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Research Article

SPECTROSCOPIC DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS, AND ANTIOXIDANT ACTIVITY, OF THE LEAVES OF *PERSEA AMERICANA*

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ABSTRACT

Objective: The aim of the present work is to determine the total phenolic content, total flavonoid content and the antioxidant activity of the leaf extracts of *Persea americana* using spectrophotometric method.

Method: Preliminary phytochemical screening was carried out using the method described by Harborne (1996). Quantitative determination of the total phenolic content and total flavonoid content of the methanolic leaf extract of *Persea Americana* was carried out using Folin Ciocalteau and Aluminum chloride colorimetric methods respectively. The in-vitro antioxidant activity was investigated by using in-vitro antioxidant models, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azinobis-(3-ethylbenzothiaoline-6-sulphonic acid) (ABTS) and nitric oxide (NO) assay.

Results: The preliminary phytochemical screening carried out revealed presence of flavonoids, saponins, tannins and glycosides, and absence of alkaloids and steroids in the methanol extract; while the n-hexane extract showed presence of saponins only. The total phenolic content was 56.43 mg Gallic acid equivalent/g extract while the total flavonoid content was 64.00 mg quercetin equivalent /g extract. Analysis of the free radical scavenging activity of the extract revealed a concentration-dependent antiradical activity resulting from the reduction of DPPH, $ABTS^{+}$ and NO radicals to non-radical forms. The scavenging activity of ascorbic acid which was used as a standard was higher than that of the leaf extract.

Conclusion: These results suggest that the leaves of *Persea americana* can be a potential source of natural antioxidant with potent antiradical activity.

Keywords: P*ersea americana,* Total phenolic content, Total flavonoid content, Antioxidant activity.

INTRODUCTION

Phenolics and flavonoids isolated from plants have been shown to possess multiple biological properties. They are essential for growth, reproduction, defense against infection and injury [1]. Researchers have shown that these compounds might be useful in the prevention of a number of diseases [2-5]. These secondary metabolites have been reported to be potent free radical scavengers [6]. Free radicals and reactive oxygen species which are formed under normal physiological conditions if not eliminated result in numerous diseases and disorders [7-8] such as cancer, cardiovascular diseases, Alzheimer's diseases, Parkinson's disease, ulcerative colitis and atherosclerosis [9-14]. The cells in the body are equipped with different mechanisms that can combat reactive oxygen species and maintain the redox homeostasis of the cell. For example, enzymes such as superoxide dismutase, catalase and glutathione peroxidase play important roles in the scavenging of free radicals and prevention of cell injury [15]. When the mechanism of antioxidant protection becomes imbalanced in the human body, antioxidant supplement may be used to help reduce oxidative damage. Medicinal plants that possess antioxidant property are very important in this respite [16]. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, atherosclerosis, heart diseases and stroke [17]. *Persea americana* is classified in the flowering plant family Lauraceae and a native of Central America. It is cultivated in tropical and Mediterranean climates throughout the world. The tree grows to 20 m, with alternately arranged leaves 12-25 cm long. The young leaves are often pubescent and reddish, later becoming smooth, leathery and dark green. The flowers are inconspicuous, greenish-yellow 5-10 mm wide. The fruit is a berry that has just a single large seed which is surrounded by a buttery pulp. The fruit is usually green when mature and turns purplish or black when ripe. The plant is remarkably versatile as to soil adaptability, doing well on such diverse types as red clay, sand, volcanic loam, or lateritic soil. It has been found healthier on nearly neutral or slightly alkaline soil than on moderately or highly acidic soil. Traditionally, it is used to treat skin problems and to induce sleep. It is also used to improve blood cholesterol and is useful in human weight control, high nutritional density, source of major antioxidants and stroke prevention [18]. Leaf poultices are also applied on wounds and the heated leaves are applied on the forehead to relieve neuralgia. The aqueous extract of the leaves has a prolonged hypertensive effect and the leaf decoction is taken as a remedy for diarrhea, sore throat and hemorrhage; it allegedly stimulates and regulates menstruation [19]. Different chemical constituents have been isolated from the plant. For example, Oberlies *et al.,* (2004) [20] isolated 1, 2, 4-trihydroxyheptadec-16-ene, 1, 2, 4-trihydroxyheptadec-16-yne and 1,2, 4-trihydroxynonadecane were isolated from the unripe fruit. Kawagishi *et al*., (1997) [21] isolated five different types of alkanols from the fruits while 1, 2, 4-trihydroxyheptadec-16-ene and its derivatives were isolated from the seeds. *Persea americana* is utilized in various domains of human activities, it may be used as food or medicinal plant. The fruit contains eleven vitamins, fourteen minerals and high percentage of oil with no cholesterol. They are also processed into oil for human consumption and used as ingredient in various cosmetics and health products [22]. This study presents the quantitative estimation of the total phenolic and flavonoid contents of the leaves of *Persea americana* by spectroscopic method. Also, evaluated were the phytohemicals present and its antioxidant potential.

