

**Research Article****PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF *PERSEA AMERICANA* LEAVE****AMUDA MUTIU OLASUNKANMI<sup>1\*</sup> , ODERINLO OGUNYEMI<sup>2</sup> **<sup>1</sup>Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria. <sup>2</sup>Department of Chemistry, Faculty of Science, Federal University Otuoke, Bayelsa, Nigeria

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**ABSTRACT**

**Objective:** *Persea americana* is a popular plant that is well known for its fruit, which is highly consumed due to its nutritional and medicinal values. The rich secondary metabolites content of *P. americana* creates an opportunity to utilize it as a functional food or standardized herbal preparations. The quality of a medicinal plant is determined by the active substances produced by the plant. Therefore, the purpose of the present study is to determine the secondary metabolites present in *P. americana* grown in uncultivated farmland in Ogbomosho, Oyo State Nigeria.

**Methods:** The dried pulverised leaves of *P. americana* from uncultivated farmland in Ogbomosho, Oyo State Nigeria were extracted with methanol by the maceration method and partitioned successively with solvents of increasing polarity. The extracts obtained were subjected to phytochemical screening and the total phenolics were determined spectrometrically using the folin-ciocalteu method.

**Results:** The phytochemical screening showed that *P. americana* is rich in various secondary metabolites such as flavonoids, saponins, tannins, and alkaloids. The total phenolic content gave 500µg/g gallic acid equivalent. The total flavonoid content obtained was 250µg/g using quercetin as standard. The anti-oxidant assay of the crude extract showed a high activity of 89.05% at a concentration of 0.05g/ml.

**Conclusion:** Total phenolic content of the plant is likely to be a key for determining the free radical scavenging and ROS reducing ability of the plant.

**Keywords:** Antioxidant, Flavonoid, Phytochemical, Phenolic, Extraction

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**INTRODUCTION**

Nature is a treasure trove of many bioactive secondary metabolites that are used in the treatment and prevention of a variety of human ailments. These secondary metabolites are also an indispensable source of inspiration in the discovery and development of novel therapeutic agents [1]. The plant kingdom has continually offered a plethora of these natural medicines and it is estimated that about 25% of our current drug arsenals is from higher plants [2, 3]. The traditional system of medicine, which principally centres on herbal medicines, plays an important role in the physical and psychological well-being for the vast majority of the world's population [4, 5]. Herbal medicine is widely accepted because of its ease of accessibility, comparative cheaper cost and relative safety profiles when compared to the synthetic alternatives [6]. For example, over 80% of the African population relies on herbal medicine [7] either solely or in combination with conventional medicines [1], and the use of herbs is increasingly gaining recognition in western countries [8].

The therapeutic effects of herbs may be attributed to their antioxidant property [9]. Natural antioxidants, such as flavonoids, coumarins, tocopherols, carotenoids, and phenolic acids, are widely distributed in medicinal plants. They exhibit a wide variety of biological effects such as anti-inflammatory, anticancer, anti-aging, antidiabetes e. t. c [10]. Although plant's antioxidant primarily function as free radical scavengers, they also interact with pro-oxidant metals and act as reducing agents [11].

*Persea americana*, commonly called avocado, is a flowering plant of Lauraceae family and a native to central Mexico. It is well known throughout the world for its fruit which is highly consumed because of its peculiar nutritional composition, antioxidant content, and biochemical profile [12]. Compound class such as carotenoids, tocopherols, phenolics (i.e., flavonoids, condensed tannins and hydroxycinnamic acids), acetogenins monounsaturated and polyunsaturated fatty acids are the main antioxidants found in avocado. Several studies have reported notable positive correlations between the phenolic contents and the antioxidant capacity of avocado [13]. Because it is well known that environmental conditions can influence the phytochemical composition and the quantity of phenolic compounds present in plants [14, 15], the current study examines the phytochemical and antioxidant profiles of *P. americana* grown in uncultivated farmland in Ogbomosho, Oyo State, Nigeria.

**MATERIALS AND METHODS****Reagents and materials**

All chemical reagents used were of analytical grade purchased from Sigma Chemical Co. London. Absorbance was measured using AJI-C03 UV-VIS spectrophotometer.

**Sample collection and identification**

Fresh leaves of *P. americana* were collected from uncultivated farmland in Aroje, Ogbomosho in Oyo State (Latitude 8° 07' 60.00"N, Longitude 4° 14' 60.00" E). The leaves were taken to a botanist in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomosho, for proper identification and classification (Voucher No. LHO 656). The leaves were then air-dried at room temperature for three weeks.

### Extraction of plant material

The dried leaves were pulverized using an electric grinder and 1.7 kg of the pulverized material was extracted by maceration with methanol (1.5 liters). The solvent was filtered off after 24 h and removed *in vacuo* to give the crude methanol extract. Thereafter, by adapting the approach of Emran and co-workers [16], the crude methanol extract was successively partitioned with increasing polarity solvents in the following order; n-hexane, chloroform, ethyl-acetate, methanol.

