Original Article

Antibacterial and Antioxidant Activities of *Persea Americana* (Mill) Lauraceae Kernel Extracts

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Abstract

Nowadays, a large number of aromatic and medicinal plants possess highly significant bioproperties, with numerous applications in medicine, pharmacy, cosmetology, and agriculture. This work aimed to conduct a phytochemical screening and assay of phytomarkers with biopharmaceutical potential and to assess the antioxidant and antibacterial activity of Persea americana Mill. kernel extracts in vitro.
P. americana plant samples were collected in May 2017 in Kinshasa. Three standard strains were selected namely *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeroginosa* ATCC 9027 for microbiological assays. The phytomarker assay was used to detect total polyphenols, and the antibacterial activity was determined using the solid diffusion method on the Mueller Hinton medium to establish the minimal inhibitory concentration. Antioxidant activity was evaluated by the DPPH, ABTS, and Phosphomolybdate techniques. The findings showed that *P. americana* kernels contain secondary metabolites such as flavonoids, polyphenols, alkaloids, tannins, and triterpenoids, which may confer interesting biological activities. Quantitative phytomarker analysis of *P. americana* extracts showed a concentration of 69.70±3.07 mg/QE of total polyphenols and 27.53±1.15 mg/QE of flavonoids. All the extracts tested had low antibacterial activity against the three strains tested. However, the methanolic extract showed better antioxidant activity. In view of the IC_{50} values of our various fractions, it is clear that *P. americana* kernels possess interesting antioxidant properties.

Keywords: Antibacterial, Antioxidant, *Persea Americana*, Kernel

Introduction

Traditional medicine has been utilized since ancient times to alleviate human illnesses. Their pharmacological activities are due to the existence of several natural compounds known as secondary metabolites. These chemicals are present in several organs and occasionally in distinct plant cells [1-3]. Nowadays, the rising adventure of microbial resistance to different compounds like antibiotics and the toxicity of synthetic antioxidants have led researchers to find an alternative to the plant world, specifically plants of medicinal and food relevance, in search of effective natural molecules devoid of any adverse effects [4, 5]. Numerous studies have highlighted the importance of secondary metabolites with effective biological activities like polyphenols and many others [5-8]. Furthermore, oxidative stress defined as a profound imbalance between pro-oxidants and antioxidants leads to irreversible cellular damage. The univalent reduction of oxygen results in the formation of activated oxygen species (AOS), like free radicals, hydrogen peroxide, etc [9, 10]. It seems that all these species are potentially toxic to the organism [11].

Recently, attention has focused on herbs as sources of antioxidants, which can be used to protect against the effects of oxidative stress [12-14]. In fact, Africa is endowed with rich plant biodiversity, especially in the central part where is located The Democratic Republic of the Congo (DRC). This latter has a huge biological richness (fauna or flora),

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unfortunately, it is not sufficiently monitored scientifically. Some of these plants are traditionally used by the population. These plant species represent a large reservoir of secondary metabolites. Among this arsenal of medicinal plants is the *Persea* genus, which is widely distributed, especially in the sub-tropical region, and the most known and consumed species is *P. americana*. This plant is widely used to treat several diseases and has been the subject of several studies to determine its chemical composition and biological properties. The current research aimed to assess the pharmacological properties of *P. americana* Mill. kernel extracts, with a focus on antioxidant and antibacterial activities.

Materials and Methods

Biological Material

Plant samples were collected in Kinshasa-East in May 2017.

Conditioning of Plant Material

In this study we used *P. americana* kernels once dried under the conditions used in traditional pharmacopeia (five days at 450 °C in ovens), the plant part is ground or pulverized to obtain a fine powder.

Microbiological Material

The three bacterial strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeroginosa* ATCC 9027), which were 24 hours old, were provided by the Microbiology Laboratory of the Faculty of Pharmaceutical Sciences at the University of Kinshasa.

Methods

Preparation of Plant Material

The harvested plant parts were oven-dried (approx. 45°C) for five days in the laboratory, then ground to obtain the powder. The powder obtained was macerated for 24 hours followed by a filtration using Whatmann n°1. The filtrate was used for chemical screening and the extractions for biological testing.

Phytochemical Screening

The phytochemical screening was carried out as reported by Inkoto *et al*. [15], Bongo *et al*. [16]. and Mbadiko *et al*. [17].

Extraction with Increasing Polarity

Fifty grams of powder (50g) were macerated in 500 mL of increasing polarity solvents (n-Hexane, Dichloromethane, and Methanol; 1:10, w/v) for 48 hours. After filtration, a rotary evaporator was used to concentrate the filtrates by evaporating the solvent to dryness using an oven at +40°C for 48 hours. The concentrated filtrates were used for further analyses.

