Evaluation of Inhibitory Zone Diameter (IZD) of crude *Spondias mombin* (Linn.) extracts (root, leaf, and stem bark) against thirty infectious clinical and environmental isolates.

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Abstract

The purpose of this research work is to evaluate the Inhibitory Zone Diameter (IZD) of crude *Spondias mombin* extracts against thirty infectious clinical and environmental organisms. The root, leaf and stem-bark of *S. mombin* were harvested and air-dried. The dried *S. mombin* was milled into powdered form using manual grinder. Powdered *S. mombin* (1kg) each of the different *S. mombin* parts was extracted with 3 L of 70% (v/v) ethanol, ethyl acetate and distilled water for 72 h at room temperature. The antimicrobial assay of crude *Spondias mombin* extracts on the test bacteria was determined by the agar diffusion method. A 0.1 ml of 1:10,000 dilutions (equivalent to 106 cfu /ml) of fresh overnight broth culture of the test bacteria was seeded on molten Mueller-Hinton agar plate. Using a sterile cork borer of 6 mm diameter, equidistant wells were bored on the agar. One millimeter of the various re-suspended extracts (7.5, 15, 30 and 60 mg / ml) was introduced into the wells. *Ofloxacin* (5 µg) was used as control. The plates were then incubated at 37°C for 24 to 48 hours. Antifungal assay of crude root, leaf, and stem bark of *Spondias mombin* extracts were determined using Agar well diffusion method. A 5- day old fungal culture on potato dextrose agar (PDA) was flooded with 2 ml of sterile distilled water containing 3% glycerol. The spores were harvested by scraping with a sterile inoculating loop. Sterile PDA plates were inoculated with 0.1 ml of the fungal spore suspension using the spread plate technique. Five wells were bored on the potato dextrose agar (PDA) plates using a 6 mm sterile cork borer. The plates were allowed to stand on the bench for 1 hour before incubating at 25°C for 5 days. Diameter of zones of growth inhibition was then measured in millimeter with a vernier caliper. Aqueous leaf extract of *S.mombin* had the zone of inhibition of 23 mm against *B. cepacia* at 60 mg/ml. The aqueous stem bark and root of *S. mombin* extracts at 60 mg/ml had the highest zone of inhibition of 23 mm, each against *C. koseri* and *K. ozaenae*. However, the aqueous Stem bark extract of *S.mombin* did not show any antibacterial activity against *M. abscessus* neither did the aqueous root extract show antibacterial activity against *E. coli.* This study revealed that the plant extracts possessed antibacterial and antifungal activities against some highly infectious clinical and environmental pathogens which justified their use in ethno-medicine for treatment of infectious diseases.

Keywords: Inhibitory zone diameter, *Spondias mombin*, Infectious clinical, Environ mental isolates, Agar well diffusion

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Introduction

Spondias mombin linn is a small tree that grows up to 20 m (60 ft.) high and 1.5 m (5 ft.) in girth, moderately buttressed; stem bark is thick, corky, deeply fissured, slash pale pink, darkening rapidly, branchlets glabrous; leaves pinnate, leaflets 5-8 opposite pairs with a terminal leaflet. It belongs to the family *Anacardiaceae*. It flowers develop between January - May and fruits between July-September, The fruits have a sharp, somewhat acid taste and are edible. The matured fruit has a leathery skin and a thin layer of pulp. The fruit pulp is either eaten fresh, or made into juice, concentrate, jellies, and sherbets. The fruit-juice is used as a febrifuge and diuretic. The roots are also used as febrifuge in Ivory Coast. The bark is used as a purgative and in local applications in the treatment of leprosy. The stem bark decoction is also used in the treatment of severe cough. It serves as an emetic, a remedy for diarrhea, dysentery, hemorrhoids and a treatment for gonorrhea and leucorrhea [1]. The decoction of the astringent stem bark is believed to expel calcifications from the bladder. The juice of crushed leaves and the powder of dried leaves are used on wounds and inflammations. The gum is employed as an expectorant and to expel tapeworms [2]. A decoction of the mashed leaves is used by the Ibos (Nigeria) for washing a swollen face. A leaf infusion is a common cough remedy or used as a laxative for fever with constipation. A leaf decoction is used in treatment of gonorrhea. The leaves are used in Ivory Coast for fresh wounds to prevent inflammation [3].