### Phytochemical screening

The qualitative phytochemical analysis of the extracts from the leaves of *P. americana* adopted the methods [17, 18] presented as follows:

#### Test for flavonoids

1 ml of 10% NaOH was added to 1 ml of the extract. A yellow colouration indicates the presence of flavonoids.

#### Test for steroids (Salkovoski test)

5 drops of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added to 1 ml of the crude extract. Red coloration indicates the presence of steroids.

#### Test for alkaloids

5 ml of 1% HCl solution was added to 2 mls of the extract in a test tube; the mixture was heated and filtered. The filtrate was used for the following tests;

To 1 ml of the filtrate, 2 drops of Mayer's reagent was added. A creamy precipitate indicates the presence of alkaloids in the extract.

To 1 ml of the filtrate, 2 drops of Wagner's reagent was added, a reddish-brown precipitate indicates the presence of alkaloids.

#### Test for saponins (frothing test)

2 ml of the extract in a test tube was vigorously shaken for about 2 min to observe if there would be frothing. Frothing indicates the presence of saponins.

#### Test for tannins

1 ml of freshly prepared 10% KOH was added to the plant extract. A dirty white precipitate indicates the presence of tannins in the extract. Alternatively, 2 drops of 5% FeCl<sub>3</sub> was added to 1 ml of the extract. A greenish precipitate indicates the presence of tannins.

#### Test for glycosides

To 1 ml of the extract in a test tube, 10 ml of 50% H<sub>2</sub>SO<sub>4</sub> was added. The mixture was heated in boiling water for 15 min, 10 ml of Fehling's solutions (I and II) was added and the mixture was boiled. A brick-red precipitate indicates the presence of glycosides in the extract.

### Determination of total flavonoid contents

The AlCl<sub>3</sub> method [18] was used for determination of the total flavonoid contents of the sample. The extract (1.5 ml) was added to 10 ml volumetric flask filled with 5 ml distilled water and 0.3 (ml) of 5% NaNO<sub>2</sub> and mixed. A reagent blank was using distilled water. After 5 min, 1.5 ml of 2% methanolic AlCl<sub>3</sub> was added. 2 ml of 1 mol dm<sup>-3</sup> NaOH was added 5 min later and then the volume was made up to 10 ml with distilled water. The mixture was shaken for 5 min and after 10 min the absorbance was taken at 420 nm. Flavonoids contents were calculated using a standard calibration curve, prepared from quercetin. The flavonoids contents were expressed as mg quercetin g<sup>-1</sup> of extract.

### Determination of total phenolic contents

Total phenolic were determine using Folin-Ciocalteu method [18]. The methanolic extracts (0.5 ml) were added to a 25 ml volumetric flask filled with 10 ml distilled water and 2.5 ml of 0.2 Folin-ciocalteu phenol reagents. A reagent blank using distilled water instead of sample was prepared. After 5 min, 2 ml of 2% Na<sub>2</sub>CO<sub>3</sub> solution were added with mixing. The solution was diluted to the volume (25 ml) with distilled water and then allowed to stand for 90 min, and the absorbance was measured at 725 nm. Total phenolic contents were calculated as mg Gallic acid g<sup>-1</sup> dry weight of sample.

### Anti-oxidant assay

1 ml of various concentrations of the extract in methanol was added to 4 ml of 0.1 mmol methanolic solution of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH). A blank probe was obtained by mixing 4 ml of 0.1 mmol methanolic solution of DPPH and 200 µl of distilled water. After 30 min of incubation in the dark at room temperature, the absorbance was taken at 515 nm against the prepared blank. Inhibition of the free radicals by DPPH in percent (I %) was calculated using this formula.

$$\text{DPPH in } 1(\%) = \left[ \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right] \times 100$$

Ascorbic acid was used as standard control. IC<sub>50</sub> (inhibition concentration at 50) denotes the concentration of sample which is required to scavenge 50% of DPPH free radicals

## RESULTS AND DISCUSSION

Table 1: Phytochemical screening of *P. americana* leaves extracts

| Extract/Fractions | Steroid | Flavonoid | Saponin | Tannin | Alkaloid | Glycoside |
|-------------------|---------|-----------|---------|--------|----------|-----------|
| n-Hexane          | –       | –         | +       | –      | +        | –         |
| Chloroform        | –       | +         | +       | –      | +        | –         |
| Ethyl acetate     | –       | +         | +       | +      | +        | +         |
| Methanol          | +       | +         | +       | –      | –        | +         |

Note: += present; – = absent

Table 2: Total phenolic and flavonoid contents of methanolic extract of *P. americana*

| Extract            | Phenolic content ( $\mu\text{g/g}$ ) | Flavonoid content ( $\mu\text{g/g}$ ) |
|--------------------|--------------------------------------|---------------------------------------|
| Methanolic extract | 500                                  | 250                                   |

