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Original Research Article

Physicochemical, GC-MS, antimicrobial, and antioxidant assays of *Persea americana* (Avocado) seed extract

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Abstract

Purpose: *To investigate the phytoconstituents, gas chromatography-mass spectrometry (GC-MS), antioxidant, and antimicrobial properties of Persea americana Mill. (Fam. Lauraceae) seeds extract and fractions.*

Methods: *The plant seeds were air-dried, extracted with methanol, and fractionated using n-hexane, ethyl acetate, and methanol, respectively. Phytoconstituents of the extract and fractions and antioxidant activity were evaluated using GC-MS and 2,2-diphenyl-1-picrylhydrazyl (DPPH) at different concentrations (500, 250, 125, and 75 mg/mL). Antimicrobial activity evaluation against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, and Escherichia coli was carried out using the agar well-diffusion method with ciprofloxacin as positive control.*

Results: *Qualitative analysis revealed the presence of alkaloids, anthocyanins, flavonoids, tannins, phenolics, carbohydrates, and terpenoids. Crude extract showed higher carbohydrate content (22.63 ± 0.73 mg/g) than fractions. Saponins were absent in extract and fractions. In vitro assays, including DPPH radical scavenging and total antioxidant capacity, demonstrated high oxidative activity of Persea americana seed extracts and fractions. The samples showed antimicrobial activities, with crude extract and n-hexane fraction having a minimum inhibitory concentration (MICs) of 31.25 mg/mL.*

Conclusion: *The findings of this study demonstrate the presence of various phytoconstituents, as well as the antioxidant and antimicrobial activities of the extracts and fractions of Persea Americana seeds. There would be need for studies to evaluate the toxicologic profile of the bioactive compounds in order to ascertain safety.*

Keywords: *Persea americana, Phytoconstituents, Antimicrobial, Antioxidant, Ciprofloxacin*

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INTRODUCTION

Medicinal plants contain substances used for therapeutic purposes or precursors for synthesizing valuable drugs [1,2]. *Persea americana* Mill. (Lauraceae) a fruit-bearing tree, growing up to 20 m (66 ft), with evergreen, dark green, elliptical leaves measuring 10 – 30 cm in length and producing small, greenish-yellow flowers, is one of the most widely cultivated

varieties of avocado pear in tropical and subtropical regions [3]. It is a fruit native to Central and South America with a unique sweet taste, numerous health and nutritional benefits, and significant popularity worldwide [4]. A known *P. americana* fruit is composed of pulp (65 – 73 %), peel (11 – 15 %), and seed (16 – 20 %) [5]. The creamy, green flesh of the fruit is widely consumed, and the seed (often referred to as the pit) has been historically underutilized and discarded as waste. Studies have investigated and highlighted the potential of *P. americana* seeds to possess high nutritional value and are valuable sources of phytoconstituents with remarkable antioxidant properties [6,7].

Phytoconstituents are naturally occurring bioactive compounds in plants that have been extensively studied for their diverse therapeutic properties [8,9]. *P. americana* seeds have drawn attention as a rich reservoir of phytoconstituents, including phenolic compounds, flavonoids, tannins, saponins, and triterpenoids, which exhibit antioxidant effects. This suggests the possibility of various health benefits when incorporated into diets or used as supplements, especially in the face of emerging resistant pathogens [10]. Antioxidants protect the body from oxidative stress caused by free radicals [11]. Antioxidants neutralize the harmful effects of free radicals and help maintain cellular health and overall well-being. Gas chromatography-mass spectrometry (GC-MS) is used to identify and quantify chemical constituents of complex mixtures [12]. By employing GC-MS, detailed information about the phytoconstituents that are responsible for various pharmacological activities helps to elucidate valuable molecular structures [13]. The combination of antioxidant properties and abundance of phytoconstituents in *P. americana* seeds presents a promising ground for applications in the food, pharmaceutical, and nutraceutical industries [14]. However, despite the growing interest in *P. americana* seeds, there remains a relative lack of comprehensive studies that delve into the specific phytochemical profiles and antioxidant potential of different *P. americana* varieties and cultivars. This study aimed to fill this gap in knowledge by conducting a detailed analysis of the phytoconstituents in *P. americana* seeds of a specific cultivar and investigating the antioxidant and antimicrobial properties of the seeds.

EXPERIMENTAL

Preparation of extract

Fresh seeds of *P. americana* were bought from the University of Nigeria, Nsukka, in August 2023. The plant was validated by a taxonomist, Mr Alfred Ozioko of the Bioresources Development and Conservation Program, Nsukka (authentication number of CEED/16310). The seed (5,000 g) was dried, pulverized into powder and cold macerated in 20 L of methanol for 72 h. The solution was filtered with Whatman No. 4 filter paper and the filtrate was concentrated to semi-solid using a rotary evaporator to obtain a dark brown slurry-like substance, which was stored in the refrigerator for further investigation.

