ANTIBACTERIAL ACTIVITIES OF *SPONDIAS MOMBIN* ON CLINICAL ISOLATES FROM UNIVERSITY OF BENIN TEACHING HOSPITAL EDO STATE NIGERIA.

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ABSTRACT

Spondias mombin has been reportedly used in the treatment of many diseases in eastern Nigeria by the natives. The composition of this world-wide cultivated plant and the reported medicinal values prompted this study, which aimed at evaluating the phytochemical and antibacterial activity of the crude leaf extracts of *Spondias mombin.* The antibacterial activity of the n-hexane and the n-butanol leaf extract of *Spondias mombin* was tested using some selected clinical isolates using standard agar diffusion and broth dilution method. The phytochemical Screening of the plants leaves was determined by standard methods. Phytoconstituents was present in both n-hexane and n-butanol solvent extracts which includes; saponins, alkaloids, carbohydrate, steroids, glycosides and tannins, the n-hexane and n-butanol extract showed increased activity (as concentration increases, activity increases and decrease in concentration leads to reduction in activity) against both clinical and standard strains with the exception of *E. coli.* in n-hexane leaf extract. Good activity was demonstrated by CPR (10µl), OFL(5µl) and GENT(5µl). Both solvents extract and gentamicin demonstrated high MIC and MBC values. The results demonstrate that the plant leaves have medicinal properties, hence, its trad-medical usage.

Keywords: Antibacterial agents, Crude extract, Infectious, Phytochemical, *Spondias mombin*

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INTRODUCTION

Plants have been the basis for medical treatments throughout human history and traditional medicine is still widely practiced till date [1]. Modern medicine recognizes medicinal plants as a form of alternative medicine, as the practice of herbalism is not strictly based on evidence gathered using the scientific method. However, since a single plant may contain many substances, the effects of taking a plant as medicine can be very complex [2]. Pathogens are causative agents of diseases, they include: bacteria, fungal virus, protozoa, helminths, which cause a wide range of diseases in man, animals and plants. They are known to cause some serious systemic infection, especially in immune-compromised patients. The incidence of this systemic infection has increased with the advent of HIV (Human Immunodeficient Virus). Management of these diseases require prolonged usage of antibiotic agents with minimal side effects on the Humans, hence there is a need for an alternative medicine. The diseases caused by these agents have serious financial, social and economic implications on man. The discovery of these antimicrobial agents in the 20th century revolutionized the treatment of infectious disease [3]. The successes were however short-lived because of the development of resistance by these microorganisms. Antimicrobial resistance is a major health challenge that has made it difficult to successfully manage infectious disease. It has led to the search for alternative therapy to manage infectious disease and one approach is the use of medicinal plants screened for their possible antimicrobial properties which are inexpensive and effective against pathogenic microorganisms [4]. It has led to the search for alternative therapy to manage infectious diseases. A new approach is the use of medicinal plants screen for their possible antimicrobial properties which is inexpensive and effective against pathogenic microorganisms [4]. Many naturally occurring compounds found in plants have been shown to possess antifungal, antibacterial and antiprotozoan activities and serve as a source of antimicrobial agents that can be used either systemically or locally [5, 6]. The associated link between the composition of this world-wide cultivated plant (*Spondias mombin*) and the reported medicinal and economic values prompted this study which aimed at evaluating the phytochemical and antibacterial activity of the leaf extracts of *Spondias mombin* plant. *Spondias mombin* belongs to the family Anacardiaceae (Linn.), is a widely cultivated economic plant that produces edible floral parts. Its local names are: Creole (gwo momben, gran monben, monben fran); Dutch (hoeboe); English (mombin plum, yellow mombin, hog plum, yellow Spanish plum); French (grand mombin, Gros mombin, mombin jaune); Fula (Chali, Chaleh, Tali); Indonesian (kedongdong cina, kedongdong cucuk); Portuguese (cajá, cajarana, cajamirim, pau). In Nigeria *Spondias mombin* could also be called Iyeye in Yoruba and Uvuru in Igbo. *S. mombin* grows very easily from stakes to make live fences and