Yield of Crude Extract

The raw extract yield is defined as the ratio between the mass of dry extract obtained and the mass of plant material processed. This yield is calculated using the following Eq. 1:

$$R(\%) = \frac{n}{N} \times 100 \quad (1)$$

Where: R(%): yield in en %
n: extract mass after solvent evaporation
N: Vegetal material mass used for extraction [18, 19].

Phytomarker Assay

Quantitative analysis samples were created by dissolving 10 mg of extract in 50 mL of methanol solvent. In the preparation of the Folin-Calciolteu reagent, the total polyphenol content and flavonoids were determined as per the protocol described by Dibacto *et al*. [20].

Biological Studies

Evaluation of Antibacterial Activity

The antibacterial efficacy of various extracts is evaluated using the solid-state diffusion method [4]. Dissolve 20 mg of the extract in 250µL of DMSO, then adjust the total volume to 5 mL with Mueller Hinton culture media for testing. The micro-dilution assay is performed in sterile, round-bottomed 96-well polystyrene microplates. Essentially, 100 µL of culture media is added to each well. Using a micropipette, 200 µL of each extract to be tested (1000 µg/ml) is placed in the wells respectively.100 µL of each extract stock solution is used for creating serial dilutions.

To prepare the bacterial suspension, three isolated colonies from the test strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027) are placed in 2 mL of saline solution for each strain. The mixture is then incubated for 24 hours to achieve a 0.5 McFarland standard. Bacterial suspensions are introduced onto Petri dishes with Mueller Hinton media using a swabbing method. Wells are created in the Mueller Hinton agar in the Petri dishes using a Pasteur pipette. Subsequently, 100µL of varying quantities of each extract are distributed into individual wells. The positive control is placed in a separate well. The cultures are cultured at 37°C for 24 hours after diffusion, and the inhibitory halos around each well are then measured.

Evaluation of Antioxidant Activity

- ABTS & DPPH

The assessment of the antioxidant activity was performed as per the protocol described by Bongo *et al*. [4] and Mbadiko *et al*. [21].

- Phosphomolybdate Test

This test was performed following Tomovska and Vllasaku [21].

Preparation of Ammonium Molybdate Reagent

100 mL of distilled water was acidified with 3.27 mL of strong sulfuric acid to prepare the ammonium molybdate reagent. Subsequently, 436.6 mg of ammonium molybdate and 436.8 mg of sodium phosphate were dissolved, and the

solution was diluted to 300 mL with water that had been distilled.

Contacting the Sample with the Reagent

In test tubes, 10 mg of *P. americana* methanolic extract (1000-25.5µg/mL) was mixed with 2 mL of ammonium molybdate reagent. After shaking, the tubes were capped with absorbent cotton, and incubated in a water bath at 950C for 1h30min. Absorbance was measured with a UV-visible spectrophotometer at 695nm. Methanol was used as the negative control, while vitamin (100-62.5) served as the positive control.

The relative antioxidant activity is calculated according to the following Eq. 2:

$$AAR = \frac{\text{Abs. sample}}{\text{Abs. Vit C}} \times 100 \quad (2)$$

Where Abs. sample: sample absorbance
Abs. Vit C: absorbance in vitamin C equivalents/g extract

Results and Discussion

Phytochemical Screening

The phytochemical content of the extracts is presented in the **Table 1**.

Table 1. Phytochemical screening of P. americana kernel

Compounds	Used part (kernel)
1. Aqueous phase	
Polyphenols	+
Flavonoids	+
Tannins	+
Anthocyanins	+
Leuco-anthocyanins	+
Alkaloids	+
Saponins	-
Bound quinones	+
2. Organic phase	
Steroids and Triterpenoids	+
Free quinones	+

(Legend: + presence, - : absence).

From the table above, *P. americana* Mill. kernels are rich in secondary metabolites, like flavonoids, steroids, polyphenols, tannins, anthocyanins, bound quinones, alkaloids, leuco-anthocyanins, triterpenoids, and free quinones, but saponins are absent. These results corroborate those published by Kosińska *et al*. [22]. In the literature, it has been shown that *P. americana* leaves contain quercetol, catechin, epi-catechin, cyanidin, procyanidin, terpenoids, catechic tannins, responsible for the anti-diarrheal effect, and essential oil (containing varying amounts of estragole, methylchavicol, α-pinene, and other terpenes) [23, 24]. The fruit pulp contains sesquiterpenes, hydroxy tryptamine, vitamins A and E, carotenoids, and carbohydrates (glucose, fructose, perseitoletmannoheptulose). The various classes of chemical constituents are monoterpenoid [25], sesquiterprnoid [26], triterpenoids [27], flavonoids [28], alkaloids [29], steroids [30, 31], carotenoids [32]. Much of the work carried out on the fruits of this plant has resulted in the discovery of molecules with interesting properties. An antifungal compound against the pathogen *Colletotrichum gloeosporioides* was discovered in 2000. It is (E, Z, Z)-1-acetoxy-2hydroxy-4-oxo-heneicosa-5,12,15-triene and was isolated from avocado idioblasts [33].

