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Antimicrobial Activity of Essential Oil from Avocado (*Persea americana*) Seed and Pulp on Some Pathogenic Organisms

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Persea americana* is regarded as a unique plant in many African countries. Its numerous parts are employed in the treatment of several diseases. The possible function mechanism of *P. americana* functions is under study.
Aims: This study was designed to investigate the phytochemical analysis and antibacterial effect of oil extracted from seed and pulp of *P. americana* using agar diffusion technique.
Methodology: The seed oil of *P. americana* was exhaustively extracted with a Soxhlet extractor from 500 g seeds and 200 g seeds of *P. americana* using di-ethyl ether as solvent. The extraction solvent was removed to obtain the oil which was then subjected to antimicrobial activity test to determine its activity against the following clinical isolates namely *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia* and *Candida albicans* using conventional antibiotics as control. Phytochemical screening was carried out following standard methods.
Results: The result of phytochemicals screening was presented with alkaloids, saponin, flavonoids, polyphenol, tannins, steroids as present. The result revealed *P. aeruginosa* to have the highest diameter of zones of inhibition of 12.00 – 30.00 mm at the concentrations of 0.1 – 0.4 g/mL respectively. *S. aureus* had diameter of zones of inhibition of 10.00 – 20.00 mm at the concentrations of 0.1 – 0.4 g/mL. *E. coli* had diameter of zones of inhibition of 10.00 – 15.00 mm at

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the concentrations of 0.1 – 0.4 g/mL respectively. *C. albicans* had diameter of zones of inhibition of 8.00 – 13.00 mm at the concentrations of 0.1 – 0.4 g/mL respectively; and *S. pneumonia* had diameter of zones of inhibition of 8.00 – 12.00 mm at the concentrations of 0.1 – 0.4 g/mL respectively. The results of the antimicrobial test on the seed oil showed appreciable antibacterial activities against the test organisms. The result of the test organisms was susceptible to conventional antibiotics.

Conclusion: Oil extract of avocado pear (*P. americana*) will be helpful to many researchers in the field of finding antibacterial activities in plant; and the oil is recommended for treatment of skin infections inherent with these organisms.

Keywords: Antibacterial; avocado; essential oil; phytochemical.

1. INTRODUCTION

"Plants have been used since ancient times by mankind either as food or as source of their medicines. *Persian americana* (avocado pear) is one of those plants currently used by indigenous persons for its nutritional value and to manage health problems. The generic name *Persea* is derived from the Greek word Dakruon meaning tear, referring to resin droplets on the bark surface of its members, while *edulis* means edible, emphasizing the importance of the nutritious fruit in the plant's cultivation. The plant belongs to the Burseraceae family whose members are characterized by an ovary of 2 to 5 cells, prominent as in ducts in the bark, wood and intrastaminal disk" [1]. "The common names of *Persea americana* are in English: African pear, African pear tree, Bush butter, Bush butter tree, Bush fruit tree, Eben tree, Native pear; and in French: Safoutier or prunier. It is cultivated in most rural communities by the peasant farmers, mostly for its edible fruits. But all parts of this tree, including leaves, bark, roots, resin, seeds and fruit pulp are used for medicinal purposes" [2].

"Persea americana is commonly known as 'avocado pear', it is known as eben among the Efik people, ube in Igbo, orumu in Benin, elemi in Yoruba, and safou in French [1,3]. They are shade loving plant species, dioecious and found in the humid tropical zone of non-flooded forest" [4]. "The fruit of *P. americana* are ellipsoidal and their size varies approximately from 4 to 9 cm long and from 2 to 5 cm wide. *P. americana* is a fruit that has seed and it is covered by pulpy pericarp, it is an edible fruit which can be eaten cooked or raw and rich in minerals, vitamins, oils, and protein" [5]. "In spite, of its nutritional values, it is highly perishable and prone to microbial attack, as it is also rich source of nutrients to microorganisms" [6].