Fractionation

The crude extract of *P. americana* seed (250 g) was subjected to column chromatography to separate the extract into its component fractions. Thereafter, Silica gel 70 - 200 mesh size was used as the stationary phase while varying solvent combinations of increasing polarity as the mobile phase. The lower part of the glass column was covered with glass wool. The slurry (prepared by mixing 150 g silica gel and 350 mL hexane) was poured down carefully into the column. The top of the glass column was left open to allow the free flow of solvent into a conical flask below. At the end of the packing process, the tap was locked. The column was left standing to stabilize for 24 h, after which, the clear solvent on the silica gel was allowed to drain down the meniscus. The wet packing method was used in preparing the silica gel column. The sample was prepared in a ceramic mortar by adsorbing 100 g of the extract in methanol to 200 g of silica gel 70 - 200 mesh size and evaporating to dryness. The dry powder was gently layered on top of the column. The column tap was opened to allow the eluent to flow at 40 drops per minute. Elution of the extract was done with solvent systems with increasing polarity using hexane, ethyl acetate and methanol.

Phytochemical analysis

Phytochemical screening of the extract and fractions was carried out as described by Evans [15].

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the methanol extract of *P. americana* seed was performed using a Bruker ScionTM GCMS with Scion 436 GC. The autosampler and gas chromatograph were interfaced with a mass spectrometer (GC-MS) equipped with an Elite-1MS (95 % dimethyl polysiloxane) fused capillary column (30 m x

0.25 mm ID x 0.25 µm). For GC-MS determination, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999 %) was used as carrier gas at a constant flow rate of 1 mL/min, with an injection volume of 0.5 EI (split ratio of 50:1). Injector temperature was maintained at 280 °C, ion source temperature was 250 °C, oven temperature was programmed from 80 °C (isothermal for 2 min), with an increase of 20 °C/min to 160 °C, then 5° C/min to 280 °C, ending at 10 min isothermal at 300 °C. Mass spectra were taken at 70 eV, scanning 0.5 sec and fragments from 50 - 500 Da. The solvent delay was 0 to 3.5 min and the total GC-MS running time was 46 min. The mass detector used in this analysis was a TQ Quadrupole Mass Spectrometer and the software adopted to handle mass spectra and chromatograms was an MS Workstation 8.

Identification of components

The relative amount of each component was calculated by comparing its average peak area to the total area. The detection employed the National Institute of Standards and Technology (NIST) library version 20. Prediction of the biological activity of compounds was based on Dr Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service / USDA. Interpretation of GC-MS was conducted using the database of NIST library having more than 62,000 patterns. Spectrum of the unknown component was compared with that of the known components stored in NIST library version 20. The name, molecular weight and molecular formula of components of test materials were determined.

Antioxidant studies

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared. Thereafter, 0.1 mM DPPH (0.1 mL) was added to crude extract, n-hexane and ethyl acetate fractions respectively. The solutions were filtered, and the tubes were incubated in the dark at room temperature for 30 min. Absorbances were measured and used to determine the antioxidant capacity of the crude extract and fractions.

Antimicrobial studies

Minimum inhibitory concentration (MIC) of the extract and its fractions was determined using the agar diffusion method [16]. Muller-Hinton agar (20 mL) was poured into 6 sterile petri dishes and allowed to solidify. Thereafter, 0.1 mL of freshly prepared bacteria stock culture was seeded into the plates and allowed to dry for 15 min. It was incubated for 48 h, and the inhibition zone diameter was measured and MIC recorded.

Statistical analysis

One-way analysis of variance (ANOVA) was done using Microsoft Excel software to establish the presence or absence of variability between the extracts and fractions at different concentrations. Values are reported as mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

RESULTS

Phytoconstituents of *Persea americana*

The results indicated the presence of alkaloids, anthocyanins, flavonoids, tannins, phenolics, carbohydrates, and terpenoids in the methanol extract, n-hexane, and ethyl acetate fractions (Table 1).

Table 1: Qualitative analysis of phytochemical constituents of *P. americana* seed extract

Secondary metabolites	Methanol extract	N-hexane fraction	Ethyl acetate fraction
Alkaloid	+	+	+
Anthocyanin	+	+	+
Flavonoids	+	+	+
Saponins	-	-	-
Tannins	+	+	+
Phenolics	+	+	+
Carbohydrate	+	+	+
Terpenoids	+	+	+

Key: + = present, - = absent

Phytochemical constituents of *P. americana* seed

The phytoconstituents in *P. americana* were quantified using standard measurement methods and the results revealed that carbohydrates were present in high amounts compared to other metabolites (Table 2).

Antioxidant capacity test

Radical DPPH scavenging

The antioxidant capacity of *P. americana* was measured for the crude extract and fractions and percentage inhibition against concentration (mg/mL) was plotted and compared with ascorbic acid used as standard (Figure 1). The study showed that the variations in % inhibition between crude extract, n-hexane, ethyl acetate,

and ascorbic acid are not statistically significant (p = 0.76).

Total antioxidant

The total antioxidant of the crude extract and fractions of *P. americana* was assayed and the graph of % inhibition against total antioxidant capacity in mg/mL of the extract and fractions was compared with ascorbic acid (Figure 2), showing dose-dependent inhibition effects and a general statistically significant difference (p = 0.04) among the samples.

