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Evaluation of Antimicrobial Activity, Phytochemical Screening and Gas Chromatography-Mass/ Spectrophotometric Profile of Essential Oil from *Persea americana & Citrus sinensis*

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Authors' contributions

This work was carried out in collaboration among all authors. Author OTO is the leading author, who designed, analyzed, interpreted and prepared the manuscript for publication. Author OTO is a researcher who researched the antimicrobial and phytochemical properties of various medicinal plants in Nigerian and Africa. Authors POU, AMYB and OOI helped during the phytochemical procedure and the chemical analysis of the plant extracts. All authors read and approved the final manuscript.

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ABSTRACT

This study aims to determine the phytochemical characteristics, structural determination of bioactive component from the leaf extract using GCMs and experimentally investigation of the antibacterial activity of essential oil from *Persea americana* and *Citrus sinensis* leaf against seven bacteria (Gram-positive and Gram-negative) which are *Staphylococcus aureus*, *Bacillus subtilis, Salmonella typhi, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis* and *Pseudomonas*

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aeruginosa known to be pathogenic to human causing high mortality among human. This study involves the extraction of essential oils from air- dried leaves of medicinal aromatic plant (*Persea americana* & *Citrus sinensis*) using soxhlet method with n- hexane as solvent. Antibacterial activity was carried out using agar well diffusion technique. Both plants essential oil shows high antibacterial property. The antibacterial sensitivity test revealed *Escherichia coli* having the widest zone of inhibition (20.0 mm) with *Citrus sinensis* essential oil at 100 mg/ml while the least zone of inhibition (5.0 mm) was recorded for *Staphylococcus aureus* at 12.5 mg/ml with essential oil of *Persea americana* however, *Pseudomonas aeruginosa* was found to be resistant to the oil. Phytochemical analysis of the plants shows the presence of active components such as Cardiac glycoside, Steroid, Anthraquinone, Flavonoid, Phenol, Alkaloid and reducing sugars. The presence of these components enhances the effectiveness of plants essential oil in the treatment of various diseases and also helps to act as an antibacterial agent. *Persea americana* essential oil was further analyzed by gas chromatography–mass spectroscopy (GC/MS), the main constituents were Squalene (45.94%), vitamin E (12.50%), Diisooctyl phthalate (7.45%) and 12-Methyl-E,E-2,13-octadecadien-1-ol (7.20%). These components aid the antibacterial activities of *Persea americana* essential oil.

Keywords: Persea americana; Citrus sinensis essential oils; antibacterial and phytochemical activity.

1. INTRODUCTION

Essential oils derived from aromatic medicinal plants have been reported to exhibit exceptionally good antibacterial or antimicrobial effects against bacteria, yeasts, filamentous fungi, and viruses. The progress of this expanding scientific field will be documented by the most important results published in the last decade. The abundant use of anti-infective agents resulted in the emergence of drug-resistant bacteria, fungi, and viruses. To overcome the increasing resistance of pathogenic bacteria, a variety of medicinal plants have been screened worldwide for their antibacterial/antimicrobial properties [1].

Despite exceptional medical advances in the development of antibiotics, bacterial infections remain an important healthcare concern due to the emergence of in-creasing bacterial resistance and the corresponding increases in healthcare costs and mortality rates. In recent years, considerable efforts have been made to control the spread of pathogens with various strategies, including the use of alternative antibacterial/antimicrobial compounds [2,3]. Essential oils (EOs) are natural products obtained from plants with proven antibacterial or antimicrobial properties against a wide range of microorganisms [4,5,6].

It is complex mixtures which are constituted by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. Due to these properties, EOs has traditionally been used to protect food against microbial deterioration [7-9].

Essential oils such as aniseed, calamus, camphor, cedarwood, cinnamon, citronella, clove, eucalyptus, geranium, lavender, lemon, lemongrass, lime, mint, nutmeg, orange, palmarosa, rosemary, basil, vetiver and wintergreen have been traditionally used by people for various purposes in different parts of the world. Cinnamon, clove and rosemary oils had shown antibacterial and antifungal activity [10]. Cinnamon oil also possesses antidiabetic property [11].

The avocado (*Persea americana*) is a tree that is native to South Central Mexico and central America [12]. Avocado pl**a**nt has been produced for many years and used mainly as a cosmetic product and raw material due to its nutrient rich unsaponifiable fraction. The recent development in technology however has led to oil that is highly suitable for the consumer market in terms of quality and health benefits. Virgin oil contains all the healthy components of avocado like β-sitosterol, α-tocopherol, lutein and chlorophyll which are well known for their anti -cholesterol and antioxidant effects. Refined oil produces oil that is more stable but is stripped of all its healthy phenolic compounds unlike avocado oil [13].

Citrus sinensis, Sweet orange is a strum belonging to the plant family Ruracea with a botanical name *citrus sinensis*. They are berries but because of their unusual structure, they are called (fresperidiums). Sweet oranges originate in southern China thousands of years ago. Now they are most popular and wide spread of the citrus fruits. *Citrus sinensis* (sweet oranges) can be grown is most parts of the tropics where there

is fairly even distribution of rainfall throughout the year. The trees can be grown from seed but it's more usual to buy budded *Citrus sinensis* from private nursery men or form official Agricultural sources. *Citrus sinensis* is a spreading ever green, sometime spiny trees up to 12 m fall with ovule elliptic leaves which are commonly 7-10 cm long dark green and routed at the base. They are carried on short articulated petioles with very narrow wins [14].