"The oil of fruits of *P. americana* is a rich source of fatty acids and triglycerides. The fatty acid compositions of fruit pulp oil of 2 cultivars of *P. americana* (cultivars 1 and 2, grown in Cameroon) were determined. Fruits significantly differed in mass, length, thickness of pulp and mass of kernel, but contained similar amounts of oil (64.7 and 62% in cultivars 1 and 2, respectively, with ratios of oil:fruit of 1.4 and 1.54, respectively). The fatty acids (palmitic, oleic, stearic, linolenic and linoleic acids) and triglycerides compositions of oils of both cultivars were similar (although cultivar 1 was richer in palmitolino-olein (18.5 compared with 14.1%) and cultivar 2 was richer in dipalmito-olein (24.6 compared with 16.2%))" [7].

"The tree is also a source of many herbal medicines. It has long been used in the traditional medicine of some African countries to treat various ailments such as wounds, skin diseases, dysentery, and fever. The extracts and secondary metabolites have been found to show antimicrobial and antioxidant activities" [8]. "A wide range of chemical constituents such as terpenes, flavonoids, tannins, alkaloids, and saponins have been isolated from the plant. The resin is sometimes burnt for lighting or used as a glue. The tree is used as an ornamental plant and is known to improve soil quality by providing large quantities of biomass" [6]. The aim of this study is to examine the phytochemical constituent and antimicrobial activities of avocado pear (*Persea Americana*) oil extract against some clinical pathogens.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Fruit Sample

Avocado pear fruit were purchased from Bisi market, Ado-Ekiti. The avocado fruit sample was cleaned with distilled water. It was sliced in

smallest size, oven-dried for two weeks at 50^{o}C and then milled into fine flour and kept inside a moisture proof polythene bag until further use.

2.2 Avocado Pear Fruit Oil Extraction

The grinded avocado pear material was placed in the extraction thimble. The weighed amount was placed in an extraction chamber which is suspended above the flask containing the solvent n-hexane and below a condenser. The flask is heated and the n-hexane evaporate is moved into the condenser where it convert into a liquid that trickled into the extraction chamber containing the plant materials. The extraction is designed so that when then solvent surrounding the sample exceeded a certain level it overflows and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the n-hexane extract is remove and n-hexane is evaporated by using rotator evaporator.

2.3 Phytochemical Screening

2.3.1 Test for alkaloids

A reaction of extract were treated with 3-5drops of wagner's reagent (1.27 g of iodine and 2 g of potassium iodine in 100 ml of water) and observed for the formation of reddish brown precipitate

2.3.2 Test for flavonoids

Two milliliter (2 mL) of extract was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicate the presence of flavonoid.

2.3.3 Test for Polyphenol

Five milliliter (5 mL) of each extract was treated with 2 ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underplayed with 1ml concentrated slphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

2.3.4 Test for saponins

To two milliliter (2 mL) of extract was added 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

2.3.5 Test for sterols

One milliliter (1 mL) of extract was treated with drops of chloroform, acetic anhydride and onc.H_2SO_4 and observed for the formation of dark pink or red colour.

2.3.6 Test for tannins

Two milliliter (2 mL) of extract was treated with 10%alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

2.4 Susceptibility Testing of Essential Oils on Organisms

The broth culture of each organism used were investigated; two were gram positive bacteria; *Staphylococcus aureus* and *Streptococcus pneumoniae*, two were gram negative bacteria; *Escherichia coli* and *Pseudomonas aeruginosa*, and fungal – *Candida albicans*. Different concentrations (0.1, 0.2, 0.3 and 0.4 g) of the extract was measured and dissolved separately in Dimethyl sulfoxide (DMSO) (0.4, 0.3, 0.2, 0.1 mL) respectively. These oil extracts were incorporated into sterilized paper disks made from No. 1 Whatman filter paper and allowed to stand for 24hrs. Nutrient agar was prepared according to the manufacturers specifications. The broth culture of each organism was inoculated into the prepared agar plates using sterilized cotton swabs as described by Ajayi and Akintola [9]. The disks with the different concentrations of oil extracts were placed, including one with water as a control, on the inoculated plates, and then incubated at 37^{o}C for 24hrs. The plates were observed for growth and the diameters of the zones of inhibition were measured in millimeter using a meter-rule.

