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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF SEED EXTRACTS OF *PERSEA AMERICANA* (AVOCADO PEAR)

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ABSTRACT
Antimicrobial activity of seed extracts of Persia amerciana against Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Corynebacterium ulcerans, Salmonella typhi, Neisseria gonorrhoea and Candida albicans was carried out using the disc diffusion technique. The methanol, ethyl acetate and chloroform extracts demonstrated promising activity against the test organisms. The activity of methanol extract was more pronounced against C. albicans (42mm) while that of petroleum ether extract was the least against E.coli (6mm). However, S. typhi and E. coli were resistant to chloroform and methanol extracts. The activity of the ethyl acetate, chloroform and methanol extracts compared favourably with that of standard antibiotic streptomycin (30µgdics^{-1}). The minimum inhibitory concentration (MIC) showed that methanol and ethyl acetate extracts had the lowest MIC value (10mg/ml) against C. albicans, indicating higher potency. Preliminary phytochemical screening revealed the presence of flavonoids, saponins, tannins, steroids alkaloids and terpenoids. The spectra of antimicrobial activities displayed by the extracts could be attributed to the presence of these phytochemicals and signifies the potential of Persia amerciana as a source of therapeutic agents. This therefore, supports the traditional medicinal use of Persia amerciana and suggests that further studies should be conducted to isolate and identify the active components of the extract.

Keywords: Phytochemical, antimicrobial screening, Persea americana, seeds, MIC

INTRODUCTION

Medicinal plants have continued to attract attention in the global search for effective antimicrobial agents that can combat resistant pathogens that have been rendering many conventional drugs obsolete in the treatment of infections (Cox, 1990). Many important drugs used in medicine today are directly or indirectly derived from plants. The most important of these bioactive constituents of plants are alkaloids, tannins, steroids, terpenoids and phenolic compounds (Hill, 1952). In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents (Krishnaraju *et al*., 2005). Thus it is anticipated that phytochemicals with adequate antimicrobial efficacy will be used for the treatment of the bacterial infections (Balandrin *et al*., 1985).

Persea americana (Lauraceae) is one of the 150 varieties of avocado pear (Pacific Health, 2005). The tree is widely cultivated in tropical and subtropical area, with a height of about 80 feet, leathery, evergreen leaves, the flowers are rarely unisexual. The seed of *Persea americana* has a diverse application in ethnomedicine, ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites to the area of skin treatment and beautification (Pamplora and Roger, 1999). *P. americana* leaves have been reported to possess anti-inflammatory and analgesic activities (Adeyemi *et al*., 2002). The seeds are rich in tannins and carotenoids and tocopherols from the fruit were shown to inhibit the *in-vitro* growth of prostate cancer cell lines (Lu *et al*., 2005) and "persin" from avocado leaves was shown to have antifungal properties and to be toxic to silkworms (Oelrichs *et al*., 1995). The effect of *P. americana* extract was evaluated on *in-vitro* rat lymphocyte proliferation (Gomez-Flores *et al*., 2008). Antioxidant activity and phenolic content of seeds of avocado pear was found to be greater than 70% (Soong and Barlow 2004). In this study, the phytochemical and antimicrobial activity of extract fractions of *P. americana* was carried out with a view to provide scientific basis on the claim by traditional healers of its uses in traditional medicine in the context of the continued search for active therapeutic agents from plants.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The fruit of *Persea americana* (Avocado pear) were collected from Southern parts of Plateau State of Nigeria. The identity of fruit was confirmed and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and a voucher specimen number 992 was deposited. The inner seed were removed from the fruit by cutting with knife, air dried and ground into powered using a porcelain mortar and pestle.

