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Persea americana Mill. (Lauraceae) Extract Exhibits Antioxidant and Antibacterial Properties

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ABSTRACT

This paper highlighted the antioxidant and antibacterial property and chemical constituents of the three parts of Persea americana. The radical scavenging activity of P. americana is dependent on the plant parts. The stem-bark ethanol extract had the highest scavenging activity. This better activity of stem-bark could be accounted to its high phenolic content. All extracts of the three plant parts exhibited inhibitory activity against S. aureus but only stem-bark extract showed inhibition against E. coli. Among the six chemicals screened, flavonoids, terpenoids, cardiac glycosides, and alkaloids were found present in all the samples. Therefore, ethanol extracts of the three plant parts of P. americana contained phytochemicals which are free radical and bacterial growth inhibitors, acting possibly as antioxidants and antibacterial.

Keywords: *Persea americana*, phytochemicals, DPPH radical scavenging assay, antibacterial.

INTRODUCTION

Since ancient time, herbs, fruit, vegetables, spices, roots, weeds and others have been used to treat various diseases. Nowadays, inspite of the synthetic drugs available in the market, people remains to believe in the power of natural and traditional folk medicines due to their less harmful side effects. Medicinal plant researches have been increased and a large amount of evidence has been collected to show their tremendous potential in several traditional health systems [1].

Persea americana, belongs to the family Lauraceae, is commonly called avocado. It is a terrestrial, erect, deciduous, evergreen tree reaching up to 15 to 20 m high. The leaves are spirally arranged, narrow to broad elliptical or obovate, and are usually pointed at the tip. Its fruit is often pear-shaped, usually shiny and green or brownish when ripe, with flesh soft, oily,

greenish or yellow pulp. In the Philippines, this plant is used in folk medicine to treat ulcer, dysentery, dyspepsia, bacterial infection, and skin diseases. *P. americana* is exhibit antihypertensive, cancer risk reduction, wound healing, hepatoprotective, analgesic, anti-inflammatory, anticonvulsant, hypoglycaemic and hypocholesterolaemic [2-8].

With the aim to establish the functional activities and to substantiate the uses of *P. americana* in folk medicine, this present work investigated the chemical components, antioxidant and antibacterial activities of the three plant parts of *P. americana* extract.

MATERIALS AND METHODS

Source of Plant Samples

The leaves, fruit rind, and stem-bark of *P. americana* were collected from Bambanaba, Cuyapo, Nueva Ecija, Philippines, and separately placed in a plastic bag with proper label. Samples were washed three times and air-dried in a shaded condition for 10 days. These were milled and prepared for ethanolic extraction and phytochemical analysis.

Ethanol Extraction

Twenty grams of each air-dried milled plant sample were soaked in 500 ml of 95% ethanol for 48 hours. These were filtered using Whatman No. 2 filter paper to separate the filtrate and plant material. Each filtrate was evaporated in a rotary evaporator to remove the solvent used. Extracts were labeled and prepared for antioxidant and antibacterial assays.

DPPH Radical Scavenging Activity Assay

The stable 2,2'-diphenyl-1-1picrylhydrazyl (DPPH) radical was used to estimate the free radical scavenging activity of the extracts, following the standard method of Shimada et al. [9]. A 100 µl of test sample in ethanol was added with 5 µl DPPH solution (5 mg DPPH powder in 2 ml of ethanol) in 96-well microtitter plates. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The inhibition of DPPH free radicals was calculated.

Estimation of Total Phenolic Content

The total phenolic content was estimated using Folin-Ciocalteu method of Slinkard and Singleton [10] with modifications. Sample solution (50 µl) was mixed 500 µl of 10% Folin-Ciocalteu reagent (Folin:Methanol, 1:1, v/v). After 2 min, 50 µl of 7.5% saturated was added and kept in the dark for 1h before absorbance was taken at 765 nm. A calibration curve was obtained using various concentrations of ascorbic acid. The total phenolic content of the sample was expressed as mg of ascorbic acid equivalents (AAEs) per gram of sample.

Antibacterial Screening

The antibacterial activities of the ethanol extracts of the three parts of *P. americana* were determined following the paper disc diffusion method of Bauer et al. [11]. Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* were cultured in 9 ml of nutrient broth (NB) medium and incubated at 37 °C. After 24 hours, the turbidity of each bacterial culture was adjusted to equal that of 0.5 McFarland standard, which approximated 1.5×10^8 ml^{-1}. The bacterial suspension was spread using a sterile cotton swab on nutrient agar plate. Six millimetre

diameter paper discs impregnated with crude extract (20 µL) and ethanol extract (20 µL), and streptomycin as standard were placed equidistantly on the medium. Plates were incubated at 37 °C, and the zones of inhibition were measured using vernier calliper after 24 hours. Each test was done in triplicate.

Phytochemical Analyses

The chemical screening of the aqueous extracts of the plants were carried out following the procedures described by Sofowora [12]. Among the phytochemicals considered include alkaloids, cardiac glycoside, flavonoids, saponins, tannins, and terpenoid. Distilled water was used a control and was used as a gauge in the changes of color/intensity of the reaction. Three replicates were laid out for each test parameter.

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA). Means were compared using Duncan Multiple Range Test (DMRT) at 5% level of significance.