Nworu et al. [4], were the first to formulate groupings of genera in the *Anacardiaceae*, dividing the family into two tribes, the *Anacardieae* and *Spondieae* [sic]. Subsequently, Mitchell et al. [5], published the tribe *Spondiadeae* was the first to formulate a relatively modern concept of *Spondias*, in which he included *Evia Blume*, *Cytheraea* Wight & Arn., and *Wirtgenia* Jung. ex

Hassk. (nom. illegit., non *Wirtgenia* Sch. Bip.).

The pulp of the fruit is sometimes eaten directly, especially when found in the forest, but is too acid to be considered attractive; it can also be boiled or dried. It is especially used for syrup, ice cream, drinks and jellies. Juices improve with keeping overnight as the mild astringency of the fresh fruit disappears. Fermented products are also good. The fruit is a good source of vitamins A and C; vitamin C quantities vary between 34 and 54 mg/g, and carotenoids are presumably present in reasonable concentrations. There is great variation in fruit quality from region to region, some being sweet and pleasant and others quite disagreeable in flavor [6]. Nworu et al. [4], reported the several uses of the plant based on oral communication and not on any recorded scientific investigation. Infusion of its leaves has been used since long time, without any report of collateral effects, due to its activity.

Materials and Methods

Chemical and reagent

All chemical reagents were of analytical grade and purchased from Sigma chemical company Limited United kindom (UK).

Microorganism for the research work

Thirty clinical and environmental microorganisms were used for this research work, which comprised 20 bacteria and 10 fungi. The breakdown of the thirty organisms used include; 10 typed bacterial isolates,10 locally isolated bacteria and 10 locally isolated fungi. The typed test bacteria included *Mycobacterium fortuitum* ATCC 6841, *Mycobacterium smegmatis* ATCC 19420, *Mycobacterium abscessus* ATCC 19977, *Mycobacterium phlei* ATCC 19240, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 35659, *Salmonella typhi* ATCC 35723, *Pseudomonas aeruginosa* ATCC 25619, and a fungus, *Candida albican* ATCC 90029. The locally isolated bacteria include *Salmonella choleraesuis*, *Salmonella arizonae*, *Proteus mirabilis*, *Aeromonas hydrophilia*, *Bacillus subtilis*, *Salmonella typhi*, *Shigella dysenteriae*, Burkholderia cepacia, *Citrobacter koseri* and *Klebsiella ozaenae*. The test fungi include *Aspergillus niger*, *Fusarium solani*, *Saccharomyces cerevisiae*, *Aspergillus flavus*, *Phytophera megakarya*, *Candida kruise*, *Rhizopus stonifer*, *Trichoderma horizionum*, *Fusarium vortercelium* and *Syncephalastrum racemosum*.

Sources of microorganisms

All typed strains used for this research work were purchased from the University of Pennsylvania, School of Medicine, Philadelphia, United States of America (USA), in an America Type Culture Collection (ATCC), and the other locally isolated bacteria and fungi were clinical organisms collected from Central Medical Laboratory (CML), Obafemi Awolowo University Teaching, Hospital (OAUTH), Ile Ife, Osun State, and the Institute of Advance Medical Research and Training (IMRAT), University College Hospital, Ibadan, Oyo State Nigeria. All isolates were from the clinical sources which include wound infection, Urinary tract infection, Upper respiratory tract infection, pneumonia cases, gastrointestinal disorder (diarrhea) and skin infection.

Authentication of test microrganisms

The identity of the test organisms was confirmed using Biomerieux API 20E Kits for bacteria as specified by the manufacturer's instruction. The test fungi were authenticated by ID 32 C system (Biomerieux, France) following the manufacturer's instructions. The yeast isolates were identified by the ID 32 C Analytical Profile Index [7].

Standardization of organisms: The organisms were standardized using a serial dilution technique i.e., the stock sample on a slant was introduced in an already prepared nutrient broth and incubated overnight (18-24 hours). A 0.1 ml of the broth was introduced into 9.9 ml of sterile distilled water to make a dilution of 1:1000 and also from the dilution; another 0.1 ml was pipetted into 9.9 ml of sterile distilled water to make a dilution of 1:10,000. It was then standardized according to National Committee for Clinical Laboratory Standards [8] by adding normal saline gradually and its turbidity compared with McFarland standard of 0.5 which was approximately (1.0×10^6 Cfu /ml). The same procedure was repeated for the fungi using potatoes dextrose broth.