enclosures. In West Nigeria the tree serves as shade and the stem for making fence. The fruits are sold in West African markets. Ripe fruits are eaten or stewed with sugar. The small fruits can be eaten or used in making juices and ice creams. The aromatic fruit of *Spondias mombin* is rich in vitamins [7]. The young leaves are cooked as greens and the green fruits pickled in vinegar and eaten like olives with salt and chili. A tea of the flowers and leaves is taken to relieve stomach ache, biliousness, and urethritis, cystitis, eye and throat inflammations. The plant is used in folkloric medicine for the treatment of various diseases. A decoction of the bark is taken in cases of severe cough with inflammatory symptoms, giving relief through vomiting [8, 9]. The powdered bark is used for treating wounds. The gum is employed as an expectorant and anti-helminthic [10]. The plant is reported to have antibacterial, anti-fungal, and anti-viral effects [11]. It also has sedative, anti-epileptic, anti-psychotic, antidiarrheal, anti-inflammatory, cytotoxicity properties [10, 12, 13]. The leaf of the species has been reported to have α-amylase inhibitory effect [12]. Although some work has been done on the pharmacological properties of crude extracts of *Spondias mombin* leaf, there are very few reports on the activities of the stem. The phytochemical, proximate, minerals and vitamins A and C compositions of *Spondias mombin* leaves were determined by Igwe *et al*. [13].

Plate 1: *Spondias mombin* plant showing leaves and seeds.

The plant accumulates phenolic compounds [11, 12]. The fruit juice is drunk as a diuretic and febrifuge. The decoction of the astringent bark serves as an emetic, a remedy for diarrhea, dysentery, hemorrhoids and a treatment for gonorrhoeae and leucorrhea. In Mexico, it is believed to expel calcifications from the bladder. The powdered bark is applied on wounds. It has been recorded to have antimicrobial [10], anti-bacterial [14], anti-fungal [9], antiviral properties [15]. Offiah and Anyanwu [16] have also reported the abortifacient activity of the aqueous extracts. A novel beta-lactamase inhibitor was isolated from a n-hexane extract of the plant by Morton & Dowling [7].

MATERIALS AND METHODS

Plant collection and identification

Spondias mombin leaves were sourced and collected from University of Benin environ and Iyowa community in Edo state Nigeria, in the month November 2016. The plant sample was taken to University of Benin herbarium and identified by Professor L. Okurobo (Botanist) in the Department of Pharmacognosy Faculty of Pharmacy, University of Benin, Benin city.

Preparation of plant material
The plant's leaves were cut or sliced into smaller pieces and air dried under shade for two weeks. The dried sample was then pulverized using Thomas willey milling machine (Gallenkamp ak, mettle, Switzerland) in the Department of Pharmacognosy, University of Benin. The powdered sample was air-dried again to reduce moisture content before storing in an air tight container prior to extraction of its contents.

Extraction of plant material
Four hundred grams (400 g) of the coarsely powdered leaf's extracts with 1000 ml of 90% acetone and 90% *n*-hexane were extracted using maceration method. The tank with the contents were tightly covered and allowed to stand for 24 hours with periodic agitation. The content was filtered with Whatman No. 1 filter paper thereafter, separating the filtrate from the marc. This was concentrated and evaporated to dryness on a thermostatic water bath at 40^0C. The yield was weighed and stored in a clean amber bottle refrigerated at -4^0 C prior to use.

Phytochemical screening
Phytochemical Screening of the n-hexane and acetone extracts were carried out using standard methods [17, 18] to screen for the presence of various chemical constituents namely: Flavonoids, Alkaloids, Anthraquinone, Carbohydrate, Tannins, Cardiac Glycosides, Glycosides, Saponins, and Steroids.

Preparation of media
Each bacteriological medium used was prepared according to the Manufacturer's specification. Sterilization was by autoclaving at 121^0C for 15 minutes. Pipettes and other glass wares were wrapped with Aluminum foil paper and sterilized in hot air oven at 160^0 C for 60 minutes.

Collection / preparation of bacteria culture
The clinical isolates were collected from patients in Microbiology Laboratory, University of Benin Teaching Hospital. Having identified the morphological features of the organisms, clinical isolates obtained were reconfirmed using biochemical test. The following clinical isolates (*Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa* and *Escherichia coli)* and their standards strains were used in this study. These pure isolates were all cultured in a nutrient agar slant and incubated at 37^0C for 24 hours before storage at -4^0 C prior to use.

