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Antimicrobial evaluation of the methanol crude extract and alkaloid fraction of *Persia americana* mill seed

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ABSTRACT

This study aimed to evaluate the antibacterial property of the methanol crude extract and alkaloid fraction of *Persia americana mill* seed against *Staphylococcus aureus, Streptococcus pyogen, Escherichia coli, Salmonella typhi*. The powdered seed of *Persia Americana* mill was extracted by cold maceration with aqueous methanol (80%v/v). Agar well diffusion method and serial dilution technique were employed in this study. The phytochemical screening conducted on the methanol extract of the plant revealed the presence of flavonoids, saponnins, alkaloids, tannins and carbohyhdrates. The result of the antimicrobial activity of study demonstrated a promising activity against the selected clinical isolates with a more significant zone of inhibition at 500mg/ml. This also revealed that *E. coli* is resistant to the antibacterial effect of the methanol extract of *Persia Americana* mill seed at lower concentration (62.5mg/ml and below) and *Staph. aureus*is more susceptible to the sample at all concentration. Methanol extract of *Persia americana* mill seed had more antimicrobial activity against *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Salmonella typhi* with zone of inhibition of16.5±0.707,18.5±1.121, 11±0.000, 17.5±0.000 respectively compared with alkaloid fraction with zone of inhibition of 13.5±3.536, 14.5±2.120, 0.±0.000, 17.5±2.820. In conclusion, the methanol crude extract and alkaloid fraction showed antimicrobial activity against clinical isolates.

Keywords: Persia americana, alkaloid, crude extract, phytochemicals

INTRODUCTION

There has been an emerging interest both general and scientific these days for discovering phytochemicals as a distinguished option for synthetic products, normally used in food, pharmaceutical and cosmetic industries. This increased interest for natural product has been used as bases of alternative source of antimicrobial agents in place of convectional drugs for the treatment of microbial infections (Bochers *et al*., 2000). Patients worry and complaint about the safety of items containing synthetic chemicals based on the fact that some of such molecules are capable of causing negative health effects (Rodriguez-Carpena *et al*., 2011). Due to the excessive and inappropriate use of conventional antibiotics in the clinical treatment of human and animal infections, they had been an increase in pathogen resistance against these compounds, turning them into less effective agents (Heuer *et al*., 2006). Thus, there is need of requirements of alternative methods for controlling pathogens. Plants are attractive alternatives because they exhibit certain secondary metabolites or constituents. Numerous medications utilized in medicine are acquired from plants (Idris *et al*., 2009). The most active of these bioactive constituents of plants are alkaloid, tannins, steroids, terpenoids and phenolics. Others include flavanoids, saponins, sesquiterpenes, cyanogenic glycosides, isoflavanoids and sulfur-containing indole derivatives. The plant material (*Persia americana* seed) used in this study contains vital phytochemicals such as catechins, procyanides, hydroxycinnamic, phenolics compounds, flavanoids, alkaloid, pro-anthrocyanidin which are responsible for its antimicrobial and antioxidant properties. *Persia americana mill*, which is commonly known as avocado pear, is an evergreen tree belonging to the family of *Lauraceae*. It is a tropical tree native to humid tropical areas of Mexico, Central America and South America but has now been successfully grown worldwide (Adodo, 1995). *Persia americana mill*, is high in antioxidant vitamins (e.g resveratol and vitamin D), heart-friendly monounsaturated fatty acids, fibres and potassium with variable data which suggests their roles in anti-cancer, anti-infection, anti-inflammatory, and life prolonging effects (Kim *et al*., 2002).

