Antimicrobial Activity of *Persea americana* Seed Extract against Bacteria and Yeast isolated from Patients with Urinary Tract Infection in Delta State University Teaching Hospital, Nigeria

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Abstract: The study was aimed at investigating the antimicrobial activity of *Persea americana* (Avocado pear) seed extracts on some clinical isolates from urine. Urine samples (500) were collected from patients with urinary tract infection (UTI) from Delta State University Teaching Hospital, Oghara, Nigeria. Bacteria and fungi were isolated and identified based on standard techniques. *Serratia*sp., *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Salmonella* sp., *Candida* sp. and *Saccharomyces cerevisiae* were isolated with *E.coli* and *Candida* sp observed to have the highest prevalence of 75.0% and71.4% respectively. Aqueous and ethanolic extracts of *P. americana* seeds were screened for phytochemicals. Concentrations of these phytochemicals (g/kg) were observed to be higher in the ethanolic than aqueous extracts. Saponin was identified with the highest concentrations of 5.08 ±0.20 and 0.6±0.12 while tannin the lowest of 0.40±0.1 and 0.15±0.20 in ethanolic and aqueous extracts respectively. Antimicrobial assays revealed that all the isolates except *Proteus* sp. and *Serratia* sp. were susceptible to the ethanolic extracts *of P. americana* while all the organisms were resistant to the various concentrations of the aqueous extract. This finding recommends further study on the use of *P. Americana* seeds as a potential antimicrobial agent in formulation of drugs for the treatment of UTIs.

Keywords: Antibiotic resistance, *Persea americana,*Fungi, Bacteria, Plant Extracts and Antimicrobial

INTRODUCTION

There is growing awareness worldwide, for using medicinal plants, over the conventional antibiotics, as an alternative for the treatment, management and prevention of infectious diseases. This is because the conventional antibiotics are fast becoming less effective due to the emergence of multidrug resistance amongst microbial strains(Ali *et al.*, 2015). This renewed interest for alternative therapy may also have been prompted by an increase in incidence of disease and opportunistic infections, especially in immune-compromised individuals which have become difficult to manage with orthodox antibiotics (Badria and Zidan, 2004). One approach to circumvent this challenge is to use plant's bio-active phytochemicals (Singh *et al.,* 2009).

The use of plants as phytotherapeutic agents are comparatively, less expensive than conventional drugs and if administered at higher concentration seldom have such drastic side effects on the host(Saraf, 2010). Plants are known to have a broad spectrum of antimicrobial activity and constitute a source of many of such compounds (Ayodele and Banuso, 2004). Most plants have been shown to possess antibacterial, antifungal and antiviral properties (Ali *et al.,* 2004). Such plants include *Thymus vulgaris*, *Cassia fistula, Xylopia aethiopica*, *Allium cepa, A. sativa* and *Anarcardium occidentalis* (Amadi *et al.,* 2007; Akpomie and Olorungbon, 2011). The most important bioactive constituents of plants are flavonoids, tannins, phenolic compounds, alkaloids, and their antimicrobial activity may reside in a variety of these phytochemicals (Gonclaves *et al*., 2005). These phytochemicals are secondary metabolites present in plants which enable them combat many disease-causing pathogens hence possess pharmacological potentials (Hussain *et al.,* 2011). *Persea americana* (avocado pear) is one of the most important fruits used as food in most tropical and subtropical countries. Different parts of

the plant are used in herbal preparations. The peel, leaves and fruits are used in the treatment of haemorrhagia, hypertension. The leaf extract effectively inhibited herpes simplex virus types I and II (Sturluson, 2017). The seeds of *P. americana* have been applied for the treatment of diverse diseases such as dysentery, skin infections, toothache (Pamplora and Roger, 1999). The seeds are equally rich in tannin and carotenoids (Lu *et al.,* 2005) and have a high antioxidant activity and phenolic content greater than 70% (Soong and Barlow, 2004).