Table 2: Phytochemical constituents of *P. americana* seed

Metabolite	Crude extract	n-hexane	Ethylacetate
Phenolics (mg/g)	0.79±0.10	0.60±0.09	0.84±0.05
Flavonoids (mg/g)	4.24±0.34	3.70±0.04	3.87±0.14
Tannins (mg/g)	1.88±0.08	1.35±0.07	2.45±0.06
Carbohydrate (mg/g)	22.63±0.73	12.04±1. 28	10.63±.09
Alkaloids (mg/g)	2.83±0.17	2.67±0.34	1.55±0.12
Anthocyanin (mg/g)	0.50±0.03	0.26±0.01	0.42±0.01
Terpenoids (mg/g)	1.28±0.03	0.96±0.03	0.33±0. 04

Values are mean ± SD

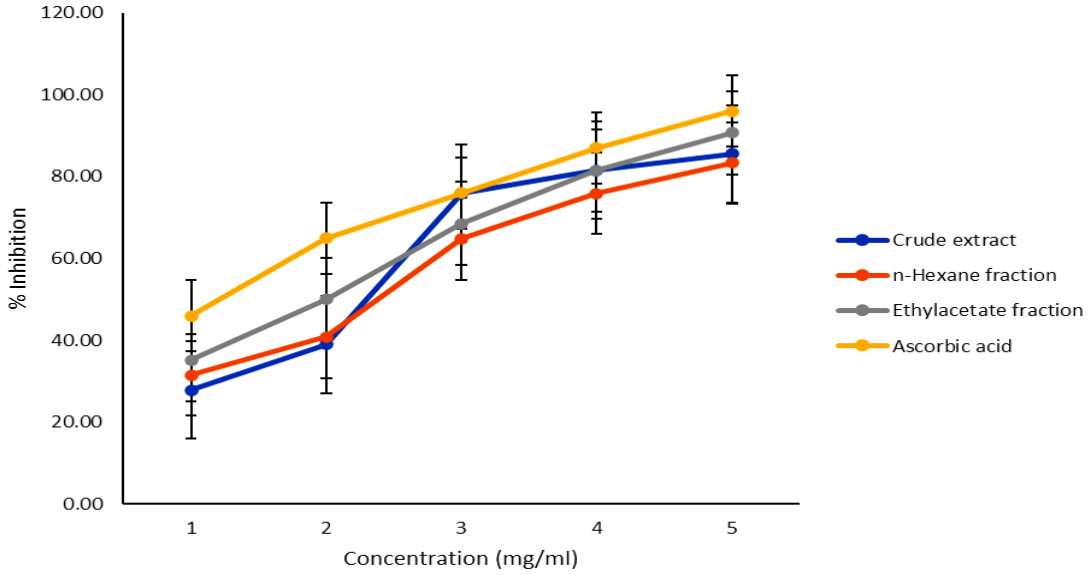

Figure 1: Antioxidant capacity of *P. americana* crude extract and fractions against DPPH. ***Key:*** 1, 2, 3, 4, and 5 represent 65.25, 125, 250, 500, and 1000 mg/mL, respectively

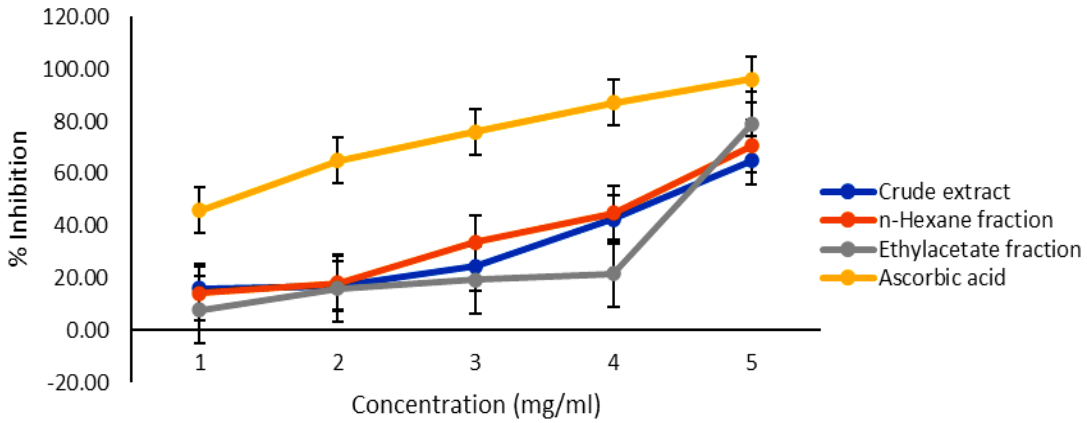

Figure 2: Plot of inhibition (%) against concentration for total antioxidant capacity. ***Key:*** 1, 2, 3, 4, and 5 represent concentrations of 65.25, 125, 250, 500, and 1000 mg/mL, respectively

Nitric oxide antioxidant activity

Nitric oxide antioxidant capacity of the crude extract and different fractions of *P. americana* compared with ascorbic acid (Figure 3), with a statistically significant (p = 0.01) difference between the samples.