Biochemical characterization of bacteria isolates
The following biochemical test were used, which includes: catalase, Voges Proskauer (VP), glucose test, citrate utilization test, coagulase, Indole, Motility, Oxidase, Urease, KEN, Methylene red (MR) and Sugar fermentation (lactose, mannitol, sucrose)

Antibacterial assay of crude extracts of *Spondias mombin* leaves
Agar well diffusion method was used as described by Adeniyi *et al*. [19] to determine the antibacterial activity of the extracts. Molten sterile nutrient agar (20 ml) was poured into sterile petri dish aseptically and allowed to set. The set nutrient agar plates were flooded with 2.0 ml of the standardized (standardization was done with normal saline and serially diluted to obtain 10^{-6} CFU/ml) stock culture (equivalent to 10^5-10^6 CFU/ml) and the excess was drained off aseptically. A sterile cork borer (diameter 8mm) was used to bore equidistant cups into the agar plate aseptically. One drop of the molten agar was used to seal the bottom of the bored hole, so that the test agent will not sip beneath the agar. Zero-point one (0.1) ml of the different concentrations (600, 300, 150, 75, 37.5) in mg/ml respectively of the extract was added to fill the bored holes aseptically. Negative control was prepared by putting 0.2 ml of sterile distilled water in one of bored hole. One-hour pre-diffusion time was allowed, after which the plates were incubated at 37^0C for 24 hours. The zones of inhibition were then measured in millimeter. This method was carried out in duplicates and the mean of the duplicate results was recorded. This was equally done for the reference drug gentamicin as a positive control.

Antibiotic sensitivity testing
This was carried out Using Kirby-Bauer CLSI modified Disc Agar Diffusion technique (DAD) [20]. One milliliter (1.0 ml) of standardized overnight culture of each isolate (containing 10^6 CFU/ml) was used to flood the surface of Mueller Hinton Agar (MHA) plates and excess drained off and allowed to dry The standard antibiotic discs were then aseptically placed at reasonable equidistance on the inoculated MHA plates and allowed to stand for 1 hour to allow diffusion of the antibiotics into the agar. The plates (prepared in duplicates for each isolate) were then incubated at 37°C for 24 h [21]. The diameter of the zone of inhibition produced by each antibiotic disc was measured and recorded.

Determination of Minimum Inhibitory Concentration (MIC)
Two-fold serial dilution of the stock solution of the extracts was made to obtain concentrations of 600 mg/ml, 300 mg/ml, 150 mg/ml, 75 mg/ml and 37.5 mg/ml. A 2 ml of each dilution was incorporated into 18 ml double strength Mueller Hinton Agar and poured into the petri dishes aseptically. Standardized inoculum of the isolate was immediately added to the discs in volumes of about 20 µl. A 20 µl aliquot of 10% sterile

distill water was added to the sterile paper disc as a negative control. The plates were left at ambient temperature for 15 minutes to allow excess pre-diffusion of organism prior to incubation at 37^0C for 24 hours. The lowest concentration that did not show any visible growth around the paper disc when compared to the control was considered as the Minimum Inhibitory Concentration [22].

Determination of Minimum Bactericidal Concentration (MBC)

This was determined by using sterile forceps to place the filter paper discs that did not show any growth from the MIC plates into sterile Nutrient broth containing inactivating agent 3 %v/v Tween 80. This was incubated at 37^0C for 24 hrs. The Minimum Bactericidal Concentration was considered as those tubes that did not show any turbidity [23].

RESULTS

The leaf extracts of *S. mombin* leave extract using n-hexane and butanol as solvents. The percentage yield for n-hexane was 7.68 % and that for butanol was 12.12 %. There was substantial difference in the yield of both solvents used (Table 1)

Table 1: Percentage extract yield of *Spondias mombin* leaves.

Plant Sample	Solvents	Yield	Percentage Yield
Spondias mombin	N-Hexane	46.1g	7.68%
Spondias mombin	N-Butanol	72.8g	12.12%

The qualitative and quantitative phytochemical screening of *S. Mombin l*eaves done with the various solvents (n-hexane and n-butanol) extracts. Phytoconstituents present were carbohydrates, glycoside, cardiac-glycoside, anthraquinonoid, saponins, flavonoids, tannins, alkaloids, steroids and triterpenes all these were found in both solvent extracts, however, much of carbohydrates, saponins, alkaloids and steroids were observed (**Table 2)**.

