Research Article

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Journal of Applied Microbiology and Biochemistry
ISSN 2576-1412

2017
Vol. 1 No. 3:12

DOI: 10.21767/2576-1412.100012

Efficacy of Essential Oils from *Persea americana* Stem Bark and Seed Extracts

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Citation: Temitope OO, Akinola MO, Aladejana OM, Ogunlade AO (2017) Efficacy of Essential Oils from *Persea americana* Stem Bark and Seed Extracts. J Appl Microbiol Biochem. Vol. 1 No. 3:12

Abstract

The aim of the study is to evaluate the Antibacterial, Phytochemical analysis of Essential Oil, From the Stem Bark and seed extracts of *Persea americana* (Avocado). This was carried out by the crude extraction of the seeds and stem bark extracts with n-hexane. The extracts were used to determine the presence of Phytochemicals. Stock cultures of test organism such as *Staphylococcus aereus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Proteus mirabilis* were used to test the antibacterial efficacy of the extracts using the agar well diffusion method. The extracts shows presence of antibacterial efficacy which was compared to antibacterial activity of a commercial antibiotic (Chloramphenical) against the test organisms. At 12.5 mg, hexane extract of avocado stem bark shows little activity against *Proteus mirabilis* (0.0 mm), *Salmonella typhi*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (0.0 mm). Qualitative phytochemical analysis of the Avocado essential oil (Stem bark) was observed at higher concentrations (12.5, 25, 50 and 100 mg) for methanol, it indicate the presence of Alkaloid, cardiac glycoside, Tannins, Saponin and Reducing sugar. Phytate extracts exhibited the highest bacterial activity. The phytochemical analysis shows the presence of phenol, saponin, reducing sugar steroids, tannin, flavonoid, alkaloid and Cardiac glycoside. From this research, Avocado seeds and stem bark when properly extracted and purified, acts as antibiotics which can be used in treatment of infections caused by pathogenic bacteria, and other various organisms such as virus and fungi.

Keywords: Antibacterial; Phytochemical analysis; Essential oil; *Persea americana* (Avocado)

Received: June 15, 2017; **Accepted:** July 03, 2017; **Published:** July 10, 2017

Introduction

Pharmaceutical and therapeutic potential of essential oils (EOs) has been known since ancient times. EOs is obtained using several techniques: water or steam distillation, solvent extraction, expression under pressure, supercritical fluid or subcritical water extractions. Nowadays, essential oils are the subject of intensive scientific research and also attract attention of cosmetic and pharmaceutical industries due to their potential as active pharmacological compounds or natural preservatives. Enormous diversity of this group of natural compounds and wide spectrum of biological properties make them attractive for many industries and new areas of application still has not been discovered [1].

Avocado essential oil is widely used by all sections of the population either directly as folk remedy or indirectly in the preparation of pharmaceuticals. Avocado essential oil is a medicinal plant used in the treatment of malaria and other ailment of human body. The avocado plant grows well in warm areas with tropical to subtropical climates. The plant at various stages does not tolerate climate that is too cold, too wet, too hot or too dry. Its flowers are very sensitive to very low temperatures and freezing temperatures tend to kill the plants. Frost and very hot weather results in a substantial loss of fruits and too much wind is also highly unfavorable.

The plant is tolerant to a wide range of soil types (acidic and alkaline) with the exception of saline conditions. It does require well aerated soils and will not survive in areas with poor drainage due to excess water. It tends to grow well on hillsides but should

never be grown near stream beds. The plant can be grown in the shade but is productive only in full sun. Fruits continuously exposed to the sun were more hardy and tolerant to conditions like high and low temperatures, had a slower rate of ripening, and more resistant to pathogen invasion than those growing in the shade. The sun exposed fruits were also higher in oil, dry matter and minerals. This supports the importance of sunlight and temperature on avocado fruit development and qualities [2].

The recent development in technology however has led to the production of Avocado essential oil that is highly suitable for the consumer market in terms of quality and health benefits. The production of various antibiotics also led to many microorganism resistant such as MRSA (Multi-Resistant Staphylococcus Aureus) which is capable resisting the activities of many or more than one antibiotics but the use of Avocado oil synergistically has led to the reduction in the activities of MRSA. 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 [3].