GC-MS data

The GC-MS analysis carried out on methanol extract of *P. americana* showed a total of 86 peaks (Figure 4).

Antimicrobial activity

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of ciprofloxacin were determined using five different microorganisms (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans*, *and Escherichia coli*). The MIC and MBC were 7.8 mg/mL for all the tested organisms.

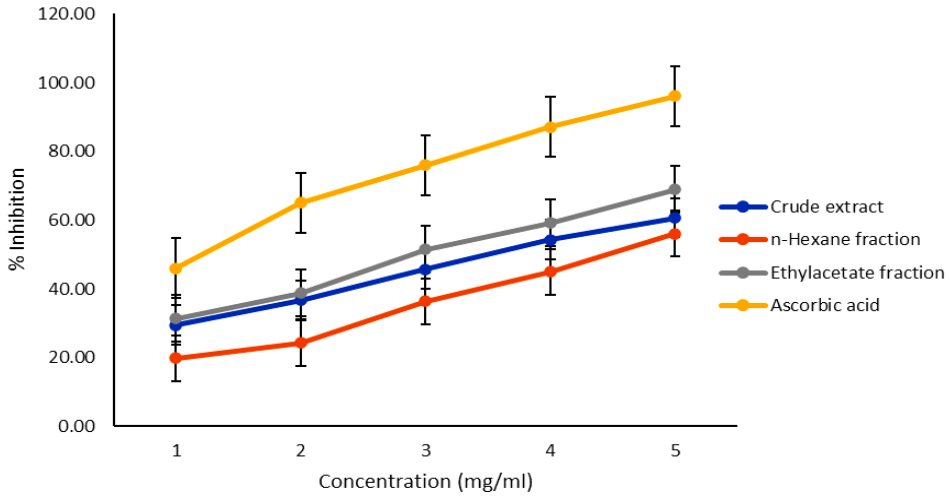

Figure 3: Plot of inhibition (%) against concentration for nitric oxide antioxidant capacity. ***Key:*** 1, 2, 3, 4, and 5 represent concentrations of 65.25, 125, 250, 500, and 1000 mg/mL, respectively