Collection of plant materials

The root, leaf, and stem bark of *Spondias mombin* tree were harvested early in the morning into a polythene bag at Oja Oba market, Ikare Akoko, Ondo State, a tropical rainforest of Ondo State, Nigeria with latitude (7.21692 North) and longitude (5.21561 East). The plant was authenticated at the herbarium of the Department of Pharmaceutical chemistry, Obafemi Awolowo University, Ile -Ife, Osun State, Nigeria and voucher was deposited. A voucher number was issue at the herbarium for proper documentation (DPC-SPM 0340) (Figure 1).

Preparation and extraction of Spondias mombin plant

The root, leaf and stem-bark of *Spondias mombin* plant were harvested and air-dried. The dried leaves were milled into powdered form using manual grinder. Powdered plant material (1 kg) each of the different plant parts was extracted with 3 L of 70% (v/v) ethanol, ethyl acetate and distilled water for 72 h at room temperature. The extraction process was repeated four times until the extract became clear. The filtrates were combined and concentrated under reduced pressure Rotatory Evaporator at 35°C to give, SMRE, SMREA and SMRAQ for root part; SMLE, SMLEA and SMLAQ for the leaf part; and SMSBE, SMSBEA and SMSBAQ for the stem-bark part. The dry extracts were kept in tightly stoppered bottles in a refrigerator at 20°C for further analysis.

Antimicrobial assay of Spondias mombin extracts

The antimicrobial activities of the *Spondias mombin* extracts were assessed on the test organisms. The test bacteria and fungi were selected on the basis of the diseases against which *Spondia mombim* was used. The antibacterial assay of the extracts was determined by the agar well diffusion method [9].

Antibacterial assay of crude *Spondias mombin* extracts (root, leaf, and bark): The antimicrobial assay of crude *Spondias mombin* extracts on the test bacteria was carried out by the agar