Essential oils from Avocado stem bark and seed are used as astringent against cold, cough and sore throat. The hexane extract of the fruit has been tested and found effective against isoniazid and ethambutol resistant *M. tuberculosis* strains *in vitro*. It also possess antioxidant properties, flavonoids present in this oil have hydroxyl radical scavenging activity, anticancer and antioxidant activity [4]. The seed is highly effective as an antifungal agent against *lentinus sajor-caju*, which causes white rot in wood hence it can be used as a preservative agent in the management of wood infected. The extract of the seed has a characteristic antibacterial and antimicrobial action against bacteria's and fungi [5].

Persea americana stem bark possesses anti-inflammatory activity and anti-fungal properties. It possesses specific activity against *Mycobacterium tuberculosis*, *Streptococcus pyogens*, *Staphylococcus aureus* and varieties of fungi. The crushed and boiled seeds of *Persea americana* is used in treating toothache and mouth sores [6]. *Persea americana* stem bark extract have been used as analgesic, anti-inflammatory, hypoglycaemic, anticonvulsant, anti-diabetic and vasorelaxant.

Research Methodology

Plants collection and identification

The plants were collected from Ijero-Ekiti in Ekiti State. The Stem bark of the plants and seed were collected on 3rd of October 2016 during the winter period and was air-dried for 8days after been identified by a taxonomist of plant science and biotechnology of Adekunle Ajasin University Akungba Akoko, Ondo State (PSB department) after which I showed it to my supervisor for approval before chopping it to smaller size. The diagram shows the place of sample collection **(Figure 1)**.

Place of sample collection

Extraction of sample: Using the soxhlet method of extraction, air dried for eight days (8) and grounded stem bark and seed of *Persea americana* and 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.

Antibacterial activity of avocado essential oil: Antibacterial activity of the essential oils was tested using agar well diffusion method. The gram-negative bacteria used were *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosae, Proteus mirabilis* and *Salmonella typhi* while gram–positive ones were *Staphylococcus aureus and Bacillus spp*. The test organisms were inoculated in nutrient broth and incubated for 4-6 hours at 37°C. 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 (McFarland No. 0.5 standard gives cell density of 1.5×10^8/ml). Antibacterial activity assay was done on Mueller Hinton agar. The media were reconstituted using distilled water and sterilized by autoclaving at 121°C for 15 minutes then dispensed into Petri dishes aseptically and left to solidify and then stored in the refrigerator at 4°C.

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°C 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

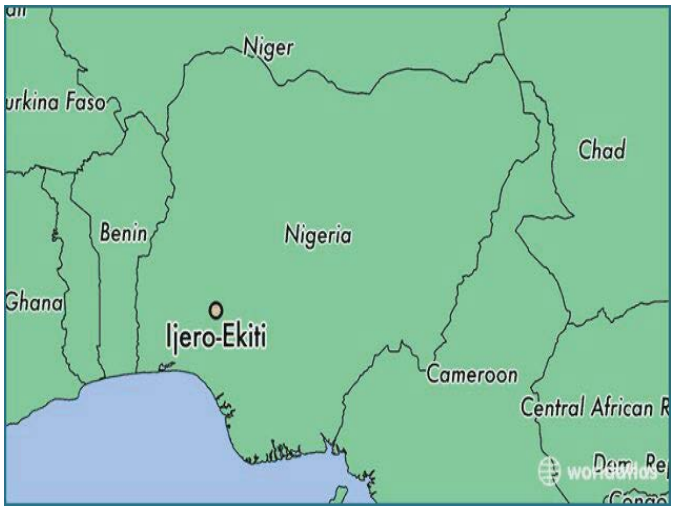

Figure 1 Map Africa showing area in which sample of avocado essential are collected in Ijero- Ekiti, Ekiti State Nigeria.

covered to avoid any possible evaporation or contamination. Chloramphenicol was used as standard controls. The inoculated plates were incubated at 37°C for 24 hr 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 [7].

