a spectrophotometer. Methanol only was used as a blank to adjust the spectrophotometer to zero absorbance and DPPH solution was used as the control. The scavenging activity of each extract was calculated as follows:

$$\% \text{ Inhibition} = [1 - (A^{\text{sample}} / A^{\text{control}})] \times 100$$

where A^{control} is the absorbance for control and A^{sample} is absorbance for test sample.

Phytochemical Analysis

The samples were analysed for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phytates, polyphenols, proanthocyanidins, saponins and tannins.

Alkaloids

The method of Harborne (2005) was used for the analysis of alkaloids in samples. 200 ml of 10 % acetic acid in ethanol was added to 5.0 g of powdered sample in 250 ml beaker. The mixture was covered and allowed to stand for 4 h. The mixture was filtered and the filtrate was concentrated on a water bath to one-fourth of its original volume, concentrated ammonium hydroxide (NH₄OH) was added drop wise to the extract until precipitation was completed and the solution was allowed to settle. The precipitate collected was washed with dilute NH₄OH and then filtered. The residue was dried and weighed. The alkaloid content was calculated using the formula: % alkaloid = Final weight of the sample / Initial weight of the extract x 100. The

experiment was replicated three times. The result was expressed as mg/100g.

Anthraquinones

Benzene (60 ml) was added to 0.5 g of sample in a beaker and stirred. The mixture was filtered into 100ml volumetric flask. 0.2% zinc dust and 59 ml of hot 5% NaOH solution were added. The mixture was heated just below boiling point for 5mins and rapidly filtered. This was then washed once in water. The filtrate was heated again with another 50 ml of 5% NaOH to develop a red colour. Standards were then prepared from 100 mg/L stock and treated in a similar way with 0.2% zinc dust and NaOH. Absorbance was read at 640nm (Harborne, 2005). The result was expressed as mg/100g.

Cardiac Glycosides

Water (40ml) was added to 1g of sample. The mixture was placed in oven at 100°C for 15mins and then filtered. To 1ml of the filtrate, 5ml of water and 2ml of glacial acetic acid were added. 1 drop of FeCl₃ and 1 ml of concentrated H₂SO₄ were then added to the mixture (Harborne, 2005). The absorbance of the mixture was read at 410nm using spectrophotometer and the result was expressed as mg/100g.

Phytates

One gram (1 g) of the sample was extracted with 0.2 M HCl. Fe³⁺ solution was added to 0.5 ml of the extract. The mixture was heated in a water bath for 30 mins, cooled and then centrifuged. 1.5 ml of 2, 2-bipyridine solution was then added to 1 ml of the supernatant. Absorbance was read at 519 nm using distilled water as blank (Harborne, 2005). The result was expressed as mg/100g.

Flavonoids

The method of Ordon Ez *et al.*, (2006) was used for the flavonoids analysis of the samples. The sample (0.5 ml) was added to 0.5 ml of 2% AlCl₃ ethanol solution. The mixture was allowed to stand for 1 h at room temperature and a yellow color indicated the presence of flavonoids. The absorbance was read at 420 nm. The samples were evaluated at a final concentration of 0.1 mg/ml. The result was expressed as mg/g using the equation: $Y = 0.0255x$, $R^2 = 0.9312$, where x is the absorbance and Y is the quercetin equivalent. The experiment

was replicated three times. The result was expressed as mg/100g.

Polyphenols

An aliquot of the extract was mixed with 5 ml of Folin-Ciocalteu's reagent (previously diluted with water at a concentration of 1:10 v/v) and 4ml of 75 g/L of sodium carbonate in a test tube. The mixture was vortexed for 15 s and left to stand for 30 min at 40 °C for color development. The absorbance was read at 765nm using spectrophotometer (Wolfe *et al.*, 2003). The results were expressed as mg/g of tannic acid equivalent using the calibration curve: $Y=0.121x$, $R^2=0.936512$, where x is the absorbance and Y is the tannic acid equivalent. The experiment was replicated three times. The result was expressed as mg/100g.

Proanthocyanidins

The extract (0.5 ml of 1 mg/ml) solution was mixed with 3 ml of vanillin-methanol (4% v/v) and 1.5 ml of hydrochloric acid. The mixture was left for 15min at room temperature and the absorbance was read at 500nm (Sun *et al.*, 1998). The result was expressed as catechin equivalent (mg/g) using the calibration curve equation: $Y = 0.5825x$, $R^2 = 0.9277$, where x is the absorbance and Y is the quercetin equivalent. The experiment was replicated three times. The result was expressed as mg/100g.