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Avocado fruit has a multipurpose value on food, medicine, cosmetics products. Its use in traditional therapy for various disease conditions has been reported. Avocado is one of such fruits which generate a large amount of waste in processing as the seed alone makes up to 25% total fruit weight. After using the pulp, the seed and peel are discarded as waste thereby causing environmental challenges. These residues (seed and peel) have been reported to been rich in polyphenol, having antimicrobial and antioxidant activities. *Persia americana mill* seed antimicrobial activities would be carried out on gram positive and gram-negative bacteria. The gram-negative bacteria tested against are *Salmonella typhi and Escherichia coli.* Infection by *Salmonella spp* is contacted by ingesting large number of viable bacteria in faecally contaminated food or water. The symptoms include diarrhoea and abdominal cramps with nausea and vomiting. The alimentary tract of man and warm-blooded animals is the natural habitat for *E. coli;* it is one of the most abundant of the intestinal flora various strains of *E. coli has* been implicated in the outbreak of diarrhoea illness and their routes of transmission has been traced to drinking of sewage contaminated water. The gram-positive bacteria tested against the plant are *Staphylococcus aureus and Streptococcus pyogenes.* This research study aimed at the evaluation of the antibacterial activities of *Persia americana mill* seed. Despite the fibres present in the pulp, which serves as a bulking agent in diarrhoea caused by *E. coli and Salmonella typhi,* if the seed is found to have activity against these same bacteria, then a powdered or extract of the avocado pear seed can be taken in cases of these infections. The exploitation of the phytochemical content of the by-product which is the seed for antibacterial compound may attach some economic value to the waste products, hence this research has a potential of benefiting the avocado farming and pharmaceutical industries.

MATERIALS AND METHOD

Apparatus

Test tubes, conical flasks, beaker, Petri-dish, Measuring cylinder, droppers, pipettes, binocular light microscope (OLYMPUS), digital weighing balance (KERO BLG 300), pressure cooker, incubator (Uniscope SM9052 laboratory incubator), rotatory evaporator, water bath, microscopic slide,

Bacteriological media

Nutrient Agar. Nutrient broth, Mueller Hinton agar, Peptone water and Mannitol salt agar were all by Titan biotech Ltd, India

Methods

Collection and identification of plant sample

The fresh ripe fruit of *Persia Americana* mill were obtained from New Benin Market in Benin City, Nigeria in November, 2018. The plant was identified by the Department of Pharmacognosy Delta State University.

Preparation of plant sample for extraction

The avocado pear fruits were neatly cut round, the seeds were collected was rinsed with distilled water and grounded into smaller pieces using a local mortar and pestle and then allowed to air dry before grinding to powder. The powdered sample was weighed and stored in a clean and air tight container for extraction purpose.

Extraction procedure

A 500g portion of the sample was extracted in 1000ml 80% methanol as solvent. The 80% methanol was prepared by making up to volume with 200ml of distilled water to 800ml of methanol. This was left for seven days the resulting extract was filtered. The extract was concentrated using rotatory evaporator and left to dry at room temperature. Then the dried extract was weighed. The alkaloid extraction was carried by the liberation of free alkaloidal which was extracted using chloroform as an organic solvent. Purification of the crude alkaloidal extract was carried out with hydrochloric acid and was followed by fractionation of the Crude alkaloid using crystallization technique.

Phytochemical Qualitative Analysis

The phytochemical tests were carried out on the aqueous extract following standard procedures as described by Sofowara(1982).

Isolation and identification of Test Organisms

With the consent of forty-one students of Delta State University, sterile swab sticks were given to them to swab their nose with it. Mannitol salt agar was aseptically prepared according to manufacturer's specifications and autoclave at 121^{o}C for 15 minutes then was poured on forty-one petri dish and allowed to cool. Each swab stick was used to streak the petri dish and incubated at 37^{o}C for 24 hours. The growths on the different plates were subcultured aseptically into Nutrient media plates using streak method described by Cheesbrough (2000), in order to isolate *Staphylococcus aureus*. The pure cultures generated were characterized and identified using their colonial description. In the same vain, sputa specimen from seven consented students of Delta State University,

were cultured in order to isolate *Streptococcus pyogenes* following the procedure described by Cheesbrough (2000) *Salmonella typhi and Escherichia coli* were obtained from the Department of Microbiology, Delta State University.
Gram Staining procedure and other biochemical test such as Hydrogen Sulphide Test, Indole Test, Catalase Test, Carbon dioxide growth test and Bile salt test were all carried out as described by Cheesbrough (2000).