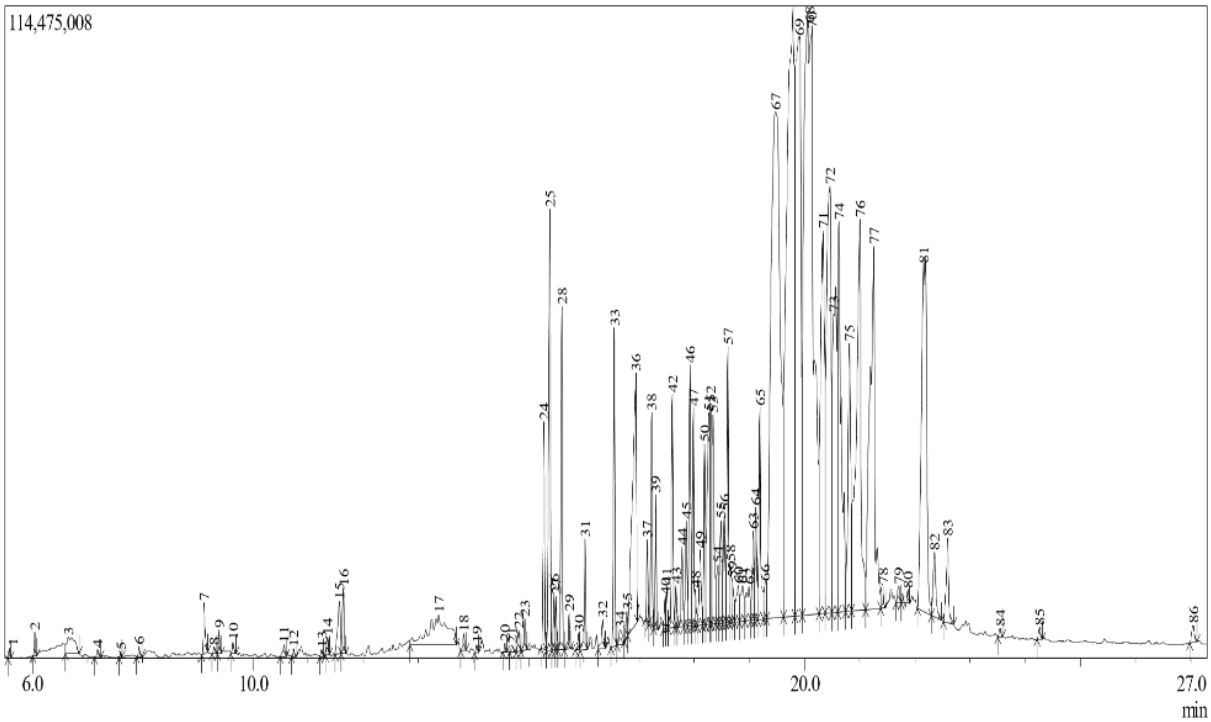

Figure 4: GC-MS spectrum of extract of *P. americana* seed

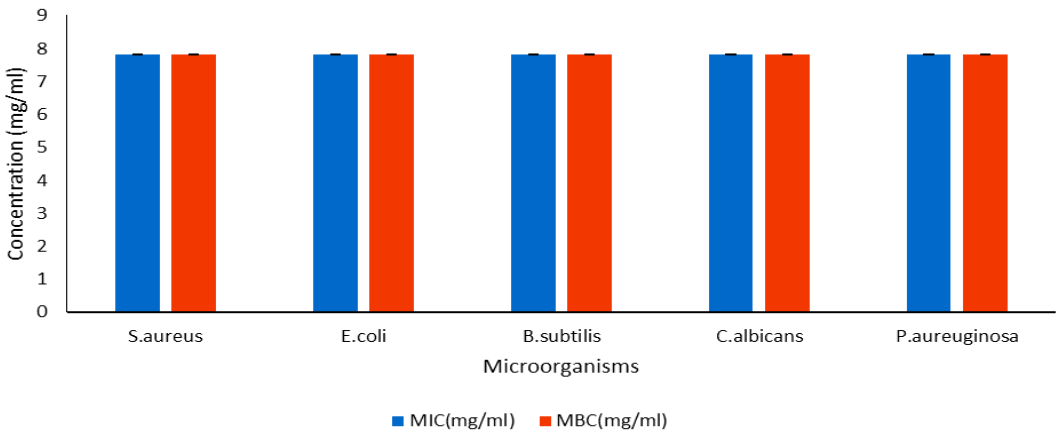

Figure 5: MIC and MBC (mg/mL) of ciprofloxacin against selected microorganisms. Values are presented as mean ± SD

Inhibition zone diameter (IZD) of ciprofloxacin

Inhibition zone diameter (IZD) for different concentrations of ciprofloxacin was measured against the microorganisms (*S. aureus, E. coli, B. subtilis, C. albicans and P. aeruginosa*) and a graph of inhibition zone diameter (IZD) was plotted against the log concentration (Figure 6). The result showed that ciprofloxacin demonstrated dose-dependent inhibition against the different organisms (Figure 6).

Minimum inhibitory concentration and minimum bactericidal concentration of crude extract of *P. americana*

The MIC and MBC of the crude extract of *P. americana* on test organisms were 31.25 mg/mL.

Inhibition zone diameter (IZD) of crude extract

The result showed there was inhibition at 500 mg/mL for all the tested organisms (*S. aureus, E. coli, B. subtillis, C. albicans*, *and P. aeruginosa*), although less at 250 and 125 mg/mL.

The MIC and MBC of n-hexane fraction

The results indicated an equal MIC and MBC of 31.25 mg/mL across the five different organisms (Figure 9).

Inhibition zone diameter of n-hexane fractions

Further analysis of the different concentrations of n-hexane fraction of the extract on *P. aeruginosa* revealed little inhibitory activity with IZD of 2.67 and 0.33 mm at 125 and 75 mg/mL, respectively and no inhibitory activity at 500 mg/mL and 250 mg/mL. However, 500 mg/mL showed the highest inhibitory activity on *E. coli* with an IZD of 20.33 mm, while the lowest concentration of the n-hexane fraction showed the highest activity with an IZD of 20 mm on *C. albicans* (Figure 10).

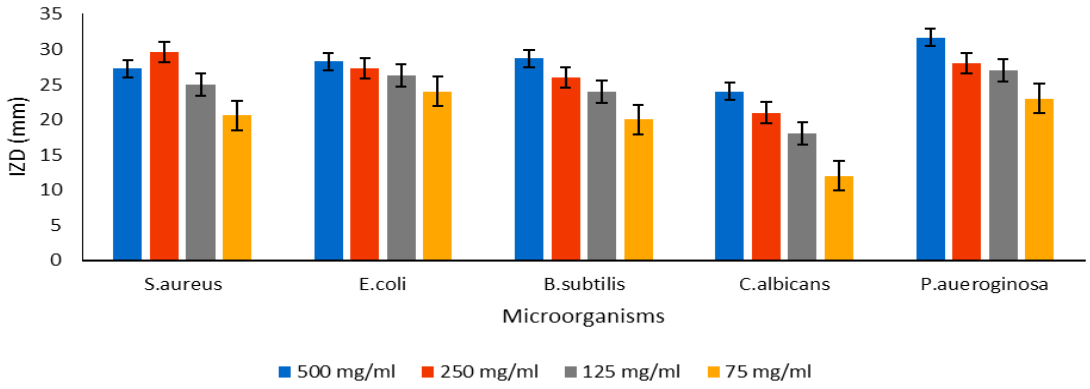

Figure 6: A graph of IZD against log concentration for ciprofloxacin. Values were presented as mean ± SD