Saponins

The powdered sample (20g) was added to 100 ml of 20% aqueous ethanol and kept on a shaker for 30 min. The sample was heated on a water bath for 4h at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 ml on a water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60 ml n-butanol was added. Then n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride (NaCl). The remaining solution was heated on a water bath. After evaporation, the sample was dried in the oven at 40°C to a constant weight (Harborne, 2005). The saponin content was calculated and expressed as

mg/100g.

Tannins

The sample (0. 20g) was added to 20 ml of 50% methanol. The mixture was shaken thoroughly and placed in a water bath at 80°C for 1h to ensure uniform mixing. The mixture was filtered into a 100 ml volumetric flask, followed by the addition of 20ml of distilled water, 2.5ml of Folin-Denis reagent and 10 ml of 17% aq. Na_2CO_3 . The solution was thoroughly mixed. The mixture was made up to 100 ml with distilled water, mixed and allowed to stand for 20 min. The bluish-green color developed from the reaction mixture of different concentrations (0-10ppm). The absorbance of the tannic acid standard solutions as well as sample was read at 760 nm using the spectrophotometer (Harborne, 2005). The results were expressed as mg/g of tannic acid equivalent using the calibration curve: $Y= 0.0593x - 0.0485$, $R^2 = 0.9826$, where x is the absorbance and Y is the tannic acid equivalent. The experiment was replicated three times. The result was expressed as mg/100g.

Proximate Analysis

The carbohydrate, crude fibre, crude protein, fat, moisture, total ash contents and energy values of samples were analysed using standard protocols (AOAC, 2006; Horwitz, 2000; Greenfield and Southgate, 1992; ASEAN, 2011) in the Laboratory of the Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria.

Determination of moisture content: The air-oven method was used for the evaluation of moisture content of samples. Crucible was washed and dried in an oven at $100\pm 5^\circ\text{C}$ until constant weight (1–2 h). The crucible was allowed to cool in the desiccator for 30 min and weighed (W_1). The thoroughly mixed sample (4 g) was transferred into the pre-weighed crucible. The weight of the sample and crucible was taken before drying (W_2). The crucible with sample was placed in the air oven preheated to $100\pm 5^\circ\text{C}$ for 2–3 h. The crucible with dry sample was transferred into the desiccator and allowed to cool for 30 min and weighed (W_3). The procedure was repeated until constant weight was obtained. The moisture content was calculated as follows:

% Moisture content = $(W_2 - W_3) / (W_2 - W_1) \times 100$.

Determination of ash content: The gravimetric method was used for the quantitative determination of ash in samples. The crucible was heated in the furnace at 500 - 550 °C for 2 – 3 h. The temperature was reduced to 180°C and the crucible was transferred into a desiccator, cooled for 30 min and weighed (W_1). 4g of sample was transferred into the pre-weighed crucible (W_2). The sample was charred over a hotplate, initially at low temperature to avoid spattering, the temperature was gradually increased until smoking ceased. The charred sample was incinerated in a furnace at 500 - 550 °C until the residue was uniformly white or neatly white, the temperature was reduced to 180°C and the sample was allowed to cool in a desiccator for 30 min, and weighed (W_3). The ash content was calculated as follows:

% Ash content = $(W_3 - W_1) / (W_2 - W_1) \times 100$.

Determination of crude fat: The Soxhlet's extraction method was used. The sample (5 g) was measured into a weighed filter paper and folded neatly; this was put inside pre-weighed thimble (W_1). The thimble with the sample (W_2) was inserted into the Soxhlet's apparatus and extraction under reflux was carried out with petroleum ether (40°C – 60°C boiling range) for 4-6h. At the end of extraction, the thimble was dried in the oven for about 30 minutes at 100°C to evaporate off the solvent and cooled in a desiccator. It was re-heated and weighed again every 30 min until a constant weight was obtained (W_3). The percentage fat was calculated as follows:

% Crude fat = $(W_2 - W_3) / (W_2 - W_1) \times 100$.