Table 2: Phytochemical screening of *Spondias mombin* leaf extract

Phytochemical constituents	n-Hexane	n-Butanol
Carbohydrates	+	++
Glycoside	+	+
Cardiac Glycoside	+	+
Anthraquinone	+	+
Saponins	+	++
Flavonoids	+	+
Tannins	+	+
Alkaloids	+	++
Steroid's	+	++
Triterpenes	+	+

Key: ++ present in large amount + present

The result for the reactions of the test bacterial to the various biochemical test conducted in the study. *S. aureus* were positive for Citrate and Coagulase test and negative to other biochemical test conducted in the study. *E. coli* was Glucose, Lactose, Mannose, Indole, and Motility Positive but negative to Sucrose, Citrate, Urease, Voges-Proskeur, Oxidase, Methyl Red and Coagulase. Test. *Ps aeruginosa*, Citrate, Motility and Oxidase positive while *S. typhi* was Glucose, Mannose, Motility, and Methyl Red positive.

Table 3: Biochemical identification of clinically selected isolates

	Biochemical test												
Isolates	**Glu**	**Lacto**	**Man**	**Suc**	**Cit**	**Urea**	**Ind**	**VP**	**Mot**	**Ox**	**Coag**	**Kcn**	**MR**
Staph. aureus	-	-	-	-	+	-	-	-	-	-	+	-	
E. coli	+	+	+	-	-	-	+	-	+	-	-	NA	+
P. aeruginosa	-	-	-	-	+	-	-	-	+	+	-	-	-
S. typhi	+	-	+	-	-	-	-	-	+	-	-	-	+

Key: Glu = Glucose; Lacto = Lactose; Man = Mannitol (mannite); Suc = Sucrose; Cit = Citrate test; Urea = Urease test; Ind = Indole test; VP = Voges-Proskeur test; Mot = Motility test; Ox = Oxidase test; Coag. = coagulase test; MR = Methyl-red test

All clinical test isolates and their standard strains were susceptible to n-hexane leaves extract and gentamicin antibiotic except *E. coli* clinical isolates that is resistant at a lower concentration of 75.00 and 37.50 mg/ml respectively (Table 3).

Table 4: Diameter of zones of inhibition of crude n-hexane extracts of *Spondias mombin* on clinical and reference bacterial isolates.

	Zone of inhibition of n– hexane leave extract (mm)				
Bacterial isolates	**600mg/ml**	**300mg/ml**	**150mg/ml**	**75mg/ml**	**37.5mg/ml**
E. coli	24.00±1.00	22.00±0.00	20.00±0.00	NA	NA
***E. coli* ATCC29214**	28.50±1.50	25.00±0.00	24.00±1.00	23.50±1.50	20.00±0.00
Gent. *E. coli*	50.00±0.00	29.00±0.00	35.00±0.00	30.00±0.00	22.00±0.00
Gent. ATCC29214	60.00±0.00	50.00±0.00	37.50±1.50	36.00±2.00	30.00±0.00
P. aeruginosa	31.50±2.50	29.50±0.50	25.00±0.00	23.50±1.50	20.00±0.00
***P. aeruginosa* ATCC29853**	30.00±0.00	25.00±0.00	24.50±0.50	21.50±0.00	15.00±0.00
Gent. *P. aeruginosa*	60.00±0.00	51.00±1.00	43.00±2.00	34.50±0.50	30.00±0.00
Gent. *P. aeruginosa* ATCC29853	50.00±0.00	40.00±0.00	25.00±0.00	23.00±2.00	20.00±0.00
S. aureus	28.00±0.00	21.00±3.00	18.00±2.00	12.50±2.50	25.00±0.00
***S. aureus* ATCC12598**	27.50±2.50	26.00±1.50	24.50±0.50	21.00±1.00	13.50±0.50
Gent. *S. aureus*	40.00±0.00	35.00±0.00	30.00±0.00	27.50±0.50	22.50±2.50
Gent. *S. aureus* ATCC12598	35.00±0.00	37.50±0.50	36.00±0.00	34.50±0.50	27.50±2.50
S. typhi	23.50±3.50	26.50±0.50	25.00±0.00	20.00±0.00	18.50±0.50
***S. typhi* ATCC14028**	30.00±0.00	26.00±1.50	24.00±1.00	21.50±1.50	17.50±2.50
Gent *S. typhi*	40.00±0.00	38.50±0.50	31.50±0.50	26.50±0.50	23.50±0.50
Gent. *S. typhi* ATCC14028	40.00±0.00	35.50±0.50	32.50±0.50	30.00±0.00	28.00±0.00

Key: ± = mean standard deviation. Cork borer diameter = 8mm.
Gent. = Gentamicin drug. NA= No activity.