2.5 Antibiotic Sensitivity Test

The antibiotic disk diffusion tests were performed for each of the test bacterial. Gram-positive and Gram-negative standard antibiotic discs were used for this test. The antibiotics susceptibility of the test bacteria were determined by disc diffusion method on Mueller-Hinton agar according to CLSI [10]. The antibiotic paper disks were aseptically, carefully and firmly placed on

the inoculated plates using sterile forceps. The plates were then inverted and incubated for 24 hours at temperature of 37 ^{o}C. After incubation, the plates were examined for growth and the diameters of zone of inhibition were measured and the results were interpreted with reference to CLSI [10].

2.6 Statistical Analysis

Three replicates of each sample were taken and experiments were repeated thrice. The statistical analysis was done by ANOVA and significance of differences between replicates were measured at 5% ($P<0.05$).

3. RESULTS AND DISCUSSION

3.1 Results

The results obtained in this study are shown in the tables below. The phytochemical constituents present in avocado pear seed and pulp is shown in Table 1. The phytochemicals screened such as alkaloids, saponin, flavonoids, polyphenol, tannins, steroids are found to be present in samples of seed in the following percentages 0.109%, 1.189%, 2.72% 0.202 mg/100g, 4.512 mg/100g and 0.105 mg/100g respectively. These phytochemicals were present in the pulp of bush pear in the following quantities; 0.121% (alkaloids), 4.355% (saponin), 2.808% (flavonoids), 0.342 mg/100g (polyphenol), 2.509 mg/100g (tannins), and 0.05 mg/100 g (steroids).

Table 2 shows the antibacterial activity of oil extracts of Bush pear pulp on *E. coli*, *P. aeruginosa*, *Staph. aureus*, *S. pneumonia* and *C. albicans*. *P. aeruginosa* had the highest diameter of zones of inhibition of 12.00 – 30.00 mm at the concentrations of 0.1 – 0.4 g/mL respectively. *S. aureus* had diameter of zones of inhibition of 10.00 – 20.00 mm at the concentrations of 0.1 – 0.4 g/mL. *E. coli* had diameter of zones of inhibition of 10.00 – 15.00 mm at the concentrations of 0.1 – 0.4 g/mL respectively. *C. albicans* had diameter of zones of inhibition of 8.00 – 13.00 mm at the concentrations of 0.1 – 0.4 g/mL respectively; and *S. pneumonia* had diameter of zones of inhibition of 8.00 – 12.00 mm at the concentrations of 0.1 – 0.4 g/mL respectively. *S. aureus* had highest diameter of zones of inhibition of 10.00 – 12.00 mm at the concentrations of 0.2 – 0.4 g/mL. *S. pneumonia* had diameter of zones of inhibition of 8.00 – 11.00 mm at the concentrations of 0.1 – 0.4 g/mL respectively. *P. aeruginosa* had diameter of zones of inhibition of 9.00 – 11.00 mm at the concentrations of 0.2 – 0.4 g/mL respectively. *E. coli* had diameter of zones of inhibition of 8.00 – 10.00 mm at the concentrations of 0.2 – 0.4 g/mL respectively; and *C. albicans* had the lowest diameter of zones of inhibition of 7.00 and 9.00 mm at the concentrations of 0.3 and 0.4 g/mL respectively.

The susceptibility pattern of the test organisms (gram positive and gram negative) against conventional antibiotics is shown in Tables 3 and 4. *Staph aureus* was susceptible to the entire conventional antibiotic discs. *S. pneumonia* was also susceptible to the conventional antibiotic discs with the exception of Septrin (SXT) and Ampiclox (APX); while C. albicans was resistant to Amoxil (AMX) (Table 5). The susceptibility pattern of gram negative bacterium *P. aeruginosa* was observed on the antibiotic discs, except Septrin (SXT) and Chloramphenicol (CH); and *E. coli* was resistant to Sparfloxacin (SP) and Augmentin (AU) (Table 4).

