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Phytochemical profile, antioxidant and antimicrobial properties of *Persea Americana* seed Mill

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ABSTRACT

Aim: The study was aimed to investigate the phytochemical profile, antioxidant, and antimicrobial efficacy of crude extracts from *Persea americana* seed.

Method and Materials: Phytochemical profile of various crude extracts obtained using different solvents in their order of polarity was determined using standard methods. All the crude extracts of *Persea americana* seed were subjected to 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Seven pathogenic microbes were used for antimicrobial activity of seed extracts using agar gel diffusion technique.

Results: The phytochemical analysis result of *Persea americana* seed extracts confirmed the presence of tannins, steroid, terpenoids, flavonoids, glycosides, and alkaloid. All seed extracts demonstrated very good antioxidant potentials against DPPH radical scavenging. The methanol seed extract showed a significant antimicrobial activity against all the tested micro-organism. The inhibitory potential of the seed extracts increases with increasing concentration, and polarity of solvent. The results exhibited good selective sensitivity against various tested pathogens.

Conclusion: It was concluded that the screened seed extracts of *Persea americana* is a good source of natural strong antioxidant,and antimicrobial agents validating the traditional use of the seed in folk medicine**.**

Keywords: Antimicrobial potency, *Persea americana* seed, Antioxidant, Phytochemicals, Methanol extract.

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Introduction

In recent years, there is a sudden shift in emergence and spread of drug-resistant pathogens with new mechanisms of resistance, there by threatening treatment of common infections (Iskandar *et al*., 2022). Human diseases and subsequent death is attributed to pathogenic microorganisms. Extracts of medicinal plants usually exhibit dose-dependent effects on microorganisms (Okeke and Ezeabara, 2019; Ezeabara and Vincent, 2021). The inhibitory activity usually shown by extracts of medicinal plant against pathogenic microorganisms justifies its varying degree of potency (Ezeabara and Egenti, 2018; Ezeabara *et al*., 2020).

Phytochemicals obtained from plants forms basis of both traditional and modern medicines which is widely utilised in drugs manufacturing (Rasool *et al*., 2020). Scientific reports established and confirmed that plants constitute about 70% of drugs currently used by humans (Savadi *et al*., 2020). About 170,000 bioactive constituents emanate from plants which forms template for many drugs. Utilisation of plants in health care delivery is due to the presence of phytoconstiuents which are inherent in them. Phenolic compounds among these bioactive molecules providea considerable interest in food, chemistry, and medicine due to their established antioxidant potency (Diouf *et al*., 2023). These phytoconstiuents are synthesised and stored in different plant part in varying concentrations (Chinelo *et al*., 2020). The utilisation of medicinal plants for medicinal purpose involves proper, accurate scientific information, and understanding of their chemical constituents which justify their therapeutic effects (Vaou *et al*., 2021).

Antioxidants are known to retard or hinder oxidation of substances with free radical scavenging ability. Polyphenols such as flavonoids, tannins, and anthocyanins are well known phytochemicals that exhibit free radical scavenging (Farag *et al*., 2020).Excess reactive oxygen species (ROS) is implicated in chronic and

degenerative diseases such as cancer, neurodegenerative, respiratory and digestive diseases development (Liu *et al*., 2018). Antioxidant has the capacity of regulating the reactive oxygen species(ROS) concentrations under a certain physiological conditions. The antioxidant can either be generated endogenously or with externally (Borel *et al*., 2022).

Persea americanaMill belongs to *Lauraceae* family (Setyawan *et al*., 2021). The genus of *Persea Americana* consists of 150 species, which 70 are grown in warmer regions of South America, and North of Central. The *Persea americana* contains a significant amount of oil in comparison to other fruits (Dabas *et al*., 2013). The fruit is a berry, consisting of a single large seed, surrounded by a buttery pulp. It contains 3-30% oil (Florida varieties range from 3% to 15%). Secondary metabolites such as lutein, α-carotene, β-carotene, zeaxanthin, neoxanthin, and violaxanthin have been isolated from *P. americana*. The plant is traditionally cultivated for food, and medicinal purposes due to its high nutritional content, and therapeutic properties. The *Persea americana* is categorised as evergreen, although few varieties will lose their leaves for a while before flowering. *Persea Americana* canopy ranges from low, dense, symmetrical to upright, and asymmetrical. The leaves are 7-41 cm in length is sometimes elliptic, oval, and lanceolate in shape (Yasir *et al*., 2010). The plant is often pubescent and reddish when young, becoming smooth, leathery, and dark green at maturity. Flowers are 1-1.3 cm in diameter and yellowish green. Although the plant is traditional used in the treatment of various diseases.

