Antimicrobial activity and preliminary phytochemical screening of oil extracted from food waste generated from *Persea americana*

BAMIDELE Florence Adebowale[1]*, OGUNDIPE Flora Olubunmi[1], OSAIDE Stella Oritsegbubemi[2], CHUCKU Godwin Josiah[1] and OLONODE Elizabeth Mayowa[1]

[1]Department of Biological Science, School of Science, Yaba College of Technology, Lagos, Nigeria.
[2]Biology Department, College of Education, Warri, P. M. B. 1251. Delta State, Nigeria.
*Corresponding author. E-mail:floxykunmi2010@yahoo.com.

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Persea americana (Mill) commonly known as avocado is a fruit consumed globally with great potentials for the pharmaceutical, food and cosmetics industries. However, the seeds and peels of this plant are commonly discarded as waste. This study was conducted to determine the antimicrobial activity and preliminary phytochemical screening of the oil from the seeds and peels of avocado against selected bacterial and fungal isolates previously characterized in the laboratory. The oil was extracted using soxhlet apparatus with n-hexane as extraction solvent. Efficacy was assessed using agar well diffusion method at various concentrations on Mueller Hinton agar. The result revealed that the extract from the seeds and peels were effective on *Penicillium notatum* (42.40 mm), *Pseudomonas aeruginosa* (35.30 mm), *Escherichia coli* (27.25 mm), *Enterococcus faecium* (23.21 mm), *Enterococcus faecalis* (21.20 mm) and *Aspergillus niger (*20 mm) respectively. The peel extract had no inhibitory effect against *Aspergillus niger* even at the highest concentration tested. Phytochemical screening of the peels showed the presence of tannins (2.43%), Saponins (7.75%), flavonoids (22.05%), alkaloids (1.21%) and cyanogenic glycosides (15.26%).The high level of flavonoids favors its antioxidant properties. The seeds contains saponins (4.14), flavonoid (4.22), alkaloid (2.25), glycoside (19.08), steroid (12.87), reducing sugar (4.20) and phenolic compound (8.12). DPPH radical scavenging value was found to be 10.753 ± 4.301 µg/ml for peels and 26.344 ± 3.495 µg/ml for seeds against standard ascorbic acid 95.11±89.784. Hence, eluting and purification of the active ingredients in the extracts can make them promising alternative to orthodox drugs; value added products for the cosmetics and food industries as well as reduction in environmental pollution.

Key words: Antimicrobial, avocado, food waste, phytoconstituents.

INTRODUCTION

In several parts of Nigeria, Avocado (*Persea americana)* is widely grown as fruit crop and used medicinally for treating many diseases by local unorthodox medical practitioners. Avocado oil has also generated growing interest among consumers due to its nutritional values, which is evidenced by an increase in the number of technological characteristics and scientific articles (Bullo, 2021). The avocado plant is erect and when they are grown on shallow soil they may not reach more than 30 ft in height while they reach up to 60 ft when grown on deep moist clay loams. The leaves are evergreen and the flowers unisexual.

Their leaf blades are multiform shape, lanceolate, elliptic, lanccolate elliptic, oblong elliptic, oval, ovate and obovate. Different varieties of avocado have been classified by different authors such as Sharma et al. (2015) and Tremocold et al. (2018) this may be due to time of production, amount of fruit, nutritional content of fruit, resistance to disease and transportation and so many other parameters. Avocado peels and

seeds contains diverse active compounds (phenolic acids and flavonoids), that acts effectively against pathogenic bacteria and fungi, demonstrating high antimicrobial activity (Vinha, 2020). The peel of avocado has been seen as waste products in Nigeria but the potentials in this products can be explored and used as value-adding natural products in pharmaceutical and food industries. Avocado oil has also been used in the production of biodegradable polymers (Faraja et al., 2018), cosmetics and culinary purposes (Ivan et al., 2020), as an anti-oxidant, anti-wrinkle and to lower blood pressure (Patel et al., 2019).

Oil extraction from avocado can be generated from the different parts of the plant. The extraction of oil can be done using solvents and several methods such as soxhlet extraction, centrifugation etc. Fixed oil and fat are present in many plants such as avocado peel, castor seed, olive, peanut, soybean, sesame, almond, cotton seed, corn, safflower, cocoa butter, linseed, sunflower, oil palm and shear butter. It is also a component of animal fats especially milk, meat and egg. The presence of these substances in human and in its food has been observed to confer some physiological functions as well assist in the prevention and or treatment of some diseases and infections. Different studies have reviewed the antibacterial potentials of fixed oils and their components.