Rodriguez-Caperna *et al.* 2011 reported that the seed extracts of *P. Americana* were more effective on gram positive bacteria than gram negative bacteria and are active against organisms such as *Cryptococcus neoformans* (Leite *et al.,* 2009); *Enterococcus faecalis, Salmonella enteritidis, Citrobacter freundii*, *Pseudomonas aeruginosa, S.* Typhimurium (Chia and Dykes, 2010).The seed oil and leaves have diverse application in ethno-medicine for the treatment of diarrhea, toothache, dysentery, intestinal parasites, skin treatment and beautification (Roger, 1999; Adeyemi *et al.,* 2002).

Despite these potential uses of the seeds, they are usually discarded hence underutilized. In view of the current trend of antibiotics resistant microorganisms, there is a need to look into the potential of *P. americana* seed extracts as an antimicrobial agent. Research into the possible therapeutic/antimicrobial activity of this underutilized agro-food waste, will also reduce the environmental waste burden it may constitute.

Therefore, this study is aimed at determining the phytochemical constituents and to investigate the antimicrobial activities of *P. americana* seeds extract against some resistant clinical bacteria and yeasts isolated from patients with UTI.

MATERIALS AND METHODS

Sample collections

The avocado fruits were purchased from the local market, Abraka, Nigeria. These fruits Flasks were thereafter covered and shaken on a rotatory shaker for a period of 7 days. were cut with a sterile knife and the seeds were detached and sliced into pieces. The seed pieces were air dried for approximately 2 weeks followed by pulverizing in a manual hand blender (Thomas Scientific, U.S.).

Urine Samples (500) each, were collected from urinary tract infection (UTI) in and outdoor-patients, at the Delta State University Teaching Hospital, Oghara, Nigeria, as well as from apparently healthy adults between March and October, 2019 . The samples were collected in sterile urine containers and taken for analysis at the Microbiology Laboratory, Delta State University, Abraka.

Identification of the microbes from urine samples

The microorganisms associated with the urine samples were isolated on Nutrient agar (NA), Potato Dextrose agar (PDA) and Cystine Lactose Electrolyte Deficient agar (CLED). The bacteria and fungi were identified based on their colonial characteristics, morphology, microscopic features and biochemical characterization (Cheesebrough, 1994). The biochemical tests conducted include Gram staining, motility, catalase, oxidase, citrate, indole and triple sugar iron.

Additionally the discrete reproductive structures were used for the identification of the fungi as well. This was performed by the germ-tube test (Ellis *etal*., 2007) where a tiny inoculum of the fungi was rubbed in the inner wall of thetest-tube containing 0.5 mL human serum. The mixture was incubated at 42°C for 3 hours. Following the incubation, a drop of the mixture was placed on a clean glass-slide, covered with a cover-slip and examined under the microscope for the presence of germ tubes.

Preparation of seed's extract

Persea americana seeds extract was prepared by adding 250g of the blended seeds to 500mL of ethanol (70%) and distilled water in conical flasks for ethanol and aqueous extraction respectively.

Following the incubation, the solutions were filtered through a 3HW 90 mm filter paper (LASEC SA, Baerenstein, Germany). The filtrate were concentrated on a water bath at 45°C for the elimination of water and ethanol, and the extracts were stored in sterile well-corked bijou bottles under refrigerated condition (4°C) until further use.

Phytochemicals analysis of *P. americana* seed extract

The qualitative and quantitative analysis of the *P. americana* seed extract was performed in accordance with the methodology described in a previous study (Sofowara, 1993).

Tannins and Phenol

Five hundred milligram of dried *P. americana* extracts was boiled in 20 mL of water and then filtered. About two drops of Ferric chloride (0.1.%) was added to the filtrate and observed for a brownish green or blue-black green coloration.