Determination of crude protein: The crude protein content of sample was analysed using Kjeldahl method. The sample (10g) was weighed into the digestion tube, 5 g of catalyst and 1 glass bead to prevent solution from bumping and 10 – 20 ml of H_2SO_4 were added to the sample in the digestion tube. The digestion tube was placed in the digester and the moisture was digested until the solution became clear and free of carbon. The clear solution was heated for another hour to completely breakdown all organic matter. 500 ml

conical flask containing 50 ml of 4 % boric acid with indicator as receiver was placed on the distillation unit. 100 ml of water and 70 ml of 50 % NaOH were added to the digests in the distillation set. The distillation of the mixture was done until all ammonia had been released or 150 ml distillate was obtained. The distilled ammonia was trapped in an ice cold condenser. The receiver flask was lowered so that the delivery tube was above the liquid surface, the distillation continued for 1 – 2 min. Finally, the delivery tube was rinsed with water and the washings allowed to drain into the flask. The distillate was titrated with the standardized 0.1 N HCL until the first appearance of the pink colour. The acid used was recorded to the nearest 0.05 ml. A blank sample was passed through the same procedure and the titre value for the blank was used to correct the titre for the original sample. The % crude protein was calculated as follows:

% Nitrogen = $(\text{ml } 0.1\text{N HCl sample} - \text{ml } 0.1\text{N HCl blank}) \times 0.0014 \times \text{N HCl} \times 100 / \text{weight of sample}$.

% Protein = % total nitrogen \times 6.25.

Determination of crude fibre: The total dietary fibre of samples was determined using enzymatic-gravimetric method. The method involves enzymatic digestion of dried defatted sample by heat stable alpha-amylase, protease and amyloglucosidase to remove starch and protein present in the sample. Ethanol is then added to the digest to precipitate soluble dietary fibre. 1g of sample was defatted in 25ml of petroleum ether. The sample was digested in 50 μ L of alpha amylase solution in a beaker. The beaker was placed in shaking water bath at 95°C and incubated for 30mins with continuous agitation. The sample was further digested in protease solution and 5ml of 0.561 N HCl solution while stirring. The pH of the solution was adjusted to 4.0-4.7 using 1 N HCl solution after which 300 μ L of amyloglucosidase solution was added to the mixture while stirring. The mixture was incubated in shaking water bath with constant agitation at 60°C for 30mins. To each digested sample, 225ml of 95% ethanol was added and pre-heated at 60°C. The solution was allowed to stand for 60min at room temperature to allow precipitate to form. The mixture was filtered and residue was washed with 78% ethanol and then transferred into a crucible. The crucible containing residue was put in oven at 100°C

overnight until a constant weight was achieved. The fibre content was expressed as %.

Determination of carbohydrate: The carbohydrate content was calculated as follows:
% CHO = 100 - (Sum of the percentages of moisture, ash, fat, protein and crude fibre).

Statistical Analysis

Analysis of variance and comparison of means was carried out using statistical analysis system (SAS) version 9. Data were expressed as Mean \pm SD. The values lower than 0.05 probability ($p < 0.05$) were accepted as statistically significant by Duncan's Multiple Range Test.

RESULTS

The anti-radical activity of *Xylopiya aethiopica* and *Persea americana* against DPPH radical and their polyphenol content are presented in Table 1. *X. aethiopica* has higher antioxidant activity (67.43 %) than *Persea americana* (48.30%). Also, *X. aethiopica* contained higher polyphenols (39.10 mg GAE/g)

than *P. americana* (24.30 mg GAE/g) at $p < 0.05$. Phytochemical screening showed the presence of alkaloids, flavonoids, proanthocyanidins, cardiac glycosides tannins, phytates, anthraquinones and saponins in both plants (Table 2). Alkaloids (1483.33 mg/100g), proanthocyanidins (16.17 mgGAE/g), tannins (968.33 mg/100g), phytates (90.00 mg/100g) and anthraquinones (58.3 mg/100g) were higher in *X. aethiopica* than in *P. americana*. On the other hand, *P. americana* contained more flavonoids (777.67 mg/100g), cardiac glycosides (41.67 mg/100g) and saponins (mg/100g) than *X. aethiopica*. Table 3 shows quantitative proximate components of the *X. aethiopica* fruit and *P. americana* leaves. Both samples contained crude ash, fats, crude protein, crude fibre, moisture, carbohydrates and energy but in varied quantity. *X. aethiopica* was richer in ash (4.47%), fats (22.43%), crude fibre (12.47%), moisture content (10.23%) and energy (2716.30Kcal) than *P. americana*. However, *P. americana* had higher contents of crude protein (21.57%) and carbohydrates (50.73%).