Fig. 1. Showing diagram of Avocado leaves *(Persea americana)*

The leaf is strongly scented, the white sweet smelling flowers are smaller than those of the group. The rounded fruits are up to 12cm in diameter. Deep yellow to orange or in humid climate remaining green when ripe. Sweet oranges (*Citrus sinensis*) are tropical crops. In a typical sweet orange, the excerpt and mesa carp are leathery and protect the juicy inner tissue deceived from the endocarp from damage and desiccation. The epidermis of the fruit has a thick cuticle and varying number of stomata, the excerpt or flavedo is a layer of irregular photosynthentically active parenchyma cells which is green in young fruit. The mesocarp is thicker than the exocarp and consists of inter cellular space. The mesocarp is known as the albedo. It is rich in vitamin C, sugar, cellulose and in pectin [14].

Citrus sinensis oil serves as a detoxifier, boost immunity, treats constipation and dyspepsia, provides relief from inflammation, relaxes muscular and nervous spasms, inhibits microbial growth and disinfects wounds, It cures acne and dermatitis, Promotes urination and eliminate toxins such as uric acid, salt and bile [15].

This study aims to determine the phytochemical characteristics, structural identification of bioactive component from the leaf extract using GC/MS and experimentally investigation of the antibacterial activity of oil essential from *Persea americana* and *Citrus sinensis* leaf.

Fig. 2. Showing diagram of orange fruit *(Citrus sinensis)*

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plants

The plants were collected from Akungba environs. Oja oba market,Ikare Akoko, Ondo State, a tropical rainforest of Ondo State, Nigeria with latitude (7.21692 North) and longitutide (5.21561 East) The leaves of the plants were collected on 20th of October 2016 during the raining period, and air-dried for 7 days in preparation for extraction after been identified by a taxonomist of plant science and biotechnology of Adekunle Ajasin University Akungba Akoko, Ondo state.

2.2 Extraction of Sample

Using the soxhlet method of extraction, air dried and grounded leaves of both *Persea americana* and *Citrus sinensis* were packed into a small bag weighing about 40 g of each samples and placed into the thimble of Soxhlet apparatus. About 250 ml of solvent (n–hexane) was placed in the round-bottom flask subjected to minimum heat using heating mantle for 3 hours. The resultant mixture of solvent and essential oil was passed through a lie big condenser cooled by a continuous flow of fresh water. The oil was then

separated using rotary evaporator and decanted into sample bottles. The procedure was repeated until a sufficient amount of oil for analysis and antibacterial test was obtained. The dried oil was weighed and the percentage yields calculated.