MATERIALS AND METHODS

Folin-Ciocalteau reagent and other chemicals used were Merck products. Genesys 10S vl. 200 2l7H311008 spectrophotometer was used for absorbance measurements.

Preparation of plant material

Fresh plant leaves were collected from the premises of Bowen University teaching hospital, Ogbomoso, Nigeria. The plant was identified by a botanist, Mrs F. A. Ogundola of Biology Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The plant leaves were air dried in the laboratory and pulverized into fine powder using a Mouliner food blender.

Extraction

500 g of the powdered leaf was sequentially extracted with n-hexane (5L) and methanol (5L).using a Soxhlet extractor. The procedure was repeated three times and the extracts were pooled together and filtered through Whatman No 1 filter paper and concentrated by distilling off the solvent under reduced pressure resulting in a dark green paste.

Phytochemical studies

An attempt was made to determine the presence or absence of different secondary metabolites in the leaf extract using the method described by Harborne (1996) [23].

Test for flavonoids

1 mL of NaOH was added to 3 mL of each extract. A yellow colouration indicates that flavonoids are present.

Test for steroids

1 mL of concentrated sulphuric acid was added to 2 mL of each of the extracts. Observation of a red colouration indicates presence of steroids.

Test for saponins

2 mL of distilled water was added to 2 mL of each extract and shaken vigorously. A persistent frothing indicates presence of saponins.

Test for tannins

2 mL of 5% ferric chloride was added to 1 mL of each extract. Observation of a greenish precipitate indicates the presence of tannins.

Test for alkaloids

1 mL of 1% HCl was added to 3 mL of each extract in a test tube. The mixture was heated for 20 mins, cooled and filtered. The filtrate was used for the following test: 2 mL of Meyer's reagent was added to 1 mL of the filtrate. Observation of a creamy precipitate indicates the presence of alkaloids.

Determination of total phenolics

Total phenolic content of the methanolic extract was determined using Folin-Ciocalteau assay [24]. A 10-fold diluted Folin-Ciocalteau reagent was prepared and 2.5 ml of it added to 2.0 ml of 7.5 % sodium carbonate. This mixture was added to 0.1 ml of the extract and incubated at 40^{o}C for 30 mins. The reaction mixture was then placed in an ultraviolet-visible spectrophotometer to measure the absorbance at 760 nm. Gallic acid was used as the standard and the total phenolic content of the extract expressed in mg Gallic acid equivalent.

Total flavonoid contents

The aluminum chloride calorimetric method was used for the determination of the flavonoid content of the plant leaf extract [25]. Quercetin was used as the standard. The extract was dissolved in DMSO and 150 µl of the solution mixed with 150 µl of 2% $AlCl_3$. The mixture was incubated for 10 min at ambient temperature. It was then placed in a UV spectrophotometer to measure the absorbance at 420 nm. The total flavonoid content was expressed as quercetin equivalent in mg/g extract.

