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Evaluation of Antimicrobial Potency and Phytochemical Screening of *Persea americana* Leaf Extracts against Selected Bacterial and Fungal Isolates of Clinical Importance

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OEA and SIA designed the study. Author SIA performed the statistical analysis. Author OEA wrote the protocol and the first draft of the manuscript. Authors OTO and OAA managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The quest for novel bioactive from natural sources informed the evaluation of the antimicrobial alongside the phytochemical composition of leaf extracts of *Persea americana* obtained from Akure, Ondo State, Nigeria.
Study Design: This study assessed the prospective antimicrobial efficacy of *Persia americana* against selected clinically relevant bacteria and fungi.
Place and Duration of Study: The study was conducted between April and September, 2015 at the Microbiology Laboratory of the Federal University of Technology, Akure, Ondo State, Nigeria.
Methodology: Clinical isolates (*Bacillus cereus, Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus, Shigella flexneri*, *Escherichia coli, Candida albicans*) and typed cultures (*Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853,

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Staphylococcus aureus ATCC 43300, *Salmonella typhi* ATCC 33489) were obtained from the Pathology and Clinical Laboratory of the Lagos State University Teaching Hospital, Lagos State, Nigeria while *Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus* were obtained from the culture collection center of the Department of Microbiology, FUTA. The authenticity of the bacterial and fungal isolates were confirmed using standard procedures. *Persea americana* leaves were collected from a building opposite BTO hall Ilesha garage, Akure, Ondo State and identified at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. Antimicrobial activities of the leaves extract were assessed on clinical and typed microbial cultures using standard microbiological procedures.

Results: The extracts displayed varying antimicrobial activities against all the test organisms with zones of inhibition ranging from 10.27 mm to 34.20 mm. The leaves extracts were effective against all the organisms; with the methanolic *P. americana* extract having the highest antibacterial activity (34.20 mm) while the acetone extract had the highest antifungal activity (12.60 mm). The phytochemical analysis revealed the presence of tannins, flavonoids, terpenoids, alkaloids and saponins.

Conclusion: This study supports the claims that *P. americana* leaves could be promising in the development of drugs to combat human diseases especially those of fungal and bacterial origin.

Keywords: Persea americana; antimicrobial; bacterial isolates; fungal isolates.

1. INTRODUCTION

The use of plants for treating diseases is as old as man. Traditional medicine is undoubtedly the main source of medical care for most developing countries around the world. In Africa specifically, indigenous plants play an important role in the treatment of a variety of diseases [1]. The use of plants and their products have a long history in folk medicine. Over the years, plants have been put into extensive use following their incorporation into traditional and allopathic medicine [2]. The secondary metabolites present in plants have been known to confer pharmacological potentials on them, thereby enabling them combat many disease-causing pathogens [3]. Infectious diseases are a prominent cause of morbidity and mortality among the general population, especially in developing countries. In recent years, pharmaceutical companies have seen the need to develop more potent antimicrobial drugs as a result of the continued emergence of multi-drug resistance microorganisms.

Antibiotics are unarguably one of the most important therapeutic discoveries that have proven effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been successfully treated by these synthetic products [4]. The resistance of these pathogens is largely due to widespread indiscriminate use of antibiotics [5,6]. The spread of multidrug-resistant (MDR) bacterial pathogens have also substantially threatened the current antibacterial therapy [7] leading to increased mortality, longer length of stays in hospitals, and higher cost of treatment and care [7,8].

A reliable method to reduce the resistance of microorganisms to antibiotics is by using antibiotic resistance inhibitors sourced from plants [9,10]. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Hence, researchers have paid more attention to safer plant-sourced medicines and biologically active compounds which are not only employed in herbal medicines but also have acceptable therapeutic index for the development of novel drugs [11,12]. The advantages inherent in the use of plant products for the control of human diseases include their cost effectiveness, biodegradability and ready availability [13].

P. americana (Avocado pear) is a medium sized tree, measuring about 9-20 meters in height. The tree is widely cultivated in tropical and sub-tropical areas of the world [14]. The seed has diverse applications in ethno–medicine, with its use ranging from treatment of diarrhoea, dysentery, toothache, intestinal parasites to skin treatment and beautification. Aside the seed, the avocado seed oil also possess several health benefits [15,16]. The anti-inflammatory and analgesic potentials of *Persea americana* leaves have also been reported [17].

