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Antibacterial and Antioxidant Activities of the Essential Oil and Methanolic Leaf Extract of *Persea americana*

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ABSTRACT
This study attempted to find out if the essential oils and methanolic crude extract of the leaves of *Persea americana* (PA) had potent antibacterial and antioxidant properties. The essential oil was extracted using steam distillation and subsequently subjected to gas chromatography –mass spectroscopy (GC-MS) analysis. The crude extract was obtained using methanol as the solvent. Subsequently, the antimicrobial activity of the essential oil and crude extract against pathogens were evaluated using standardized assays while the antioxidant activity of the essential oil and crude extract was evaluated using DPPH and FRAP assay. The GC-MS showed 1R-α-pinene, terpinolene, caryophyllene, camphene, isopulegol, longiverbenone, isoaromadendrene and humulene as the main constituents in *Persea americana* respectively which are constituents on the leaf's extract. The crude extract showed higher inhibitory activity (15.00±1.23 mm) on *S. aureus* compared to the essential oil (11.00±1.11 mm). The same applies for *P. aeruginosa* where the crude extract and essential oil inhibited the enzyme by 23.00±1.02 mm and 19.00±1.12 mm respectively, also *Salmonella typhi* where the crude extract and essential oil inhibited the enzyme by 24.00±1.24 mm and 18.00±1.32 mm respectively. *P. americana* volatile oil demonstrated significant ability to scavenge free with an IC_{50} value of 72.22 µg/mL in the DPPH assay. Additionally, the FRAP assay revealed that the *P. americana* volatile oil possessed notable reducing power of 4.42 µg/g further highlighting its antioxidant capacity. *P. americana* crude extract also demonstrates significant free radical scavenging activity in the DPPH assay with an IC_{50} value of 61.22 µg/mL and the FRAP assay showed its notable reducing power of 4.10 µg/g. Overall, the results indicate that the *P. americana* crude extract has stronger antioxidant properties compared to the essential oil. While in the FRAP assay, the essential oils showed more antioxidant potency.

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INTRODUCTION

Natural products are substances that are found in nature and are not greatly modified by man. They could come from plants, animals, fungi or micro-organisms. Natural products are characterized by a very diverse chemical composition and can contain a wide variety of substances (Jalil *et al*., 2024). Over hundreds of thousands of years, natural selection and evolutionary processes have altered the usefulness of natural products, giving them unique pharmacological or biological actions and significant structural diversity. In order to preserve the freshness and shelf life of food, natural products and their derivatives are also frequently utilized as food additives in the form of herbs and spices, antibacterial agents, and antioxidants. In actuality, natural and organic items are used in practically every aspect of our life, from the energy we use to drive our cars to the plastics and rubber goods, health and beauty products, and even the clothing we wear (Abozenadah *et al*., 2017).

Essential oils (EOs) are multipurpose natural substances that have important antibacterial, antioxidant, antifungal, and therapeutic qualities. As such, they are useful in a variety of sectors, including agriculture, healthcare, and food preservation (Omidian *et al*., 2025).

The avocado, or *Persea americana* mill is a member of the family *Lauraceae*, its excellent nutritional and therapeutic qualities have led to its historic cultivation for both food and medicine. In traditional medicine, *Persea americana* (PA) is a common medicinal herb (Muñoz-Redondo *et al*., 2022). Some of the biological virtues of *P. americana* are purported to be antiviral, antioxidant, antimicrobial, anti-inflammatory, antihyperglycemic, analgesic, antiulcer, hypertensive, antihepatotoxic, anticonvulsant, and vasorelaxant. Also, the body weight is influenced by it, and different toxicological studies have shown favorable results. These qualities are due to presence of many naturally occurring phytochemicals such as tannins, alkaloids, phenols, saponins and flavonoids (Ngbolua, 2019). They also contain a number of