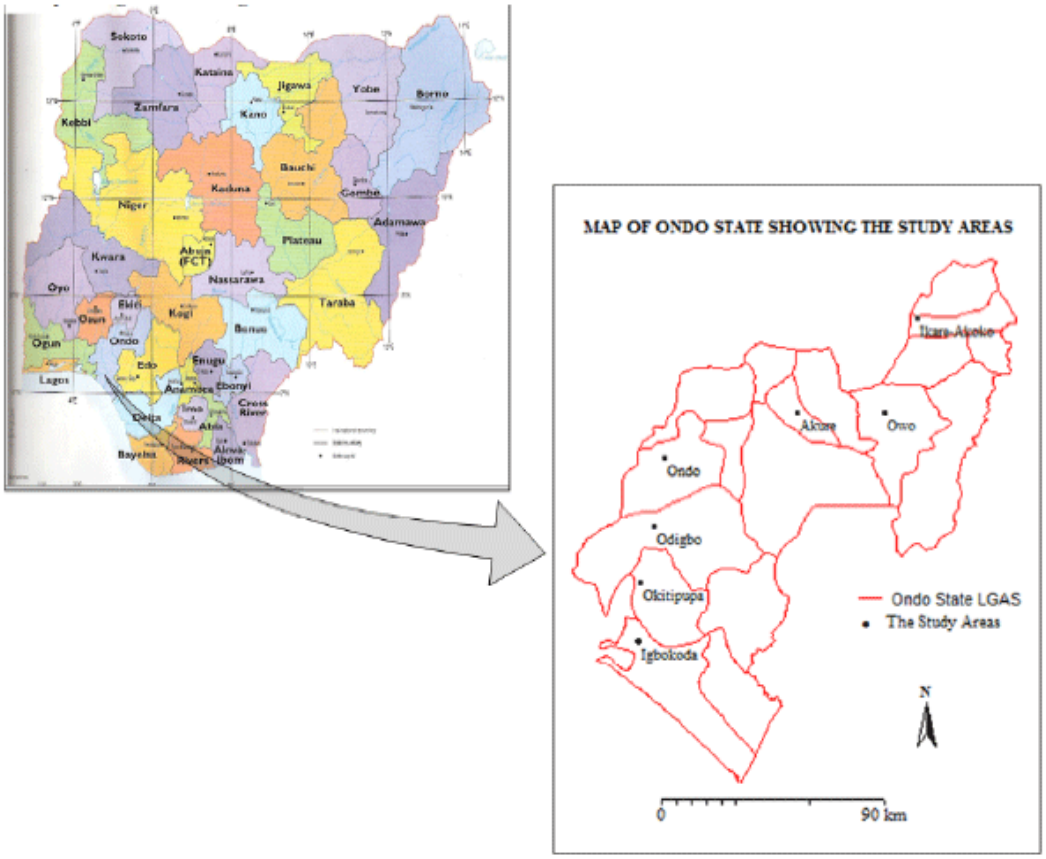

Figure 1. *Map of Nigeria indicating Ikare Akoko, Ondo.*

diffusion method [9]. A 0.1 ml of 1:10,000 dilutions (equivalent to 10^6 cfu /ml) of fresh overnight broth culture of the test bacteria was seeded on molten Mueller-Hinton agar plate. Using a sterile cork borer of 6 mm diameter, equidistant wells was made in the agar. One millimeter of the various re-suspended extracts (7.5, 15, 30 and 60 mg/ml) was introduced into the wells. Ofloxacin (5 µg) was used as control. The plates were allowed to stand on the bench for 1 hour, to allow pre-diffusion of the extracts before incubation. The plates were then incubated at 37°C for 24 to 48 hours. The zones of inhibition were measured to the nearest millimeter (mm) using a transparent ruler.

Antifungal assay of crude of *Spondias mombin* extracts (root, leaf, and stem bark): Antifungal assay of crude root, leaf, and stem bark of *Spondias mombin* extracts was done using Agar well diffusion method. A 5-day old fungal culture on potato dextrose agar (PDA) was flooded with 2 ml of sterile distilled water containing 3% glycerol. The spores were harvested by scraping with a sterile inoculating loop. Sterile PDA plates were inoculated with 0.1 ml of the fungal spore suspension using the spread plate technique. Five wells were bored on the potato dextrose agar (PDA) plates using a 6 mm sterile cork borer. The first, second, third and fourth well were filled with 60, 30, 15 and 7.5 mg/ml of the extracts, respectively, while the fifth well was filled with fluconazole which served as control. The plates were allowed to stand on the bench for 1 hour before incubating at 25°C for 5 days. Diameter of zones of growth inhibition was then measured in millimeter with a vernier caliper [10].

Determination of minimum inhibitory concentration (MIC) of crude *Spondias mombin* extracts (root, leaf, and stem bark): A serial dilution of the extracts ranging from 1:10 to 10.009 was made. The bacterial strain was cultured in Muller Hinton broth and suspended in 5 ml peptone water. To the suspension, 5 ml of each extract concentration was added into Muller Hinton broth and then 1.0 ml of standardized broth culture containing 1.0×10^6 cfu/ml was introduced into each test tube and then incubated at 37°C for 18–24 hrs. Following incubation, turbidity was examined; the concentration at which no turbidity was observed was regarded as the MIC value [11].

Determination of minimum bactericidal concentration (MBC) of crude *Spondias mombin* extracts (root, leaf, and stem bark): Suspensions from the MICs were used for the MBC determination. A bacterial streaking of equal streaks was made from the MIC test tubes onto Mueller-Hinton agar plates and the procedure was repeated all through the required numbers of the corresponding isolates. The isolated organism on the Mueller-Hinton agar was incubated at 37°C for 18–24 hrs. After incubation, the plates were observed; the concentration that exhibited no bacterial growth was considered as the MBC [11].