Of note is the study of Dhifi et al. (2016), who pointed out that the important features of avocado oils is their hydrophobicity which allows them to break into lipids of bacteria. They noted that this feature permits the disrupting of the cell membrane structure, and making it more permeable. Paula et al. (2020) in their review on the bioactive compounds and healthy benefits of avocado fruit pointed out that its pulp and wastes (seeds, leaves, and peel) has shown antibacterial effect on *Escherichia coli* and *Staphylococcus aureus.*

Some of the important phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. which are distributed in various parts of the plants. Nature is a unique source of structures of high phytochemical diversity representing phenolics (45%), terpenoids and steroids (27%) and alkaloids (18%) as major groups of phytochemicals. Although, these compounds seem to be non-essential to the plant producing them, they play a vital role in survival by mediation of ecological interactions with competitors, protect them from diseases, pollution, stress, UV rays and also contribute to colour, aroma and flavour with respect to the plant. The metabolites produced by the plants to protect themselves against biotic and abiotic stresses have turned into medicines that people can use to treat various diseases (Shaikh and Patil, 2020). Plants of important medicinal value like the avocado peels are often cheaper, locally available and easily consumable (Olutayo et al., 2013)

By-products such as peel and seed are domestically generated from avocado fruits after consumption. Hence, it is very essential to reutilize these waste products in order to curtail their negative impacts in the environment and for the purpose of adding value to the consumers seeing that they are source of important phytochemicals and antioxidants. Therefore, this study was conducted to determine the antimicrobial activity and preliminary phytochemical screening of the oil from the peels and seeds of avocado against selected bacterial and fungal isolates. In order to achieve maximum productivity, use of avocado by-products which are cheap and have no adverse impact on the environment should be exploited.

MATERIALS AND METHODS

Collection of samples

50 Samples of avocado fruit were purchased from Oyingbo market, Lagos state and was thoroughly washed with under clean running water. The peel, pulp and seeds were separated. Thereafter, the peels and seeds were further washed to remove the sticky pulps from the inner layers. They were air dried at ambient temperature for 5 days, further drying in an electric oven before being pulverized using a blender

Preparation of samples

The extraction using the hexane solvent was used in this experiment. The soxhlet extractor was used for the continuous extraction of the oil from

the avocado seed and peels using n-hexane as the solvent. 200 ml of n-hexane (Analytical grade, 203-77-6, Merck Germany) was dispense into a conical flask, 100 g of the pulverized peel inside a muslin cloth was placed in the heating mantle and left to run for two 2 h at a temperature of 600°C. The extracted oil it was then placed over a rotary evaporator for 5 mins at a temperature of 70°C (to concentrate the extract and remove traces of residual solvents from the solution) as described by Sunmonu et al. (2017). 200 g of the pulverized avocado seed sample was hydro distillated using a Clevenger apparatus. The oil distilled was collected and kept until ready to use.

Antimicrobial activity

Antibacterial activity was evaluated using the bacterial isolates *E. coli* and *Pseudomonas aeroginosa, Enterococcus faecalis*, *Enterococcus faecium* ,*Penicillum notatum*, and *Aspergillus niger*. These isolates were obtained from Microbiology laboratory of Yaba College of Technology, Yaba, Lagos. They were maintained on appropriate agar and kept in the refrigeration at 4°C before the experiment.

Agar well diffusion method as described by Zamirah et al. (2013) was used to determine the antimicrobial activity of the extracts. The inoculums were standardized to 0.5 McFarland using a densitometer and were aseptically inoculated onto the Mueller-Hinton agar. Wells of 5 mm diameter were made with a sterile cork-borer on Mueller Hinton plates that had been previously seeded with the bacterial culture. Each well was aseptically filled with 20, 40 60, 80 and 100 µl of the extract using a micropipette. The plates in duplicate were incubated at 37°C for 24 h. The diameter of zones of inhibition was measured in millimeter (mm).

Fungi

The agar well diffusion method was used to test antifungal activity of the extract as described by Alastruey-izquierdo et al. (2015) with slight modification. The inoculums were standardized to 0.5 McFarland using a densitometer and were aseptically inoculated onto the Mueller-Hinton agar that has been supplemented with 2% glucose and 0.5 mg/L Methylene blue dye. Using a sterile corkborer, equidistance holes of 0.5 mm were made on the agar medium, then the extract, positive control (Gentamicin 10 µg), negative control (2% DMSO) were introduced into their appropriate holes. This was carried out in duplicates. Plates were incubated at 25°C for 48 h and results taken.

Phytochemical screening of extract

These analyses were carried out as described by Dai et al. (2015).