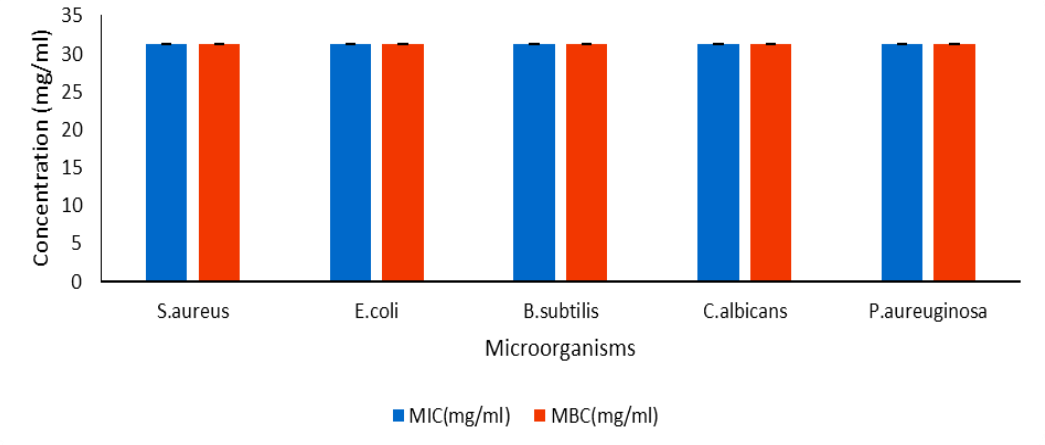

Figure 7: Graphical representation of MIC and MBC (mg/mL) of crude extract. Values are presented as mean ± SD

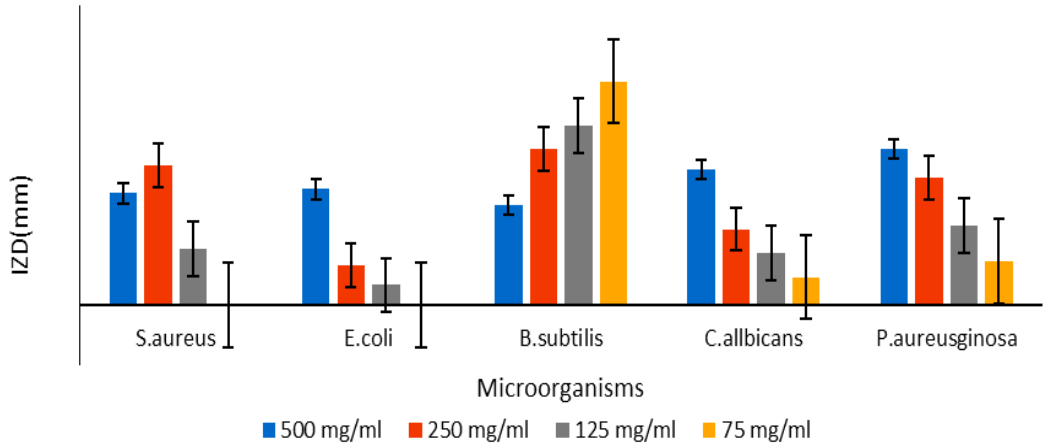

Figure 8: A graph of IZD against log concentration for the test organisms (crude extract of *Persea americana*). Values are presented as mean ± SD

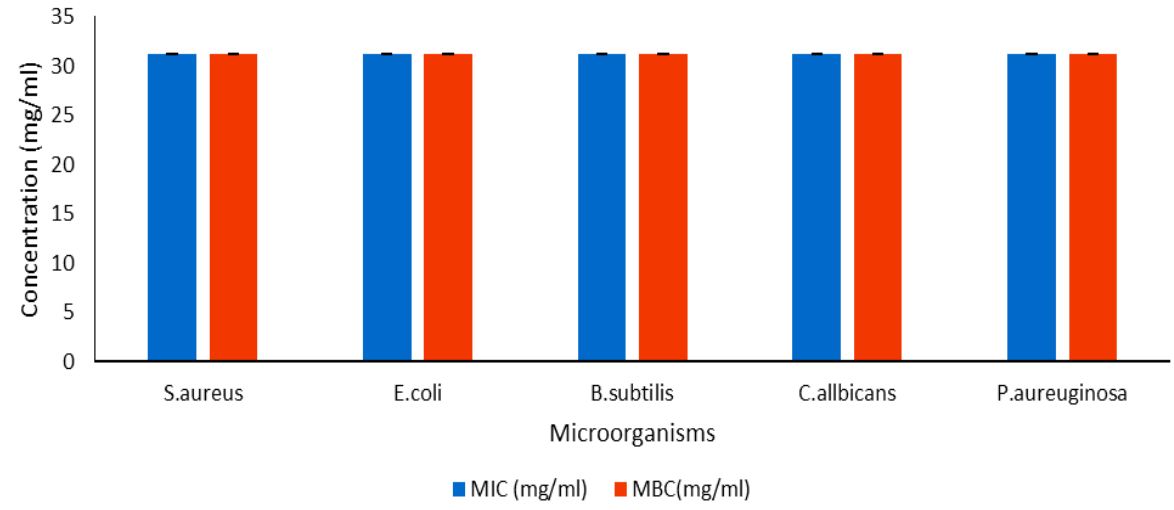

Figure 9: Graphical reprsentation of MIC and MBC(mg/ml) of n-hexane fraction of *Persea americana*. Values are presented as mean ± SD