However, there are untapped scientific investigations into the seed of *Persea americana*, despite its historical usage in treatment of oxidative stress related diseases, and antimicrobial potency which is attributed to the phytoconstiuents. Therefore, the present study aim to thoroughly examine the phytoconstiuents of the seed extracts, evaluate their antimicrobial, and antioxidant activities against different microbial pathogens.

Materials and Methods

Chemicals and reagents: Dichloromethane, methanol, ethanol, hexane, ethyl acetate, 2,2-diphenyl-1-picrylhydrazyl, hydrochloric acid, sulphuric acid, methanol, ammonium hydroxide, ferric chloride, gallic acid, sodium hydrogen carbonate, aluminium chloride, and quercetin. All the chemicals were analytical grade and purchased from Merck Limited (Mumbai, India). Filter paper from Whatman Company, Catalogue No. 8174900 and nutrient agar and plastic petri dish were purchased from Sharlau Chemie Company, Korea.

Plant collection: Fresh seed of *Persea americana* were freshly collected from Omoku market in Ogba Ebagama Nodini Local Government Area, Rivers State, Nigeria in the month of August, 2020. The seeds were identified, and authenticated immediately after harvesting.

Preparation of seeds sample: The fresh seeds of *Persea americana* were washed thoroughly with distilled water, cut into smaller segments and shade dry for two weeks in a well-ventilated room. The dried seeds of *Persea americana* were grounded and stored in a sealed glass jars at room temperature.

Extraction: The powdered seed sample (30 g) was extracted by maceration technique with 500 mL of methanol at ambient temperature for 24 hour. The ethanol extract was filtered through Whatman no. 4 paper. It was concentrated with rotary evaporator at 40 °C to dryness and stored in refrigerator.

Calculation of extraction yield (% yield): The yield (%, w/w) from dried seed extract was calculated as: Yield (%) = (W_1 x 100) /W_2 W_1 is the weight of the extract after lyophilisation of solvent and W_2 is the weight of the powdered material.

Phytochemical Screening: The preliminary phytochemical profile was done in accordance with standard protocol described by (Ali and Hossain, 2015; Thusa & Mulmi, 2017). The different extracts were screened for metabolite such as anthraquinones, terpenoids, cardiac glycosides, alkaloids, saponins, flavonoids, tannins, and carbohydrate.

Terpenoids: Aqueous seed extract 5 mL of was mixed with 2 mL of chloroform in a test tube with 3 mL of concentrated H_2SO_4 added carefully, the mixture form a layer. The interface of the mixture forms a reddish brown coloration confirming the presence terpenoids if present.

Steroids: (Salkowski test): The crude extract obtained from *Persea americana* seed was mixed with chloroform and few drops of conc. tetraoxosulphate vi acid, properly shaked and allowed to stand for some minutes. Appearance of red coloration at the lower layer confirmed the steroids.

Flavonoid: Small quantity of *Persea americana* seed extracts was dissolve in 10% of sodium hydroxide (NaOH) and Hydrochloric acid (HCl). A yellow

solution which turned colourless on HCl addition justifies the presence of flavonoids.

Anthraquinones: Powdered extracts was shaken with 10 mL of benzene. The solution was filtered and 5 mL of 10 % NH_4OH solution was added to the filtrate. A pink, red or violet color in the ammonical (lower) phase indicated the presence of anthraquinones

Test for Tannins: Crude extract of *Persea americana* seed was dissolved in distilled water follow by two drops of 5% $FeCl_3$. Formation of a dirty-green precipitate is an indication of the presence of tannins in the extract.

Test for alkaloids: The crude extract of *Persea americana* seed was added to 2ml of 1% hydrochloric acid and heated gently. Mayer's reagent was added to the mixture leading to the formation of turbidity which precipitates indicating the presence of alkaloids.

Test for glycosides

Liebermann's test: *Persea americana* seed crude extract was mixed with 2ml of chloroform and 2ml of acetic acid. The resulting mixture cooled in ice with carefully addition of concentrated H_2SO_4. The change in colour from violet to blue to green confirms the presence of steroidal nucleus constituting the glycone portion of glycoside.

Test for saponins: Crude extract of *Persea americana* seed was dissolved in 5ml of distilled water in a test tube and shaken vigorously. The formation of stable foam indicates the presence of saponins.

Test for phenols: The crude extract of *Persea americana* seed was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration formation indicates the presence of phenols.

DPPH radical scavenging assay: The DPPH assay was done using method described by (Dzoyem *et al*., 2015). Briefy, 900 μL of DPPH solution (0.2 mM) prepared in methanol was mixed with the different seed extracts 100 μL at various concentrations such as (12.5 to 200 μg/mL). It was incubated in dark at ambient temperature for 30 min. Absorbance of the mixture was examined with spectrophotometer at wave length 517 nm. The positive control and negative control used was ascorbic acid and methanol, respectively. The extracts without DPPH serve as a blank.