Antibacterial Sensitivity Test
Preparation of culture media
Mueller- Hilton agar was prepared following manufacture's instruction and autoclaved at 121^{o}C for 15 minutes. This was allowed to cool to 40^{o}C and then poured into 8 sterile petri dishes labeled according to the concentration of extract to be used (500, 250, 125, 62.5, and 31.25) in a clockwise manner with control label at the middle of the plate and allowed to cool in an aseptic environment. Four swab sticks were labeled *Staph, Strep, s. typhi and E. coli* and each swab stick was dipped in a corresponding labeled broth bottle (*Staph, Strep, S. typhi and E. coli*) and streak was made on agar plate.

Preparation of Serial Dilution
Five test tubes previously sterilized were used for the serial dilution of plant. 2ml of sterile water was placed in a test tube and 1ml in the other four test tubes. Exactly 1g of the methanol extract or alkaloid fraction was added to the test tube containing 2ml of sterile water and shaken very well till the extract dissolved then with the aid of a pipette 1ml was transferred into another test tube containing 1ml of the solvent, this was shaken and 2ml transferred to the next test tube. This was done continuously until the last test tube had 2ml of solution in it. The concentrations of extract in each of the tubes were 500, 250,125, 62.5 and 31.25mg/ml.

Preparation of wells for inoculation
A well flamed sterilized standard cork-borer was used to bore 6mm holes according to the number of labeled concentrations on the freshly prepared Mueller Hilton agar plates containing the clinical isolates.

Application of plant extracts
1ml of the various extract contractions (500 mg/ml 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml) were used to fill the wells in the agar plates with the aid of sterile pipette under aseptic condition. This was done for both the methanol extract and alkaloid fraction.

Incubation of plates
The plates were allowed to stand on the table undisturbed for 20minutes after the application of extracts and solvent (negative control plates). The plates were incubated at 37'C for 24 hours.

Measurement of zones of inhibition
Zones of clearance round each well were measured in millimeter (mm) using a meter. The diameter of the (6mm) was subtracted from the zone of inhibition measured and recorded as the zone of inhibition. The mean, Standard deviation and Standard mean error for the zone of inhibition measure were calculated and recorded.

Determination of minimum inhibitory concentration (MIC)
A double fold serial dilution ranging from 1.95-12.50g/ml of the alkaloid fraction was prepared. 3.8g of Mueller Hilton agar was dissolved 100ml of distilled and was autoclaved at 121^{o}C for 15 minutes. 1ml of each concentration was poured into 6 labeled petri dish and 9ml each of the agar was poured into the 6-petri dish and the plate was rocked clockwise and anticlockwise on the table to allow a good diffusion of the extract into the agar which left to solidify. Four swab sticks were used, one for each clinical isolate to streak the agar plate according to the label on the agar plate. After this, the plate was left to stand for 15 minutes before incubating at 37^{o}C for 24 hours. After the incubation period, growth on any part of the plate indicates lack of susceptibility of that organism to the alkaloid fraction at that particular concentration while no growth of organism shows susceptibility of that organism at the particular concentration of no growth.

RESULT

Table 1: Characteristics and Identities of the Test Bacteria

Parameter	*S. areus*	*S pyogen*	*S. typhi*
Catalase test	+	-	+
Indole test	-	-	-
Carbondioxide growth test	-	+	-
Bile test	-	+	-
Hydrogen sulphide test	-	-	+
Gram staining reaction	+	+	-
Shape	Cocci	Cocci	Rod

+= Positive ; - = Negative

Table 2: Qualitative Phytochemical Screening of the Seed Extract

Phytochemical	*Persea americana* Seed
Alkaloid	+
Tannins	+
Flavonoids	+
Saponins	+
Steroids	+
Carbohydrate	+
Cardiac Glycosides	+

+= Present ; - = Absent

Table 3: Phytochemical constituents of *Persia americana* mill seed extract

Parameters	Value(mg/100g)
Alkaloid	0.84 ± 0.03
Tannins	0.36 ±0.04
Flavanoids	1.87± 0.41
Saponnins	17.18 ±0.81
Cardiac glycosides	0.08± 0.01
Steriods	0.04±0.00
Carbohydrates	8.91 ±0.41