Corral-Aguayo *et al*. [34], demonstrated that avocado is endowed with antioxidant properties. This is due to total soluble phenols, vitamin C, β-carotene, and total carotenoids. The aqueous extract is 95 times more antioxidant than the lipophilic one, these are phenols (24.2mg/100g FM) and vitamin C (58%) that are mostly responsible for avocado's antioxidant activity. Ramos [35], showed that the methanolic extract of the kernel, followed by fractionation, enabled the separation and identification of three compounds of chlorogenic acids and their isomers, quinic acid, salodrosides, pro-antocuacinedins B1 and B1. *In vitro* tests of these compounds have shown them to have stimulatory inhibitory effects on human keratinocytes and fibroblasts. Other bioactive phytochemicals have been found to improve hypercholesterolemia, inflammation, diabetes, and hypertension. In addition, insecticidal, fungal, and antimicrobial effects were once again demonstrated [36-39].

Extraction Yield with Solvents of Increasing Polarity

The extraction yield of metabolites is presented in **Figure 1** below.

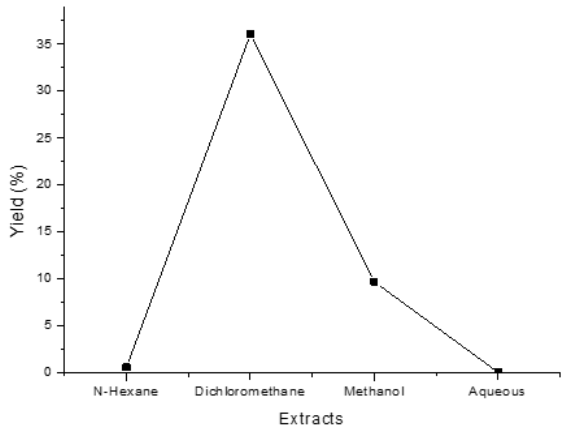

Figure 1. Extraction yield of different metabolites using different solvents

The figure shows that for the increasing polarity extraction of our samples, starting from 25g of *P. americana* Mill. kernel

powder, the yield obtained for the n-hexane fraction is 0.6%. For the dichloromethane fraction, the yield was 36.08%, and for the methanol fraction, the yield was 9.68%. The aqueous fraction gave a yield of 0.04%.

Antibacterial Activity of P. Americana Extracts

The antibacterial activity of the extracts is presented in **Table 2**.

Table 2. Antibacterial activity of P. americana kernel

Fractions	MIC (µg/mL)							
	2000	1000	500	250	125	62.5	31.25	M/C (µg/mL)
Escherichia coli ATCC 8739								
n-Hexane	+	+	+	+	+	+	+	›2000
Dichloro	+	+	+	+	+	+	+	›2000
Methanol	+	+	+	+	+	+	+	›2000
Aqueous extract	+	+	+	+	+	+	+	›2000
Staphylococcus aureus ATCC25923								
n-Hexane	+	+	+	+	+	+	+	›2000
Dichloro	+	+	+	+	+	+	+	›2000
Méthanol	+	+	+	+	+	+	+	›2000
Aqueous extract	+	+	+	+	+	+	+	›2000
Pseudomonas aeruginosa ATCC 9027								
n-Hexane	+	+	+	+	+	+	+	›2000
Dichloro	+	+	+	+	+	+	+	›2000
Méthanol	+	+	+	+	+	+	+	›2000
Aqueous extract	+	+	+	+	+	+	+	›2000

(Legend: +: bacterial growth; -: growth inhibition; ATCC: American Type Cell Collection, MIC minimum inhibitory concentration)

The table shows that all the bacterial strains tested are less sensitive to *P. americana* Mill. kernel extracts (MIC greater than or equal to 2000 µg/mL). This antibacterial activity is, however, low, and may be due to the phenolic and other compounds present in the extracts tested. *S. aureus* is a gram-positive bacteria with a thick cell wall that bioactive chemicals from P. americana Mill. would target pharmacologically. In contrast, *E. coli* has an outer membrane that hinders the penetration of chemical compounds into the cell [40, 41].