The total phenols content was determined by boiling the dried samples with 50 mL of ether for 15 minutes followed by mixing 5 mL of the ether extract with distilled water (10 mL). Further, the mixture was added to 2mL ammonium hydroxide and concentrated amyl alcohol (5 mL), incubated for 30 minutes at room temperature, observe for colour development and finally the absorbance was measured at OD_{505}. Tannin was estimated by adopting the methodology of Van-Burden and Robinson (1981). Five hundred milligram of the dried sample was mixed with 50 mL of water and shaken on a rotatory shaker for 1 hour. After incubation, the mixture was filtered and 5 mL of the filtrate was mixed with 2 mL of $FeCl_3$ (0.1 M) in HCl (0.1N) and potassium Ferro cyanide (0.008 M). The absorbance was measured at OD_{120} within 10 min of the reaction.

Saponin

Saponin was detected by mixing 2 g of the powdered plantwith20ml of distilled water, incubated on water bath and filtered. Following the filtration, 10 mL of the filtrate was mixed with 5mL of distilled water and shaken vigorously until a stable froth was formed. The froth obtained was then mixed with 3 drops of olive oil, shaken vigorously and observed for the formation of emulsion, indicating the presence of Saponin.

Saponin was estimated quantitatively in accordance to a previous study (Obadoni and Ochuko, 2001). One hundred milliliter of aqueous ethanol (20%) was added to 20 g of grounded seed samples, heated on water bath (55°C) for 4 h with constant stirring. Following the filtration of this mixture, the residue was re-extracted with 200 mL ethanol (20%) and the extract was further concentrated on a water bath at 90°C and mixed vigorously with shaking with addition of 20 mL of diethyl ether. The aqueous layer was recovered and the purification process was repeated. The purified aqueous layer is mixed with 60 mL of n-butanol and washed twice with 10 mL of aqueous sodium chloride (5%). Further, the solution was heated to evaporate on water bath, followed by drying in the oven to a constant weight to calculate the Saponin content.

Flavonoid

The flavonoid was identified by adding diluted ammonia solution (5 mL) to a portion of the aqueous filtrate of the dried seed extract, followed by addition of concentrated H_2S0_4. A yellow coloration that disappeared on standing is indicative of the presence of flavonoids.

The quantitative estimation of flavonoid was performed as previously described (Bohm and Kocipai-Abyazan, 1994). The dried seed extract was extracted repeatedly with 100 mL of aqueous methanol (80%) at 26 ± 2°C. followed by filtration of the solution using Whatman No 42 filter paper (125mm). The filtrate was evaporated to dryness on a water bath to a constant weight in order to calculate the flavonoid content.

Alkaloids

The dried plant extract was mixed with ammonia, followed by extraction with chloroform solution. The chloroform extract was mixed with dilute hydrochloric acid and the acid layer was mixed with few drops of Wagner's reagent and observed for a reddish brown precipitate.

The alkaloids quantitative analysis was performed following previously described protocol (Harborne (1973). Five grams of the dried samples was mixed with 200 mL of 10% acetic acid in ethanol, covered and incubated for 4 hours at room temperature. Following the incubation, the solution was filtered and concentrated to a decoction (one quarter of the original volume) on a water bath. Further, ammonium hydroxide was added drop-wise to the decoction until the precipitation occurred. The precipitate was collected, washed with dilute ammonium hydroxide and filtered. The residue obtained was dried to constant weight in order to estimate the alkaloid content.

Terpenoids

Terpenoids was determined bySalkowski test. A portion (5mL) of the extract was mixed with 2mL chloroform and 3mLof concentrated H_2S0_4. A reddish-brown coloration at the interface confirmed the presence of terpenoids.

Glycoside (Keller-Killani test)

The qualitative estimation of glycoside was performed by the Keller-Killani test. The dried extract (5 mL) was mixed with 2mL of glacial acetic acid and a drop of ferric chloride solution followed by the addition of 1 mL concentrated H_2S0_4. A brown ring at the interface indicated a deoxysugar, depicting cardenolides (glycosides). A violet ring occasionally appeared below a greenish ring, which might form gradually throughout the thin layer.