Table 4: Mean Zone of Inhibition and Standard Deviation **of** Antibacterial Activity of Methanol Extract of *Persia Americana* mill Seed Against Clinical Isolates

Crude Extract Treatment Concentration of *P. american* Seed (mg/ml	Zone of inhibition of bacteria (mm)								Mean zone of inhibition (mm) ± Standard deviation			
	E.coli		*Staph*		*E. coli*		*Strep*		*E.coli*	*Staph*	*Strep*	*S. typhi*
Plate	I	II	I	II	I	II	I	II				
500	11.00	11.00	17.00	20.00	16.00	17.00	17.50	17.50	11.00±0.00	18.50±2.12	16.50±0.70	17.50±0.00
250	10.00	10.00	14.00	18.00	15.00	12.00	15.00	13.00	10.00±0.00	16.00±2.82	13.50±2.12	14.00±1.41
125	9.00	9.00	17.00	16.00	11.00	11.00	13.00	10.00	9.00±0.00	16.50±0.70	11.00±0.00	11.50±2.12
62.50	NIL	NIL	15.00	14.00	11.00	11.00	12.00	11.00	0.00±0.00	14.50±0.70	11.00±0.00	11.50±0.70
31.25	NIL	NIL	17.00	16.00	10.00	10.00	11.00	11.00	0.00±0.00	16.50±0.70	10.00±0.00	11.00±0.00
Ciprofloxacin	65.00	64.00	43.00	43.00	40.00	45.00	58.00	60.00	64.50±0.70	43.00±0.00	42.50±3.53	59.00±1.41

TABLE 5: Mean Zone of Inhibition and Standard Mean Error of Antibacterial Activity of Methanol Extract of *Persia americana* Mill Against Clinical Isolates.

Crude Extract Treatment Concentration of *P. American* Seed (mg/ml)	Zone of inhibition of bacteria (mm)								Mean zone of inhibition (mm) ± Standard mean error			
	E.coli		*Staph*		*Strep*		*S. typhi*		*E.coli*	*Staph*	*Strep*	*S. typhi*
Plate	I	II	I	II	I	II	I	II				
500	11.00	11.00	17.00	20.00	16.00	17.00	17.50	17.50	11.00±0.00	18.50±1.50	16.50±0.50	17.50±0.00
250	10.00	10.00	14.00	18.00	15.00	12.00	15.00	13.00	10.00±0.000	16.00±2.00	13.50±1.50	14.00±1.00
125	9.00	9.00	17.00	16.00	11.00	11.00	13.00	10.00	9.00±0.000	16.50±0.50	11.00±0.00	11.50±1.50
62.5	NIL	NIL	15.00	14.00	11.00	11.00	12.00	11.00	0.00±0.000	14.50±0.50	11.00±0.00	11.50±0.00
31.25	NIL	NIL	17.00	16.00	10.00	10.00	11.00	11.00	0.00±0.000	16.50±0.50	10.00±0.00	11.00±0.00
16.52	NIL	NIL	15.00	13.00	9.00	10.00	9.00	9.00	0.00±0.000	14.00±1.00	9.50±0.50	9.00±0.00
Ciprofloxacin	65.00	64.00	43.00	43.00	40.00	45.00	58.00	60.00	64.50±0.50	43.00±0.00	42.50±2.50	59.00±1.00

Table 6: Mean Zone of Inhibition and Standard Deviation of Antibacterial Activity of Alkaloid Fraction of *Persia Americana* mill Seed Against Clinical Isolates.