Extraction
The powdered material (500g) was extracted exhaustively using continuous extraction method with (700ml) each of petroleum ether (60-80^{0}C) (PE), chloroform (CF), ethyl acetate (EA) and methanol (ME) in a soxhlet extractor (Harborne, 1973). The various extracts were concentrated in vacuo at 40^{0}C using a rota vapour, after which 5.23g, 3.42g, 3.25g and 11.34g of the extracts referred to as (PE), (CF), (EA) and (ME) were obtained.

Phytochemical screening
Phytochemical screening of the extract and fractions were carried out to identify the constituents, using standard phytochemical methods as described by Trease and Evans (1989) as well as Sofowora (1993). The screening involves detection of alkaloids, flavonoids, steroids, terpenoids, saponins, tannins, anthraquinones, cardiac glycosides and cynogenetic glycosides.

Test organisms
Ten clinical isolates including nine bacteria (*Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis*, *Streptococcus pyogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Corynebacterium ulcerans, Salmonella typhi, Neisseria gonorrhoea*) and one fungus (*Candida albicans*) were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, Nigeria. The bacterial isolates were maintained on nutrient agar and sub-cultured every three days. An inoculum of each isolate was suspended in 5 ml of Mueller Hinton broth (MHB) and incubated overnight at 37 ºC. The overnight cultures were diluted with Mueller Hinton broth and adjusted to give a concentration of bacterial cells equivalent to a McFarland 0.5 standard prior to the bacterial testing (Samie *et al.*, 2005).

Determination of antimicrobial activity
The disc diffusion method was used (Nostro *et al.,* 2000). Stock solutions (100 mg mL^{-1}) of each extract were prepared using the diluents. Discs (6 mm diameter) were prepared using Whatman filter paper and sterilized by autoclaving at 0 ^{o}C. The blank sterile discs were placed on the inoculated Mueller Hinton Agar (MHA) surface and impregnated with 15µL of stock solutions (1500µg dics^{-1}). An antibiotic disc of streptomycin (30µg dics^{-1}) was used as control. The plates were incubated at 37ºC for 24h. All tests were performed in duplicate and the antimicrobial activity was expressed as the mean diameter of inhibition zones (mm) produced by the plant extracts.

Determination of Minimum inhibitory concentration [MIC]
Minimum inhibitory concentration was carried out using broth dilution as previously reported by Lennette *et al.,* (1974). Dilutions (10-90 mgmL^{-1}) of concentrations of extract and fractions that exhibited sensitivity against the test organisms were prepared using test tubes containing 9 ml of double strength broth. The test tubes were inoculated with (0.2 ml) suspension of the standardized inocula and incubated at 37ºC for 24h for bacteria while at 22^{o}C for 48h for fungus. MICs were recorded as the lowest concentration of extract showing no visible growth of the broth.

RESULTS
The preliminary phytochemical screening showed that the seed extracts were contain steroids and terpenoids in all the four solvent extracts while tannins, cardiac glycoside and alkaloids are present in only methanol and ethyl acetate extracts. However, anthraquinones and cynogenetic glycoside were absent in all the extracts (Table 1). The PE extracts exhibited activity on all the test organisms producing zone of inhibition ranging from 6-27mm, the CF extract also showed a range of 12-37mm and the EA extract had 16-40mm while the ME extract showed a range of 15-42mm. The activity of methanol extract was more potent against *C. albicans* (42mm) while that of petroleum ether extract was least against *E. coli* (6mm). However, *S. typhi* and *E. coli* were resistant to chloroform and methanol extracts. The result of the minimum inhibitory concentration (MIC) showed that methanol and ethyl acetate extracts had the lowest MIC value (10mg/ml) against *C. albicans,* thus indicating a high potency.

Table 1: Phytochemical screening of *Persea americana* seed extracts.