The percentage inhibition of DPPH radical scavenging (%I) was evaluated using the formula:

$$\%I = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

The concentration of each seed extracts necessary to scavenge 50% of radicals (IC_{50}) was evaluated by simply plotting percentages of inhibition against each sample concentrations.

Antimicrobial assay: The antimicrobial assay of *Persea americana* seed crude extracts was carried out with agar gel diffusion method (Hossain *et al*., 2014). Amoxicillin was used as standard to compare the activity of the *Persea americana* seed extracts. Negative control was dimethyl sulfoxide and Whatman filter was utilised as disc in this experiment. All the crude extracts were separately dissolved and diluted with dimethyl sulfoxide, producing solution with concentrations 3.00, 2.50, 2.00, 1.50, 1.00, and 0.25 mg/mL. The paper discs were soaked in different polarities of the crude samples of *Persea americana* seed. The paper disc was placed on nutrient agar plate already seeded with the organism. The plates were incubated at 37 °C for 24 h. Growth of inhibition zone was measured after plate incubation. Each experiment was done in triplicate. *Persea americana* seed extracts were examine with seven (7) different microbe, such as *Bacillus subtilis, Bacillus cereus, Salmonella typhi, Pseudomonas aeruginosa, Proteus mirabilis, E. coli,* and *C. albicans.*

Results and Discussion

Percentage of yield: The *Persea americana* seed were collected, washed, shade dried, and powdered. The powdered *Persea americana* seed was extracted with methanol and concentrated. The extraction yield of12.11 %was obtained for the ethanol extract. The methanol crude extract (6 g) was partitioned with different polarities of solvents such as hexane, ethyl acetate, dichloromethane, and ethanol. The percentages yield obtained from the partitioned *Persea americana* ethanol seed extract yielded 2.1 %, 3.7%, 2.5%, 12.11%, and 5.9% (Fig 1). The highest yield from *Persea americana* seed was in ethanol while hexane extract gave the lowest yield. The order of yield rate was methanol>ethanol>ethylacetate>dichloromethane> hexane which largely depend on the polarity index of the solvent used in the extraction process, and nature of extractable constituents available in the seed (Dellavalle *et al*., 2011).

Phytochemical screening: The results of qualitative phytochemicals analysis of *Persea americana* seed extracts were shown (Table 1). The results established that all seed extracts contained steroid and saponins. Alkaloids and Anthraquinones were found in ethanol and methanol seed extracts but

absent in dichloromethane, ethyl acetate and hexane seed extracts. Terpenoids were present in all extracts except ethyl acetate.

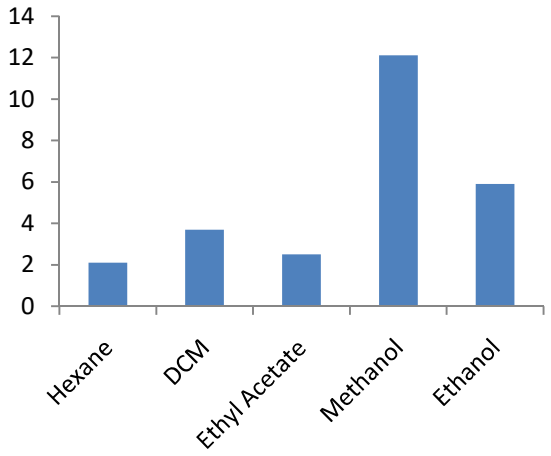

Fig 1. Percentage of 30 g crude extracts of *Persea americana* seed

Flavonoids were absent in hexane, dichloromethane, and tannins were present in dichloromethane and methanol seed extracts. Furthermore, the phytoconstiuents are known to possess various biological activities, such as antioxidant, antimicrobial, antiplasmodial, anti-inflammatory, and anticancer activities (Abeysinghe *et al*., 2021). The results established pharmacological relevance of the seed extracts with potency to demonstrate therapeutic and physiological activities due to the availability of secondary metabolites (Ali & Hossain, 2015; Thusa & Mulmi, 2017). The availability of these phytoconstiuents has pharmacological and medicinal relevance to humans (Yasir *et al*., 2010). Alkaloids are known to possess antiasthma, antibacterial, antimalarial and anticancer pharmacological potency. The works of (Sunday *et al*., 2022) illustrated high presence of tannins, alkaloids, saponins, and flavonoids in the peels of *Persea americana.* Owusu Boadi *et al*. (2015) established that leaves of *P. Americana* contained steroids, tannins, saponins, alkaloids, terpenoids, flavonoids, and glycosides. The preliminary phytoconstiuents profile serves as good variable in the investigation of bioactive constituents which serves as a lead in new drug development (Frag *et al*., 2020).