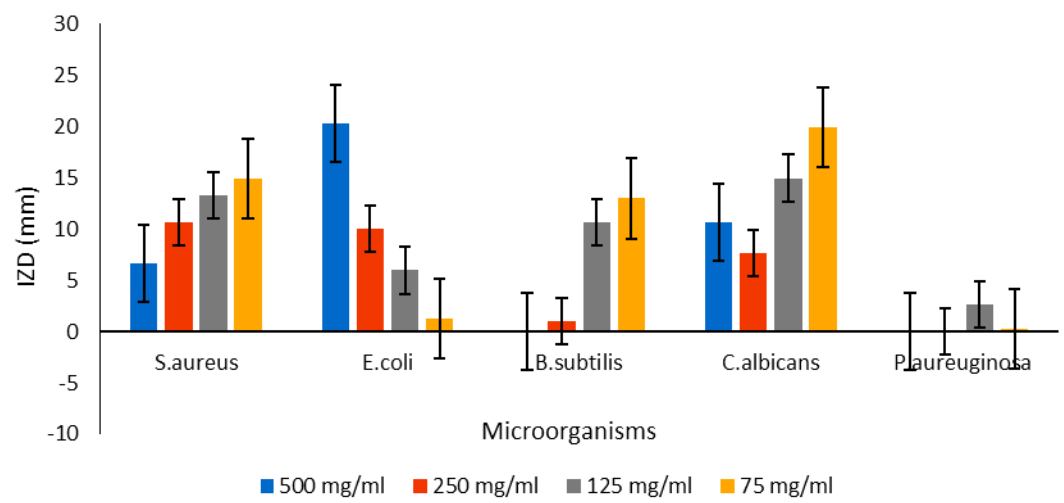

Figure 10: IZD of *P. americana* (n-hexane fraction) against log concentration for the test organisms. Values are mean ± standard deviation

Antimicrobial activity of ethyl acetate fractions

The ethyl acetate fraction showed its highest MIC on *P. aeruginosa* at 250 mg/ml, followed closely by *S. aureus* at 125 mg/ml MIC with an MIC of 31.25 mg/ml each for *E. coli*, *B. subtilis* and *C. albicans* (Figure 11).

Inhibition zone diameter (IZD) of ethyl-acetate fraction

The results showed maximum inhibition of *E. coli* at 500 mg/mL. Interestingly, the lowest concentration (75 mg/mL) ethyl-acetate fraction of the extract showed the highest inhibitory activity on the organisms except for *S. aureus* (Figure 12).

DISCUSSION

The qualitative analysis revealed the presence of various secondary metabolites, including alkaloids, anthocyanins, flavonoids, tannins, phenolics, carbohydrates, and terpenoids in the methanol extract, n-hexane and ethyl acetate fractions of *Persea americana*. However, saponin was absent in all. Previous studies [7] revealed that *P. americana* is rich in diverse phytochemicals, known for their potential health-promoting properties. Furthermore, the crude extract exhibited a relatively high concentration of flavonoids, soluble carbohydrates, and phenolics, indicating strong antioxidant potential. The n-hexane fraction also displayed antioxidant activity but at slightly lower levels. The ethyl-acetate fraction showed a similar profile to the crude extract.

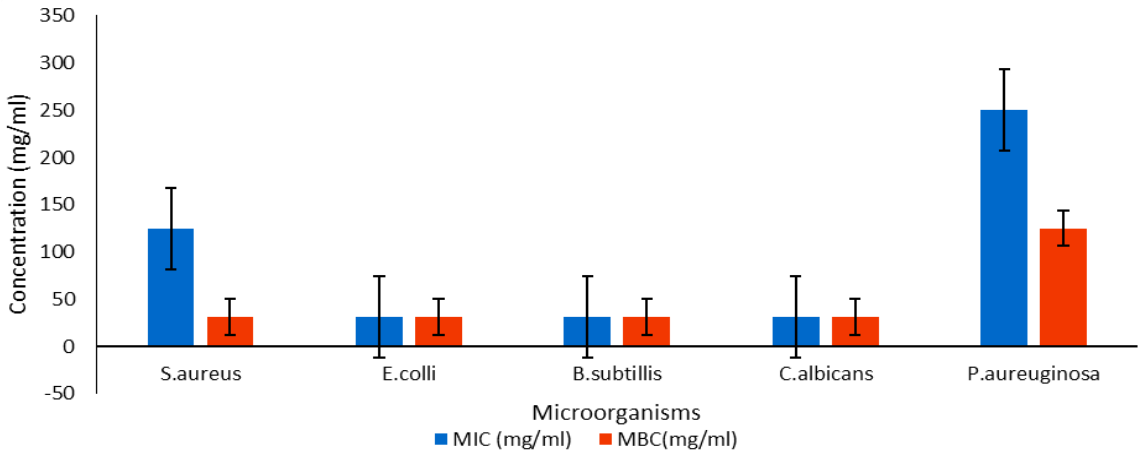

Figure 11: Graphical representation of MIC and MBC of Ethylacetate fraction of *Persea americana*. Values are presented as mean ± standard deviation