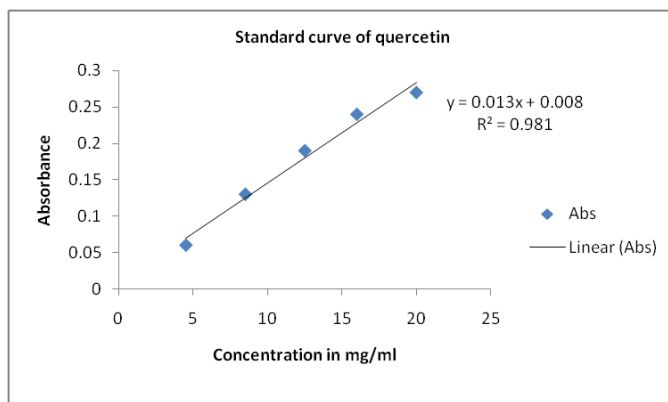


Fig. 1: Standard curve for quercetin

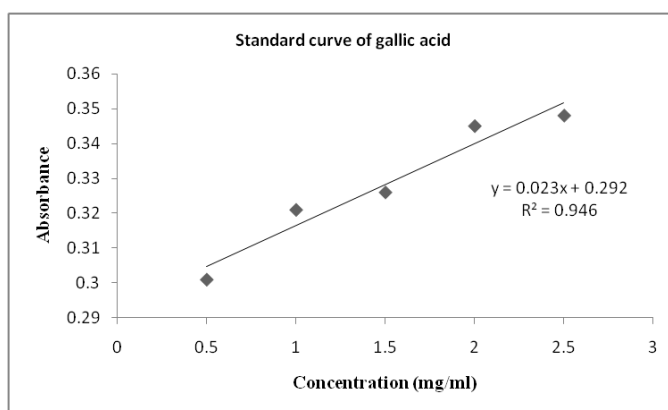


Fig. 2: Standard curve for gallic acid

Table 3: Antioxidant analysis

| S. No. | Absorbance | Concentration | % Inhibition/IC <sub>50</sub> |
|--------|------------|---------------|-------------------------------|
| 1      | 0.224      | 0.15          | 81.18/6.21                    |
| 2      | 0.30       | 0.05          | 89.08/3.36                    |
| 3      | 0.320      | 0.20          | 73.11/8.67                    |
| 4      | 0.54       | 0.10          | 87.06/7.35                    |

## DISCUSSION

The phytochemical screening of the extracts (table 1) revealed the presence of flavonoids, saponins, alkaloids, tannins, glycosides, steroids in the leaves extracts of *P. americana*. This result is in agreement with the previous analysis of *P. americana* leaves extracts obtained from Akure, Ondo State, Nigeria by Ajayi *et al.*, [19], except for the absence of steroids in their extracts, which is present in our methanol fraction. Similarly, our findings correspond with the analysis of avocado leaf extract from Indonesia by Rahman *et al.*, [12] with the exception that alkaloid is lacking in their extract.

Furthermore, only saponins and alkaloids were found present in our n-hexane extract, whereas flavonoids, tannins, glycosides and steroids were all absent. From the chloroform, ethyl acetate, and methanol fractions, the type of flavonoid present were analysed to be flavones and flavonols.

The present study revealed (table 2) the phenolic content of the leave of *P. americana* in terms of mg gallic acid equivalent/g dry weight of the sample (standard plot:  $y = 0.023x + 0.292$ ,  $R^2 = 0.946$ ), the value was 500  $\mu\text{g/g}$  gallic acid equivalent/g. Plant phenolics are diverse group of secondary metabolites and are utilized as wound and infection protectants. Phenolics have been identified to have several biological activities such as antioxidants, antimutagenic, anticarcinogenic, anti-inflammatory and antimicrobial activities in human [20, 21].

Using the standard curve of quercetin ( $y = 0.013x + 0.008$ ,  $R^2 = 0.981$ ), the flavonoid contents of the leaf of *P. americana* was found to be 250  $\mu\text{g/g}$  quercetin equivalent/g of dry sample weight.

Table 3, indicate free radical scavenging potency of the methanolic extract, which was carried out using a UV-spectrometer at an absorbance of 470 nm with different concentration ranging from 0.20-0.5g. The results shows that the extract is more potent at a lower concentration (0.05g) with %I of (89.08%) up to the higher concentration (0.20g) with %I (73.11%), which is less potent in that trend.

## CONCLUSION

The results obtained indicate that *P. americana* leaves extracts contain a good amount of phenolic and flavonoid content and can be used as a natural source antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age-associated oxidative stress. Additionally, phytochemicals can have complementary and overlapping mechanism of action in the body, including anti-oxidant effects, modulation of detoxification enzymes, stimulation of the immune system and modulation of hormone metabolism.

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## AUTHORS CONTRIBUTIONS

AMO conceptualised the study, collected the plant material, coordinated and performed the experimental. OOO provided intellectual leadership. Both authors participated in the preparation of the manuscript and reviewed the final version.

## CONFLICT OF INTERESTS

Declared none

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