Results

Antimicrobial activities of Spondias mombin extracts

Tables 1 and 2 shows the diameter of zones of growth inhibition of leaf, stem bark and root crude extracts of *S. mombin* on the test bacteria and fungi at concentrations of 60, 30, 15 and 7.5 mg/ml, respectively. Aqueous leaf extract of *S. mombin* had

Table 1. *Diameter of zones of inhibition of crude aqueous extracts of Spondias mombin on the test bacteria.*

Diameter of zone of growth inhibition (mm)																
	LEAF					ROOT					STEM BARK					
Bacteria	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Ofloxacin(5 µg)	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Ofloxacin(5 µg)	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Ofloxacin(5 µg)	MIC (15 mg/ml) , MBC (7.5 mg/ml)
Mycobacterium fortuitum	17	9	7	3	28	18	7	4	2	25	17	10	6	4	27	
Mycobacterium smegmatis	18	11	7	4	27	17	7	3	2	25	19	6	4	2	24	
Mycobacterium abscessus	19.0)	6	4	2.0)	27	10	4	2	0	32	19	5	3	4	32	
Mycobacterium phlei	14	6	2	1	24	14	6	3	1	25	20	6	4	2	29	
Staphylococcus aureus	15	10	6	4	23	15	10	7	3	25	14	10	5	2	29	
Escherichia coli	17	13	7	3	25,0	12	6.0.	3	2	26	21	6	3.0)	0	24	
Klebsiella Pneumoniae	13	7	4	3	23	13	7	5	2	25	19	7	4	2	27	
Salmonella typhi	17	11	6	2	22	13	8	5	3	25	19	17	6	3	26	
Pseudomonas aeruginosa	19	12	7	4	21	14	10	6	3	20	17	11	7	2	23	
Salmonella choleraesuis	12	9	7	5	20	13	10	8	6	20	15	10	9	9	19	
Salmonella arizonae	21	18	17	5	21	21	18	17	5	22	21	18	15	9	22	
Proteus mirabilis	21	19	17	8	22	18	16	15	6	23	18	17	9	6	19	
Aeromonas hydrophilia	19	14	6	2	20	15	11	7	2	20	13	6	4	2	18	
Bacillus subtilis	17	12	9	5	18	17	10	7	4	20	19	10	7	4	19	
Salmonella typhi	12	9	5	3	1.5	19	13	7	4	20	18	12	7	4	17	
Shigella dysenteriae	18	15	10	4	21	20	19	15	9	22	21	18	12	6	20	
Burkholderia cepacia	23	19	8	3	26	21	17	8	5	25	18	10	6	2	25	
Citrobacter koseri	22	10	7	1	26	23	18	12	5	25	23	20	10	7	21	
Klebsiella ozaenae	20	16	9	3	21	23	20	15	5	19	20	19	15	6	19	

*P value- <0.0001, P value summary**** *Differences significant (P <0.05), significantly different standard deviations (P <0.05), MIC (1mg/ml), MBC (7.mg/ml)*

Table 2. *Diameter of zones of inhibition of crude aqueous extracts of Spondias mombin on the test fungi.*

Diameter of zone of growth inhibition (mm)																
	LEAF					ROOT					STEM BARK					
Fungi	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Fluconazole (5 µg)	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Fluconazole(5 µg)	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Fluconazole(5 µg)	MIC: Minimum Inhibitory Concentratio-30 mg/ml),
Aspergillus niger	17	15	8	4	20	19	15	9	5	21	19	13	8	3	18	
Candida albican	18	8	6	3	26	11	8	5	2	25	17	8	4	2	27	
Fusarium Solani	18	16	12	7	19	15	12	8	3	17	19	17	10	8	20	
Saccharomyces cerevisiae	19	15	10.0)	6	22	18	15	13	7	20	18	15	10	7	22	
Aspergillus flavus	18	15	10	7	20	18	14	9	5	23	18	14	8	4	21	
Phytophera megakarya	5.0)	2	1	0	19	10	5	2	0	19	0	0	0	0	20	
Candida kruise	19	15	10	6	22	20	15	8	3	22	18	17	10	5	20	
Rhizopus stonifer	7	5	2	1	15	15	12	10	7	16	0	0	0	0	16	
Trichoderma horizionum	10	5	2	0	18	18	15	11	9	20	0	0	0	0	15	
Fusarium vortercelium	18	15	10	9	21	16	12	7	3	19	20	17	12	10	22	
Syncephalastrum Racemosum	17	12	9	4	22	16	12	8	3	23	15	12	7	2	20	