Determination of DPPH radical scavenging activity

The free radical scavenging activity was evaluated by the DPPH assay described by Gulcin [26]. 1 ml of the extract dissolved in methanol was added to 1 ml of a 0.25mM solution of DPPH in methanol. The mixture was allowed to stand for 30 min in the dark and the absorbance measured at 517 nm using a UV spectrophotometer. Ascorbic acid was used as the positive control. Free radical scavenging activity from the sample was calculated according to the formula:

$$\% \text{ of DPPH scavenging} = [(A_0 - A_1)/A_0] \times 100\%$$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample.

ABTS assay

2, 2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was used to measure the antioxidant potential of the plant leaves. 0.17mM ABTS, 40µg/ml extract, 25µg/ml ascorbic acid and phosphate buffer (pH = 7.4) were mixed together according to the method described by Miller and Rice-Evans (1996) [27]. 3.5 ml of the mixture was placed in a UV-vis spectrophotometer and the absorbance recorded at a wavelength of 734 nm. The antioxidant potential of the leaf extract was measured against the standard.

Nitric oxide radical scavenging assay

Different concentrations of the extract were prepared and each one was added to sodium prusside (10 mM) in phosphate buffered saline. Ascorbic acid dissolved in methanol was also added to the mixtre and incubated at room temperature for 150 min. Later, 0.5 ml of Griess reagent was added and the absorbance measured at 546 nm [28].

RESULTS

Phytochemical studies

Preliminary phytochemical investigation revealed the presence of the secondary metabolites flavonoids, saponins, tannins and glycosides and absence of alkaloids and steroids in the methanol extract; while the n-hexane extract showed presence of saponins only as shown in table 1.

Table 1: Preliminary screening of secondary metabolites from leaf extracts of *Persea americana*

S. No.	Secondary metabolite	Name of test	methanol	n-hexane
1	Flavonoids	NaOH	+	-
2	Steroids	Salkowski	-	-
3	Saponins	frothing	+	+
4	Alkaloids	dragendorf	-	-
5	Tannins	Gelatin	+	-
6	Glycosides	Molisch	+	-

(+) positive, (-) negative

Total phenolic and flavonoid contents

The spectroscopic determination of total phenolic content from the methanolic leaf extract was carried out using Folin-Ciocalteau reagent and linear Gallic acid standard curve ($y = 0.023x + 0.292$, $R^2 = 0.946$. The flavonoid content was determined using the aluminum colorimetric assay. Quercetin was used as the standard with a standard curve ($y = 0.013x + 0.008$, $R^2 = 0.981$). The total phenolic content obtained is 56.43 ± 1.15 mg GAE/g extract while the total flavonoid content obtained in this study is 64.00 ± 0.23 mg QRT/ g extract (Table 2).

Table 2: Total phenolic and flavonoid contents of the methanolic leaf extract of *Persea americana*

Plant part	Total Phenolic content (mg Gallic acid equivalent/g extract)	Total Flavonoid content (mg quercetin equivalent/g extract)
leaves	56.43	64.00

