

ANTIBACTERIAL AND CYTOTOXICITY PROPERTIES OF LEAF EXTRACT OF *PERSEA AMERICANA*

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ABSTRACT

The crude methanolic extract obtained from the leaves of *Persea americana* was screened for phytochemical constituents, in vitro antibacterial activity using agar diffusion method and preliminary cytotoxicity test using the larvae of the brine shrimp, *Artemia salina*. Phytochemical screening gave positive results to flavonoids, cardiac glycosides, saponins and tannins. At a concentration of 20mg/ml, its exhibited antibacterial activities on all the studied bacterial. Streptomycin was included as a reference compound at concentration of 1mg/ml. The inhibition of growth of almost all the microorganisms compared moderately with that of streptomycin. In addition, the extract inhibited the growth of *Escherichia coli* (NCIB 86), which was not sensitive to streptomycin. The *Persea americana* extract was found to exhibit lethality against the brine shrimps. It was however found to be relatively toxic as the extract had an LD₅₀ value lower than 1000µg/ml.

KEY WORDS: *Persea americana*, Phytochemical, *Escherichia coli*, streptomycin.

INTRODUCTION

Persea americana (Mill.) (Lauracea), a native of tropical America (Mexico), is now widely cultivated throughout the tropics and subtropics of the world. Commonly known as Avocado pear, Alligator pear or Mexican avocado. It is an evergreen tree, 14-20 m high with large spreading and flat-topped crown and deeply fissured brown corky bole. The branches are grey and also fissured but the twigs are green and smooth (FAO, 1986). The bark, fruit and leaf are used in traditional medicine in South and Central America, West Indies and Africa for the treatment of various ailments such as menorrhagia (Watt and Breyer-Bradnijk, 1962), hypertension, stomach ache, bronchitis, diarrhoea and diabetes (Ramirez *et al.*, 1988). Oil extracted from the flesh of Avocado fruit is employed in making hair creams, hand lotion, facial creams and fine soap. The seed yields a milky fluid with the odour and taste of almond. Because of its tannin content which turns red on exposure, indelible red brown or blackish inks are made from it in the days of Spanish conquest.

Estragole has been isolated from the essential oil (Gonzalez-Coloma *et al.*, 1992), sitosterol, campesterol, and psitosterols (Phytochemical and Ethnobotanical database, 2003). Unripe avocados are said to be toxic. Two resin derived from the skin of fruit are toxic to guinea pig by subcutaneous and peritoneal injection. Ingestion of avocado leaves and/or bark has caused mastitis in cattle, horses, rabbits and goats (Standler *et al.*, 1991). Large doses have been fatal to goat. Cardiomyopathy in ostriches due to avocado intoxication has also been reported (Burger *et al.*, 1994). In continuation of the search for medicinal plants with antimicrobial and brine shrimp lethality potentials in our laboratory, the present work was designed to evaluate the antibacterial activity and cytotoxicity test on brine shrimp larvae of the crude extract of *Persea americana*.

MATERIAL AND METHODS

Plant Materials

The fresh leaves of *Persea americana* were collected in October 2001 at Fagboto's compound, Ilesa road, Akure, Ondo-State, Nigeria. Mr. S. T. Arannilewa of the Department of Biology, Federal University of Technology, Akure, Nigeria, authenticated voucher sample. It was deposited in the herbarium (Code number OB 002) of Biochemistry Department, Federal University of Technology, Akure, Nigeria.

Preparation of the crude extracts of the leaves of *Persea americana*

Persea americana leaves were air-dried for 21 days at room temperature. The air-dried sample was ground to a mesh size of 1 mm. A 350 g sample of each of the powdered materials was soaked in 1000 ml of a mixture of methanol and water (4:1) for 96 hours. This was filtered and concentrated to a small volume to remove the entire methanol using rotary evaporator. The small volume was later freeze-dried and kept in the freezer at 4 °C for further studies.

Phytochemical Screening

The methanolic extract was screened for the presence of alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, flavonoids and phlobatanins according to the methods described by Sofowora (1993).

Antibacterial activities and Minimum Inhibitory Concentration

Antibacterial activity and the minimum inhibitory concentrations of the plant extract was studied using agar disc-diffusion method (Schillinger and Kucke, 1989).