Antimicrobial activities assay

The *P. americana* seed extract was assessed for its antimicrobial activities against the urine isolates. Pure single colony of each bacterial and fungal isolates were inoculated in 5 mL of the Nutrient and Potato Dextrose broth respectively. Following the inoculation, the bacterial and fungal cultures were incubated at 37°C, 24 h and 25^0C, 72 h respectively. The grown cultures were adjusted to a concentration of 10^6 cells/mL MacFarland standard in 20 mL molten Nutrient Agar (bacteria) and Potato Dextrose Agar (fungi), gentle shaking and poured in petri dishes (100 mm x 15 mm). The plates were air-dried under laminar air flow (Esco Technologies, Pennsylvania, USA).

The Disc diffusion assay was performed in accordance with a previous study with minor modifications (Ilozue *et al.,* 2014)..The discs were prepared with Whatman no 1 filter paper (110mm), sterilized at 160^0C for 1 hour, allowed to cool and infused with the seed extracts. The impregnated discs were laid down on the prepared media plates and incubated at37^0C and 25°Crespectively, until a well-developed lawn of the potential bacterial and fungal isolates was observed. The antimicrobial activity was visualized as zone of growth inhibition around the impregnated disc and was expressed as the mean diameter of zones of inhibition (mm).

Determination of Minimum Inhibitory Concentration of *P.americana* seed extracts

This was carried out using double strength broth dilution with nutrient broth. Two milliliter of the nutrient broth was put into test tubes and 1ml of the seed extract was introduced and serially diluted. 0.5ml of standardized test organism (10^7) was incorporated into each test tube and incubated at 37^0C for 24hours and 25^0C for bacteria and fungi respectively. Dilutions of concentrations of extracts that exhibited activity against the test organisms were streaked on a sterile nutrient agar and incubated for 24hours (Khan and Omotoso, 2003).

Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of *P. americana* seed extracts

Bacteria and fungi from the MIC tube were streaked on a sterile nutrient and Malt Extract Agar plates respectively, and

incubated at 37^0C and 25^0C (Nostro *et al*., 2002) observing for growth.

Determination of Antibiotic Susceptibility of Isolates

This test was carried out using the agar disc diffusion method (Bauer *et al*., 1996). Mueller-Hinton agar plates were inoculated with each isolate suspended in sterile saline solution using sterile cotton swab stick. The plates were left for about 30minutes after which the discs were aseptically transferred to the plates using sterile forceps. Plates were incubated at 37^0C for 24h and examined for zones of inhibition around the disc (Slevamohan and Sandhya, 2012)

RESULTS

Bacterial and fungal identification

Macroscopic/microscopic examinations and the biochemical tests (Table 1 and Table 2) identified 20 bacterial and 6 fungal isolates from the UTI samples. The bacterial isolates(Table 1) were Gram negative rods and identified as *E. coli* along with the genera of *Klebsiella*, *Salmonella*, *Proteus* and *Serratia* with a prevalence of 35%, 15%, 15%, 20% and 10% respectively (Table 3). The fungal isolates were detected as *Candida* sp. (Spherical, cream colored, clusters of blastocondia) and *Saccharomyces cerevisiae* (Spherical to elongated, pinkish, reticulated)(Table 2) with prevalence of 20% and 5% respectively (Table 3). The biochemical tests revealed that all the bacterial isolates were positive for catalase and glucose and negative for oxidase excluding the *Proteus* sp

Phytochemical composition of *P.americana* seed

Phytochemicals such as Phenol, alkaloid, flavonoid, saponin, terpenoid, steroid and glycoside were detected in the ethanolic extracts of the seed. Similar, phytochemical composition profiles were obtained for seed's aqueous extract however, the reducing sugar could not be identified (Table 4). The quantitative analysis revealed that the ethanolic extract possess higher concentration of all the phytochemicals determined in comparison to the aqueous ones. Saponin exhibited the highest concentration for both extracts with a concentration of 5.08 ± 0.20 and 0.6 ± 0.12 for ethanol and aqueous extracts respectively (Table 4).

As shown in table 5, three of the bacterial isolates exhibited multi-drug resistance. All the bacterial isolates were resistant to septrin except *Klebsiella sp*. *Klebsiella* sp and *Salmonella* sp were susceptible to the antibiotics. The *Candida* sp and *S. cerevisae* were susceptible to the antifungal agent (fluconazole).