In herbal medicine, infusion, concoction and extracts made from any part of this plant are

effective against hypertension, cancer, menstrual problems, inflammation and wounds [18,19]. With a view to enriching knowledge on the antimicrobial potentials of *P. americana*, the present study investigated the Secondary metabolites composition and Antimicrobial potency of leaf extract of *Persea americana* against selected bacterial and fungal isolates of clinical importance.

2. METHODS

2.1 Collection of Leaves

Persea americana leaves were collected from a building opposite BTO hall Ilesha garage, Akure, Ondo State, Nigeria. (Latitude: 7.3064N, Longitude: 5.12227E) in April, 2015. The leaves identity was authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. They were thereafter air dried, ground, and labeled for easy identification. Further analyses were carried out on the leaves at the laboratory of the Department of Microbiology, Federal University of Technology, Akure.

2.2 Ethical Clearance

Ethical clearance was obtained from the relevant regulatory bodies before the clinical isolates and typed cultures were obtained.

2.2.1 Collection of test organisms

Clinical isolates (*Bacillus cereus, Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus, Shigella flexneri*, *Escherichia coli, Candida albicans*) and typed cultures (*Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATC 43300, *Salmonella typhi* ATCC 33489) were collected from the Pathology and Clinical Laboratory of Lagos State University Teaching Hospital, Lagos State, while *Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus* were obtained from the culture collection center of the Department of Microbiology, FUTA. The isolates' viability were verified after which they were sub-cultured onto nutrient agar for bacteria, and sabouraud dextrose agar for fungi. The plates were afterwards incubated at 37℃ for 24 hours and 27℃ for 48-72 hours for the bacterial and fungal isolates respectively.

2.3 Identification of Test Organisms

The authenticity of the bacterial isolates was confirmed by morphological and biochemical tests. The appearance of each colony on the agar media and characteristics such as shape, edge, colour, elevation and texture were observed as described by Olutiola et al. [20]. Relevant biochemical tests were carried out as described by [21].

The fungal isolates were identified based on morphological and microscopic characteristics of moulds as described by Devarshi et al. [22]. The morphological characteristics of the moulds were based on the size, colour and aerial mycelia growth while the microscopic characteristics were determined using the simple staining method called "wet mount" with lactophenol-cotton-blue stain. This involved picking mycelia growth from the culture plates with a sterile inoculating loop and teasing out properly on a grease free slide to which a drop of sterile distilled water had been placed. Two drops of cotton-blue in lactophenol were thereafter added to the preparation and covered with a clean cover slip. The prepared slides were afterwards viewed under the x40 objective lens of a light microscope.

2.4 Preparation of Leaf Extracts

Powdered *P. americana* leaves (100g) were weighed separately into two different containers and 2000 mL of 100% acetone and methanol were added to either container respectively. The containers were covered with aluminum foil, allowed to stand for 3 days with continuous stirring for extraction to take place. The extracts were thereafter obtained by filtering the solution using a funnel fitted with a filter paper. The filtrates were afterwards evaporated to dryness at 50℃ in a rotary evaporator (RE-52A; Union Laboratory, England) at 90 rpm under reduced pressure.

2.5 Determination of Antimicrobial Activity of *Persea americana* Leaves Extracts

Antimicrobial activity of the leaves extracts was determined by the agar well diffusion method (Schinorf et al. [23]. Stock cultures were maintained on slopes of nutrient agar and pure cultures were prepared by transferring a loopful of cells from the stock cultures to plates of

Nutrient broth (NB) for bacteria and Sabouraud dextrose broth (SDB) for fungi. Incubation was thereafter carried out at 37℃ for 24 hours and 25℃ for 48-72 hours for the bacterial and fungal isolates respectively.