Cytotoxicity Test

Seawater was put in a soap case (partitioned into dark and light area). Brine shrimp eggs were added to the dark side and covered. The set up was left in a well lit place for 48 hours. The hatched eggs, which swarm to the lit side, were used for the bioassay. 20 mg of each of the extracts was dissolved in 2 ml of sea water. From this solution, 500, 50 and 5µl were transferred into vials and made up to 5 ml. The corresponding concentrations were 1000, 100 and 10 µg/ml, respectively. Ten (10) brine shrimps (nauplii) were transferred into each of these vials using Pasteur pipette. Replicates of each of the dose levels were prepared, using seawater as control. Number of survivors, deaths, and nauplii with sluggish movement were recorded, 24 hours later. Data were processed (Using probit analysis) to estimate LC₅₀ values at 95% confidence interval for statistically significant comparisons of potencies; LC₅₀ less than 1000µg/ml was considered as potent/toxic (Gupta *et al.*, 1996).

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Table 1: Phytochemical constituents of crude extracts of leaves of *Persea americana*

Metabolites	<i>Persea americana</i>
Saponins	+
Tannins	+
Flavonoids	+
Anthraquinones	-
Alkaloids	-
Cardiac glycosides	
Cardenolides	+
Steroidal ring	+
Deoxy sugar	+

Statistical Analysis

Data obtained were expressed as mean \pm SD (Zar, 1984).

RESULTS AND DISCUSSION

Persea americana exhibited antibacterial activities against *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia marseculans*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Pseudomonas fluorescens* (Table 2). Antibacterial effects of this plant extract against *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* suggest that it may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhoea in man and skin diseases (Roger *et al.*, 1990). The inhibitory activity of *Persea*

americana against these pathogenic bacteria may also support the use of leaf decoction as a remedy for diarrhea, sore throat infections and hemorrhage (Perry, 1980). The plant was able to inhibit the growth of *E. coli* which was not sensitive to *Streptomycin*, a standard broad spectrum antibiotic. These antibacterial activities are likely due to the presence of the secondary metabolites present in the plant (Table 1).

Brine shrimp lethality is a general bioassay, which is indicative of cytotoxicity, various pharmacologic actions, antibacterial activities and pesticidal effects (MacLaughlin *et al.*, 1991). Extract of *Persea americana* was found to be potent against brine shrimps. The brine shrimps lethality was found to be concentration-dependent. This result indicates the ability of this plant extract to kill cancer cells in cell cultures, kill various pests, and exert a wide range of pharmacologic effects. It has been observed that LD₅₀ values for general cytotoxicities are about one-tenth LD₅₀ values in the brine shrimp test (MacLaughlin *et al.*, 1991). *Persea americana* had its LD₅₀ value (25.03) less than 100 ppm, which indicated that it has high pharmacologic actions (Gupta *et al.*, 1996). This high activity of *Persea americana* against brine shrimps is in agreement with the study of Oberlies *et al.* (1998) who isolated cytotoxic and insecticidal constituents of an unripe fruit with the aid of brine shrimps lethality assay. The presence of tannins, saponins and cardiac glycosides may be responsible for the observed lethality activity of this extract. The brine shrimps lethality further supports the antibacterial activity of *Persea americana*.

Phytochemical screening of extract revealed the presence of tannins, saponins, flavonoids and cardiac glycosides in *Persea americana*. Cardiac glycosides are cardioactive compounds belonging to triterpenoids class of compounds. Their inherent activity resides in the aglycone portions of their sugar attachment. Their clinical effects in cases of congestive heart failure are to increase the force of myocardial contraction (Brian *et al.*, 1985). They exert their hypotensive effect by inhibiting Na⁺ - K⁺ATPase. They also act directly on the smooth muscle of the vascular system. They exert a number of effects on neural tissue and thus indirectly influence the mechanical and electrical activities of the heart and modify vascular resistance and capacitance. The presence of cardiac glycosides in this plant extract thus gives credence to their popular use in the treatment of hypertension.