Table 1: Polyphenol Component and Antioxidant activities of *Xylopiya aethiopica* and *Persea americana*

| Parameters | Botanicals | |
|-----------------------------------|-------------------------------|-------------------------------|
| | <i>X. aethiopica</i> | <i>P. americana</i> |
| Polyphenols (mg GAE/ g) | 39.10 \pm 0.26 ^a | 24.3 \pm 0.21 ^b |
| % inhibition of DPPH ⁺ | 67.43 \pm 0.15 ^a | 48.30 \pm 0.40 ^b |

Values expressed as mean \pm S.D; n= 3. Means with different alphabet across the row are significantly different from each other at $p < 0.05$.

Table 2: Phytochemical components of fruits of *Xylopiya aethiopica* and leaves of *Persea americana*

| Parameters (mg/100g) | Botanicals | |
|----------------------|---------------------------------|--------------------------------|
| | <i>X. aethiopica</i> | <i>P. americana</i> |
| Alkaloids | 1483.3 \pm 16.07 ^a | 760.0 \pm 13.23 ^b |
| Tannins | 968.3 \pm 12.58 ^a | 246.7 \pm 12.58 ^b |
| Cardiac glycosides | 21.7 \pm 5.77 ^b | 41.7 \pm 5.77 ^a |
| Anthraquinones | 58.3 \pm 7.64 ^a | 11.7 \pm 2.89 ^b |
| Flavonoids | 431.7 \pm 10.41 ^b | 776.7 \pm 10.41 ^a |
| Proanthocyanidins | 6.2 \pm 1.26 ^a | 2.8 \pm 0.58 ^b |
| Phytates | 90.0 \pm 5.00 ^a | 51.7 \pm 2.89 ^b |
| Saponins | 341.67 \pm 10.41 ^b | 610 \pm 13.23 ^a |

Values expressed as mean \pm S.D; n= 3. Horizontally, means with different alphabet are significantly different from each other at $p < 0.05$.

Table 3: Proximate composition of *Xylopia aethiopica* fruit and of *Persea americana* leaf

| Parameters (%) | Botanicals | |
|------------------|---------------------------|---------------------------|
| | <i>X. aethiopica</i> | <i>P. americana</i> |
| Moisture content | 10.2±0.21 ^a | 9.3±0.15 ^b |
| Proteins | 19.6±0.15 ^{ab} | 21.6±0.21 ^a |
| Fats | 22.4±0.21 ^a | 6.5±0.15 ^b |
| Ash | 4.5±0.25 ^a | 3.4±0.15 ^b |
| Crude Fibre | 12.5±0.15 ^a | 8.5±0.15 ^b |
| Carbohydrates | 30.8±0.21 ^b | 50.7±0.21 ^a |
| Energy (Kcal) | 2716.2±60.32 ^a | 2299.0±17.93 ^b |

Values expressed as mean ± S.D; n= 3. Horizontally, means with different alphabet are significantly different from each other at p<0.05.