For the determination of the antifungal effect of the extract, fungal spores' suspensions were prepared from 3-day old cultures that grew on a Sabouraud dextrose agar. Molten Mueller Hinton agar was prepared according to manufacturer's specification, poured into sterilized Petri plates and left to solidify. An aliquot of the respective fungal cultures (100 µL) was thereafter evenly spread on the surface of the solidified Mueller Hinton agar plates. Wells of about 7mm diameter were bored in the agar using sterile cork borers and varying concentrations of the extracts (100, 50, 25, 12.5, 6.25 and 3.125 mg/mL) were prepared by dissolving different amount of the extracts in different volumes of 30% tween 20. The prepared extracts were then sterilized by passing them through a Millipore membrane filter (0.22 µm). About 100 µL of the sterile extract were then introduced into the bored agar wells with the aid of a micropipette. Same procedure was employed for the determination of the antibacterial effect of the extract, except that a 24 hour old broth culture of each of the test bacterium was used Cotrimoxazole, nystatin and gruseofluvin were used as positive control for fungi and reference antibiotic disc; ciprofloxacin (10 µg), rocephin (25 µg), gentamicin (10 µg), pefloxacin, (10 µg), erythromycin (10 µg) for bacteria. Tween 20 (30%) was however used as negative control. The plates were thereafter incubated at 37ºC for 24 hour for bacteria, while the fungal plates were incubated at 26 ± 1ºC for 48 to 72 hours. Inhibition zones around the isolates were measured in millimeters and all experiments were carried out in triplicates.

2.5.1 Determination of minimum inhibitory concentration of the extract

Varying concentrations of the extracts (100, 50, 25, 12.5, 6.25 and 3.125mg/mL) were prepared by dissolving different amount of the extracts into different volumes of 30% tween 20. The concentration with the least inhibitory effect on the isolates was taken as the Minimum Inhibitory Concentration.

2.6 Phytochemical Screening of Leaves Extracts of *Persea americana*

The qualitative and quantitative phytochemical analyses of the plants extracts were determined using standard protocols as described by [24] and [25].

2.6.1 Qualitative phytochemical analysis of leaves extracts of *Persea americana* test for alkaloid

The extracts (0.5 g each) were stirred with 5 mL of 1% aqueous hydrochloric acid (HCl) for two minutes on a steam water bath. The mixtures were filtered and few drops of Dragendorff's reagent were added. The sample was then observed for colour change.

2.6.1.1 Test for saponin

The persistent frothing test for saponin was used. Distilled water (30mL) was added to 1g of each of the plant extracts. The mixture was vigorously shaken and heated on a steam water bath. The sample was thereafter observed for the formation of froth.

2.6.1.2 Test for phlobatannin

The leaves extracts (0.2 g) were dissolved in 10 mL distilled water each and filtered. The filtrates were boiled with 2% HCl solution and observed for deposition of red precipitate which indicates the presence of phlobatannin.

2.6.1.3 Test for tannin

The method of [24] was adopted. Each the extracts (0.5 g) were dissolved in 5 mL distilled water, boiled gently and cooled. One mL of each solution was dispensed in test tubes and 3 drops of 0.1% ferric chloride solution were added. The preparation was thereafter observed for brownish green or blue black colouration.

2.6.1.4 Test for terpenoids

Five mL of each leaves' extracts was mixed in 2 mL of chloroform, and 3mL concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. Each solution was then observed for reddish brown colouration which confirms the presence of terpenoids.

2.6.1.5 Test for steroid

Acetic anhydride (2 mL) was added to 0.5 g of each extracts and filtered. Sulphuric acid (2 mL) was added to the filtrate. A colour change from violet to blue or green indicates the presence of steroid.

2.6.1.6 Test for flavonoids

Diluted ammonia solution (5 mL) was added to portions of aqueous filtrate of each plant extracts. This was then followed by the addition of concentrated sulphuric acid. Yellow colouration that disappears on standing indicates the presence of flavonoids.

2.6.1.7 Test for anthraquinone

Borntrager's test was used for the detection of anthraquinone. The extract (0.5 g) was shaken with 10 mL of benzene. The mixture was filtered, 5 mL of 10% ammonia solution was added to the filtrate and thereafter shaken. The presence of pink red or violet colour in the ammonia layer of the preparation indicates the presence of free anthraquinone.

2.6.1.8 Test for cardiac glycosides

The following tests were carried out to screen for the presence of cardiac glycosides in each of the extract.

2.6.1.9 Legal's test

Each extract was dissolved in pyridine and a few drops of 2% sodium nitroprusside and few drops of 20% NaOH were added. A deep red coloration which fades to a brownish yellow indicates the presence of cardenolides.

2.6.1.10 Salkowski's test

Each extract was mixed with 20 mL of chloroform and filtered. This was followed by the addition of 3mL of concentrated H_2SO_4 to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicates the presence of steroidal ring.

2.6.1.11 Keller- Killiani's test

Each of the extracts (0.5 g) was dissolved in 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was then under laid with 1 mL of concentrated H_2SO_4. It was observed for a brown colouration at the interface indicating the presence of a deoxy sugar which is characteristic of cardenolides. It was also observed for violet ring which may appear below the brown ring while in the acetic acid layer. The presence of a green ring formed just above the brown ring which can gradually spread throughout this layer, also indicates the presence of cardiac glycosides.