The ethanolic extract of *P. americana* seed was more efficient as an antimicrobial agent than the aqueous extract. The efficiency was at high concentrations (100%) and 80%). There was no inhibition at the lower concentrations (Table 6).

Table 1: Microscopic and biochemical characterization of bacterial isolates

Shape	Gram characteristics	Motility	Biochemical characterizations									Bacterial Identity
			Catalase	Oxidase	Citrate	Indole	H_2S	Acid	Gas	Lactose	Glucose	
Rod	-	-	+	-	+	+	+	-	-	+	+	*Serratia* sp
Rod	-	+	+	-	+	+	-	+	+	+	+	*Klebsiella* sp
Rod	-	+	+	+	+	-	-	+	+	-	+	*Proteus* sp
Rod	-	+	+	-	-	-	+	-	+	-	+	*Salmonella* sp
Rod	-	-	+	-	+	-	+		-	-	+	*Escherichia coli*

('+'= presence; '=' = negative)

Table 2: Identification of fungal isolates

Microscopic	Macroscopic	Biochemical test	Fungi identication
Spherical and sub-spherical, composed of hyphae and pseudohyphae, budding, grape like clusters of blastoconidia at the septa. Chlamydospores are present at the end of hyphae.	Cream colored globose and yeast like, rough, filamentous border.	Glucose (+), Maltose (+), Raffinose (+)	*Candida albicans*
		…..	
Spherical to elongated budding, yeast-like cells with blastoconidia	Smooth convex and pasty pink, some reticulated		*Saccharomyces cerevisiae*

('+'= presence; '=' = negative)

Table 3: Occurrence of isolates in the urine samples.

Isolates	Number (%) Prevalence	
	From UTI Patients (N=500)	From Apparently Healthy Individuals (N=500)
Escherichia coli	175(35.0)	23(4.6)
Klebsiella sp	75(15.0)	2(0.4)
Salmonella sp	75(15.0)	4(0.8)
Proteus sp	125(20.0)	5(1.0)
Serratia sp	50(10.0)	3(0.6)
Candida albicans	125(20.0)	4(0.8)
Saccharomyces sp	25(5.0)	0(0.0)

Table 4: Phytochemical content (g/kg) of *P. americana* seed. Results were expressed as the mean of triplicate determinations ± standard deviation**.**

Phytochemical constituent	Ethanol	Aqueous
Phenol	+ (0:42±0.19)	+ (0.16±0.10)
Tannin	+(0.40±0.1)	+ (0.15±0.20)
Alkahoid	+(1.14±0.14)	+ (0.28±0.12)
Flavonoid	+(1.80±0.31)	+ (0.21±0.12)
Saponin	+(5.8±0.20)	+ (0.6±0.12)
Terpenoid	+(ND)	+ (ND)
Steroid	+(ND)	+ (ND)
Glycoside	+	+ (ND)
Reducing sugar	+	+ (ND)

(+ = present; ND = Not determined)

Table 5(a): Antibiotic Susceptibility of bacterial and fungal isolates with zones of inhibition (mm)

Isolates	SXT	CH	SP	CPX	AM	AU	CN	PEX	OFX	S	FLU	R(%)
E. coli	8.12±3.78*	16.2±3.42*	18±3.82*	16.5±3.82*	8.12±3.78*	16.5±3.82*	18.0±3.82*	20±3.82*	00	00	ND	40
*Proteus*sp	00	15.5±091	8.12±3.78	15.2±2.89	18.0±2.97	15.2±0.89	00	00	00	8.12±3.78*	ND	60
Salmonella sp	00	18.00±2.82	16.5±2.82	15.20±2.91	18.0±2.82	18.00±2.82	00	16.50±2.82	18. ±2.82	15.2±2.91	ND	20
*Serratia*sp	8.12±3.78*	16.2±3.42*	8.22±0.70	16.20±3.40	15.2±0.89	15.20±0.89	00	14.40±0.65	14.00±0.62	16.2±0.89	ND	30
*Klebsiella*sp	18. ±3.82*	15.2±0.89	16.50±3.82	16.50±3.82	18.00±3.88*	16.50±0.89	00	16.2±3.42*	14.8±0.71	16.2±3.42*	ND	10
*Candida*sp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	18	0
S. cerevisae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	22	0