Different pattern of susceptibility was observed in n-butanol leave extract of *S. mombin*, as both clinical isolates and standard strains were all susceptible to the various concentration of the leaf extract but resistance was equally observed with both clinical and standard strains at a concentration of 37.50 mg/ml. However, all clinical and their standard strains were susceptible to gentamicin antibiotics as depicted in table

Table 5. Diameter of zones of inhibition of crude n-butane extracts of *Spondias mombin* on clinical and reference bacterial isolates.

Bacterial isolates	**Zone of inhibition of n–butane leave extract**				
	600mg/ml	**300mg/ml**	**150mg/ml**	**75mg/ml**	**37.5mg/ml**
E. coli	23.50±1.50	20.00±0.00	19.00±0.00	15.00±0.00	NA
***E. coli* ATCC29214**	30.00±0.00	25.00±0.00	20.00±0.00	12.00±0.00	NA
Gent. *E. coli*	50.00±0.00	29.00±0.00	35.00±0.00	30.00±0.00	22.00±0.00
Gent. ATCC29214	60.00±0.00	50.00±0.00	37.50±1.50	36.00±2.00	30.00±0.00
P. aeruginosa	18.50±1.50	17.00±1.00	14.00±0.00	10.00±0.00	NA
***P. aeruginosa* ATCC29853**	25.00±0.00	20.00±0.00	19.00±0.00	15.00±0.00	NA
Gent. *P. aeruginosa*	60.00±0.00	51.00±1.00	43.00±2.00	34.50±0.50	30.00±0.00
Gent. *P. aeruginosa* ATCC29853	50.00±0.00	40.00±0.00	25.00±0.00	23.00±2.00	20.00±0.00
S. aureus	20.00±0.00	15.00±0.00	13.00±0.00	12.00±0.00	NA
***S. aureus* ATCC12598**	30.00±0.00	16.00±0.00	15.00±0.00	13,50±0.50	NA
Gent. *S. aureus*	40.00±0.00	35.00±0.00	30.00±0.00	27.50±0.50	22.50±2.50
Gent. *S. aureus* ATCC12598	35.00±0.00	37.50±0.50	36.00±0.00	34.50±0.50	27.50±2.50
S. typhi	17.00±0.00	18.50±0.50	16.00±1.50	15.00±0.00	NA
***S. typhi* ATCC14028**	20.00±0.00	20.00±0.00	17.00±0.00	10.00±0.00	NA
Gent *S. typhi*	40.00±0.00	38.50±0.50	31.50±0.50	26.50±0.50	23.50±0.50
Gent. *S. typhi* ATCC14028	40.00±0.00	35.50±0.50	32.50±0.50	30.00±0.00	28.00±0.00

Key: ± = mean standard deviation.
Cork borer diameter = 8mm
NA= No Activity. Gent = Gentamicin drug used

All isolates and their standard strains were not susceptible to CAZ, AMP, CTR and CXC, *S. typhi* and its standard strains and *Ps. aeruginosa* clinical isolate were resistant to GENT antibiotics while *E. coli* and *Staph. aureus* and their standard strains were all susceptible to GENT. It was equally observed that all clinical bacterial and their standard strains were susceptible to CPR, except the clinical bacterial that shoed resistant to CPR. Clinical isolates (*S. aureus*, *Ps. Aeruginosa* and *E. coli*) were resistant to OFL, other organisms and their standard strains were susceptible, as shown in the table below.

Table 6: Susceptibility profile of clinical and reference bacterial isolates using convectional multi disc drug

	Zone of inhibition (mm) antibacterial agents										
Organism	CAZ (30µg)	CRX (30µg)	GENT (10µg)	CPR (5µg)	OFL (5µg)	AUG (30µg)	NIT (300µg)	AMP (10µg)	CTR (30µg)	ERY (5µg)	CXC (30µg)
S. typhi	-	-	R (10)	S (28)	S (30)	-	-	-	-	-	-
ATCC14028	-	-	R (10)	S (25)	S (22)	-	-	-	-	-	-
S. aureus	-	-	S (20)	-	-	-	S (22)	-	-	S(20)	-
ATCC12598	-	S (20)	S (30)	S (30)	S (28)	S (20)	-	-	-	-	-
Ps. aeruginosa	-	-	-	S (30)	-	-	-	-	-	-	-
ATCC29853	-	-	S (15)	S (30)	S (25)	-	-	-	-	-	-
E. coli	-	-	S (25)	-	-	-	S (20)	-	-	R(10)	-
ATCC29214	-	-	S (20)	S (23)	S (30)	-	-	-	-	-	-