2.6.1.12 Lieberman's test

About 20 mL of acetic anhydride was added to 0.5 g of the extract and filtered. This was then followed by the addition of 2 mL of concentrated H_2SO_4 to the filtrate. There was a colour change from violet to blue or green which indicate the presence of steroids nucleous. (i.e. a glycone portion of the cardiac glycosides).

2.6.2 Quantitative phytochemical screening of leaves extracts of *Persea americana*

2.6.2.1 Tannin determination

This was done according to the method of Association of Official Analytical Chemists [26] (1990), with some modifications. A 0.20 g of the sample was added to 20 mL of 50% methanol. This was shaken thoroughly and placed in a water bath at 80℃ for 1 hour to ensure uniform mixing. The extract was filtered into a 100mL volumetric flask, followed by the addition of 20mL of distilled water, 2.5 mL of Folin-Denis reagent and 10 mL of 17% aq. Na_2CO_3 (Sodium carbonate). The mixture was made up to 100 mL with distilled water, mixed and allowed to stand for 20 minutes. A bluish-green colour developed at the end of the reaction mixture of different concentrations ranging from 0 to 10 ppm. The absorbance of the tannic acid standard solutions as well as sample was measured after colour development at 760 nm using the spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). Results were expressed as mg/g of tannic acid equivalent using the calibration curve: $Y = 0.0593x - 0.0485$, $R = 0.9826$, where x is the absorbance and Y is the tannic acid equivalent.

2.6.2.2 Saponin determination

Quantitative determination of saponin was done using the method of [27]. The powdered sample (20 g) was added to 100 mL of 20% aqueous ethanol and kept in a shaker for 30 minutes. The samples were heated over a water bath for 4 hours at 55℃. The mixture was then filtered and the residue re-extracted with another 200 mL of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 mL over the water bath at 90℃. The concentrate was transferred into a 250 mL separating funnel and extracted twice with 20 mL diethyl ether. The

ether layer was discarded while 60 mL n-butanol was added to the retained aqueous layer. The n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride and the remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven at 40℃ to a constant weight. The saponin content was calculated using the formula:

$$\text{Saponin } (\%) = \frac{\text{final weight of sample}}{\text{initial weight of extracts}} \text{x} 100$$

2.6.2.3 Alkaloids determination

Alkaloids were quantitatively determined according to the method of Harborne [25]. Two hundred milliliters of 10% acetic acid in ethanol was added to 5 g powdered extract, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide was added drop wisely to the extract until the precipitation was completed and the whole solution was allowed to settle. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content was determined using the formula:

$$\text{Alkaloid } (\%) = \frac{\text{final weight of sample}}{\text{initial weight of extracts}} \text{ x } 100.$$

2.6.2.4 Steroid determination

Steroid content of the plant sample was determined using the method described by [24]. A portion of 2 mL was taken from a solution of 2.5 g of powdered plant material prepared in 50 mL of distilled water after vigorous shaking for 1 hour. The extract solution was washed with 3 mL of 0.1M NaOH (pH 9) and later mixed with 2 mL of chloroform and 3 mL of ice cold acetic anhydride followed by the cautious addition of two drops of concentrated H_2SO_4. The absorbance of both sample and blank were measured using a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215) at 420 nm.

2.6.2.5 Cardiac glycosides determination

Cardiac glycoside content in the samples was evaluated using Buljet's reagent as described by El-Olemy et al*.* [28]. The samples were purified using lead acetate and Disodium hydrogen phosphate (Na_2HPO_4) solution before the addition of freshly prepared Buljet's reagent (containing 95 mL aqueous picric acid + 5 mL 10% aqueous sodium hydroxide [NaOH]).

2.6.2.6 Determination of total flavonoid content

The total flavonoid content of the extracts was determined using a colorimeter assay developed by [29]. The extract (0.2 mL) was added to 0.3 mL of 5% sodium nitrate ($NaNO_3$) at zero time. After 5min, 0.6 mL of 10% aluminium chloride ($AlCl_3$) was added, and after 6 min, 2 mL of 1M NaOH (sodium hydroxide) was added to the mixture followed by the addition of 2.1mL of distilled water. Absorbance was read using a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215) at 510 nm against the reagent blank, and the calibration curve was prepared by using rutin methanolic solutions at concentrations of 12.5 to 100 µg ml^{-1}. Total flavonoid was expressed as mg rutin equivalents per gram of dried extract (mg RE g^{-1}).