Antioxidant determination

The antioxidant activity of each extract were evaluated on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)- free radical activity, in comparison with Ascorbic acid standard, by a slightly modified method employed by Atewolara-Odule et al. (2020). Ascorbic acid standards (concentrations 0.2, 0.4, 0.6, 0.8 and 1.0) were prepared from stock solution in triplicates using suitable dilution. 0.1 mM of DPPH was prepared in methanol and 1 ml of this solution was mixed with 3 ml of the standard solutions in test tubes. These solutions were shaken, then they were allowed to stand for 30 mins and absorbance was measured at 517 nm using UV-VIS Spectrophotometer. Methanol (3 ml) with DPPH solution (0.1 mM, 1 ml) was used as control. Methanol was used as blank. The same procedure as with the Ascorbic acid standard was carried out with each of the extract concentrations.

RESULTS

Antibacterial activity of the extract showed that the higher the concentration the greater the inhibition zones (table 1). Gentamicin act as the positive control while 2% DMSO was used as the negative control. The activity of extracts on fungal isolates in table 2 showed From the table, no significant inhibition zone was noticed at 20 µg concentration and the higher the concentration, the higher the zones of inhibition. Amphotericin B as positive control and DMSO-Dimethyl sulfoxide (2%) as negative control. ualitative analysis of the oil extracted from the peels and seeds of avocado, revealing the

Table 1. Antibacterial susceptibility test of extracts showing inhibition zones measured in mm.

Isolates	CP (20 µl)	CS (20 µl	CP(40 µl)	CS(40 µl	CP(60 µl)	CS(60 µl	CP(80 µl)	CS(80 µl	CP (100 µl)	CS(1000 µl)	(+)Control (Gentamicin 10 µl)	(-)Control (DMSO)
Escherichia coli	15	27	20	22	11	17	20	24	25	27	30	5
Pseudomonas sp.	17	18	21	20	16	17	20	22	30	35	21	5
Enterococcus faecalis	15	10	20	22	19	19	19	23	18	21	21	5
Enterococcus faecium	19	19	20	12	20	17	21	20	15	23	26	5

Key: CP= Concentration of peel oil, CP= Concentration of seed oil, DMSO= Dimethyl sulfoxide

Table 2. Antifungal Susceptibility Test of Extract Showing Inhibition Zones Measured in mm.

Isolates	CP (20 µl	CS (20 µl	CP (40 µl	CS (40 µl	CP (60 µl	CS (60 µl	CP (80 µl	CS (80 µl	CP (100 µl	CS (100 µl	Amphotericin B	DMSO(2%)
Aspergillus niger	6	8	8	11	10	14	11	17	10	20	23	5
Penicillum notatum	8	9	13	13	20	17	25	34	40	42	23	5

presence of Terpenoids as the most abundant (+++) in both samples. Qualitative analysis of the oil extract revealed that flavonoids had the highest value of 22.01 in peels, while the highest value of 19.08 was found in cardiac glycoside in the seed oil (table 4).

DISCUSION

This work aims at determining the antimicrobial and the phytochemical activities of the fixed oil extracted from *Persea americana* (Mill) peel and seeds. This work was carried out between the month of February and April 2021. The colour of the oil from the peel and seed was greenish which were in agreement with the emerald green colour reported by Aletan *(*2018). This green colour is attributed to high levels of chlorophylls and carotenoids in the oil, thus indicating high level of chlorophyll which is a natural source of magnesium, a natural substances for removing heavy metals such as mercury and lead from major organ in the body. It therefore shows that avocado peel and seed oil can be used to detoxify the body.

Table 1 and 2 shows the antimicrobial activity carried out on the extract using two isolates of Gram positive (*E. faecalis* and *E. faecium*) and two isolates of Gram negative bacteria (*E. coli* and *Pseudomonas* sp.) along with two fungal isolates (*P. notatum* and *A. niger).* Gentamicin antibiotic and Amphotericin B sensitivity disc were used as a positive controls with DMSO (2%) as negative control. It was observed that the isolates vary in their susceptibility pattern to the extract and to the positive controls as well. Furthermore, the result shows that the strongest antimicrobial activity of the extract was shown at concentration 100µl against *P. notatum*, *E. coli*, Pseudomonas sp. *E. faecalis*, *E.s faecium* and *Aspergillus niger* with zones of 40, 25, 25, 21,18 and 0 for peels and 42, 27, 35, 21, 23 and 20 mm for seeds respectively. The weakest activity was at concentration of 20µg against all the test isolates.

Table 3 shows the qualitative analysis of the oil, revealing the presence of terpenoids as the most abundant (+++). The result agrees with the work of Olutayo et al. (2013), who

Table 3: Qualitative analysis of the extract from the peels and seeds oil.