phytochemicals that may prevent cancer (Muñoz-Redondo *et al*., 2022). Investigations were conducted into the chemical makeup of *P. americana* leaves, fruit, and seeds. The findings demonstrated the presence of phytochemicals like flavonoids, phenols, and saponins in significant amounts in the examined samples. The proximate content showed that the fruit of *P. americana* has more energy and fat; the seed has more energy, fat, and protein; and the leaf has more ash, fiber, and protein (Arukwe *et al*., 2012). Based on the result obtained by Nasri *et al*., 2022, p. *American* leaf essential oil was reported to be potential source of bioactive phytochemicals for medical and cosmetic use and that report could also be considered as the first report on *Persea americana* grown in Morocco. Another research conducted by Ilesanmi and others in the year 2022, showed that the antimicrobial test on the seed essential oil of *p. americana* showed appreciable antibacterial activities against the test organisms. The activity on the test organisms was comparable to conventional antibiotic drugs. According to Nouara, 2024, the essential oil *p. americana* was effective against Results both Gram-positive and Gram-negative bacteria. The oil could inhibit the mycelial growth of all tested fungi, with *Cladosporium* sp. and *Alternaria* sp. being the most sensitive species. Noura concluded that the essential oil *P. americana* proved to be an effective antimicrobial and antioxidant agent, that could be used as a natural perspective to control foodborne pathogens and lipid peroxydation. This research seeks to add to the body of knowledge relating to the antioxidant and antibacterial properties of *p. americana.* This is because very few studies have been done on the antioxidants and antibacterial properties of its essential oil.

MATERIALS AND METHODS

Samples Collection

The healthy leaves of *Persea americana* (Avocado) were collected from its tree found within the University of Lagos, Nigeria. The botanical identification and authentication were done in the Herbarium of the Department of Botany, University of Lagos, Nigeria with authentication number LUH: 7410.

Plant Extraction

The air dried *Persea americana* (200 g each) was loaded into a Clevenger typed steam distillation apparatus, previously washed with liquid detergent and distilled water. The steam distillation was carried out continuously for 3 h (Njoku *et al*., 2021). The extract (volatile oil, hexane and water) was collected in a glass vial and dried with anhydrous sodium sulfate to remove the water and evaporated to remove the *n*-hexane. The dried oil was transferred to a weighed glass vial, and the actual yield of volatile oil was determined. The oil was placed in a sealed vial and stored at 4 °C in a refrigerator until GC-MS analysis. The crude extract was prepared by cold maceration in methanol for 72 hours. The extract was concentrated and dried and stored in an amber colored vial for further analysis.

GC–FID and GC-MS Analyses of Volatile Oils

A gas chromatograph (Varian CP-3800) fitted with a dimethylpolysiloxane (100%) column (CP Sil-5 CB: 50 m length × 0.25 mm i.d. × 0.4 μm film thickness) and a flame ionization detector (FID) was used to evaluate the volatile oil samples (Varian, the Netherlands). At 16 psi of input pressure, nitrogen served as the carrier gas. In split mode, samples (0.2 μL) were injected at a split ratio of 1:100. After holding the column at 60 °C for five minutes, it was ramped up to 220 °C at a rate of 5 °C per minute and held there for three minutes. After that, the temperature increased to 250 °C at a rate of 5 °C per minute and maintained there for four minutes. The temperatures of the injector and detector were both set to 250° C and 300 °C respectively. The gas chromatography/mass spectrometry (GC-MS) analysis was performed on Perkin Elmer Turbo mass Clarus 600 Instrument (ionization energy: 70 eV; mass range: 40-500 amu) with an Elite-5 column (5 % phenyl and 95 % dimethylpolysiloxane) (30 m length, 0.25 mm internal diameter and 0.25 μm film thickness) (PerkinElmer, USA). The carrier gas was helium (1 mL/min). Initial temperature was 60 °C (1 min) increasing to 240 °C at 6 °C/min and held at 240 °C for 6 min, and then further increased to 250 °C at 10 °C/min. The final stage was 10 min at 250 °C. The oven temperature was programmed from 50 °C to 250 °C at a dynamic rate of 5 °C/min and held at 250 °C for 15 min. Samples were injected (0.1 μL) in a split less mode.

Identification of Volatile Oil Constituents

The identification of components was performed by comparing the retention indices (RI) of the GC peaks with those of saturated n-alkanes (C8–C30) (Aldrich, St. Louis, MO, United States) and those reported in the literature (Njoku *et al*., 2017) and by comparing the mass spectra of the peaks with those reported in the literature (Jennings, 2012a) and the NIST library. Percentages of peak areas were calculated from GC–FID response without correction factors: RI values for all components were calculated by using a homologous series of *n*-alkane. Mixtures (C7-C30) were injected under similar conditions as the samples, and the computers were matched with the NIST libraries. The components were identified by comparing the mass spectra of the peaks with those reported in the literature (Jennings, 2012a) and the NIST library, as well as by comparing the retention indices (RI) of the GC peaks with those of saturated n-alkanes (C8–C30) (Aldrich, St. Louis, MO, United States) and those reported in the literature (Njoku et al., 2017). Peak area percentages were computed using the GC-FID response without correction factors: A homologous sequence of n-alkane was used to obtain the RI values for each component. With the NIST libraries, the computers were matched, and mixtures (C7-C30) were injected under conditions identical to the samples.