*Note: MIC: Minimum Inhibitory Concentratio-30 mg/ml), MFC: Minimum Fungicidal Concentration -15 mg/ml, P value- < 0.0001, P value summary**** *Significantly different standard deviations (P <0.05)*

the zone of inhibition of 23 mm against *B. cepacia* at 60 mg/ml. The aqueous stem bark and root of *S. mombin* extracts at 60 mg /ml had the highest zone of inhibition of 23 mm each against *C. koseri* and *K. ozaenae*. However, the aqueous Stem bark extract of *S. mombin* did not show any antibacterial activity against *M.abscessus* neither did the aqueous root extract show antibacterial activity against *E. coli*. The aqueous leaf and root extracts of *S.mombin* did not show any zone of growth inhibition against *P. megakarya* and *T.horizionum* at 7.5 mg/ml (Table 1). The MIC and MBC) of the aqueous leaf, root and Stem bark extracts on all the susceptible test bacteria was 15 mg/ml and 7.5 mg/ml, respectively (Table 1). Meanwhile, the

MIC and MFC of the aqueous leaf, root and stem bark extracts on all the susceptible test fungi were 30 mg/ml and 15 mg/ml, respectively (Table 2).

The antibacterial activity of crude ethyl acetate extracts of *S. mombin* (Leaf, Stem-bark and Root) at concentrations of 60, 30, 15 and 7.5 mg/ml is represented in Tables 3 and 4. The diameter of zones of growth inhibition varied with the various test bacteria with significant activity observed at 60 mg/ml, with the highest inhibition zone of 30.0 mm against *S. choleraesuis*. The root extract exhibited considerable level of antimicrobial activity with the zone of inhibition of 23.0 mm each against *C. koseri* and *B.cepacia* (Table 3). The ethanolic extracts (Leaf, root and stem bark) demonstrated varied antibacterial and antifungal activity against the test bacterial and fungi (Tables 5 and 6). However, the leaf extract at all the concentrations used did not show any zone of antifungal inhibition against *R. stonifer*, likewise against *P. megakarya* at 15. 0 and 7.5 mg/ml, respectively (Table 6).

Discussion

The purpose of this research work is to evaluate the Inhibitory Zone Diameter (IZD) (antibacterial and antifungal activity) of crude *Spondias mombin* extracts against thirty clinical and environmental organisms. Scientific compilation of studies on antimicrobial activity of medicinal plants will enhance understanding of the extent of research that has been undertaken over the years, to elucidate the antimicrobial potential of medicinal plants. Such study could arouse interest on medicinal plants with potential antimicrobial activity from which new antimicrobial molecules could possibly be put to use, which is under the scope of this research work. Several researchers

Table 3. *Diameter of zones of inhibition of crude ethyl acetate extracts of Spondias mombin on the test bacteria.*

Diameter of zone of growth inhibition (mm)																
	LEAF					ROOT					STEM BARK					
Bacteria	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Ofloxacin (5 µg)	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Ofloxacin (5 µg)	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Ofloxacin (5 µg)	MIC (15mg/ml) , MBC (7.5mg/ml)
Mycobacterium fortuitum	15	10	6	4	29	12	8	4	2	26	20	8	6	4	28	
Mycobacterium smegmatis	17	13	7	3	28	14	9	6	3	28	17	13	7	3	28	
Mycobacterium abscessus	13.0(	8	4	2	34	10	4	2	0	32	18	6	4	2	35	
Mycobacterium phlei	14	6	3	1	25	11	7	4.0)	20	27	18	6	3	2	28	
Staphylococcus aureus	17	11	7	4	25	16	8	4	2	27	14	17	4	2	26	
Escherichia coli	15	10	6	5	24	12	6	3	3	26	10	5	3	1	27	
Klebsiella Pneumoniae	13	7	2	6	26	14	7	3	4	24	15	8	4	3	23	
Salmonella typhi	18	10	6	2	24	16	8	4	2	23	15	19	3	1	25	
Pseudomonas aeruginosa	16	11	6	3	20	19	13	9	3	23	18	13	7.0)	2	22	
Salmonella Choleraesuis	13	9	6	4	20	14	9	7	6	19	12	10	8	5	20	
Salmonella arizonae	20	18.0)	14	7	20	21	19	17	5	26	21	18	13	8	19	
Proteus mirabilis	20	19	15	6	17	22	19	16	8	19	19	18	12	8	22	
Aeromonas hydrophilia	19	12	10	4	22	15	9	5	3	19	16	17	12	9	20	
Bacillus subtilis	18	15	7	4	19	19	14	8	3	16	18	14	7	3	20	
Salmonella typhi	16	8	5	2	18	19	14	8	6	19	10	6	3	1	16	
Shigella dysenteriae	20	18	15	5	20	18	15	14	6	19	20	18	15	4	20	
Burkholderia cepacia	18	15	6	2	23	19	18	7	4	26	20	18	7	3	24	
Citrobacter koseri	23	20	18	9	24	20	10	4	25	20	15	19	6	2	24	
Klebsiella ozaenae	20	17	9	2	23	23	20	16.0)	7	23	20	16	9	4	15	