** = Statistically Significant*

***Key**: SXT = Septrin/Clotrimoxazole; CH = Chloramphenicol; Sp= Spafloxacin;*
CPX = Ciprofloxacin; AM = Amoxacillin; AU = Augmentin;
CN = Gentamycin; PEF = Pefloxacin; OFX = Tarivid;
S = Streptomycin; FLU = Fluconazole; R = Resistance
ND=Not Determined

Table 5(b): Summary of the Statistical Analysis of Antibiotic Susceptibility of bacterial and fungal isolates zones of inhibition Summary of ANOVA Result

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1583.023	6	263.837	5.371	<0.0001	2.231
Within Groups	3438.669	70	49.124			
Total	**5021.691**	**76**				

Since the F value (5.371) higher than the F crit (2.231), there shows a significant variation between the isolates

Table 6: Antibiotic activity of extracts of *P.americana* seed on isolates from urine samples

Isolates	Conc (%)	100		80		60		40		20		10	
		e	A	E	a	e	a	e	a	e	a	e	a
E. coli	00	18±2.82	00	15.5±0.91	00	00	00	00	00	00	00	00	00
Proteus sp	00	16.20±3.42	00	00	00	00	00	00	00	00	00	00	00
Salmonella sp		15.50±0.91	00	15.50±0.91	00	00	00	00	00	00	00	00	00
Klebsiella sp		16.20±3.42	00	00	00	00	00	00	00	00	00	00	00
Candida sp		16	00	15.50±0.91	00	00	00	00	00	00	00	00	00
S. cerevisae		14.50±1.91	00	14	00	00	00	00	00	00	00	00	00

***Key**: Minimum Inhibitory Concentration* ***a** = aqueous extracts;* ***e** = ethanol extract*
The various concentrations of the aqueous extract did not inhibit any of the isolates.

DISCUSSION

The study was carried out to determine the antimicrobial activity of *P. americana* seeds on isolates from patients with urinary tract infection. The urine from healthy individuals were free of these isolates. This could be attributed to urinary tract normally being sterile and resistant to bacterial contamination due to acidity of urine, vesicoureteral valve and immunologic and mucosal barriers. Contamination arises when bacteria from other anatomical sites ascend the urethra to the bladder and sometimes from ureter to the kidney (Lindsay *et al*., 2019). The organisms isolated in this study were similar to those isolated by Behzadi *et al.* (2010). They isolated *E. coli, K. pneumoniae, P. mirabilis* and *Lactobacillus* sp. They found *E. coli* and *K. pneumoniae* being most prevalent. Martin *et al.* (2019) isolated similar organisms which included *E. coli* (32.2%), *S. aureus, P. mirabilis, K. pneumoniae* being the most prevalent. Catin *et al.*(2017) isolated *E. coli* from patients with UTI and postulated that most UTI are caused by *E. coli* in the gut which eventually spread to the urinary tract.

The yeast isolated in this study were *S. cerevisae* and *Candida* sp. *Candida* is a normal flora of the human body so may have being a contaminant in the urine. Most previous studies on UTI reported presence of *Candida* sp. but very few on *S. cerevisae*. Behzadi*et al.* (2010) associated UTI with *Candida albicans.* The presence of these organisms may be due to their ability to overcome body's defense, poor personal hygiene, blocked urine, suppressed immunity, as well as use of antibiotics which can disrupt natural flora of the bowel and urinary tract.

Phenol, Tannin, Alkaloid, flavonoid, saponin and terpenoid were present in both the ethanol and aqueous extracts but the concentrations were higher in the ethanolic extract. The higher concentrations in the ethanol extracts may be due to the phytochemicals being more soluble in ethanol than in water. Uzel*et al.* (2005) reported that tannin and flavonoids were extracted with ethanol and water whereas saponins and alkaloids were not. Difference in polarity of the extracting solvents may be responsible for the differences in solubility of the phytochemicals. Idris*et al.* 2009 detected flavonoids, saponins,tannins, steroids, alkaloids and terpenoids in the seed extracts of *P. americana* but not reducing sugar, phenol and glycoside.