Anti-Microbial Screening of the Extracted Volatile Oils and Crude Extract

The invitro antimicrobial activity of the extracted oil and the crude methanol extract of T. occidentalis was carried out against five bacterial strains: *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* using the agar well diffusion method (Osinubi et al., 2026) with minor modifications. These bacterial strains are clinically relevant strains known to display multidrug resistance to conventional antibiotics. Gentamicin (2 mg/mL) was the positive control, while hexane was the negative control to ensure that the activities were not solvent induced. Nutrient agar plates were seeded with 0.1 mL of an overnight culture of each bacterial strain (equiv. to 107 -108 CFU/mL). The seeded plates were allowed to set, and a standard cork borer (diameter: 7 mm) was used to cut uniform holes on the surface of the agar. 10 μL each of the essential oil and the crude extract (2 mg/mL) were used. Each extract was introduced into the wells and set in an incubator at 30°C for 24 h. After this, the clear area around each well was measured indicating the zone of inhibition (mm).

Antioxidant Assay

DPPH Free Radical Scavenging Assay

The antioxidant properties of the essential oil were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, with minor modification (Osinubi et al., 2024, Odusina et al., 2023). DPPH solution (0.1 mM, methanol) was freshly prepared, and the solution (1 mL) was added to 1 mL of the extracted T. occidentalis essential oil and crude extract separately. Each mixture was shaken together and incubated for 30 min. After incubation, UV-Vis analysis was run at a wavelength of 517 nm. Ascorbic acid served as the positive control. Percentage scavenging activity was calculated using equation 1.

DPPH Scavenging effect

$$(\%) = [(A0 - A1)/ A0] * 100 \quad (1)$$

Where A0 = absorbance of control and A1 = absorbance of respective extract (Crude and Oil). The IC_{50} value was defined as the concentration of the extract required to achieve 50 % inhibition of DPPH radical activity.

Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP assay was carried out following (Fernandes et al., 2016) protocol. Acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and ferric chloride solution (20 mM) were mixed in a ratio of 10:1:1 (v/v/v) to obtain freshly prepared FRAP reagent. The T. occidentalis extracts were mixed with the FRAP reagent and incubated for 30 min at 37 °C after which absorbance was measured at 593 nm on a UV-Vis Spectrophotometer. Gallic acid was used as positive control. The percentage FRAP Scavenging activity was determined using equation 2.

FRAP Scavenging effect

$$(\%) = [(Ae - Ab)/ (Ac - Ab)] \; X \; 2 \quad (2)$$

Where Ab = Absorbance of blank reacted with water and Ae = Absorbance of respective extracts (Crude and Oil) Ac = Absorbance of positive control, reacted with ascorbic acid. Leftover reagents were discarded that is, all reagent used must be freshly prepared.

Data Analysis

All experiments were done in triplicate. Data analysis was achieved with Microsoft Excel 2010. Results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Chemical Composition

Steam distillation resulted in a yield of leaf volatile oil of 1.10 % (w/w, on the basis of the weight of dry material). The analysis by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) identified 28 volatile compounds, of which about 14 were present in trace amounts (Table 1) accounting for 94.00 % of the total extracted oil, which were identified by matching retention times of available authentic standards, retention indices (RIs), and mass spectra in the NIST 17 database (Table 1). The major components in the volatile oil of *Persea americana* were humulene (20.44 %), terpinolene (19.62 %), caryophyllene (19.27 %), 1R-α-Pinene (7.11 %) and terpinene (4.24 %). The chemical component of the oil obtained in this research had similarities and variance in major components compared to those obtained in earlier research. For instance, Nasri *et al*., (2022) reported estragol the major component of leaf essential oils *p. Americana*. In another research, the oil from this plant had were estragole (61.86%), sabinene (15.16%) and 1R-α-pinene (14.25%) (Nasri et al., 2022). In the year 2007, Ogunbinu and co-workers researched on the essential oil of *Persea americana*, they found out that the major components are β-Caryophyllene (43.9%), valencene (16.0%) are the germacrene D (5.9%), ot-humulene (5.0%) and δ-cadinene (4.8%). (Ogunbinu *et al*., 2007). The chemical composition of *Persea americana* from this research shows the presence of some highly bioactive compounds. The bioactivity of the essential oil and crude extracts may be due to the presence of these compounds. These assertions will be further discussed and validated in the discussions below.