2.6.2.7 Total phenolic content determination

The total phenolic content of the extracts was determined by the method of Singleton et al. [30] About 0.2 mL of each of the extracts was mixed with 2.5 mL of 10% Folin ciocalteau's reagent and 2 mL of 7.5% sodium carbonate (Na_2CO_3). The reaction mixture was incubated at 45^{o}C for 40 minutes, and the absorbance was measured at 700 nm in the spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). A calibration curve was plotted for the standard of gallic acid. Total phenolic content was expressed as mg gallic acid equivalents per gram of dried extract (mg GAE g^{-1}) using the linear equation obtained from standard gallic acid calibration curve.

3. RESULTS

The extracts recovered using both solvents were found to be rich in various phytochemicals such as alkaloids, saponins, tannins, flavonoids and cardiac glycosides. The result is depicted by Table 1. Acetone extracts of the *P. americana* leaves had the highest inhibitory effect on *S. typhi* (27.47± 0.23 mm) and the least (10.27±0.15 mm) on *Shigella dysenteriae*. As for the methanol extract, *Staphylococcus aureus* ATCC 43300 had the highest inhibition zone (34.20±0.12 mm) while *Bacillus subtilis* had the least (12.33± 0.18 mm) Table 2 gives a representation of these. The antimicrobial activity

of the bacterial isolates when challenged with the commercial antibiotics is shown in Table 3. Ciprofloxacin exerted the highest inhibitory effect (14.10±0.12 mm) on *S. aureus* ATCC 43300 and the least (11.20±0.12 mm) on *Escherichia coli* ATCC 35218. Rocephin had the highest inhibitory effect (49.97±36.60 mm) on the clinical *E. coli* isolate and the least (12.50±0.12) on *S. aureus*. Highest zones of inhibition were obtained with ciprofloxacin, pefloxacin, and erythromycin for *S. aureus* ATCC 43300, *Escherichia coli* ATCC 35218 and the clinical *S. aureus* isolate respectively. For the antifungal activity of the extracts, at a concentration 50 mg/mL, *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* had a higher inhibition zone with acetone extract than they did with the methanol extract. The isolates however had a comparatively wider inhibition zone when challenged with commercial antifungal drugs. The result is depicted by Table 4. The minimum inhibitory concentration of the extracts ranged from 0.391 to 1.562 as shown in Table 5.

3.1 Minimum Inhibitory Concentration (MIC) of *P. americana*

The minimum inhibitory concentration (MIC) for all the leaves extracts ranged between 0.391-1.562 mg/mL.

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

Phytochemicals	PAM	PAA
Saponin	+	+
Tannin	+	+
Alkaloid	+	+
Flavonoid	+	+
Steroids	-	-
Terpenoids	+	+
Phlobatannin	-	-
Anthraquinone	-	-
Cardiac glycosides		
Legal test	+	+
Keller kiliani	+	+
Salkowski	+	+
Liberman test	+	+

Keys: PAA: Acetone leaf extract of Persea americana; PAM: Methanol leaf extract of Persea americana

Table 2. Antibacterial activity of leaves extracts of *Persea americana*

Test organism	PAA	PAM
Salmonella typhi (ATCC 33489)	15.43 ± 0.15^{b}	20.60 ± 0.12^{c}
Salmonella typhi	27.47 ± 0.23^{c}	28.27 ± 0.12^{d}
Staphylococcus aureus (ATCC 43300)	19.40 ± 0.12^{a}	32.40 ± 0.26^{d}
Staphylococcus aureus	26.43 ± 0.18^{a}	34.20 ± 0.12^{d}
Escherichia coli (ATCC 35218)	15.47 ± 0.15^{a}	23.57 ± 0.24^{c}
Escherichia coli	23.47 ± 0.24^{c}	24.43 ± 0.15^{d}
Pseudomonas aeruginosa (ATCC 27853)	17.50 ± 0.17^{c}	18.47 ± 0.20^{d}
Shigella dysenteriae	10.27 ± 0.15^{a}	17.43 ± 0.26^{c}
Bacillus cereus	21.63 ± 0.18^{c}	25.50 ± 0.21^{d}
Bacillus subtilis	14.43 ± 0.18^{b}	12.33 ± 0.18^{a}

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a row are significantly different at (p < 0.05).