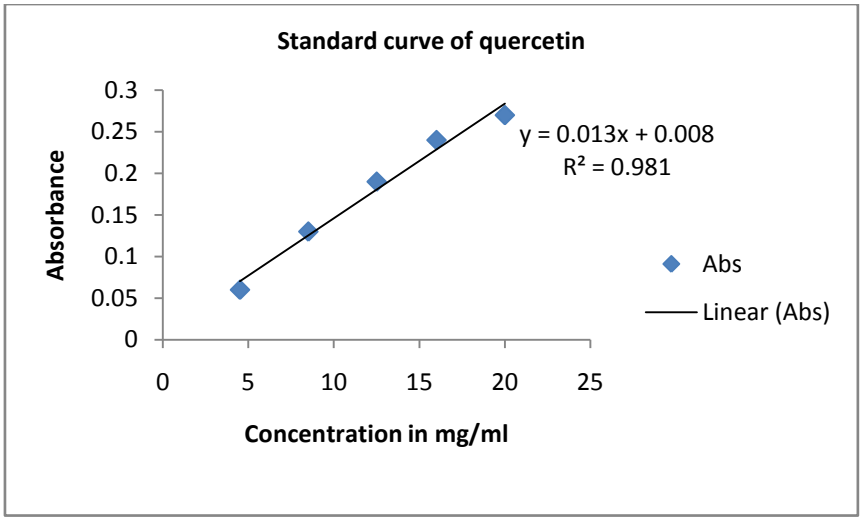

Fig. 1: Standard curve for quercetin

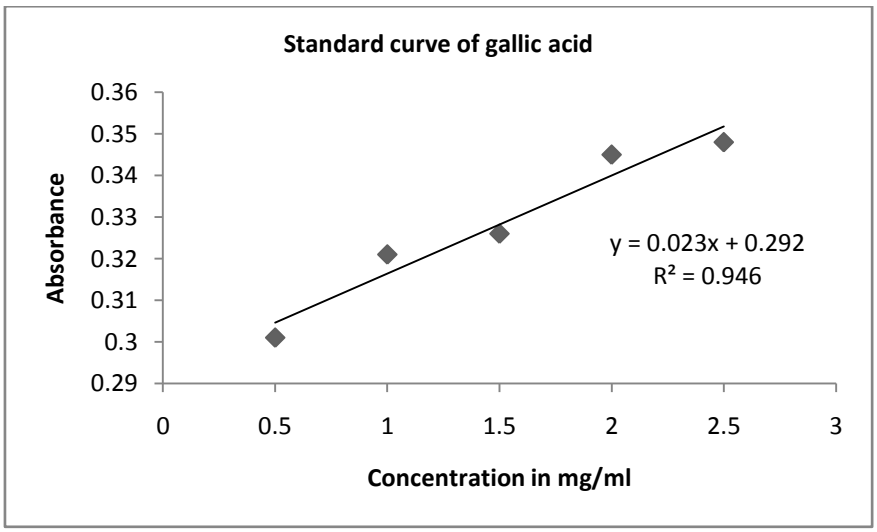

Fig. 2: Standard curve for Gallic acid

In-vitro antioxidant assay

The in-vitro screening of the methanolic leaf extract for possible radical scavenging antioxidant activity was carried out by employing DPPH, ABTS and NO with ascorbic acid as standard. The results revealed that the leaves possess a concentration-dependent antiradical activity resulting from the reduction of DPPH (fig.3), ABTS (fig. 4) and NO (fig. 5) radicals to non radical forms. These results were observed to be lower than that of ascorbic acid which was used as the standard.