Key: R = Resistance S = Susceptible
- = No activity NIT= Nitrofurantoin AMP= Ampicillin CAZ= Ceftazidime CTR= Ceftriaxone
CXC= Cloxacilin ERY= Erythromycin GEN= Gentamicin Cefuroxime CPR= Ciprofloxacin

OFL= Ofloxacin AUG= Amoxicillin/Clavulanate

Spondias mombin n-hexane and n-butanol leaf extract all demonstrated good inhibitory activity on all clinical isolates and their standard strains used in this study with both solvent extracts showing a very high MBC values at higher concentrations and all clinical isolates and their standard strains were susceptible to gentamicin at a low concentration at MIC and MBC (Table 6).

Table 7: Minimal Inhibitory Concentration (M.I.C) and Minimum Bactericidal Concentration (M.B.C) of *Spondias mombin* leaves extract of the various solvents

Organism	**N-Hexane extract mg/ml**		**N-Butanol extract mg/ml**		**Gentamicin Mg/ml**	
	MIC	MBC	MIC	MBC	MIC	MBC
S. typhi	37.50	75.50	150.00	>600.00	1.33	1.14
***S. typhi* ATCC14028**	37.50	75.00	150.00	>600.00	1.33	2.00
S. aureus	37.50	75.50	75.00	300.00	1.33	1.14
***S. aureus* ATCC12598**	37.50	75.00	75.00	300.00	1.33	1.66
P. aeruginosa	150.00	300.00	600.00	>600.00	1.33	2.00
***P. aeruginosa* ATCC29853**	150.00	300.00	150.00	300.00	1.33	2.60
E. coli	150.00	300.00	600.00	>600.00	1.33	1.14
***E. coli* ATCC29214**	150.00	600.00	300.00	>600.00	1.33	2.60

DISCUSSION

There was substantial difference in the yields obtained from n-butanol (12.12 %) and n-hexane (7.68 %) and solvent extract, this is not surprising since butanol is generally able to dissolve multivariable type of component; polar and non – polar, simple and complex chemical compounds compared with n- hexane which is able to extract mainly lipophilic compound present in *S. mombin* extract. All the extracts contain carbohydrate, cardiac glycoside, tannin, saponins, triterpenes, anthraquinone, flavonoid, resin, and steroid. The secondary metabolites are generally found as components of plant [24]. It also confirms finding made by Abdullahi *et al*. [25]. The secondary metabolites have been reported to possess appreciable inhibiting activities against various organisms [26]. These phytocompounds have been confirmed to have antibacterial activity [27, 28, 29]. Many workers have reported the presence of plant's chemicals in *S. mombin* these include steroid and cardiac glycoside, which are useful in the treatment of diseases associated with Humans heart [30]. Presence of saponins in all extract suggests the ability of *S. mombin* to play the role of antidiarrheal and anti-hemorrhagic agent as described by Asqwith and Butler [31]. Tannins have been reported to hasten the healing of wounds and inflamed mucous membrane [32]. Saponins, though are hemorrhagic on red blood cells, are harmless when taken orally and they have beneficial properties of lowering cholesterol level in the body [33]. Glycoside and flavonoid are known to inhibit tumor growth and protect against gastrointestinal infection [34].

The different solvent extracts exhibited different level of antibacterial activities which were also dependent on the nature of the test organism. Generally, it is observed that Gram-positive bacteria are susceptibility due to the nature of their cell wall component that possesses peptidoglycan with teichoic acid [35] hence, the susceptibility of the Gram-positive clinic isolates to both extracts. *Staphylococcus aureus* were susceptible to n-hexane extracts at a very low concentration of 37.5 mg/ml. This is of great importance as it has been reported that this organism has developed resistance to many antibiotics, which sometime makes its clinical management difficulty [36]. High activity of antibacterial agent at low concentration is very essential for chemotherapeutic purposes in order to avoid toxicity in patient. However, the Gram-negative bacteria possess lipopolysaccharides and lipoproteins in their cell wall structures, hence a hindrance of antimicrobial agents but on the contrary, appear to be more susceptible, this may be due to spectrum of activity of the plant extract as they were able to penetrate the lipid bilayer of the Gram-negative organisms. The reference drug used (gentamicin) which had a remarkable activity on all microorganisms used in the study which is as a result of the fact that gentamicin is not a drug that can easily be abused by drug user, as it is administered intramuscular. The n-butanol solvent leaf extract of *S. mombin* has the ability to extract intermediate bioactive compounds from plant material, however, a remarkable antibacterial activity was exerted on the Gram-negative and Gram-positive organisms at a higher concentration. Both solvent extracts had a good inhibition on all the Enterobacteriaceae organisms. The lipophilic compound present in n-hexane extract has the propensity to induce lipopolysaccharides leakage, this might be the reason why it penetrates the