Table 1: Chemical Composition of Persea Americana

Name	[a]RI	[b]RI	Area %
1R-α-Pinene	929	932	7.11
Terpinolene	1079	1075	19.62
D-Limonene	1018	1022	2.97
γ-Terpinene	1050	1048	4.24
Terpinen-4-ol	1164	1164	2.68
Terpineol	1143	1147	2.73
Caryophyllene	1419	1415	19.27
Humulene	1451	1453	20.44
Alloaromadendrene	1460	1462	2.90
α-Guaiene	1444	1448	1.68
Sabinene	967	962	1.64
Verticiol	2106	2108	1.32
cis-Muurola-4(15),5-diene	1454	1456	1.27
Cyclofenchene	886	882	1.23
Cadina-1(10),4-diene	1469	1466	1.19

[a]RI: Retention index determined relative to n-alkanes (C7-C30) on the HP-5ms column. [b]RI: literature retention indices (Njoku, *et al*., 2021;)(*Koenig, et al.,* 1998*)*.(Jennings, 2012b), (Adams, 2017).

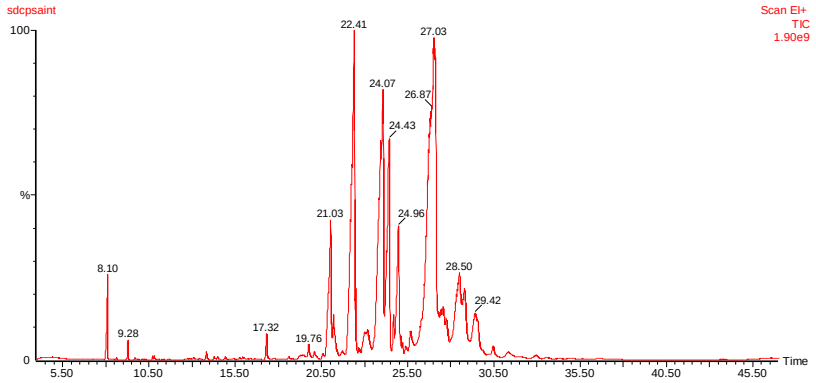


Figure 1: Total Ion Chromatogram of Persea Americana

Result of Antimicrobial Analysis of *P. Americana*

The antibacterial activity of the extracts varied by microorganism (Table 2-3). The essential oil had considerable activity against some gram positive human pathogenic bacteria. From the zones of inhibition (Z.I) obtained the oils from *P.americana* had the highest Z.I of 19.00±1.12 on *P. aeruginosa*, compared to 18.00±1.32 and 11.00±1.11 on *Salmonella typhi* and *S. aureus* respectively. Similarly, from the zones of inhibition (Z.I) obtained, the crude extracts from *P.americana* had the highest Z.I of 24.00±1.24 on *Salmonella typhi*, compared to 23.00±1.02 and 15.00±1.23 on *P. aeruginosa* and *S. aureus* respectively. It is important to note that the essential oils and the crude extract had competitive activity when compared to the standard antibiotic drug gentamycin.

Nasri et al. (2022) opined that *Persea americana* had promising antibacterial activities against gram-positive bacteria (Staphylococcus epidermidis, Staphylococcus aureus). They also posit that avocado leaf essential oils are in fact a potential source of bioactive phytochemicals for medical and cosmetic use (Nasri *et al*., 2022).

In another research by Ilesanmi and co-workers, the oil from *Persea americana* from a soxlet extraction showed appreciable antibacterial activities against the test organisms.(Ilesanmi *et al., 2022)*. The activity of any plant matrix may be linked to the presence of the secondary metabolites present in them. The essential oils from this research had some components that may be said to be responsible for the kind of biological activity elicited. For instance, one of the major components in the essential oil of the leaf essential oil of *P. americana* is caryophyllene. Research has shown that caryophyllene is a strong antibacterial agent. According to Moo et al. (2020), the minimum inhibitory concentration of β-caryophyllene for *B.cereus* was 2.5% (v/v). Beta-caryophyllene at minimum inhibitory concentration showed complete bactericidal activity in 2 hours killing kinetics. The results suggested that β-caryophyllene could change the membrane permeability and integrity of *B. cereus*, resulting in membrane damage and the leakage of intracellular contents, and ultimately cell death (Moo *et al.*, 2020). It is interesting to note that when compared to the standard drug gentamycin both the oil and crude extract showed encouraging antibacterial activity (Table 2 and 3).

Table 2: Antibacterial Activity of the Essential Oils of PA

(1)	Microorganisms	(2)	Diameters of zone of inhibition(mm)		
(3)		(4)	Volatile oil	(5)	Antibiotics
(6)	*S. aureus*	(7)	11.00±1.11	(8)	13.00±0.18
(9)	*P. aeruginosa*	(10)	19.00±1.12	(11)	20.00±1.30
(12)	*Salmonella typhi*	(13)	18.00±1.32	(14)	20.00±1.32

Results of antibacterial capability were reported in mean ± SD of triplicate values.