Qualitative method of phytochemical analysis of avocado essential oil (stem bark and seed)

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.

Test for alkanol:

TLC method 1: 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 [8].

TLC method 2: Wet the powdered test samples with a half diluted NH4OH and lixiviated with EtOAc for 24 hr at room temperature. Separate the organic phase from the acidified filtrate and basify with NH_4OH (pH 11-12). Then extract it with chloroform (3X), condense by evaporation and use for chromatography. Separate the alkaloid spots using the solvent mixture chloroform and methanol (15:1). Spray the spots with Dragendorff's reagent. Orange spot shows a positive result [9].

Test for anthraquinone:

Borntrager's test: Heat about 50 mg of extract with 1 ml 10% ferric chloride solution and 1 ml 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.

Borntrager's test: Add 1 ml of dilute (10%) ammonia to 2 ml of chloroform extract. A pink-red colour in the ammoniacal (lower) layer.

Test for cardiac glycosides:

Kellar- Kiliani test: Dissolve 50 mg of methanolic extract in 2 ml of chloroform. Add H_2SO_4 to form a layer. Brown ring at interphase shows is a positive result.

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. Separatethe glycosides using EtOAc-MeOH-H_2O (80:10:10) solvent mixture. The colour and hRf values of these spots can be recorded under ultraviolet (UV254 nm) light [9].

Test for flavonoid:

Shinoda test: To 2-3 ml of methanolic extract, add a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid. Pink red or red coloration of the solution, shows is a positive result.

TLC method: Extract 1 g powdered test samples with 10 ml methanol on water bath (60°C/ 5min). 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 (UV254 nm) light [9].

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.

Test for saponin:

Frothing test / Foam test: Add 0.5 ml of filtrate with 5 ml of distilled water and shake well. Persistence of frothing shows is a positive result [10].

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 [9].

Test for steroid: TLC method Extract two grams of powdered test samples with 10 ml methanol in water bath (80°C/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°C/6 min). The color (Greenish black to Pinkish black) and hRf values of these spots can be recorded under visible light [9].

Test for tannin: Braemer's test 10% alcoholic ferric chloride will be added to 2-3 ml of methanolic extract (1:1) Dark blue or greenish grey coloration of the solution [10].

Quantitative methods of phytochemical analysis of avocado essential oil (stem bark and seed)

Test for saponins: About 20 grams each of dried plant samples of avocado seed and stem bark 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°C, 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°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether were added and then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. 60 ml 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 [11].

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 [11].

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 [11].

Test for alkaloids : Five grams of the plant sample were weighed into a 250 ml beaker and 200ml 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.

Results

Antibacterial activity of *Persea americana* of stem bark and seed of essential oil

Table 1 shows the zone of inhibition of test organisms by the essential oil extract of *Persea americana* stem bark and see (avocado) expressed in mm with *Escherichia coli* showing the widest zone of inhibition of (20.0 mm) while the least zone of inhibition (6.0 mm) was recorded for *Staphylococcus aureus*. The essential oil of *Persea americana* act as a strong anti-bacterial agent against, *Escherichia coli, Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Bacillus spp. in vitro*. The oil showed activity against both gram+ positive and gram-negative bacteria. **Table 2** shows the essential oil of *avocado seed* evaluated for anti-bacterial properties were found to be active against the entire gram-positive and gram-negative bacteria *Salmonella typhi* and *Escherichia coli as* shown by the inhibition zones in the **Table 3**. *Pseudomonas aeruginosa 1 shows* no inhibition zones. Among the gram-negative bacteria, the oil was very active against *Escherichia coli*. On the average, antibacterial activity of essential oil was more observed on gram positive bacteria (mean zone of inhibition: 14.0 mm) than gram negative (12.0 mm). In **Table 4**, qualitative phytochemical analysis of *Persea americana* stem bark reveals the presence of alkaloids, cardiac glycoside, tannins, saponins and reducing sugar using methanol, but in avocado seed it reveals the presence of alkaloids, phenol, tannins, saponin and flavonoids using methanol. In **Table 5**, qualitative phytochemical analysis of *Persea americana* stem bark reveals the presence of alkaloid, cardiac glycoside, anthraquinone, phenol, tannins, saponin, flavonoids and reducing sugar, but with avocado seed all but flavonoid was absent using ethyl acetate.