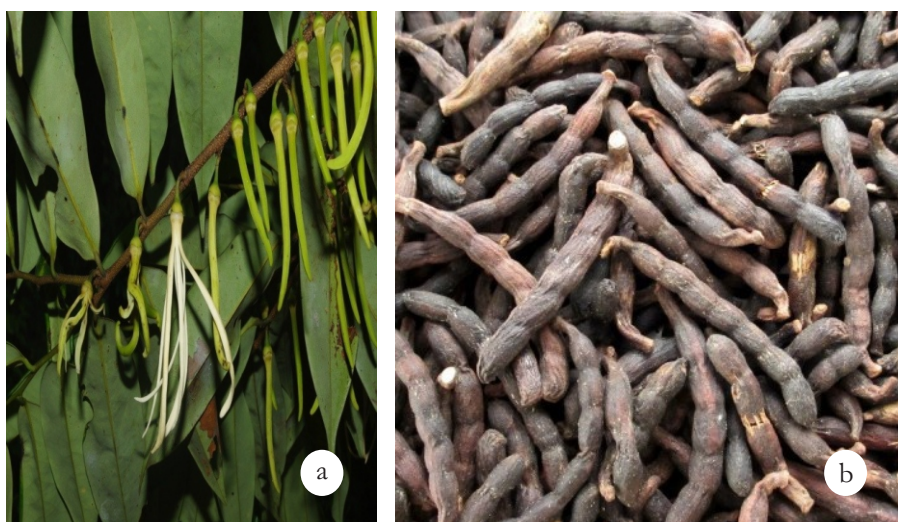


Figure 1: *Xylopia aethiopica* (Dunal) A. Rich
(a) Leaves and young fruits, (b) Mature fruits

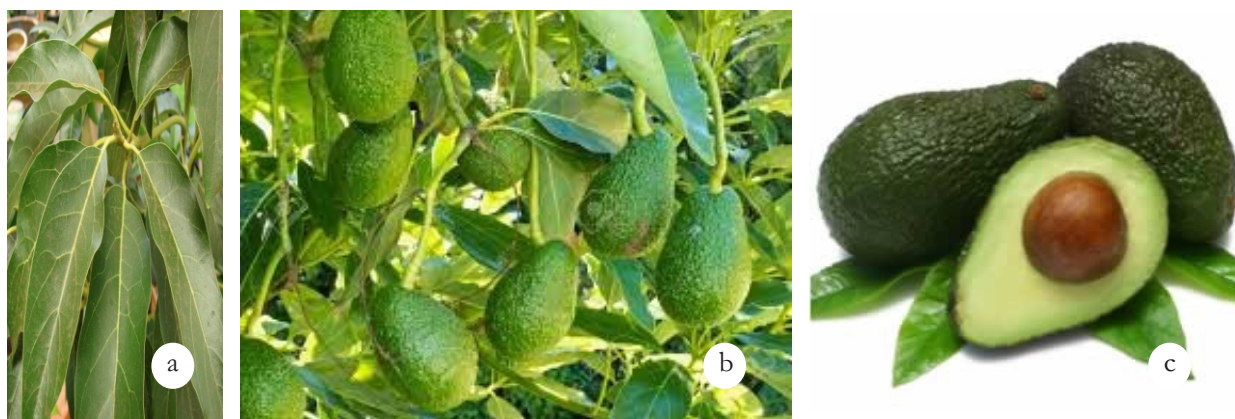


Figure 2: *Persea americana* Mill.
(a) Leaves, (b) Young fruits, (c) Mature fruits

DISCUSSION

The antiradical activity of the extract against DPPH⁺ was observed to be directly proportional to the total phenol content of the two plants. This suggests that the more the phenolic content, the higher the antioxidant activity. It was observed that *X. aethiopica* showed more antioxidant activity (67.43%) than *P. americana* (48.30%). The higher antioxidant activity displayed by *X. aethiopica* than *P. americana* agrees with the work of Amarowicz *et al.* (2000) who also reported a positive correlation ($r=0.966$; $P=0.002$) between antioxidant activity and total phenolic content. Tannic acid, a naturally occurring plant polyphenol was reported to exhibit an effective antioxidant activity in six different *in vitro* antioxidant assays including DPPH radical assay (Gulcin *et al.*, 2009). The antioxidant activity of *X. aethiopica* has been previously reported by Adefegha and Oboh (2012). *X. aethiopica* showed more antioxidant properties than synthetic antioxidants such as BHT (49%) and BHA (64%). Also, *X. aethiopica* contained significantly higher polyphenols (39.10 mg GAE/g) and this might be responsible for the higher antioxidant activity observed. Apart from antioxidant potentials, polyphenols have also been shown to have antibacterial, anti-inflammatory, anti-allergic, antiviral antineoplastic activity (Ojiako and Igwe, 2008). The effect of various extraction solvents on antioxidant activity of *P. americana* seed (Adeboyejo *et al.*, 2016) and leaf (Boadi *et al.*, 2015) has been reported. In this study, the antiradical activity displayed by *P. americana* (48.30%) is lower than those of *X. aethiopica* (67.43%) and BHA (64%), however, it is comparable with that of synthetic antioxidants such as BHT (49%). Furthermore, the little or no side effect of plant derived antioxidants makes them safer in administration compared to synthetic antioxidants that have been reported to be carcinogenic, no matter their low antiradical activities. In literature, there are experimental evidences that reactive oxygen species (ROS) play a major role in the pathophysiology of hypertension. The excessive production of ROS could lead to renal dysfunction and vascular damage (Sinha and Dabla, 2015). Whereas clinical data suggests that antioxidant rich diets reduce blood pressure and cardiovascular risk (Kizhakekuttu and Widlansky, 2010).