Table 2: Antimicrobial activities of extracts of leaves of *Persea americana*

Microorganism	Zone of Inhibition (mm)		MIC (mg/ml)
	Pa (20 mg/l)	Sp (1 mg/l)	
Bacteria			
<i>Staphylococcus aureus</i> (NCIB 8588)	14 \pm 0.4	21 \pm 0.3	0.63 \pm 0.3
<i>Bacillus stearothermophilus</i> (NCIB 8222)	9.0 \pm 0.2	23 \pm 0.2	0.63 \pm 0.1
<i>Micrococcus luteus</i> (NCIB 196)	2 \pm 0.4	25 \pm 0.2	0.63 \pm 0.1
<i>Serratia marseculans</i> (NCIB 1377)	10 \pm 0.2	20 \pm 0.4	0.30 \pm 0.2
<i>Pseudomonas aeruginosa</i> (NCIB 950)	20 \pm 0.2	21 \pm 0.4	0.63 \pm 0.2
<i>Clostridium sporogenes</i> (NCIB 532)	10 \pm 0.2	25 \pm 0.2	0.63 \pm 0.2
<i>Escherichia coli</i> (NCIB 86)	22 \pm 0.2	0.0	1.25 \pm 0.1
<i>Klebsiella pneumoniae</i> (NCIB 418)	23 \pm 0.2	10 \pm 0.2	0.63 \pm 0.2
<i>Bacillus cereus</i> (NCIB 6349)	20 \pm 0.2	28 \pm 0.2	0.63 \pm 0.2
<i>Pseudomonas fluorescens</i> (NCIB 3756)	12 \pm 0.2	30 \pm 0.2	1.25 \pm 0.2
Fungus			
<i>Candida albicans</i> (LIO)	0	-	-

Values are mean \pm SD; n=3, MIC, minimum inhibitory concentration; LIO, locally isolated organism;

NCIB, collection of industrial Bacteria; O, resistant; Pa, *Persea americana*; Sp, *Streptomycin*

Table 3: Effects of extract of leaves of *Persea americana* on brine shrimps

Extract	Conc. ($\mu\text{g/ml}$)	No of subject	No of living	No of death (Ppm)	LD ₅₀
<i>P. americana</i>	1000	10	1.01	9.3	25.0 \pm 3.04
	100	10	3.31	6.5	
	10	10	5.71	4.0	

Values are mean \pm SD of 3 replicates.

Saponins are glycosides of both triterpenes and steroids having hypotensive and cardiac depressant properties. Saponins bind to cholesterol to form insoluble complexes; dietary saponins in the gut of monogastric combine with endogenous cholesterol excreted via the bile. This prevents cholesterol reabsorption and results in a reduction of serum cholesterol (Cheeke, 1971). Saponins have been found to be potentially useful for the treatment of hypercholesterolemia which suggests that saponins might be acting by interfering with intestinal absorption of cholesterol (Malinow *et al.*, 1977a 1977b). The presence of saponins in this plant may account for proper management of excess cholesterol synthesised *de novo* or exogenous cholesterol (if any) by preventing the excessive intestinal absorption of this compound and thus reduce the risk of cardiovascular diseases such as hypertension and hence may be responsible for its hypotensive properties (el-Sandany *et al.*, 1991).

The presence of tannins in *Persea americana* serves a number of medicinal purposes. The binding of tannins with proteins participates in the anti-diarrhic activity of tannins by protecting the digestive organs from injurious attack. It is well known that plants with anti-diarrhic activity inhibit intestinal motility to exert their activities. It also participates in the anti-haemorrhagic effects of tannin-rich drugs. The detoxification of the snake venoms bacterial toxins by persimmon tannin is another activity most probably based on the strong binding activity of tannin (Asquith and Butler, 1986; Murdiat and McSweeney, 1987). Tannins have been shown to inhibit the growth of HIV and herpes simplex virus (Okuda *et al.*, 1991). Tannins are also used in the treatment of bruises and superficial wounds (Trease and Evans, 1985). This suggests that *Persea* could also be used in the treatment of diseases like diarrhoea and viral diseases.

Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent antioxidant activity against the superoxide radical. Its consumption has been documented not to be associated with coronary heart disease mortality (Hertog *et al.*, 1993). This may be as a result of its antioxidant activity and subsequent inhibitions of LDL oxidation known to have been attributed to the dietary and supplemental intake of flavonoids and other micronutrients. Epidemiologic studies indicate an inverse relationship between intake of dietary flavonoids and coronary atherosclerotic disease (Knekt *et al.*, 1996). The presence of flavonoids in this plant may give support to its therapeutic effects. The results of the present study showed that the plant extract could be useful in the treatment of some diseases caused by the above named pathogenic bacteria.

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