Discussion

Several researchers had reported that plants contain antimicrobial substances. Many plant species yield biological active products that are capable of exerting physiological effects upon microorganisms and other living things. Plants of this nature (e.g. avocado essential oil) exist and serve as drugs.

The phytochemical analysis shows the presence of phenol, saponin, steroids, reducing sugar cardiac glycoside flavonoid, alkaloid and glycoside. The presence of these components has been linked with the antibacterial activity of avocado stem bark and seeds and plants that contain them in higher amount are considered to be superior in their antimicrobial activity.

The result of antibacterial activity of the extract against selected human pathogens indicated that the plant sample was active against a wide variety of human pathogenic bacteria. Methanol extracts exhibited the highest inhibitory effect followed by n-hexane. This result agrees with the findings made by where ethanol extract proved active in inhibition of the tested organisms than other extraction solvents.

The low inhibition effect shown by other solvent extracts as

Table 1 Antibacterial activity of *Persea americana* essential oil stem bark on (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Bacillus spp, Salmomella typhi, Pseudomonas aeruginosa* Zone of Inhibition (mm).

Microorganism	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Chloramphenical (Control) 30 µg
E. coli	20.0	16.0	13.0	10.0	29.0
S. typhi	19.0	13.0	0.0	0.0	23.0
K. pneumoniae	17.0	12.0	0.0	0.0	12.0
P. mirabilis	11.5	9.0	6.0	0.0	9.0
P. aeruginosa	7.0	0.0	0.0	0.0	12.0
S. aureus	17.0	11.0	9.0	6.0	26.0
Bacillus subtilis	14.0	9.0	8.0	0.0	20.0

Table 2 Antibacterial activity of *Persea americana* essential oil Seed on *(E. coli, K. pneumoniae, P. mirabilis, S. typhi, P. aeruginosa.*

	Essential oil concentration				
Microorganism	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Chloramphenical (Control) 30 µg
E. coli	19.0	15.0	10.0	7.0	30.0
S. typhi	17.0	14.0	0.0	0.0	17.0
K. pneumoniae	17.0	12.0	0.0	0.0	15.0
P. mirabilis	12.0	9.0	7.0	0.0	11.0
P. aeruginosa	9.0	0.0	0.0	0.0	10.0
S. aureus	18.0	12.0	7.0	6.0	22.0
Bacillus subtilis	11.0	9.0	7.0	0.0	13.0

compared to methanol and n-hexane could be due to the fact that these components are more soluble in n- hexane and methanol than in water.

However, most of the gram negative organism e.g. *E. coli* showed high susceptibility than most of the gram positive. The higher susceptibility of the gram negative bacteria is difficult to explain in the study considering the observation.

Gram negative bacteria appear to be more resistant to antimicrobial agents than the gram positive bacteria. This resistance has been observed to reside in the complex cell wall and cell membrane structure. More so, more antibacterial activities were observed with high concentration of the extracts than at lower concentrations. Activity even at low concentration indicates high potency of the extract against the microorganism [12].