lipopolysaccharides of the Gram-negative organism and exert it effect. However, *E. coli* was resistant to n-hexane extract at a concentration of 75.00 and 37.50 mg/ml respectively, also resistance was equally observed on the Gram-positive bacterial. All clinical isolates used in the study were resistant at a lower concentration of 37. 50 mg/ml except the standard strains that were susceptible to *S. mombin* leaf extract of n-butanol this might be due to the differences in mechanism of drug entering in the bacterial cell. All organisms used in this study were susceptible to the reference drug used (Gentamicin). Generally, the degree of the antibacterial activity of the plants extracts varies from one test organisms to another, thus larger zones of inhibition were produced by the susceptible organisms than the resistant isolates. However, these activities are concentration dependent, irrespective of the test organism involved. This variation in level of the activity among the extracts could be due to the differences in solubility of the active ingredient in each solvent used on one hand, and the constitutional or structural variability of the tested organisms on the other hand.

The n-hexane extract of *S. mombin* leaf had a lower MIC values (37.5 mg/ml – 150 mg/ml) and MBC values (70.00 mg/ml – 300.00 mg/ml) against clinical isolates compared with MIC of n-butanol extract that displayed higher values (75.00 – 600 mg/ml) with its MBC higher than >600 mg/ml, which contradict the report of Tona *et al.* [37]. When biocidal concentrations were considered, *S. mombin* extract's leaf extracts MBCs were very high compared to those of the standard (gentamicin) antibiotic agent used in this study. This observation is a common occurrence when one is dealing with crude extract having a lot of constituents that play little or no role in the antibacterial activity of the crude extract [36]. Furthermore, these impurities may even antagonize the antibacterial activity of the active constituent.

The result of this extract had shown activity against Gram-positive and Gram-negative bacteria, indicating a broad spectrum of activity, *S. mombin* which has been widely reported [38, 39]. The susceptibility pattern of bacterial isolates to standard antibiotic multidisc (Oxoid) was interpreted following the guideline of the Clinical Laboratory Standard Institute [40]. Some clinical isolates and all their standard strains were susceptibility to CPR(5µl) with the exception of *E. coli* and *Staph. aureus* that showed completely resistant. Same pattern was observed with OFL(5µl) with the exception of *Ps. aeruginosa, E. coli* and *Staph. aureus* Also, all test showed a remarkable susceptibility to GENT (10µl) with the exception of *S. typhi* which might be due to the fact that gentamicin is a drug that cannot be abused as it is taken intramuscularly. The susceptibility profile of both clinical isolates and standard strains to multidisc antibiotic revealed that, the organism were all resistant, due to indiscriminate use of drugs both on human and livestock, a remarkable susceptibility profile was demonstrated by the organism under investigation on GENT, CPR and OFL , however, these drug all have broad spectrum of activity and used to treat both upper and lower respiratory tract infection, urinary tract infection, pelvic inflammatory infection and gentamicin mainly has a large spectrum of activity against both Gram positive and negative organism [41]. CRX and AUG were more effective on standard strain *S. aureus* and has no activity on the *S. aureus* clinical isolates, this might be due to mis-used of drugs hence the development of resistance by this organism.

CONCLUSION

The hexane and butanol extract of *Spondias mombin* leaf have demonstrated a high antimicrobial activity against both clinical and standard strains. The active constitutes of the extract that showed this activity were not identified but the presence of flavonoids, alkaloids and tannins in the plant gives credence on the herbal use of the plant as a remedy to diseases.

RECOMMENDATION

Spondias mombin leaf extracts can be used tradio-medically for treatment of *Staph. aureus* which is one of the organisms associated with a problem of antimicrobial resistant in clinical settings.

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