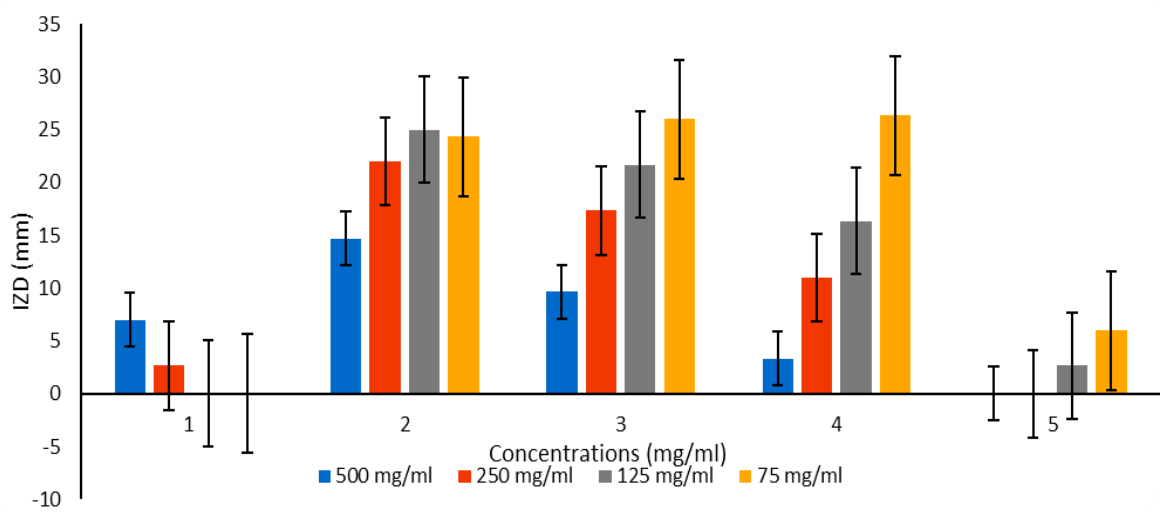

Figure 12: A graph of IZD against log concentration of ethyl-acetate fraction. Values are presented as mean ± standard deviation (SD)

The DPPH antioxidant assay demonstrated that *P. americana* extract and fractions possess the ability to scavenge free radicals, with the ethyl acetate fraction consistently showing the highest DPPH radical scavenging activity. This suggests the plant's potential to protect against oxidative stress and related health issues [17].

However, it is important to note that ascorbic acid, a well-known antioxidant, outperformed the extract and fractions, which serve as a reference for antioxidant capacity. The total antioxidant capacity results further confirmed the antioxidant potential of *P. americana* crude extract and fractions, with the ethyl-acetate fraction demonstrating strong activity at higher concentrations. While the crude extract also showed antioxidant capacity, the ethyl–acetate fraction showed consistently higher activity, while the n-hexane fraction showed the least effect. Ascorbic acid remained the most potent antioxidant, reinforcing its role as positive control.

The antibacterial potential of *P. americana* was also assessed because of its medicinal characteristics, which have been shown in previous studies. The plant is widely recognized for its antimicrobial, anti-inflammatory, anti-tumor, and anti-diabetic characteristics [18]. Using the agar diffusion method, different concentrations of the extract and fractions (500, 250, 125, and 75 mg/ml) were applied to the test organisms to evaluate the IZD. The phytoconstituents have varied solubility in various solvents because of polarity changes that impact the degree of activity [18]. The MBC and MIC of 7.80 mg/mL showed how effective ciprofloxacin is as a conventional medication. The inhibitory zone diameter measurement for ciprofloxacin confirms its effectiveness as a conventional therapy for treating both gram-negative and gram-positive organisms.

The MIC and MBC of the n-hexane fraction were fairly comparable to the standard drug, ciprofloxacin. The n-hexane fraction was not very effective against *P. aeruginosa* compared to *E. coli* and *S. aureus,* which demonstrated greater IZD. The ethyl acetate fraction did not show any inhibitory activity at 250 and 500 mg/mL against *P. aeruginosa*. Also, *S. aureus* was resistant to the ethyl acetate fraction. The antimicrobial activity that *P. americana* extract and fractions exhibited is attributed to the presence of secondary metabolites like flavonoids, tannins, and steroids identified in the phytochemical screening [19]. Gram-negative bacteria such as *E. coli* usually show high intrinsic resistance to most antimicrobial agents, however, the activity of *P. americana* extract and its fractions against *E. coli* was substantially similar to previous findings [20].

CONCLUSION

P. americana furnishes an array of secondary metabolites that contribute to its antioxidant and antimicrobial activities. However, there is a need for further studies, including the isolation of the bioactive compounds and evaluation of the toxicological profile, in order to ascertain its safety.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

None required.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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