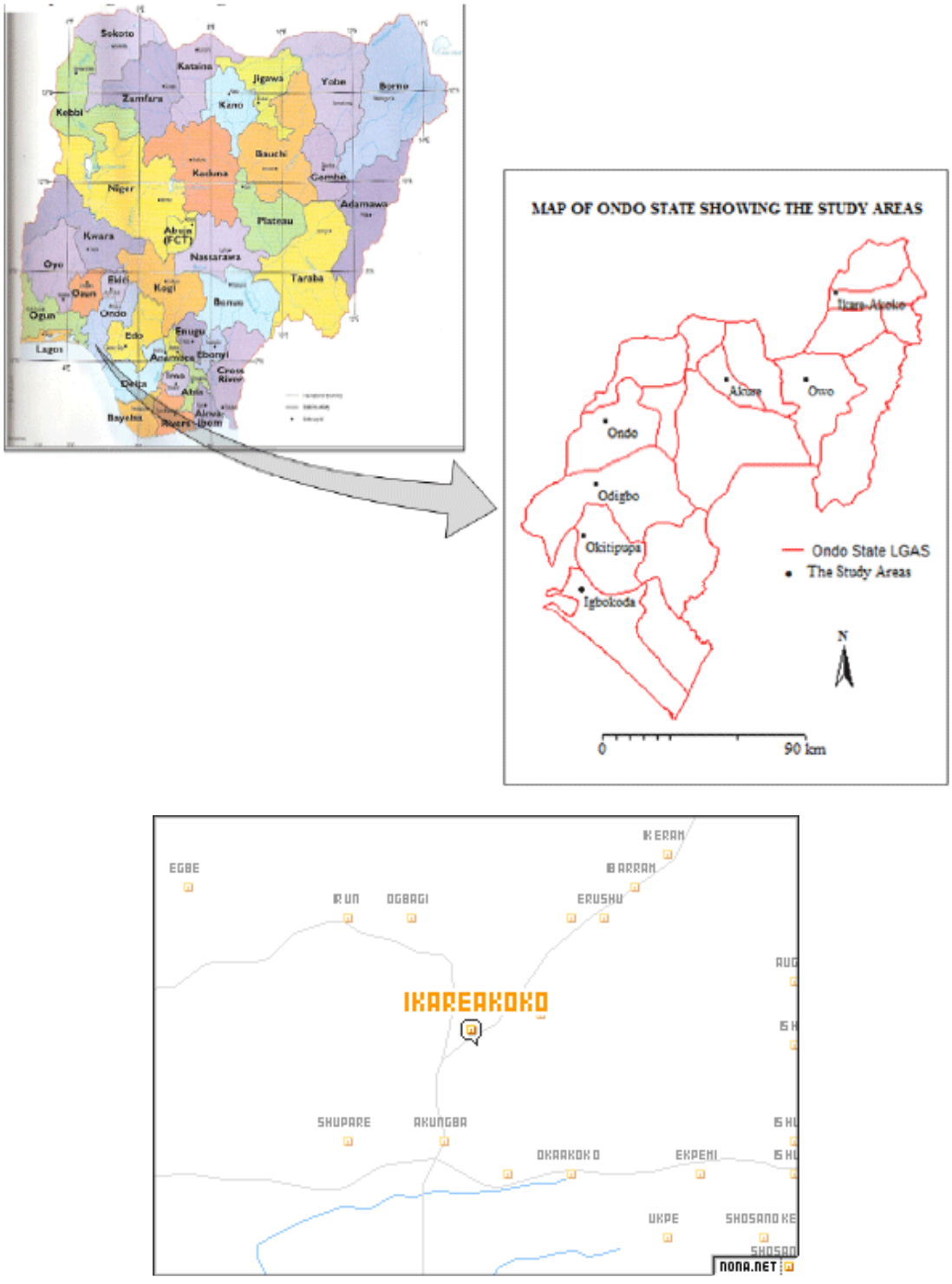


Fig. 3. Map of Nigeria indicating Ikare Akoko, Ondo

2.2.1 Microorganisms used for the research work

The organisms used for the research work are as follow**s** *E.coli, S. typhi, K. pneumoniae, P. mirabilis, P. aeruginosa, S. aureus, Bacilus spp*. All organisms are from clinical source.

2.2.2 Antibacterial assay

Antibacterial activity of the essential oils was tested using agar well diffusion method [16].The Gram-negative bacteria used were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosae, Proteus mirabilis and Salmonella typhi* while Gram–positive bacteria were *Staphylococcus aureus and Bacillus subtilis*. The test organisms were inoculated into nutrient broth and incubated for 4-6 hours at 37℃. To standardize the microbial inoculums for the susceptibility test, a Barium sulphate standard equivalent to McFarland No. 0.5 standards or its optical equivalent was used (Mc Farland No. 0.5 standard gives cell density of 1.5x108/ ml). Antibacterial activity assay was done on Mueller Hinton agar. The media were reconstituted using distilled water and sterilized by autoclaving at 121℃ for 15 minutes then dispensed into Petri dishes aseptically and left to solidify and then stored in the refrigerator at 4℃. The freshly grown microbial cultures were inoculated on solid media. The blank sensitivity discs were divided into three Bijoux bottles and sterilized in the oven by air-drying at 160℃ for 1 h. Test oils (1 ml) was impregnated into sterile blank disc and placed aseptically into the inoculated Petri dish. All these procedures were done in duplicate. The individual Petri dishes were covered to avoid any possible evaporation or contamination. Chloramphenicol was used as standard controls. The inoculated plates were incubated at 37℃ for 24hr before the activity was determined. The activity of the test oils was established by the presence of measurable zones of inhibition (mm). The essential oil was tested for anti-bacterial activity [17].

2.3 Characterization of Sample

2.3.1 Phytochemical analysis

2.3.1.1 Qualitative method of Phytochemical analysis of Persea americana and Citrus sinensis essential oil

2.3.1.1.1 Test for reducing sugars

One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; A brown colour with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars.

2.3.1.1.2 Test for Alkaloid (TLC method)

Solvent system: Chloroform: methanol: 25% ammonia (8:2:0.5). Spots can be detected after spraying with Dragendorff reagent Orange spot shows is a positive result [18,19].

2.3.1.1.3 Test for anthraquinone (Borntrager's test)

Heat about 50 mg of extract with 1ml 10% ferric chloride solution and 1ml of concentrated hydrochloric acid. Cool the extract and filter. Shake the filtrate with equal amount of diethyl ether. Further extract the ether extract with strong ammonia. Pink or deep red coloration of aqueous [20-22].

2.3.1.1.4 Test for cardiac glycosides (TLC method)

Extract the powdered test samples with 70% EtOH on rotary shaker (180 thaws/min) for 10 hr. Add 70% lead acetate to the filtrate and centrifuge at 5000 rpm/10 min. Further centrifuge the supernatant by adding 6.3% Na_2CO_3 at 10000 rpm/10 min. Dry the retained supernatant and re-dissolved in chloroform and use for chromatography. Separate the glycosides using EtOAc-MeOH-H_2O (80:10:10) solvent mixture. The color and hRf values of these spots can be recorded under ultraviolet (UV 254 nm) light. [22].

2.3.1.1.5 Test for Flavonoid (TLC method)

Extract 1g powdered test samples with 10 ml methanol on water bath (60℃/ 5 min). Condense the filtrate by evaporation, and add a mixture of water and EtOAc (10:1 mL), and mix thoroughly. Retain the EtOAc phase and use for chromatography. Separate the flavonoid spots using chloroform and methanol (19:1) solvent mixture.The color and hRf values of these spots can be recorded under ultraviolet (UV 254 nm) light. [19,20].