Phyto-Marker Assay in the Kernels of P. Americana Mill

In order to assess the content of secondary metabolites in our sample, we assayed total polyphenols and flavonoids **(Table 3)**.

Table 3. Chemical composition of P. americana MILL. extracts in phyto-markers

Extracts	Secondary Metabolites	
	Total Polyphenols (µg GAE/g)	Flavonoids (µg QE/g) (% ratio)
***P. americana* Mill.**	69.70 ± 3.07	27.53 ± 1.15 (39.49)

(GAE: gallic acid equivalent; QE: quercetin equivalent; Ratio: ratio of flavonoids to total polyphenols).

This table shows that *P. americana* Mill. extract has a high polyphenol content. However, it should be noted that the metabolites sought in this plant pass easily into polar solvents (methanol). Furthermore, this plant is rich in flavonoids (ratio equal to 0.39), and it is reported that flavonoids can therefore be selected as more physico-chemically stable phyto-markers, making them more suitable for further phytochemical study. In the literature, flavonoids have been shown to have anti-sickling activity [42], and the antioxidant activity is also attributed to polyphenols [43].

Antioxidant Activity

The antioxidant activity of *P. americana* kernel of different fractions and the phosphomolybdate reagent is presented in **Table 4**.

Table 4. Antioxidant activity of P. americana kernel extract

Extracts	IC50 (µg/mL)	
	ABTS	DPPH
Methanol fraction	12.22±2.67	11.94±4.45
Aqueous extract	83.95±10.67	1104.08±10.95
Vitamine C	2.94±0.27	1.52±0.19
Antioxidant activity with phosphomolybdate reagent		
Concentration (µg/mL)	**Antioxidant activity (%)**	**Equivalent in vitamin C (mg/g extract)**
1000 µg/mL	63.65 ± 0.49	636.5 ± 4.90
500 µg/mL	61.32 ± 0.45	613.2 ± 4.50
250 µg/mL	56.06 ± 0.00	560.6 ± 0.00
125 µg/mL	21.05 ± 0.00	210.5 ± 0.00
62.5µg/ml	15.55±0.00	155.5±00
31.25µg/ml	7.35±0.00	73.5±0.00

It is observed that the methanol fraction of *P. americana* Mill. kernels showed IC_{50} values of less than 100 µg/mL in the ABTS and DPPH tests, while the aqueous fraction displayed an IC_{50} value of less than 100 µg/mL in the ABTS test. The radical-inhibiting capacities of different extracts varied significantly in each type of test. In addition, the inhibitory

concentration 50 (IC_{50}) value obtained in the DPPH test with the methanol fraction showed greater activity than the aqueous fraction against ABTS. The variation in activity can be attributed to the different reaction mechanisms. The ABTS reagent reacts with hydrophilic and lipophilic molecules, whereas the DPPH° reagent exclusively reacts with hydrophilic chemicals [44]. Our fractions exhibited lower activity compared to Vitamin C used as a positive control. However, the observed activity is noteworthy when compared to other plant species [45]. There is therefore evidence that polyphenols found in foods or medicinal plants are capable of modulating oxidative stress [46].

On the other side, the antioxidant activity of *P. americana* Mill. Kernels with the phosphomolybdate reagent varies according to extract concentration, ranging from 1000µg/mL to 31.25µg/mL. It should be noted that the activity of the extract of this plant tested is greater at higher concentrations, as we can observe. In the literature, it has been reported that the antioxidant activity of plants used in traditional pharmacopeia is attributed to phenolic compounds [47, 48]. The presence of these constituents with this property in this plant could justify its use by the population in traditional medicine [49].

Conclusion

This work aimed to conduct a phytochemical screening and evaluate the *in vitro* antioxidant and antibacterial properties of *P. americana* Mill. kernel extracts. All the extracts tested had low antibacterial activity against the three strains tested. However, the methanolic extract showed better antioxidant activity. In view of the IC_{50} values of our various fractions, it is clear that *P. americana* kernels possess interesting antioxidant properties.

The findings show that this species commonly used in traditional medicine appears to be biologically active, and ethnobotanical studies could therefore be an interesting biological approach that opens up new prospects for the discovery of new drugs for the treatment of ailments caused by oxidative stress. It would therefore be interesting to extend the range of antioxidant and antibacterial tests of the different fractions by chromatography, as well as the isolation and characterization of active compounds in a view to identifying the molecules responsible for these biological activities.

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