Table 1. Qualitative and quantitative phytochemical screening of avocado pear seed and pulp

Parameters	Seed		Pulp	
	Qualitative	**Quantitative**	**Qualitative**	**Quantitative**
Alkaloids	+	0.109%	+	0.121%
Saponin	++	1.189%	+	4.355%
Flavonoids	++	2.72%	++	2.808%
Polyphenol	++	0.202 mg/100g	+	0.342 mg/100g
Tannins	+	4.512 mg/100g	++	2.509 mg/100g
Steroids	-	0.105 mg/100g	+	0.05 mg/100g

- *Not present* **+** *Fair* **++** *Moderate*

Table 2. Antibacterial activity of oil extract of avocado pulp against test bacterial and fungal species

Test organisms	Diameter of zones of inhibition (mm)							
	Concentration (g/mL)							
	0.1		0.2		0.3		0.4	
	Pulp	Seeds	Pulp	Seeds	Pulp	Seeds	Pulp	Seeds
E. coli	10.00±0.01^{a}	0.00±0.00^{a}	10.00±0.01^{a}	8.00±0.01^{a}	14.00±0.01^{a}	9.00±0.03^{b}	15.00±0.02^{b}	10.00±0.01^{a}
P. aeruginosa	12.00±0.02^{a}	0.00±0.00^{a}	15.00±0.01^{b}	9.00±0.03^{b}	20.00±0.01^{a}	10.00±0.01^{a}	30.00±0.01^{a}	11.00±0.03^{a}
S. aureus	10.00±0.01^{a}	0.00±0.00^{a}	13.00±0.03^{c}	10.00±0.01^{a}	13.00±0.02^{a}	10.00±0.02^{b}	20.00±0.02^{b}	12.00±0.01^{a}
S. pneumoniae	8.00±0.03^{b}	8.00±0.01^{a}	8.00±0.02^{a}	9.00±0.03^{a}	10.00±0.01^{a}	10.00±0.02^{b}	12.00±0.03^{a}	11.00±0.02^{a}
C. albicans	8.00±0.02^{c}	0.00±0.00^{a}	10.00±0.01^{a}	0.00±0.00^{a}	10.00±0.02^{a}	7.00±0.04^{c}	13.00±0.02^{b}	9.00±0.03^{b}

Values are mean ± SD of triplicate determination. Samples with different superscripts within the same column were significantly (p≤0.05) different

Table 3. Antibiotic sensitivity of Gram positive bacterial isolates

Organism	Diameter of zones of inhibition (g/mL)							
	Conventional antibiotic discs							
	CPX	RD	CN	AML	S	SXT	ERY	PEF
S. pneumoniae	13.00±0.02^{a}	15.00±0.02^{b}	14.00±0.02^{b}	13.00±0.02^{b}	21.00±0.02^{b}	0.00±0.00^{c}	18.00±0.02^{b}	21.00±0.02^{b}
Staph. aureus	21.00±0.01^{a}	19.00±0.03^{d}	22.00±0.01^{b}	22.00±0.01^{b}	21.00±0.02^{a}	20.00±0.02^{a}	21.00±0.02^{a}	20.00±0.01^{c}

Values are mean ± SD of triplicate determination. Samples with different superscripts within the same column were significantly (p≤0.05) different
CPX – Ciprofloxacin; RD – Rifampicin; CN – Gentamycin; AMX – Amoxil; S – Streptomycin; SXT – Septrin; ERY – Erythromycin; PEF – Pefloxacin

Table 4. Antibiotic sensitivity of Gram negative bacterial isolates

Organism	Diameter of zones of inhibition (g/ml)								
	Conventional antibiotic discs								
	SXT	S	CH	SP	CPX	AM	AU	CN	PEF
E. coli	12.00±0.02^{c}	13.00±0.01^{c}	13.00±0.02^{b}	0.00±0.00^{a}	6.00±0.03^{a}	5.00±0.04^{a}	0.00±0.00^{a}	9.00±0.03^{c}	12.00±0.02^{c}
P. aeruginosa	0.00±0.00^{a}	15.00±0.02^{b}	0.00±0.00^{a}	15.00±0.01^{c}	22.00±0.02^{c}	21.00±0.03^{b}	16.00±0.02^{b}	15.00±0.03^{b}	21.00±0.02^{c}