2.3.1.1.6 Test for Phenol

Phenol test Spot the extract on a filter paper. Add a drop of phosphomolybdic acid reagent and expose to ammonia vapors. Blue coloration of the spot, shows is a positive result [20].

2.3.1.1.7 Test for Saponin (TLC method)

Extract two grams of powdered test samples with 10 ml 70% EtOH by refluxing for 10 min. condense the filtrate, enrich with saturated n-BuOH, and mix thoroughly. Retain the butanol, condense and use for chromatography. Separate the saponins using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. Expose the chromatogram to the iodine vapors. The colour (yellow) and hRf values of these spots were recorded by exposing chromatogram to the iodine vapour [19,23].

2.3.1.1.8 Test for Steroid (TLC method)

Extract two grams of powdered test samples with 10 ml methanol in water bath (80℃/15 min). Use the condensed filtrate for chromatography. The sterols can be separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The color and hRf values of these spots can be recorded under visible light after spraying the plates with anisaldehyde- sulphuric acid reagent and heating (100℃/6 min). The color (Greenish black to Pinkish black) and hRf values of these spots can be recorded under visible light [19].

2.3.1.1.9 Test for Tannin (Braemer's test)

10% alcoholic ferric chloride will be added to 2-3ml of methanolic extract (1:1) Dark blue or greenish grey coloration of the solution [20,23]

2.3.1.2 Quantitative methods of phytochemical analysis of Persea americana and Citrus sinensis essential oil

2.3.1.2.1 Test for Saponin

About 20 grams each of dried plant samples were ground and, put into a conical flask after which 100 ml of 20% aqueous ethanol were added. The mixtures were heated using a hot water bath. At about 55℃, for 4 hour with continuous stirring, after which the mixture were filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90℃. The concentrate was transferred into a 250 ml separatory funnel and 20 rnl of diethyl ether were added and then shaken vigorously. The aqueous layer were recovered while the ether layer was discarded. The purification process was repeated three times. 60 rnl of n-butanol were added. The combined n-butanol extracts were washed twice with 10 m1 of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material [24].

2.3.1.2.2 Test for Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrate were later transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weigh [25].

2.3.1.2.3 Test for Tannins

About 500 mg of the plant sample were weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M FeCl in 0.1 M Hcl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract [25].

2.3.1.2.4 Test for Alkaloids

Five grams of the plant sample were weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was then be added, the reaction mixture were covered and allowed to stand for 4 hour. This was filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution were allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass [26].

2.3.1.2.5 Total Phenol (Spectrophotometric methods)

2 g each of the samples were defatted with 1ml of diethyl ether using a soxhlet apparatus for 2 hours. The fat free samples were boiled with 50 ml of ether for the extraction of the phenolic components for 15 minutes. 5 ml of the extracts

were pipetted into 5 mL flask and then 10 ml distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes. For color development. This was measured at 505 nm [26,27].

2.3.2 GC-MS analysis of *Persea americana* leaves and *Citrus sinensis* essential oil

Persea americana oil was analysed using GC/MS (Shimadzu capillary GC-quadrupole MS system QP 5000) with two fused silica capillary column DB-5 (30 µm, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) which was operated in EI mode at 70 eV. Injector and detector temperatures were set at 220℃ and 250℃, respectively. One micro-liter essential oil solution in hexane was injected and analyzed with the column held initially at 60℃ for 2 min and then increased by 3℃/min up to 300℃. Helium was employed as carrier gas (1 ml/min). The relative amount of individual components of the total oil is expressed as percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds, or by retention indices (RI) and mass spectra [27,28].

3. RESULTS

Table 1 shows the Antibacterial activity of *Persea americana essential oil* against selected clinical organisms. The zone of inhibition of test organisms against essential oil extract from *Persea americana* leaf (avocado) expressed in mm with *Escherichia coli* showing the widest zone of inhibition of (18.0 mm) while the least zone of inhibition (5.0 mm) was recorded for *Staph aureus.* The essential oil from *Persea americana* act as a strong antibacterial agent against *Staphylococcus aureus, Escherichia coli, Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Bacillus subtilis in-vitro.* The essential oil extract from *Persea americana* shows activity against both Gram+ positive and Gram-negative bacteria. On the average, antibacterial activity of essential oil was more pronounced on Gram positive bacteria (mean zone of inhibition: 14.0 mm) than Gram negative (12.0 mm).

Table 2 shows the Antibacterial activity of *Citrus sinensis essential oil* against selected clinical organisms .The antibacterial properties were found to be active against the Gram-positive and two Gram-negative bacteria. *Salmonella typhi a*nd *Escherichia coli as* shown by the inhibition zones in the Table 2. The microbe, *Pseudomonas aeruginosae* was resistant to the oil since no inhibition zones were observed*.* Among the Gram-negative bacteria, the oil was very active against *Escherichia coli*. . On the average, antibacterial activity of essential oil was more pronounced on Gram positive bacteria (mean zone of inhibition: 13.0 mm) than Gram negative (11.0 mm).