Alkaloid Extract Treatment Concentration of *P. American* Seed (mg/ml)	Zone of inhibition of bacteria (mm)								Mean zone of inhibition (mm) ± Standard deviation			
	Strep		*Staph*		*E. coli*		*S. typhi*		*Strep*	*Staph*	*E. coli*	*S. typhi*
Plate	I	II	I	II	I	II	I	II				
500	11.00	16.00	16.00	14.00	NIL	NIL	19.50	19.50	13.50±3.53	14.50±2.12	0.00±0.00	17.50±2.82
250	8.00	9.00	11.00	15.00	NIL	NIL	13.00	11.00	8.50±0.70	13.00±2.82	0.00±0.00	12.00±1.41
125	8.00	6.00	7.00	7.00	NIL	NIL	8.00	7.00	7.00±1.41	7.00±0.00	0.00±0.00	7.50±0.70
62.5	6.00	4.00	6.00	7.00	NIL	NIL	8.00	9.00	5.00±1.41	6.50±0.70	0.00±0.00	8.50±0.70
31.25	5.00	3.00	7.00	6.00	NIL	NIL	7.00	7.00	4.00±1.41	6.50±0.70	0.00±0.00	7.00±0.00
Ciprofloxacin	64.00	65.00	43.00	43.00	40.00	45.00	58.00	60.00	64.50±0.70	43.00±0.00	42.50±3.53	59.00±1.41

Table 7: Mean Zone of Inhibition And Standard Mean Error of Antibacterial Activity of Alkaloid Fraction of *Persia Americana* mill Seed Against Clinical Isolates.

Alkaloid Extract Treatment Concentration of *P. American* Seed (mg/ml)	Zone of inhibition of bacteria (mm)								Mean zone of inhibition (mm) ± standard mean error			
	Strep		*Staph*		*E. coli*		*S. typhi*		*Strep*	*Staph*	*E. coli*	*S. typhi*
Plate	I	II	I	II	I	II	I	II				
500	11.00	16.00	16.00	14.00	NIL	NIL	19.50	19.50	13.50±2.50	14.50±1.50	0.00±0.00	17.50±2.00
250	8.00	9.00	11.00	15.00	NIL	NIL	13.00	11.00	8.50±0.50	13.00±2.00	0.00±0.00	12.00±1.00
125	8.00	6.00	7.00	7.00	NIL	NIL	8.00	7.00	7.00±1.00	7.00±0.00	0.00±0.00	7.50±0.50
62.5	6.00	4.00	6.00	7.00	NIL	NIL	8.00	9.00	5.00±1.00	6.50±0.50	0.00±0.00	8.50±0.50
31.25	5.00	3.00	7.00	6.00	0.00	0.00	7.00	7.00	4.00±1.00	6.50±0.50	0.00±0.00	7.00±0.00
Ciprofloxacin	64.00	65.00	43.00	43.00	40.00	45.00	58.00	60.00	64.50±0.50	43.00±0.00	42.50±2.50	59.00±1.00

Table 8: Minimum Inhibitory Concentration (MIC) (mg/ml) of the Test Bacteria

Organism	125	62.50	31.25	15.65	7.81	3.91	1.95
Salmonella typhi	-	-	-	15.65	7.81	3.91	1.95
Staphylococcus aureus	-	62.50	31.25	15.65	7.81	3.91	1.95
Streptococcus pyogenes	-	-	31.25	15.65	7.81	3.91	1.95
Escherichia coli	-	62.50	31.25	15.65	7.81	3.91	1.95

Table 9: The mean of methanol extract and alkaloid fraction **a**t varying concentrations

Concentration mg/dl	Extract	Mean (mm)	STD. Deviation	STD. Mean Error
500	Methanol	15.88	1.68	10.54
	Alkaloid	11.38	7.77	3.89
250	Methanol	13.38	2.49	1.25
	Alkaloid	8.38	5.91	2.95
125	Methanol	12.00	3.19	1.59
	Alkaloid	5.38	3.59	1.80
62.50	Methanol	9.25	6.36	3.18
	Alkaloid	5.00	3.63	1.81
31.25	Methanol	9.38	6.87	3.18
	Alkaloid	4.39	3.19	1.59