DPPH radical scavenging activity: The antioxidant activity of *P. Americana* seed was determined using the DPPH method. The proton radical scavenging ability is a vital attribute of antioxidants. Hydrogen donating potency of antioxidants molecules adds to its free radical scavenging nature. It was indicated that the IC_{50} values of respective extracts in different *in vitro* models (Table 2). Each values represents SD± mean (n=4).The DPPH radical scavenging activity and IC_{50} values of *Persea americana* seed extracts ranged from 25.31 ±0.13μg/mL for hexane and 52.99 ±0.23 μg/mL for methanol seed extracts, respectively. Generally, all the seed extracts, exhibited increasing antioxidant activity in order of increasing polarity index of the extracting solvent. The antioxidant activity observed in various seed extracts is attributed to the availability of various phenolic compounds confirmed from phytochemical profile results. These phenolic compounds are simply antioxidants agents which act by hydrogen donating properties of their phenolic group hydroxyls (Borel *et al*., 2022). Furthermore, phytoconstiuents such as phenolic compounds usually form chelate with metal ions which involved in ROS production (Iqbal *et al*., 2015).

Table 1. Qualitative phytochemical screening of seed extracts of *Persea americana*

Secondary Metabolites	Hexane	Seed Extracts Ethyl acetate	DCM	Ethanol	Methanol
Terpenoid	+	-	+	+	+
Steroid	+	+	+	+	+
Flavonoids	-	+	+	+	+
Anthraquinones	-	-	+	+	+
Tannins	-	-	+	-	+
Alkaloids	-	-	+	+	+
Glycosides	-	-	-	+	+
Saponins	+	+	+	+	+

The symbols + indicate present while – signifies absent

Table 2. IC_{50} (μg/mL) different values of *Persea Americana* seed extracts of against DPPH

Extract	(IC 50 in μg/mL) DPPH radical scavenging activity
Hexane	22.31 ±0.13
Ethyl acetate	29.47 ±0.20
Dichloromethane	30.56 ±0.17
Ethanol	40.79 ±0.07
Methanol	52.99 ±0.23
Ascorbic acid	29.49±0.00

Antimicrobial result Persea americana seed extracts: The antimicrobial activity of the crude extracts from *Persea americana* seed (Table 3) established some degree of sensitivity against Gram-positive and Gram-negative pathogenic bacterial strains. The activities were determined by measuring various zone of inhibition (mm) of different *Persea americana* seed extracts. The methanol seed extracts of *Persea americana* showed the highest activity with a zone of inhibition diameter of 12.8 mm, followed by dichloromethane seed extracts with 10.0 mm for *Salmonella typhi.* The hexane seed extract of *Persea americana* showed no activity index with increasing concentration.

Table 3: Antimicrobial activities of Persea americana seed extracts

Mean Zone of	Inhibition (mm)				
Bacterial strain	PASHE	PASEE	PASDE	*PASEE	PASME
Salmonella typhi	R	S	S	R	S
Bacillus subtilis	R	R	R	S	S
Proteus mirabilis	S	S	S	R	S
E. coli	R	S	R	S	S
Bacillus cereus	R	R	S	R	S
P. aeruginosa	R	S	S	S	S
C. albicans	R	S	R	R	S

Key word: R = Resistance, B=sensitive, PASHE: *Persea americana* seed hexane extract, *PASEE: *Persea americana* seed ethyl acetate extract, PASDE: *Persea americana* seed dichloromethane extract, PASEE: *Persea americana* seed ethanol extract, and PASME: PASEE: *Persea americana* seed methanol extract.

The variation in the degree of activity indices may be attributed to different phytoconstiuents present in each *Persea americana* seed extracts. This is because different solvents have different degrees of solubility for different phytoconstiuents (Gopalakrishnan *et al.*, 2012). The different antimicrobial activities of seed extracts of *Persea americana* may be due to the presence of phytoconstiuents like phenolics, alkaloids, flavonoids, saponins, tannins, steroids, and triterpenes, which are known for their antimicrobial potency leading to cell membrane damage, causing cell death by its disruption.

Conclusion

It was concluded that the phytochemical profile of *Persea americana* revealed the presence of high quantity of saponins, alkaloids, flavonoids, tannins, terpenoids, and steroid. All the seed extracts of *Persea americana* except hexane showed the lowest antioxidant activity. Methanol extracts exhibited the highest antioxidant activity. However, the seed exhibit the ability to serve as a natural source of antioxidants. The results of antimicrobial assay of *Persea americana* seed extracts against drug-resistant pathogens established selective sensitivity. All the tested seed extracts of *Persea americana* exhibited good potency against various pathogens with methanol extracts howing the highest inhibition zone. Further studies are necessary to identify, isolate the antioxidant, and antimicrobial active compounds which could serve as a potential drug.

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