Tables 3-6, Qualitative Phytochemical analysis of *Persea americana* reveals the presence of alkaloids, cardiac glycoside, tannins and saponins using the four solvents while anthraquinone was not detected using methanol as solvent but was found using ethyl acetatae, dichloromethane and n-hexane. Steroid and flavonoid, phenol and reducing sugars were

Table 1. Antibacterial activity of *Persea americana essential oil* against selected clinical organisms

Microorganism	Essential oil concentration				Control
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Chloramphenical 30 µg
E. coli	18.0	15.0	12.0	8.0	30.0
S. typhi	12.0	8.0	2.0	1.0	25.0
K. pneumoniae	10.0	6.0	3.0	0.0	11.0
P. mirabilis	12.0	9.0	6.0	0.0	8.0
P. aeruginosa	9.0	5.0	2.0	0.0	12.0
S. aureus	15.0	11.0	8.0	5.0	26.0
Bacilus subtilis	13.0	9.0	7.0	1.0	20.0

Note: ND means not detected, (-) indicates negative while (+) indicates positive, Inhibition (mm)

Table 2. Antibacterial activity of *Citrus sinensis essential oil* against selected clinical organisms

Microorganism	Essential oil concentration				Control
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Chloramphenical 30 µg
E. coli	20.0	15.0	10.0	7.0	30.0
S. typhi	17.0	14.0	9.0	3.0	25.0
K. pneumoniae	10.0	7.0	2.0	1.0	15.0
P. mirabilis	8.0	4.0	3.0	0.0	11.0
P. aeruginosa	0.0	0.0	0.0	00.	10.0
S. aureus	15.0	12.0	8.0	3.0	20.0
Bacillus subtilis	11.0	8.0	5.0	2.0	15.0

Note: ND means not detected, (-) indicates negative while (+) indicates positive, Inhibition (mm)

negative in ethyl acetate extract, dichloromethane extract and methanol extract respectively.

Plate 1. Antibacterial activity of *Citrus sinensis* essential oil against *E.coli*

Plate 2. Antibacterial activity of *Persea americana* essential oil against *Bacillus subtilis*

Table 4, Alkaloids, Reducing sugars, Tannins and Saponin were present in *Citrus sinensis* using the four extracts (methanol, ethyl acetate, dichloromethane and n-hexane). Cardiac glycoside, steroids and phenol were negative in dichloromethane extract while flavonoid is negative in ethyl acetate extract.

Tables 5 and 6 Quantitative Phytochemical analysis of *Persea americana* and *Citrus sinensis essential oil*, revealed the presence of alkaloid, phytate, phenol, tannins, saponins and flavonoids in methanol, ethyl acetate and n-hexane extract except for flavonoid which was not detected in *Citrus sinensis* using methanol extract. while phytates were found to be the most abundant in ethyl acetate extract of *Persea americana* (29.37%).

Table 7; The oil was dominated by hydrocarbons, characterized by a high percentage of squalene (45.94%). Considering components with concentration of ≥ 2%, the other major components were: - Oxirane, [(hexadecyloxy)methyl] (2.34%), Tetrate tracontane 2.46%), Hexatriacontane (3.58%), Cis-9-hexadecanoic acid(4.02%). Appreciable amounts were: - Diisooctyl phthalate (7.45%), 12-methyl-e,e-2,13-octadecadien-1-ol (7.20%), vitamin E (12.50%), and squalene (45.94%).

4. DISCUSSION

Essential oils are potential source of antibacterial compounds especially against pathogenic bacteria. In vitro studies in this work showed that essential oils inhibit bacterial growth but their effectiveness varied. The antibacterial activity of essential oils has been previously reviewed and classified as strong, medium or weak of antibiotics [29].

Table 3. Qualitative phytochemical analysis of *Persea americana*

Sample	Solvent	Alkaloid	Cardiac glycoside	Steroids	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids	Reducing sugar
Persea americana	Methanol	+ve	+ve	+ve	ND	+ve	+ve	+ve	+ve	-ve
Persea americana	Ethyl-acetate	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve
Persea americana	Dichloro methane	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
Persea americana	N-hexane	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve

Note : (+ve) means positive, (-ve) means negative and ND means not detected

Table 4. Qualitative phytochemical analysis of *Citrus sinensis*

Sample	Solvent	Alkaloid	Cardiac glycoside	Steroids	Anthraquinone	phenol	Tannins	Saponin	Flavonoids	Reducing sugar
Citrus sinensis	Methanol	+ve	-ve	+ve	ND	+ve	+ve	+ve	+ve	+ve
Citrus sinensis	Ethyl-acetate	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Citrus sinensis	Dichloromethane	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
Citrus sinensis	N-hexane	+ve	+ve	+ve	ND	+ve	+ve	+ve	+ve	+ve

Note : (+ve) means positive, (-ve) means negative and ND means not detected

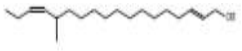
Table 5. Quantitative phytochemical analysis of *Persea americana*

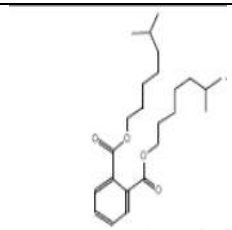
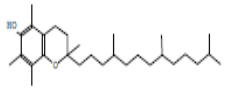
Sample	Solvent	Alkaloid	Phytate	Phenol	Tannins	Saponin	Flavonoid
Persea americana	Methanol	13.87	9.78	9.70	7.52	7.61	1.67
Persea americana	Ethyl-acetate	20.93	29.37	24.72	17.38	20.31	2.78
Persea americana	N-hexane	10.93	20.37	14.72	15.38	10.31	5.78