S/No.	Phytochemicals	Extracts			
		Methanol	**Ethyl acetate**	**Chloroform**	**Petroleum ether**
1	Carbohydrates	+	+	-	-
2	Flavonoids	+	+	-	-
3	Steroids	+	+	+	+
4	Terpenoids	+	+	+	+
5	Saponins	+	+	+	+
6	Tannins	+	+	-	-
7	Anthraquinones	-	-	-	-
8	Cardiac glycoside	+	+	-	-
9	Alkaloids	+	+	+	-
10	Cynogenetic glycosides	-	-	-	-

Key: + = present - = absent

Table 2: Determination of antimicrobial activity of *Persea americana* seed extracts

	Zone of inhibition (mm)				
Test organisms	PE	CF	EA	ME	Streptomycin (30µg dics^{-1})
S. aureus	16	32	37	37	27
S. pyogenes	15	32	35	35	22
C. ulcerans	7	12	20	15	NT
B. subtilis	27	31	32	32	30
S. typhi	8	0	16	0	0
E. coli	6	0	16	0	0
K. pneumoniae	22	24	27	22	20
P. aeruginosa	16	28	22	32	NT
N. gonorrhoea	17	34	33	27	NT
C. albicans	9	37	40	42	NT

Key: PE= petroleum ether, CF= chloroform, EA=ethyl acetate, ME=methanol, NT=Not tested

Table 3: Minimum inhibitory concentration (MIC) of *Persea americana* seed extracts

	Concentration (mg/ml)			
Test organisms	PE	CF	EA	ME
S. aureus	40	20	20	20
S. pyogenes	40	20	20	20
C. ulcerans	50	40	30	40
B. subtilis	30	20	20	20
S. typhi	50	NT	40	NT
E. coli	50	NT	40	NT
K. pneumoniae	30	30	30	30
P. aeruginosa	40	30	30	20
N. gonorrhoea	40	20	20	30
C. albicans	50	20	10	10

PE= petroleum ether, CF= chloroform, EA=ethyl acetate, ME=methanol, NT=Not tested

DISCUSSION

The solvent extracts had demonstrated antimicrobial activity against the test organisms. The ethyl acetate extract was more potent with activity against all the test organisms especially against *S. aureus* (37mm), *S. pyogenes* (35mm), *C. ulcerans* (20mm) and *C. albicans* (40mm). The petroleum ether extract however, demonstrated the least activity against all the test organisms. Differences in polarity among the various solvents are perhaps responsible for the differences in solubility of plant active principles, hence variation in degree of activity. The activity of ethyl acetate extract against the Gram negative [*E. coli* (16mm) and *S. typhi* (16mm)] is impressive because Gram-negative bacteria tend to have higher intrinsic resistance to most antimicrobial agents (Ndukwe *et al.*, 2005). Demonstration of low MIC value (10 mg/ml) especially by the ethyl acetate and methanol extracts (Table 3) is an indication that the phytoconstituents of the plant have therapeutic potential. The activity of the ethyl acetate, chloroform and methanol extracts based on the zones of growth inhibitions, compared with or even surpassed that of standard antibiotic (streptomycin at 30µgdics^{-1}) as shown in Table 2.

Flavonoids, saponins, tannins, steroids and terpenoids were present in one extract or the other (Table 1). Flavonoids are known to be synthesized by plants in response to microbial attack. Hence, it should not be surprising that they have been found to be effective antimicrobial substances against a wide array of microorganisms, when tested *in-vitro*. Their activity is probably due to their ability to react with extracellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria (Cowan, 2002). Tannins are also reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically, tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins (Westendarp, 2006).

The spectra of antimicrobial activities displayed by these extracts could perhaps be explained by the presence of flavonoids, tannins, saponins and steroids. The purified components may have even more potency with respect to inhibition of microbes. The result of the present study signifies the potential of *P. americana* as a source of therapeutic agents, which may provide leads in the ongoing search for antimicrobial agents from plants. Further studies on the phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential(s) of the plant to inhibit several pathogenic microbes. In conclusion, the activity exhibited by the extracts against clinical microbial isolates that are associated with various infectious diseases, may provide scientific justification for the ethnomedicinal uses of the plant.

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