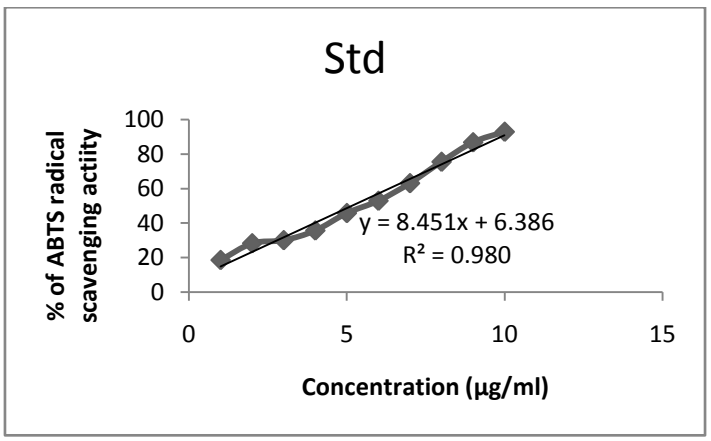

Fig. 3a: Inhibition of ABTS radical by the standard

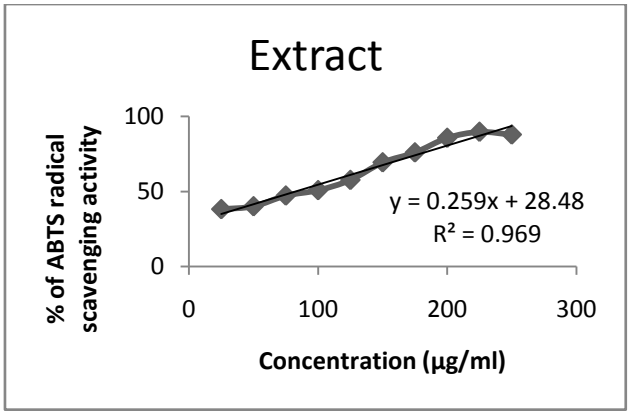

Fig. 3b: Inhibition of ABTS radical by the extract

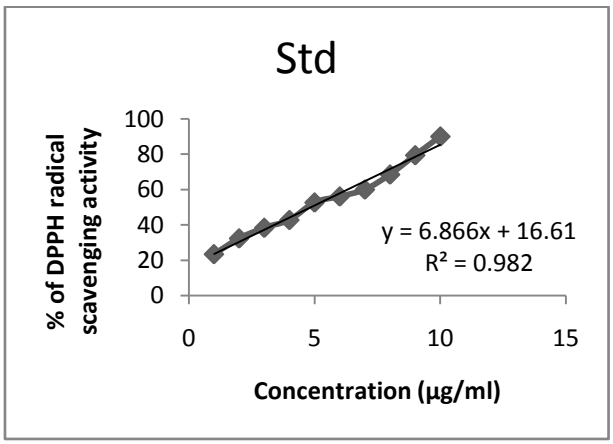

Fig. 4a: Inhibition of DPPH radical by standard

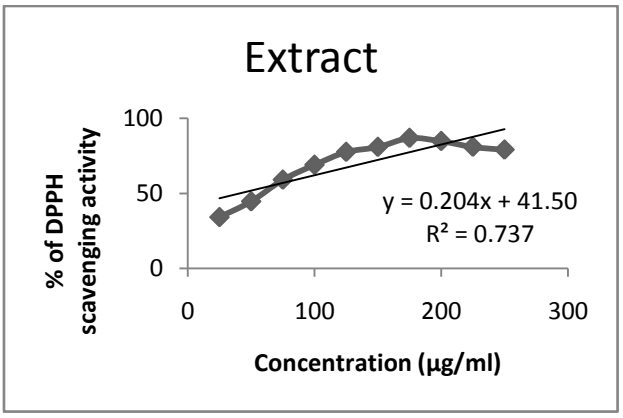

Fig. 4b: Inhibition of DPPH radical by extract

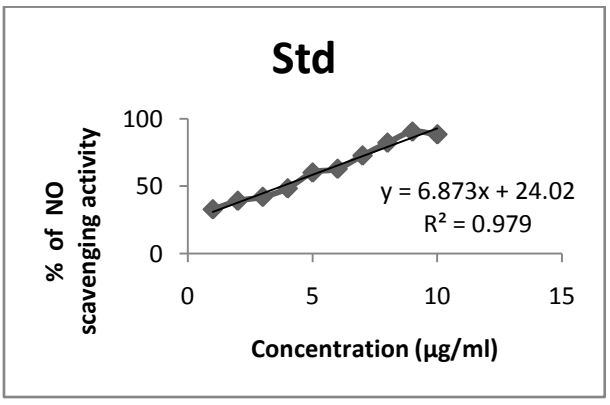

Fig. 5a: inhibition of nitric oxide radical by standard

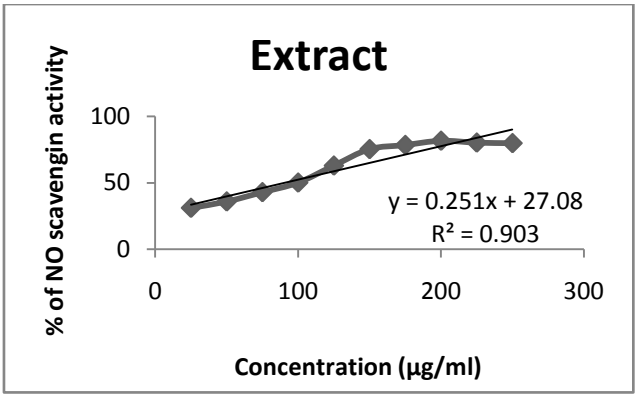

Fig. 5b: Inhibition of nitric oxide radical by extract

DISCUSSION

Various plant parts have been used for the treatment and prevention of many ailments and diseases from time immemorial. This is as a result of the presence of secondary metabolites in those plants. The present study was carried out to determine the pharmacological effects of the leaves of *Persea americana*. The results suggest that the high level of antioxidant activity is as a result of the presence of secondary metabolites such as phenolic compounds. Phenolics present in fruits and vegetables have received considerable attention because of their potential antioxidant activities while flavonoids are the most diverse group of natural products and probably the most important natural phenolics [27-29]. These compounds possess a broad spectrum of chemical and biological activities including free radical scavenging properties, inhibitory effects on mutagenesis and carcinogenesis in humans when ingested daily from a diet rich in vegetables and fruits [30]. Phenolic compounds from plants are considered to be good natural antioxidants [31, 32]. At certain concentrations, phenolic compounds tend to slow down the rate of conjugated diene formation.

Free radicals are very reactive chemical species such as $O_2^{\bullet}$. $OH^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$, H_2O_2. O,* which are produced in an organism during oxygen metabolic reactions or are induced by exogenous damage [33] that contribute to the development and maintenance of cellular life [34]. Body cells and tissues are continuously threatened by the damage caused by these free radicals due to reaction with endogenous molecules such as DNA, proteins and lipids. An antioxidant is a substance which significantly delays or inhibits an oxidation process. The antioxidant activity is measured indirectly by determining the inhibition