Table 6. Quantitative phytochemical analysis of *Citrus sinensis*

Sample	Solvent	Alkaloid	Phytate	Phenol	Tannins	Saponin	Flavonoids
Citrus sinensis	Methanol	8.59	6.43	6.56	10.38	10.41	ND
Citrus sinensis	Ethyl-acetate	20.33	15.53	25.38	18.71	4.38	8.52
Citrus sinensis	N-hexane	20.33	15.53	20.38	10.71	4.38	10.52

Table 7. Chemical composition of *Persea americana* leaves essential oil

	Molecular weight	Retention time	Base peak	Height % present in oil	Structures	Molecular formular
Cis-9-hexadecanoic acid	326	19.592	311.25	4.02		$C_{19}H_{38}O_2Si$
12-Methyl-E,E-2,13-octadecadien-1-ol	280	20.790	87.05	7.20		$C_{19}H_{36}O$
Eicosane	282	21.901	57.05	1.65		$C_{20}H_{42}$
Tetratetracontane	618	23.420	57.05	2.46		$C_{34}H_{70}$
Pentatriacontane	492	24.144	57.05	1.63		$C_{35}H_{70}$
Hentriacontane	450	22.675	57.05	1.92		$C_{36}H_{74}$
Phytol	396	18.595	57.05	1.85		$C_{20}H_{40}O$

	Molecular weight	Retention time	Base peak	Height % present in oil	Structures	Molecular formular
Diisooctyl phthalate	390	22.190	149.05	7.45		$C_{24}H_{38}O_4$
Squalene	410	24.285	69.05	45.94		$C_{30}H_{50}$
2-methyloctacosane	408	23.420	57,05	1.69		
Vitamin E	430	27.199	2.99	12.50		$C_{29}H_{50}O_2$
1,6,10,14,18,22-Tetracosahexaen-3-ol	426	25.100	69.05	1.61		
Oxirane,[(hexadecyloxy)methyl]	298	23.420	57.05	2.34		$C_{19}H_{38}O_2$
Hexatriacontane	506	29.234	57.05	4.15		$C_{36}H_{74}$

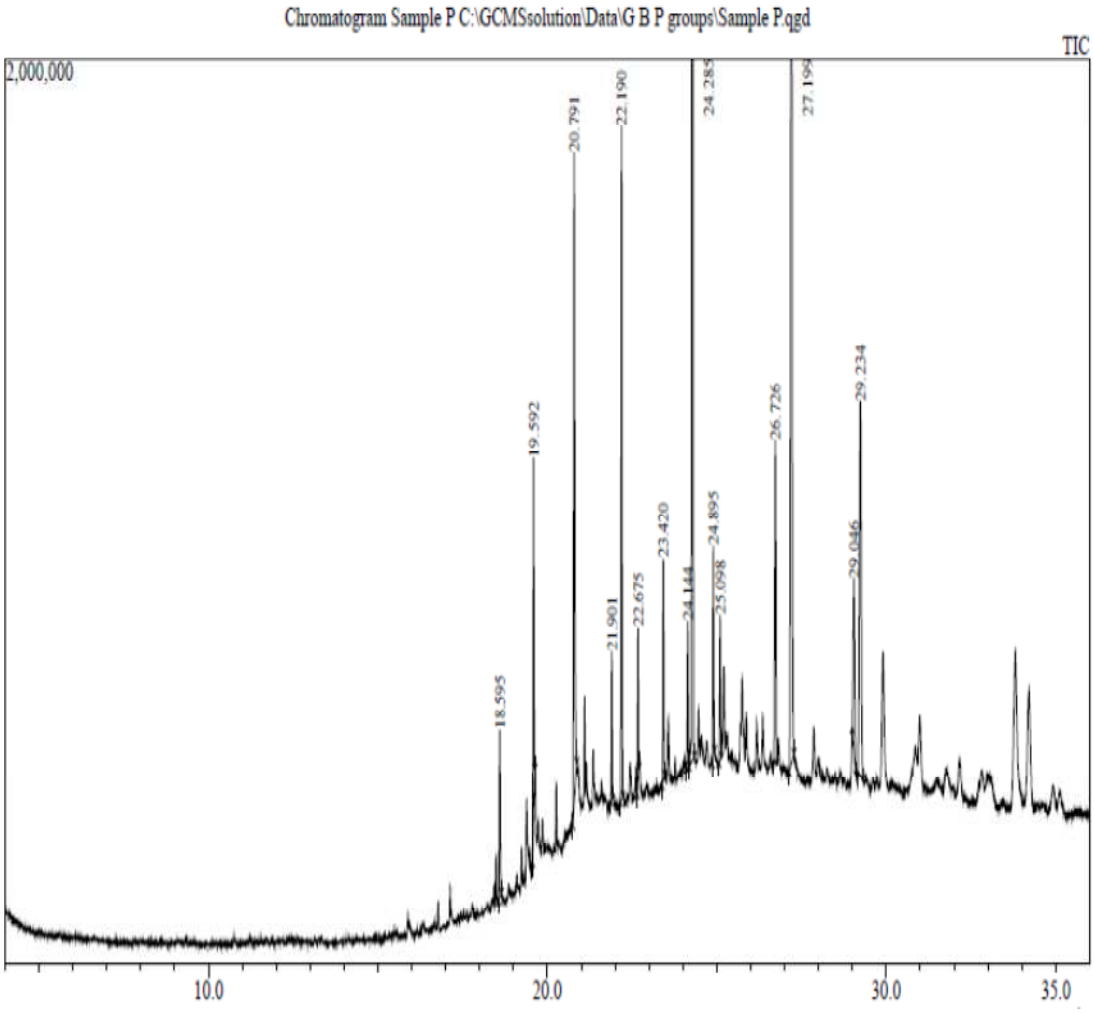


Fig. 4. Spectra of gas chromatography/mass spectophotometry analysis of *Persea americana*