*Note: P value-<0.0001, P value summary**** Significantly different standard deviations -(P <0.05), MIC (30 mg/ml), MFC (15 mg/ml)*

Table 4. *Diameter of zones of inhibition of crude ethyl acetate extracts of Spondias mombin on the test fungi.*

Diameter of zone of growth inhibition (mm)																
	LEAF					ROOT					STEM BARK					
Fungi	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Fluconazole (5µg)	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Fluconazole (5µg)	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Fluconazole (5µg)	MIC: Minimum Inhibitory Concentratio-30mg/ml),MFC: Minimum Fungicidal Concentration -15mg/ml
Candida albican	19	9	5	3	28	14	7	4	2	26. 0	11	5	3	0	25	
Aspergillus niger	19	17	10	5	23	18	15	8	5	21	20	18	15	9	22	
Fusarium solani	20	18	15	9	22	18	15	10	6	20	19	15	10	8	21	
Saccharomyces cerevisiae	17	10	5	1	18	19	17	12	8	20	18	15	9	5	19	
Aspergillus flavus	12	8	5	3	18	19	15	10	6	23	10	5	2	1	20	
Phytophera megakarya	8	5	2	0	18	3	2	0	0	20	10	0	0	0	17	
Candida kruise	18	13	8	4.0)	20	18	13	10	7	20	19	15	9	6	22	
Rhizopus stonifer	9	6	4	1	12	7	4	3	0	12	10	7.0)	5	1	10	
Trichoderma horizionum	10.0)	7	5	2	12	18	15	10	8	20	19	10	3	1	21	
Fusarium vortercelium	17	12	9	5	21	21	16	10	4	22	20	15	9	3	21	
Syncephalastrum racemosum	19	10	6	2	21	17	15	10	4	30	19	16	10	4	23	

*Note: P value-<0.0001, P value summary**** Significantly different standard deviations -(P < 0.05), MIC: Minimum Inhibitory Concentratio-30mg/ml, MFC: Minimum Fungicidal Concentration -15 mg/ml*

Table 5. *Diameter of zones of inhibition of crude ethanolic extracts of Spondias mombin on the test bacteria.*

Diameter of zone of growth inhibition (mm)																
	LEAF					ROOT					STEM BARK					
Bacteria	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Ofloxacin 5 µg	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Ofloxacin 5 µg	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Ofloxacin 5 µg	MIC (15mg/ml) , MBC (7.5mg/ml)
Mycobacterium fortuitum	12	9	7	3	28	14	7	4	2	25	23	10	6	4	27	
Mycobacterium smegmatis	14	11	7	4	27	10	7	3	2	25	11	6	4	2	24	
Mycobacterium abscessus	13	6	4	2	27	10	4	2	0	32	17	5	3	4	32	
Mycobacterium phlei	14	6	2	1	24	14	6	3	1	25	10	6	4	2	29	
Staphylococcous aureus	15	10	6	4	23	15	10	7	3	25	14	10	5	2	29	
Escherichia coli	17	13	7	3	25	12	6	3	2	26	12	6	3	0	24	
Klebseilla pneumoniae	13	7	4	3	23	13	7	5	2	25	11	7	4	2	27	
Salmonella typhi	17	11	6	2	22	13	8	5	3	25	15	10	6	3	26	
Pseudomonas aeruginosa	19	12	7	4	21	14	10	6	3	20	17	11	7	2	23	
Salmonella choleraesuis	30	25	20	10	19	16.R	8	5	3	20	17	12	9	6	20	
Salmonella arizonae	18	15	10	8	19.0)	20	18	17	9.R	25	20	17	12	6	18	
Proteus mirabilis	21	19	13	6	21	20	15	7.0)	3	21	19	17	8	5	21	
Aeromonas hydrophilia	17	10	5	2	18	16	12	8	5	18	22	8	3	1	16	
Bacillus subtilis	18	15	9	4	20	16	12	7	4	18	12	8	3	2	15	
Salmonella typhi	16	10	8	3	19	17	12	7	4	18	15	9	5	2	18(I)	
Shigella dysenteriae	20	16	10	5	20	21	19	15	5	21	18	15	9	4	19	
Burkholderia cepacia	21	18	7	3	24	19	11	6	2	23	15	12	10	7	18	
Citrobacter koseri	21	20	18	8	24	23	19	10	6	25	21	17	10	7	23	
Klebsiella ozaenae	23	19	15	10	25	23	19	15	10	23	21	18	15	10	23	