RESULTS AND DISCUSSION

Antioxidant Effect of *P. americana*

Antioxidants are protective agents that help the human body to reduce oxidative damage. Oxidative damage is caused by free radicals related to aging and diseases such as cancer. Following the DPPH radical scavenging assay, the antioxidant property of the three plant parts of *P. americana* extracts was determined. Table 1 shows the results of the assay. Among the plant parts, stem-bark extract had the highest scavenging activity (61.82%), followed by leaves extract. The fruit-rind recorded the lowest activity (53.96%). These results indicate that the radical scavenging property of *P. americana* is dependent on the plant parts. Moreover, these plant parts contained phytochemicals which are free radical inhibitors or scavengers, acting possibly as primary antioxidants. Their ethanolic extracts might react with free radicals by terminating the free radical chain reactions and reducing power [13,14]. The methanolic extract of avocado seeds showed antioxidant activity in a 2, 2'-azobis (2, 4-dimethylvaleronitrile) (AMVN)-induced methyl linoleate peroxidation assay [15].

Table 1. Radical scavenging activity and total phenolics of the three plant parts of *P. Americana*

P. americana	**Radical Scavenging Activity (%)**	**Total Phenolics (mg AAE / g sample)**
Leaves	58.28[b]	279.49[b]
Fruit-rind	53.96[c]	218.58[c]
Stem-bark	61.82[b]	285.05[a]
Cathechin	90.35[a]	-

In the mean column, means having the same letter of superscripts are not significantly different from each other using DMRT at 5% level of significance.

Total phenols are one of the major naturally occurring antioxidant. Ethanol extract of the three plant parts of *P. americana* contained varying amounts of phenolics (Table 1). Similarly, stem-bark extract recorded the highest phenolic content with 285.05 mg AAE / g sample. This was followed by leaves extract (279.49 mg AAE / g sample) and fruit-rind (218.58 mg AAE / g sample). The highest content of total phenols stem-bark might be the key components accounting for their better results found in DPPH radical scavenging activity. Therefore, total phenolic is the

one responsible for the remarkable antioxidant properties of the three samples studied. Phenols such as BHT and gallate were known to be effective antioxidants [16]. Kim et al. [17] isolated and demonstrated the presence of compounds persenone A and B with unique antioxidant properties in *P. americana* fruit.

Antibacterial activity of *P. Americana*

The antibacterial activity of the three parts of *P. americana* was also studied. The diameter zone of inhibitions of the three plant parts of *P. americana* ethanol extract against the two reference pathogenic bacteria *E. coli* and *S. aureus* is presented in Table 2. Apparently, all plant parts exhibited inhibitory activity against *S. aureus* but only stem-bark extract showed inhibition against *E. coli*. Stem-bark ethanol extract had 9.52 mm diameter zone of inhibition against *E. coli* and 13.79 mm zone of inhibition against *S. aureus*. This inhibitory effect of P. americana could be attributed to its bioactive chemical components. The 1,2,4,-trihydroxyheptadec-16-ene,1,2,4-trihydroxyheptadec-16-yne and 1,2,4,-trihydrononadecane isolated from the unripe fruits of *P. americana* showed cytotoxic effects against the panel of cancer cell lines [18]. Moreover, persin [15; (2R,12Z,15Z)-2-hydroxy-4-oxoheneicosa-12,15-dienyl acetate], a constituent of *P. americana* leaves showed toxic activity in acinar epithelium of lactating mammary gland and the myocardium of livestock [19] and antifungal effect against *Colletotrichum gloeosporioides* [20]. Furanoid constituents are attributed to the antibacterial, antifungal, and insecticidal activities of avocado [21].

Table 2. Diameter zone of inhibition of ethanol extract of the three plant parts of *P. americana* against *E. coli* and *S. aureus in vitro*.

P. americana	Diameter zone of inhibition (mm)	
	E. coli	***S. aureus***
Leaves	0.00^{c}	11.27^{b}
Fruit-rind	0.00^{c}	9.80^{c}
Stem-bark	9.52^{b}	13.79^{b}
Streptomycin	28.61^{a}	32.73^{a}

In the mean column, means having the same letter of superscripts are not significantly different from each other using DMRT at 5% level of significance.

Phytochemical Constituents of *P. americana*

Phytochemicals are natural chemicals present in a variety of plant based foods. The phytochemical components of the three plant parts of *P. americana* are summarized in Table 3. Among the six chemicals screened, flavonoids, terpenoids, cardiac glycosides, and alkaloids were found present in all the samples. These chemicals play an important role in the various functional activities of *P. americana*, particularly antioxidant and antibacterial properties. Tannin was not found in leaves and fruit-rind whereas saponin was absent in stem-bark. Several flavonoids isolated from the leaves and seeds of *P. americana* such as quercetin showed virustatic effects by inhibiting HIV syncytium formation and viral p24 antigen formation [22]. Many alkaloids derived from medicinal plants show biological activities such as anti-inflammatory, antimalarial, antimicrobial, cytotoxicity, antispasmodic and pharmacological effects [23-27]. Cardiac glycosides have been used to treat congestive heart failure and cardiac arrhythmia [28]. In conclusion, the three parts of *P. americana* contain phytochemicals that work together for its medicinal importance particularly antioxidant and antibacterial.

Table 3. Phytochemical composition of the three plant parts of *P. Americana*

Phytochemicals	*P. americana*		
	Leaves	Fruit-rind	Stem-bark
Flavonoids	+	+	+
Terpenoids	+	+	+
Cardiac Glycosides	+	+	+
Saponins	+	+	**0**
Alkaloids	+	+	+
Tannins	**0**	**0**	+

+ positive; 0 negative

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