rate of oxidation processes in the presence of an antioxidant [35]. When the ABTS reacts by losing an electron, the product obtained quickly reacts with ethanol or hydrogen donors to form a colourless ABTS. This reaction is pH independent. A decrease in the concentration of the product obtained is linearly dependent on the antioxidant concentration. The leaf extract showed good scavenging activity for ABTS cation in a dose dependent manner. 2, 2-diphenyl-1-picryl hydrazyl (DPPH) is an organic stable radical in its crystalline form and in solution. It is widely used for the determination of the antiradical activity of a given compound or extract. It is considered to be a model for a lipophilic radical. As a stable radical at room temperature, it can accept an electron or hydrogen radical to become a stable diamagnetic molecule [36]. The antioxidant activity of a given compound or extract is often associated with its free radical scavenging activity [37]. The results show that the leaves possess a significant scavenging effect; but this effect was lower than that of ascorbic acid. Nitric oxide plays an important role in various types of inflammatory processes in the body. The generated nitric oxide radical was inhibited by the extract in a dose dependent manner [38]. Flavonoids have been found to have an additive effect to endogenous scavenging compounds and can interfere with three or more different free radical producing systems [39]. Korkina and Afanas'ev [40] observed that flavonoids can prevent injuries caused by free radical by reacting with the reactive compound of the radical. This was achieved due to the high reactivity of the hydroxyl groups on the flavonoids. By directly scavenging radicals flavonoids can inhibit LDL oxidation in vitro thereby protecting the LDL particles which could result in the prevention of atherosclerosis [41].

CONCLUSION

The present study revealed that the leaves of *Persea Americana* contain significant amount of phenols and flavonoids. Therefore the leaves of *P. americana* can be used as a natural source of antioxidant and as a great therapeutic agent for the prevention or slowing down of age associated oxidative stress related to degenerative diseases. However further work is required on the isolation and characterization of the antioxidant constituents present in the leaves.

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