Table 3: Antibacterial Activity of the Crude Extract of PA

(15)	Microorganisms	(16)	Diameters of zone of inhibition(mm)		
(17)		(18)	Crude extract	(19)	Antibiotics
(20)	*S. aureus*	(21)	15.00±1.23	(22)	17.00±0.1
(23)	*P. aeruginosa*	(24)	23.00±1.02	(25)	24.00±1.12
(26)	*Salmonella typhi*	(27)	24.00±1.24	(28)	25.00±1.44

Results of antibacterial capability were reported in mean ± SD of triplicate values.

Result of DDPH and FRAP Antioxidant Analysis of PA

Table 4 and 5 show the comparative antioxidant activity of essential oil and crude extract of *P. americana*. This property was determined by FRAP and DPPH scavenging activity. Conversely, Crude extract (CE) showed higher DPPH scavenging activity than Essential oil (EO) with slightly lower IC_{50} values (IC_{50} values; EO: 72.22 ± 2.16 and CE:61.22 ± 3.12 µg/mL). Both extracts showed higher DPPH scavenging activity than standard. The two exhibited a high antioxidant activity, which is in concordance with the findings of other researchers. Ferreira and colleagues in 2013 reported that extracts obtained from the seeds of *Persea americana* had higher in *vitro* DPPH assay antioxidant activity (43 %) than the extracts obtained from the skin (35 %) and the fruit pulp (23 %) (Vinha *et al*., 2013). The antioxidant activity of the extracts of *Persea americana* is due to the existence of phytogenic compounds, that is., polyphenols and flavonoids, as reported by Abd Elkader *et al*., 2022 in another research. Antioxidant activity was increased in concentration dependent manner. Avocado fruit

extract at a concentration of 1000 µg/mL scavenged 95% of DPPḢ while leaf extract rummaged 91.03% of free radicals compared with Vit C and BHT (Abd Elkader *et al*., 2022). Moreover, FRAP assay revealed that the EO had higher antioxidant activity than the CE (Table 5). EO showed significantly higher capacity (4.42 ± 0.02 µg/g) than CE (4.10 ± 0.02 µg/g). The presence of the terpenes in the predominant amount increases the electron donating ability of the oil compared to the crude extract which is suspected to contain electron donating potential limiting components hence, limiting the electron donating potential of the sample in its crude form. These compositional differences may account for the differences in antioxidant activity. This is supported by recent mechanistic studies which have showed that the antioxidant potential of medicinal plants depends on their phenolic and flavonoid constituents i.e. amount of hydroxyl groups (Platzer *et al*., 2022). While ascorbic acid showed slightly higher activity, both the oil and extract performed well, highlighting their potential as natural antioxidants. Overall, the volatile oil exhibited stronger antioxidant properties than the crude extract, suggesting its value in combating oxidative stress.

Table 4: DPPH and FRAP Antioxidant Assay of the Volatile Oil from PA.

(29) Antioxidant Assay	(30) PA (Volatile oil)	(31) (Positive control)
(32) DPPH Assay (µg/mL)	(33) 72.22 ± 2.16	(34) 74.24 ±. 1.24
(35) FRAP Assay (µg/g)	(36) 4.42 ± 0.02	(37) 5.32 ± 0.02

Results of antioxidant capability were reported in mean ± SD of triplicate values.

Table 5: DPPH and FRAP Antioxidant Assay of the Crude Extract of PA

(38) Antioxidant Assay	(39) PA (Crude Extract)	(40) (Positive control)
(41) DPPH Assay (µg/mL)	(42) 61.22 ± 3.12	(43) 74.24 ±. 1.24
(44) FRAP Assay (µg/g)	(45) 4.10 ± 0.02	(46) 5.32 ± 0.02

Results of antioxidant capability were reported in mean ± SD of triplicate values.

CONCLUSION

This study found that *Persea americana* essential oil and crude extract have strong antimicrobial and antioxidant properties, making them useful for various applications. The antibacterial activity, antioxidant and chemical composition of *Persea americana* leaf essential oil make it a potential active ingredient in the treatment of pathogenic diseases. The ability of the essential oils and crude extracts to elicit the stated activities might be due to the presence of some biologically active components and phytochemicals. Further studies on minimum inhibitory concentration and minimum bactericidal concentration (MIC/MBC) and phytochemical screening are required to ascertain the therapeutic potency of this plant.

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