DISCUSSION

The clinical isolates used in this study were all bacteria both the gram-positive bacteria and gram-negative bacteria. About 80% of the world population in Africa depends on traditional medicine for primary health care(Willcox and Bodeker, 2004). *Persia americana* (Avocado) is among the useful plants used for traditional medicine in Africa. Different parts of this plants extracted with different types of solvents have been used by researchers to investigate their properties. In this present study, methanol was used as solvent for extraction of vital secondary metabolites from the seed of avocado pear because it's capable of extracting bioactive compounds such as flavanoids, saponnins, alkaloid, carbohydrates and tannins found in plants. The result of the phytochemical screening of the methanol extract of *Persia americana mill* seed showed the presence of flavanoids, saponnins, alkaloid, carbohydrates, tannins, cyanogenic glycosides and steroids which are shown in Table 3, all of which have been found *in vitro* to have antibacterial property. Saponnin showed more intensity in the plant extracts. Flavanoids are known to be produced in response to microbial attack to plants (Evbuomwan and Inetianbor, 2017) these effects could be a major source of the antibacterial activity of avocado pear seed methanol extract for treatment of infections. This result obtained from the present study agreed with the reports of earlier researchers such as Idris *et al*., (2009) who reported similar phytochemicals from the methanol extract of avocado pear seed but in addition reported the presence of terpenoids and the absence of anthraquinone and cyanogenic glycosides. llozue *et al.,* (2014) in their works reported the presence of flavanoids, alkaloid, steroid, saponnin, tannin and cardiac glycosides which agrees with the result obtained in this present study. The antimicrobial activities of these plant extract in this study could therefore be attributed to the presence of these phytochemicals. This confirms the reports of Cowan (2002) and Anozie (1986) who stated that the antimicrobial 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. The present study revealed the inhibitory effect of the methanol extract and showed a significant difference in *E. coli< Strep< S. typhi< Staph* with their mean zone of inhibition of 10, 11.92, 12.42, 16mm respectively. This is in agreement with the findings of Idris *et al*., (2009) and Ilozue *et al*., (2014) which showed that *Staphylococcus aureus* is more susceptible to the methanol extract of sample when compared to other organism while that of the alkaloid fraction showed mean zone of inhibition in the range of *E. coli <strep< staph< S.typhi* at 0.00, 7.6, 9.5, 10.5mm respectively. This implies that *E. coli* does not have susceptibility to the alkaloid fraction at any concentration worked with. This however may not imply that the organism was resistant but could mean that higher concentrations if used could have could better results as suggested by Akharaiyi *et al*.(2011) and also due to the lipopolysaccharide contained in the cell envelop of *E. coli* being gram-negative bacteria, the penetration of the antimicrobial agents contained in the plant extract limited compared with the Gram-positive bacteria. This was in line with the works of Tegos *et al*., (2002), Ahmadu *et al*., (2006) and Tagoe and Gbadago (2010) whose findings emphasized this. The antibacterial activity of ciprofloxacin against all clinical isolates was higher than the antibacterial activity of the extract. In comparing the methanol extract and the alkaloid fraction of sample mean zone of inhibition against the clinical isolates at the same concentrations, it was seen that the alkaloid fraction had a lower effect on the clinical isolates when compared with the same concentration with the methanol extract this could be as a result of other secondary metabolites found in the methanol extract. From the mean plot it can be deduced that the activity of the treatment is dependent on the concentration with some concentration having the same effect with the exception to *S. typhi* of the alkaloid fraction and *S. aureus* of the methanol extract whose activity was independent to the concentrations. The antibacterial sensitivity test of methanol extract and alkaloid fraction carried out on clinical isolates, *S. typhi, S. aureus, E. coli, Strep* were best inhibited at 500mg/ml. For the MIC test result, it can be conceded that Infections caused by *Salmonella typhi* and *Streptococcus pyogenes* could be well treated by administering dose of *Persia americana* mill seed even at low doses of 31.25mg/ml and 62.5mg/ml respectively. While for *Staphylococcus aureus* and *Escherichia coli* higher concentrations are required for the effective eradication of the bacterial.

CONCLUSION

The study evaluated the efficacy of methanolic extract of *Persia americana mill* seed against gram positive and gram-negative bacteria from clinical isolates namely: *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella typhi.* It is clear that avocado pear seed possess antimicrobial property against *Salmonella typhi* and *Streptococcus pyogenes* even at low concentration while it is effective at a high concentration against *Staphylococcus aureus* and

Escherichia coli in the treatment of infections caused by these bacteria. This study supports the folklore use of the plant by local users to treat infection and shows the plant as economical and safe in treatment of microbial infections.

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