Values are mean ± SD of triplicate determination. Samples with different superscripts within the same column were significantly (p≤0.05) different
SXT – Septrin; S – Streptomycin; CH – Chloramphenicol; SP – Sparfloxacin; CPX – Ciprofloxacin; AM – Amoxacillin; AU – Augmentin; CN – Gentamycin; PEF – Pefloxacin

3.2 Discussion

"The seed and pulp of bush pear was observed to contain alkaloids, saponin, flavonoids, polyphenol, tannins, steroids. However, some of the phytochemicals present in the fruit oil such as flavonoids, alkaloids, and tannins as reported" by Ajibesin [6], Okwu and Nnamdi [11], and Duru et al. [12] were either absent or not detected in the seed oil extracted. The significant antimicrobial properties of the oil extract could be attributed to the presence of these bioactive compounds. Tannins exert their antimicrobial effects through mechanisms such as membrane disruption, binding to proteins, enzyme inhibition, substrate deprivation and metal ion complexation [13]. Alkaloids produce antimicrobial effects by interfering with processes such as deoxyribonucleic acid (DNA) replication and ribonucleic acid (RNA) transcription which are vital to microbial functioning [14]. Saponins are classes of glycosides which demonstrate antifungal properties [13]. Synergistic interactions between some of these chemical groups may produce greater activity against pathogenic microorganisms.

"The results of the antimicrobial test on the oil extract of *P. americana* pulp showed good, noticeable, and remarkable activity against the test *P. aeruginosa* as compared with the standard antibiotics. The seed oil showed appreciable antibacterial activities against the test organisms. This corroborates the earlier findings on antimicrobial efficacy of the crude extracts of *P. Americana"* [6]. However, the susceptibility pattern obtained is higher than report by Anyam et al. [15] "where ethyl acetate, chloroform, hexane and methanol extract were tested against similar clinical isolates. Antibacterial activity of these plants may be due to the saponins, quinones, cardiac glycosides, terpenoids, and phenol identified in the plants". Meanwhile, Bhat and Al-Daihan [16] in their research has reported similar phytochemicals.

"Several researchers have reported that plants contain bioactive substances" [17,18,19]. "The results of the present study corroborate the reports of previous workers. The results demonstrates that oil extracts of *P. americana* seed and pulp had significant *in vitro* antimicrobial activity on both gram positive and gram negative bacteria. However the degree of inhibition by the extracts varied with oil extract of seed being more effective than the oil extract of pulp. This is possibly due to the better solubility of the bioactive components in oil compared to water". This is in agreement with earlier report by Ibekwe et al. [20].

The fact that the oil extracts were active against both gram positive and gram negative bacteria tested indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing food preservative or therapeutic substances that will be active against food spoilage and multidrug resistant organisms. The gram negative bacteria *Pseudomonas aeruginosa* was more susceptible to the activity of *P. americana* seed extract compared with some of the gram positive bacteria tested such as *Staph. aureus* and *S. pneumonia*.

Thus, susceptibility of this gram positive isolates is a good indication of high efficacy against these bacteria. Furtherance to this oil developed from the avocado pear will rapidly control the infections caused by these bacteria in humans. The resistivity of some gram negative organisms obtained in this study may be due to the inherent resistance of some gram negative organisms to a large number of antimicrobial agents. This is an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds in *P. americana* seed and pulp extract.

4. CONCLUSION

The oil extracted with n-hexane as solvent from *Persea americana* has shown to be composed mainly of alkaloids, saponins, flavonoids, polyphenol, tannins, steroids. The oil was found to have antimicrobial properties, which leads us to believe that the use of oil extract of the plant in traditional medicine may be justified. The results of the present study showed that the oil extracts of avocado pear (*Persea americana*) possess potential antibacterial activity against some organisms and it is believed that these findings reported for oil extract of *P. americana* will be helpful to many researchers in the field of antibacterial activities in plant. And oil extract of avocado pear is recommended for treatment of skin infections inherent with these organisms.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not

intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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