The chemical composition of plants has been reported to be influenced by soil type, organic and inorganic composition, the type and number of microorganisms as well as interactions among these and other factors.

The presence of flavonoids can be attributed to flavonoid being synthesized by plants in

response to microbial attack. Omodamino *et al.*(2016) showed the presence of alkanoids, flavonoids, saponins, steroids, tannins and phenol in extracts of *P.americana* seed.

The bacterial isolates were found to be resistant to at least one of the conventional antibiotics with many of them showing multi-drug resistance. The *Candida* sp. and *S. cerevisae* were susceptible to fluconazole. The resistance against the antibiotics may have been acquired through misuse of antibiotics, not adhering to the recommended dosage, self-prescription and prolonged usage which are common practices in Nigeria. Evidence have shown that increased antibiotics usage may result in higher prevalence of resistant microorganisms while reduced antibiotic use showed lower resistant rates (Kim *et al.,* 2011). The ethanolic avocado seed extracts exhibited antimicrobial activity on all the isolates at a high concentration [100% and 80%].

The antibiotic resistance of some of the organisms may be attributed to factors such as prolonged misuse and indiscriminate use of antibiotics, antibiotic use in livestock feed (Laxmarayar *etal.,* 2013; Milach*et al.,* 2016). This has constituted a serious challenge to treatment of many infectious diseases. The use of plant derivatives is seriously being investigated as an alternative to solve the problem of antibiotic resistance. The efficacy of antimicrobial agent that could overcome this resistance need to be discovered (Quing*et al.,* 2017). The antimicrobial activity of *P. americana* has been studied extensively. Adelina*et al.*(2013) found the chloroform and ethanolic extracts to be amoebicidal, antimicrobial and giardicidal. The glycolic extract was reported by Jesus *et al*., 2015 to be active against *C. albicans* biofilm*, Cryptococcus neoformis*, and *Malassezia pachydermatis.* Guzman- Rodriguez (2013) reported activity of defensin from *P. americana* against *E.* coli and *S. aureus*.

.Many of the organisms which were resistant to conventional antibiotics were inhibited at 100% and 80% concentrations of the ethanolic extracts. The seeds of *P. americana* are rich in phenol which are fungicidal and antimicrobial activities (Dabias*et al.,* 2013). Ogundare and Oladejo (2014) reported a relatively high zone of inhibition (6-12mm) on *S. aureus* for leaf and bark extracts of *P. americana* .Yulian*iet al.* (2017) reported that ethanol extract *of P. americana* seed showed antimicrobial activity against Gm-ve and Gm-ve bacteria with the highest inhibition zone at 100% and weakest at 0 and 40%.

CONCLUSION

Many of the test organisms such as *E. coli*, *Proteus*sp and *Serratia* sp, *C. albicans* and *S. cerevisae* which were resistant to conventional antibiotics were observed to be sensitive to the ethanol extract of *P. americana* at high concentrations. The ethanolic extract was able to inhibit *C. albicans* which has been found to be very resistant to most of the plant extracts tested. *P.*a*mericana* seed extracts therefore has potent antibacterial and antifungal activity. The ethanol extract was more potent indicating ethanol as a better solvent in extracting phytochemicals from *P. americana*. *P. americana* can be used as a potential source of antimicrobial agent on susceptible organisms isolated in this study thus can be used as therapeutic and preservative agents. Further work can be done on its activity against other organisms and other solvents can be used for the extraction and their results compared with those of this study. Purification and activity of individual groups of the bioactive components on several resistant pathogenic and spoilage microorganism can be studied.

Author contributions

OOA and EDA conceived the study and designed the experiment. KEO and ACG conducted all the experiments. SG drafted the manuscript. EDA, SG, EA, KEO and KGA read and edited the manuscript.

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