Keys: FEA: PAA: Acetone leaf extract of Persea americana; PAM: Methanol leaf extract of Persea americana

Table 3. Antibacterial activity of commercial antibiotics

Test organism	Zone of inhibition (mm)				
	CPX	R	CN	PEF	E
Salmonella typhi (ATCC 33489)	12.27 ± 0.15^{a}	14.30 ± 0.12^{c}	13.60 ± 0.17^{b}	15.40 ± 0.12^{d}	14.43 ± 0.20^{c}
Salmonella typhi	12.40 ± 0.23^{a}	14.37 ± 0.20^{c}	14.53 ± 0.23^{c}	13.50 ± 0.17^{b}	14.33 ± 0.15^{c}
Staphylococcus aureus (ATCC 43300)	14.40 ± 0.12^{b}	15.50 ± 0.17^{c}	15.33 ± 0.15^{c}	13.30 ± 0.12^{a}	16.47 ± 0.15^{d}
Staphylococcus aureus	15.43 ± 0.20^{d}	12.50 ± 0.12^{c}	11.30 ± 0.17^{b}	10.57 ± 0.18^{a}	15.30 ± 0.12^{d}
Escherichia coli (ATCC 35218)	11.20 ± 0.12^{b}	14.27 ± 0.15^{c}	0.00 ± 0.00^{a}	15.40 ± 0.17^{d}	14.40 ± 0.12^{c}
Escherichia coli	12.50 ± 0.23^{a}	49.97 ± 36.67^{a}	0.00 ± 0.00^{a}	14.30 ± 0.17^{a}	14.47 ± 0.20^{a}
Pseudomonas aeruginosa (ATCC 27853)	15.43 ± 0.15^{c}	16.33 ± 0.18^{d}	6.43 ± 0.20^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Shigella dysenteriae	14.40 ± 0.12^{b}	14.27 ± 0.15^{b}	18.50 ± 0.12^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Bacillus cereus	12.33 ± 0.18^{b}	14.53 ± 0.20^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Bacillus subtilis	14.33 ± 0.15^{d}	15.50 ± 0.12^{e}	12.40 ± 0.17^{b}	11.57 ± 0.15^{a}	13.27 ± 0.15^{c}

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a row are significantly different at (p < 0.05).

Keys: CPX: Ciprofloxacin (10 ug); R: Rocephin (25 ug); CN: Gentamicin (10 ug); PEF: Pefloxacin (10 ug); E: Erythromycin (10 ug)

Table 4. Antifungal activity of leaves extracts of *Persea americana* (50 mg/mL) and commercial antifungal drugs (1 mg/mL)

Test organism	PAA	PAM	CLOT	GRIS	NYST
Candida albicans	12.33 ± 0.18^{d}	7.40 ± 0.12^{b}	16.65 ± 0.68^{e}	20.50 ± 0.29^{g}	6.40 ± 0.21^{a}
Aspergillus niger	7.20 ± 0.12^{c}	5.47 ± 0.18^{b}	22.33 ± 0.33^{f}	21.67 ± 0.33^{f}	17.47 ± 0.32^{e}
Aspergillus flavus	12.60 ± 0.12^{d}	10.63 ± 0.15^{c}	25.00 ± 0.15^{f}	9.77 ± 0.15^{b}	18.73 ± 0.22^{e}
Aspergillus fumigatus	10.63 ± 0.15^{c}	5.47 ± 0.20^{a}	35.67 ± 0.44^{f}	9.33 ± 0.44^{b}	20.57 ± 0.30^{e}

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a row are significantly different at ($p < 0.05$).

Keys: PAA: Acetone leaf extract of Persea americana; PAM: Methanol leaf extract of Persea americana CLOT: Cotrimazole; GRIS: Griseofulvin; NYST: Nystatin

Table 5. Minimum inhibitory concentration (mg/mL) of leaves extracts of *Persea americana*

Test organism	PAA	PAM
Salmonella typhi (ATC 33489)	1.563	0.391
Salmonella typhi	0.391	0.391
Staphylococcus aureus (ATCC 43300)	0.391	0.781
Staphylococcus aureus	0.781	0.391
Escherichia coli (ATCC 35218)	0.781	0.391
Escherichia coli	0.391	0.781
Pseudomonas aeruginosa (ATCC 27853)	1.563	0.391
Shigella dysenteriae	0.781	1.562
Bacillus cereus	0.391	0.391
Bacillus subtilis	1.562	1.562

Keys- PAA: Acetone leaf extract of Persea americana; PAM: Methanol leaf extract of Persea americana

4. DISCUSSION

The quest for plants with medicinal properties continues to receive global attention [31]. This study investigated the phytochemical components alongside the antimicrobial properties of *Persea americana* with a view establishing the scientific basis of its traditional medicinal uses.