The presence of phytochemicals observed in fruits of *X. aethiopica* agrees with the report of previous authors (Okwari *et al.*, 2013; Omeh *et al.*, 2015). *P. americana* has also been reported to contain many phytochemicals including tannins, flavonoids, terpenoids, and phytosterols (Ross, 1999). Some phytochemicals have been implicated in the antioxidant activity of their plant source by either acting singly or by interacting with polyphenols. For example, Maiza-Benabdesselam *et al.* (2007) reported the antiradical activity of alkaloids extracts of two Algerian *Fumaria* spp. against DPPH radical. The alkaloids (Isoquinoline alkaloids, stylophine, protopine, fumaritine, fumaricine, fumarophycine, fumariline and fumarofine) also had effective reducing power and inhibited lipid peroxidation of linoleic acid emulsion (Maiza-Benabdesselam *et al.* 2007). Amarowicz *et al.* (2000) reported that crude tannins from canola and rapeseed hull showed significant antioxidant activity ($P=0.01$). The antioxidant activity of canola hull crude tannins was significantly ($P \leq 0.025$) stronger than those of rapeseed due to its higher total phenolics and tannins contents. Pande *et al.* (2014) reported that saponins and tannins extracted from leaves of *Tridax procumbens* showed good to moderate antioxidant activity. In this study, *X. aethiopica* fruit was observed to be richer in phytochemicals than *P. americana* leaf especially polyphenols. Phytochemicals are known to exert their beneficial effects in the management of many chronic diseases such as hypertension by reducing the circulating levels of cholesterol or by inhibiting anti inflammatory and antiplatelet activities (Upadhyay and Dixit, 2015). The health benefits of Dietary Approaches to Stop Hypertension (DASH) are partially attributed to the phytochemicals and might extend beyond cardiovascular disease risk reduction (Most, 2014).

The findings on nutritional components of the two plants are in line with the reports of previous authors (Omeh *et al.*, 2014; Nwaogu *et al.*, 2008; Arukwe *et al.*, 2012). Omeh *et al.* (2014) reported that the proximate composition of fibre in *X. aethiopica* was 14.51% which is comparable to 12.47% reported in this study. This study also revealed that *P. americana* is richer in crude protein (21.57%) and carbohydrates (50.73%) than *X. aethiopica*. This result agrees with the work of

Nwaogu *et al.* (2008) who reported high crude protein (18.55%) and carbohydrates (47.35%) in the seed extract of *P. americana*. This study also observed that the % composition of crude protein in leaves (21.57%) of *P. americana* is lower than that of its seed (39.01%) as reported by Parameswaran and Murthi (2014). Plant protein has been reported to have beneficial effect in the management of blood pressure. A blood pressure lowering effect of protein may have important public health implication (Altorf – van der Kuil, 2010). Of importance is the crude fibre content of *X. aethiopica* reported in this study. Plant fibre is an important part of diet and decreases serum cholesterol level, risk of coronary health disease, hypertension, diabetes, colon and cancer (Liu *et al.*, 1999). The levels of carbohydrates in the investigated samples may indicate that both *X. aethiopica* fruits and *P. americana* leaves would be beneficial in production of energy to power the cells and tissues of the body on consumption (Arukwe *et al.*, 2012). Overall, pharmacological effects of medicinal plants have been attributed to presence of phytochemicals and nutrients in such plants.

CONCLUSION

Xylopiya aethiopica fruits and of *Persea americana* leaves could be good sources of natural antioxidants due to their significant antiradical activity against DPPH radical observed in this study. The nutritional components could have supplemented the phytochemicals in the antioxidant activity. Furthermore, this study justifies the use of the two plants in ethnomedicine in the management of hypertension by providing scientific information on the phytochemical and nutritional components of samples that could be responsible for the observed antioxidant activity and plausible hypotensive effect.

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