The *Persea americana* essential oil also shows activity against both Gram- positive and Gram-negative bacteria. On the average, antibacterial activity of essential oil was more effective on gram positive bacteria (mean zone of inhibition: 14.0 mm) than gram negative whose mean zone of inhibition is (12.0 mm). Due to the complex structure of gram-negative bacteria having a thick peptidoglycan layer of 2-3 nm which is thinner in gram-positive bacteria enabling hydrophobic molecules to easily penetrate the cells and act on both the cell wall and the cytoplasm [30]. Phenolic compounds present in the *Persea americana* essential oils show antibacterial activity against Gram-positive bacteria. Their effect depends on the amount of the compound present; At low concentrations, they interfere with enzymes involved in the production of energy, and at higher concentrations, they denature proteins. It is likely that antibacterial activity is due to different mechanisms of action that implies several targets in the cell [31].

The current study shows the role of essential oil from *Persea americana* as a strong antibacterial agent against *Staphylococcus aureus, Escherichia coli, Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Bacillus subtilis in-vitro*, and may be considered a useful lead in the search of new drugs. The essential oil of *Citrus sinensis* evaluated for anti-bacterial properties were found to be active against the entire Gram-positive and two Gram-negative bacteria *Salmonella typhi* and *Escherichia coli as* shown by the inhibition zones. The microbe *Pseudomonas aeruginosae* was resistant to the *Citrus sinensis* oil since low inhibition zones were

observed. Among the gram-negative bacteria, the *Citrus sinensis* oil was very active against *Escherichia coli*. On the average, antibacterial activity of *Citrus sinensis* essential oil was more effective on gram positive bacteria (mean zone of inhibition: 13.0 mm) than gram negative (11.0 mm) because the bacterial cell wall of gram positive bacteria allows hydrophobic molecules to easily penetrate the cells thus, the susceptibility of gram-positive bacteria.

The Tables summarizes the microbial inhibition by the crude oil from *Persea americana* and *Citrus sinensis* which shows antibacterial activities against the tested organisms. crude oils from *Citrus sinensis* exhibit better antibacterial activity against most bacteria at higher concentration except for *Pseudomonas aureginosa* which shows lesser activity to the *Persea americana* oils activity. Thus reveals the susceptibility of both Gram positive and negative bacteria at higher concentration. All microorganism were susceptible at the highest concentration of the *Persea americana* essential oil ranging from *Escherichia coli, Staphylococcus aureus, Bacillus subtilis Proteus mirabilis, Salmonella typhi, Pseudomonas aureginosa and Klebsiella pneumonia* susceptible microorganism to the *Persea americana* essential oil ranges from *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis,* and *Proteus mirabilis* at the highest concentration. While at a lower concentration, the antibacterial activity of the *Persea americana* oil is less effective. A more significant inhibition was seen with a higher essential oil concentration. At low concentrations, a very limited inhibitory effect was observed on the growth of microorganisms. With increasing *Persea americana* essential oil leaves concentration, an obvious inhibitory effect on the growth of *Escherichia coli* and *Staphylococcus aureus,* was significantly increased.

When the efficacy of the plant extracts were compared using the agar well diffusion method, *Citrus sinensis* oil extracted had the highest inhibition zone (20.0 mm) at the highest concentration of 100mg/ml against *Escherichia coli*, followed by inhibition zone of (17.0 mm) and (10.0mm) against *Salmonella typhi and Klebsiella pnuemoniae* respectively, while the other concentrations (50 mg/ml, 25 mg/ml, 12.5 mg/ml) were relatively effective the effectiveness of *Citrus sinensis* oil is due to its high content of ascorbic acid which is believed to stimulate the production of white blood cells primarily neutrophil which attacks foreign antigens and boost production of antibodies [32].

Persea americana oil extract was effective with the highest inhibition zone of (18.0 mm) against *Escherichia coli* at 100 mg/ml, followed by (15.0 mm and 13.0 mm) against *Staph aureus and Bacillus subtilis,* respectively. While *at* a concentration of 12.5 mg/ml shows the least inhibition zone of (5.0 mm) against *staphylococcus aureus* .

Persea americana has higher antibacterial effect against the isolates compared to *Citrus sinensis*. *Persea americana* has a high zone of inhibition (18.0 mm) against *Escherichia.coli* at 100 mg/ml and lowest inhibition zone (5.0 mm) against *Staph aureus* at 12.5 mg/ml. This research revealed that *Persea americana* and *Citrus sinensis* oils exhibited strong activity against the selected bacterial strains. Several studies have shown that *Persea americana* and *Citrus sinensis* oils had strong inhibitory effects against various pathogens [33].