Escherichia coli was the most susceptible bacterium with avocado stem bark, an observation that may be attributed to the presence of single membrane of the organism which makes it more accessible to permeation by active principles of the extract of avocado essential oil (bark). In contrast *Pseudomonas aeruginosa* shows the least susceptibility to the extract. This may be due to the fact *Pseudomonas aeruginosa* has intrinsic resistance from a restrictive outer membrane barrier and trans-envelope Multidrug resistance pumps (MDRs), which is in agreement with previous observation. Whereas moderate antimicrobial activity of avocado essential oils extract was observed on *Escherichia coli*. These suggest that the avocado extract of the stem bark is broad spectrum in activity and that its mode of action may be due to inhibition of cell wall synthesis. Similar findings have been reported by and the n-hexane extracts of Avocado stem bark shows the strongest activity than the seed. The gram positive bacteria were more susceptible to the extract than the gram-negative bacteria, possibly because of the presence of the outer membrane that serves as an effective barrier and gram negative species. In addition, since the zones inhibitions are equal or greater than the standard, it shows that the test organisms are sensitive to the avocado extract. The strong activity of the extract on *Escherichia coli* and *Staphylococcus aureus* suggests that it may be used for the treatment of wound infection and diarrhea caused by these organisms. This work also revealed the potential use of extracts of Avocado essential oil (seed and stem bark) for use in the control of medically important organisms such as *Salmonella typhi* (causative agent of typhoid fever) and *Pseudomonas aeruginosa*. Similar results have been obtained with extract seed and stem bark of avocado essential oil when tested against *Staphylococcus aureus, Escherichia coli, Bacillus subtlis, Pseudomonas aeruginosa*.

The anti-bacterial activity observed in this study was concentration dependent. The MIC values obtained for the entire test organism were very high, ranging from 25 to 100 mg/ml, when compared to the value of 0.01-10 ug/ml usually recorded for typical antibiotics. This difference may be due to the fact that the extract used was in the pure form and would definitely contain substances which do not have antibacterial activities.

The 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.

The qualitative phytochemical analysis of *Persea americana* seed and stem bark 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 [10]. Tannin is non-toxic; it also generates physiological responses in animals that consume them [13]. Saponin has also been reported to have anti-inflammatory, cardiac depressant and hyper-cholesterolemic.

Tannins are used as antidiarrheal, saponin are glycosides of

Table 3 Qualitative phytochemical analysis of *Persea americana* stem bark.

Sample	Solvent	Alkaloid	Cardiac Glycoside	Steroids	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids	Reducing sugar
Persea americana	Methanol	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve
Persea americana	Ethyl-acetate	+ve	+ve	±ve	+ve	+ve	+ve	+ve	+ve	+ve
Persea americana	Dichloromethane	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
Persea americana	N-hexane	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve

Table 4 Quantitative phytochemical analysis of *Persea Americana* stem bark.

Sample	Solvent	Alkaloid	Oxalate	Phytate	Phenol	Tannins	Saponin	Flavonoid
Persea Americana	Methanol	3.50	3.55	4.25	2.47	3.48	3.44	3.21
Persea Americana	Ethyl-acetate	2.45	1.95	2.20	2.10	2.38	2.45	1.92
Persea Americana	N-hexane	2.45	1.92	2.20	2.10	2.38	2.45	1.92

Table 5 Quantitative phytochemical analysis of *Persea americana* seed.

Sample	Solvent	Alkaloid	Oxalate	Phytate	Phenol	Tannins	Saponin	Flavonoid
Persea Americana	Methanol	17.30	17.27	6.49	7.71	1.25	1.20	2.97
Persea Americana	Ethyl-acetate	3.58	3.20	3.50	3.55	3.00	3.58	3.20
Persea Americana	N-hexane	3.58	3.20	3.50	3.55	3.00	3.58	3.20

triterpenes, steroid alkaloid found in plant are useful for lowering cholesterol and displays analgestic properties [14].

In **Table 4** anthraquinone were not detected in Avocado seed but was detected in its stem bark extract using methanol while in **Table 5**, Anthraquinone was also not detected in *both plant part* using n-hexane as solvent. As indicated in **Tables 4 and 5** qualitative phytochemical analysis of medicinal plant 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 medicinal plant to be effective in treating heart diseases, menstrual and fertility issues (cardiac glycoside and phenols) respectively [15].

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 stem bark of *Persea americana* and *seed* shows 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 stem bark of *Persea americana* and *seed* against these microorganisms 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 [16-22].

Recommendation

i. The shelf life of essential oil should also be determined, in order to assess their rate of deterioration if it is to be used as medicine.

ii. Essential oils could have greatest potential use as a food preservative since they have been known to inhibit bacteria, fungi and yeast, therefore research on this could be carried out.

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