S/N	Qualitative analysis		Peel oil	Seed oil
		METHOD		
1	Alkaloids	Mayer's Test	-	-
		Dragendorff's Test	Trace	Trace
		Wagner's Test	-	Trace
2	Saponins	Frothing Test	++	+++
3	Reducing Sugar	Fehling's Test	+	+
4	Anthraquinones	Borntrager's Test	-	-
5	Cardiac glycosides	Keller Killani's Test	++	+++
6	Terpenoids	Liebermaan-Burchard	+++	+++
7	Steroids	Salkowski's Test	++	+++
8	Phenolic Compounds	Lead acetate Test	++	+
9	Tannins	Ferric chloride Test	+	-
10	Flavonoids	Shinoda's Test	+	+

Table 4. Quantitative analysis of peel and seed oil (mg/g).

S/N	Phytoconstituents	Peels oil	Seed oil
1	Alkaloids	1.21	2.25
2	Saponins	7.75	4.14
3	Reducing Sugar	11.91	4.20
4	Cardiac glycosides	15.26	19.08
5	Steroids	7.27	12.87
6	Tannins	2.43	-
7	Phenolic comps	17.29	8.12
8	Flavonoids	22.01	4.22

worked on the Phytochemical and antioxidant properties of some Nigerian medicinal plants. Slightly different from this result is the work of Vinha (2020), who reported that the peel of the Hass avocado varieties collected from Porto area of Portugal had the highest carotenoid content, which could be attributed to the variety and cultivars of avocado fruit used.

Table 4 shows the quantitative analysis of the extract, which reveals that flavonoids possess the highest mean value of 22.01 for peel and cardiac glycoside for seeds. This is in support with the work of Aletan (2018), who carried out the proximate and physiochemical analysis of the fruit and oil of Avocado pear purchased from Ogun state, Nigeria. Tremocoldi et al. (2018) demonstrated that "Hass" and "Fuertes" varieties peel presented superior phenolic contents. Thus, the differences could be attributed to the methods of extraction and extracting solvent used in the various analyses. Furthermore, the result of flavonoids in this work agrees with that of Nwaoguikpe et al. (2011) and Aletan (2017) in his work on proximate and physicochemical analysis of the fruit and oil of avocado pear found tannins, phenols, flavonoids, steroids in the three parts of the fruit. Saponins and alkaloids were found in the skin and seed but not in the flesh. Terpenoids were however found only in the flesh. Phlobatannin, anthraquinone and cardiac glycosides were however not found in the extracts from any part of the fruit, this is in agreement with this present work as phlobatannin and anthraquinone were not found. But on the other hand, cardiac glycosides were found in the extracts. This difference may be as a result of species differences in the avocado used for the various studies or climatic condition and soil type that is used for the cultivation of the fruits.

The apparent fact from several studies is that phytochemicals abound in avocado pear fruit (Both in the peel, pulp and seed. Sharma et al. *(*2015), in his work pointed out that the tannins, phenols, flavonoids, steroids which are present in the three parts of the avocado fruit are known to

possess anti-diabetic effects acting through various mechanisms. Furthermore, terpenoids found in the flesh have shown inhibitory effects on various forms of tumors. Alkaloids present in the seed and skin can exacts anti-arrhythmic effects, antihypertensive effects, anticancer and anti-malarial activity. Saponins, also detected in the skin and seed can enhance antibody production, inhibit tumor growth, and prevent hyper-lipidemia and liver injury.

The absorbance of the extracts at different concentrations decreases with equivalent decrease in concentration and shows higher antioxidant capacity than the ascorbic equivalent (AAE) per ml. Generally, the Avocado peel has greater antioxidant activity followed by seed and then the pulp as supported by Rodroguez-carpena et al. (2011). It is possible that the higher antioxidant capacity of the peel may be related to the higher content of antioxidant compounds. DPPH was chosen for it rapid, stable, simple and inexpensive attributes in antioxidant assay. Also, this could be as a result of the type, variety, degree of ripeness, climate and cultivation, processing and storage processes. Different varieties of avocado show different morphological characteristics and yields in their peels, Paula et al. (2020), in their work, reported that peel, seed, and leaf of avocado shows higher total polyphenols content than the pulp. On the other hand, they reported the lowest TPC from extraction with hexane extract.

The work of Bhuyan et al. (2019) on avocado peel, has shown some total carotenoids content (TCC) from 0.89 to 2.6 mg/100 g. They highlighted that carotenoids, lutein, zeaxanthin, and carotene found in the peel of the avocado are potent free radical scavengers. The activities of the fixed oil and their constituents can be singly or multiply targeted on a substrate (microorganism). However, they identified the lack of *in vivo* studies invalidating the antimicrobial activity of avocado. By and large, these results has demonstrate the potential of the non-edible parts of the avocado as a rich source of bioactive compounds. Instead of being wasted as trash, they could constitute an inexpensive source of carotenoids in the cosmetic which makes them an essential ingredient of several dermatological formulations. They can also be harnessed as a source of compounds used as food additives or functional food ingredients. Results showed the pure form of oil that can be employed for nutritional and industrial purposes and for pharmaceutical formulations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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