The qualitative Phytochemical analysis of *Persea americana* and *Citrus sinensis* using methanol , dichloromethane, n-hexane and ethyl acetate shows the presence of varying chemical components such as the Cardiac glycoside, Steroids, Phenol, Anthraquinone, Flavonoid and reducing sugars while Alkaloid, tannins and Saponin is present (+) in both plants. Alkaloids are important drug source and have been reported to possess antimicrobial, antioxidant activity [34]. Tannin is non-toxic, it also generates physiological responses in animals that consume them [35]. Saponin has also been reported to have anti-inflammatory activity, cardiac glycoside, a depressant and hyper-cholesterolemic. Saponin and steroid also have relationships with sex hormones like oxytocin which regulates the onset of labour in pregnant women and subsequent release of milk [36]. Tannins are used as antidiarrheal, saponin are glycosides of triterpenes, steroid alkaloid found in plant are useful for lowering cholesterol and displays analgestic properties [37].

Anthraquinone were not detected in both plant extract using methanol while in Table 4, Anthraquinone was also not detected in *Citrus sinensis* using n-hexane as solvent. Qualitative Phytochemical analysis of *Persea americana* and *citrus sinensis* reveals the presence of certain components which are absent in quantitative Phytochemical analysis using same solvent and

vice-versa. The presence of these components has made *Persea americana* and *Citrus sinensis* to be effective in treating heart diseases, menstrual and fertility issues (cardiac glycoside & phenols) respectively [38].

it can be deduce that the quantitative phytochemical analysis of *Persea americana* and *Citrus sinensis* using ethyl acetate and n-hexane gives high yield in percentage compared to methanol. The extraction technique with organic solvents is based on the distribution balance or selective dissolution of the oil within two immiscible phases [39]. Although *Persea americana* has a high yield in alkaloid (13.87%), *Citrus sinensis* also had high yield in saponin (10.41%) using methanol while flavonoid was not detected in methanol extract of *citrus sinensis*. alkaloid and saponin yielded (10.93%) and (4.38%) respectively.

Gas chromatography and Mass spectrophotometer reveals 15 active compounds with different isomers present in *Persea americana* oil. The total percentage yield of chemical compound present equals 100%. GC-MS analysis revealed Squalene and vitamin E to be the major constituent of *Persea americana* oil. These compounds were the predominant active compound found in *Persea americana*. Squalene act by exerting anti-carcinogenic effect through decrease in farnesyl pyrophosphate (FPP) levels in cells of which prenylation of FPP are required for oncogene activation [40,41]. It also reduces colon cancer [42].

Earlier studies suggested that the antibacterial activity of *Persea americana* oil was probably due to Squalene and vitamin E which are effective against skin wrinkling and slows the process of ageing [43]. Reports state that the healing properties may be linked with lysyl oxidase which initiates cross linking in collagen and inhibits enzyme activity. The vitamin content of the oil also helps to prevent skin burns and accelerate healing process [43].

Squalene shows a height percentage of (45.94%) with a retention time of 24.285 while vitamin E having a high retention time of 27.199 gives a height percentage of (12.50%). With the height percentage of Squalene, it is regarded as the major active component of *Persea americana* oil. Eicosane serve as precursor for prostaglandin while cis-9-hexadecanoic which occurs as triglycerides is used as excipient in pharmaceuticals, for drug formulation and helps to reduce blood pressure [44]. While Disooctyl phthalate stabilizes membranes of red blood cells enabling blood product storage. Tetratetracontane possess inhibitory activity and help to maintain cellular osmotic conditions. 2-methyloctacosane is an acyclic hydrocarbon which possesses anti-inflammatory activity. oxirane,(hexadecyloxy) methyl plays a vital role in maintaining the body's antioxidant defense while 1,6,10,14,18,22- Tetracosahexaen-3-ol is a triterpenoid which possesses anti-inflammatory, antiarthritic and antimicrobial properties. 16-hentriacontane promotes the excretion of urine through its effect on the kidney, it also reduces inflammation. Hexatriacontane possesses antimicrobial property inhibiting the growth of microorganisms while, Pentriacontane balances overall body function thus, stimulates the function of gastrointestinal tract. Phytol is an acyclic diterpene alcohol used as a precursor for the manufacture of vitamin E and K. It also inhibits the growth of *Staphylococcus aureus* and blocks the teralogenic effects of retinol [45]. The results also show that essential oils varied significantly in their antibacterial potential. These differences may be attributed to differences in nature and/or concentration of chemical constituent in the different plant species and in their relative solubility in water and extracts used. Major active constituents present in the plants essential oil.

5. CONCLUSION

This study indicates that essential oils serve as an important source of antibacterial compounds that may provide renewable sources of useful antibacterial drugs against bacterial infections in human. The results of this study present essential oils as good antibacterial agents to combat pathogenic microorganisms. The essential oils from the leaf of *Persea americana* and *Citrus sinensis* showed varying degrees of antibacterial activity against clinical isolates. From the study, it can be inferred that essential oil extract shows significant growth inhibiting effects on Gram- positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*). The efficacy of leaf oil of *persea americana* and *citrus sinensis* against these micro organisms provides a scientific ground for the application of the herb in the prevention and treatment of bacterial infections caused by various pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*, which have the ability of developing resistance to antibiotics.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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