Results from the phytochemical screening revealed the presence of secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, saponins and cardiac glycosides and the absence of steroids, phlobatannin and anthraquinone. The phytoconstituents obtained in this study are in consonance with those obtained by [32] and [33] in their respective studies. These secondary metabolites have been reported to be of pharmacological importance [34,35] and [36]. For instance, Alkaloid-containing plants have been used by humans since ancient times for therapeutic and recreational purposes [36]. Alkaloids can act as antimalarial, anticancer, antiasthma and antibacterial pharmacological constituents in humans. Most of the known functions of alkaloids are related to protection. In addition, the presence of alkaloids in plants prevents insects and chordate animals from eating them [37].

Cardiac glycosides have been adapted for the treatment of congestive heart failure and cardiac arrhythmia*. In vitro*, tannins have been reported to show antiviral, antibacterial and antiparasitic effects, they have also been reported to hasten the healing of wounds and inflamed mucus membranes [38,39]. Saponins have been reported to be widely used in the pharmaceutical industry as adjuvants to enhance absorption of other drugs by increasing their solubility or by interfering in the mechanisms of absorption [40]. Their use as raw material for the synthesis of steroidal drugs has also been documented [41].

Terpenoids are known to exhibit anti-inflammatory, anticancer and antimalarial activities [42,43]. Tannins on the other hand have been used to combat diarrhoea [44]. The antioxidant properties of *P. americana* is enhanced by the presence of tannins [45] while saponins have documented use as dietary supplements and nutraceuticals [46]. In addition to lowering blood cholesterol level, saponins have proven amphipathic properties which aid the penetration of proteins through the cell membranes. Glycosides which are also an essential phytoconstituent of *P. americana* are renowned for their antibiotic properties and have been reported to have wide range of pharmacological activities including antimalarial, antiasthma, anticancer, vasodilatory, antiarrhythmic, and analgesic effects [47]. The therapeutic potentials of *P. americana* based on its rich phytochemical composition can thus not be overlooked [48].

The activities of leaf extracts of *Persea americana* against some pathogenic organisms have been previously investigated [49]. The results from this study showed that the extracts displayed varying and appreciable antimicrobial activities. The antimicrobial activities of these extracts may be attributed to the presence of

bioactive such as phenols, flavonoids, tannins, and terpenoids [50].

The anti-inflammatory [51], antifungal [52] and antibacterial activities [53] of *P. americana* leaves have been previously reported.

There were variations in the reaction of Gram-negative and Gram-positive bacteria to the extracts of *P. americana*. The differences in their variation are mainly due to their cell wall structure. The cell wall of Gram-positive bacteria consists of a single layer while that of Gram-negative consists of a multi-layered structure bounded by an outer cell membrane [54]. Various reports have shown that bacteria are more sensitive to antimicrobials than fungi and this could be as a result of the difference in their cell wall transparency [55]. The observed comparatively higher potency of the plant extracts when compared with the commercial drugs activities could be suggestive of a better effect when both are used synergistically [56]. The appreciable antimicrobial activity of the extract as observed in this study justifies its ethnobotanical uses for the treatment of various microbial infections. Demonstration of low Minimum Inhibitory Concentration value by the methanol extract is also indicative of the therapeutic potentials of the phytochemicals.

5. CONCLUSION

This work has demonstrated that leaves extract of *Persea americana* exhibits considerable antimicrobial activities. The methanol and the acetone extracts of the plants extracts showed antimicrobial potentials which justify its ethnobotanical uses in the treatment of diseases caused by pathogenic organisms such as *Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* and *Bacillus cereus*.

The results from this study suggest that these plants could be promising candidates for drugs development and thus validate its tribal claims as a cure for some human ailments. Further pre-clinical and clinical studies are required to establish the usefulness of this extract in the treatment of human ailments. Also, further work is aimed at the isolation and characterization of the bioactive present in the extracts, along with their mechanisms of action.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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