*Note: P-value-<0.0001, P value summary**** *Significantly different standard deviations (P 0.05), MIC (15 mg/ml), MBC (7.5 mg/ml)*

Table 6. *Diameter of zones of inhibition of crude ethanolic extracts of Spondias mombin on the test fungi.*

Diameter of zone of growth inhibition (mm)															
	LEAF					ROOT					STEM BARK				
Fungi	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Fluconazole (5 µg)	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Fluconazole (5 µg) Control	60 mg/ml	30 mg/ml	15 mg/ml	7.5 mg/ml	Fluconazole (5 µg)
Candida albican	18	8	6	3	26	11	8	5	2	25	18	8	4	2	27
Aspergillus niger	17	15	8	5	19	20	19	15	8	23	18	15	6	3	20
Fusarium solani	20	18	13	8	23	20	15	8	3	19	17	15	12	9	18
Saccharomyces cerevisiae	18	15	10	5	21	19	15	10	6	22	18	15	12	5	19
Aspergillus flavus	22	18	10	6.0)	23	21	18	13	10	22	18	13	10	7	20
Phytophera megakarya	2	1	0	0	20	8	5	2	0	21	3	1	0	0	18
Candida kruise	19	18	9	3	20	17	13	8	3	21	20	18	9	5	21
Rhizopus stonifer	0	0	0	0	10	10	8	5	2	12	9	5	2	1	10
Trichoderma horizionum	10	5	2	1	10	12	10	6	2	15	18	15	10	8	23
Fusarium vortercelium	21	18	15	12	23	18	17	14	11	20	20	19	15	12	23
Syncephalastrum racemosum	19	16	10	4	24	17	15	10	5	20	16	10	6	4	18

*Note: P-value-<0.0001, P value summary*** *Significantly different standard deviations (P<0.05), MIC: Minimum Inhibitory Concentratio-30 mg/ml), MFC: Minimum Fungicidal Concentration -15 mg/ml.*

had reported that plants contain antibacterial and antifungal substances. Many plant species yield biological active products that are capable of providing physiological activity against microorganisms. Plants of this nature (e.g. *Spondias mombin*) exist and serve as therapeutic antimicrobial drugs [12].

In this study, all the plant parts (leaf, root, and stem bark) assayed possessed varying degree of antimicrobial activities. The crude aqueous stem bark, root and leaf extracts of *S. mombin* were less active against the test bacteria and fungi compared to ethanol and ethyl acetate extracts. This may be attributed to partial solubility

of the active components of *S. mombin* in water. The crude ethyl acetate extracts of all the plant parts used possessed significant antimicrobial property. This suggests that ethyl acetate is the best solvent of extraction for *S. mombin*. however, the observed variation in the diameter of zones of growth inhibition of the test bacteria and fungi may be due to the differences in their cell wall compositions and arrangement. It can be deduced that the process of extraction in antimicrobial studies is critical as it determines to a large extent the result of this study i.e. the solvent used for extraction play an important role during the process of as assessing the antimicrobial activity of *Spondias mombin* [13].

Antimicrobial studies indicated that both the ethyl acetate and ethanol leaf, root and stem extracts of *Spondias mombin* inhibited the growth of the microbes but at varied levels and the inhibition was extracts concentration dependent (Tables 3-5). The stem and leaf extracts of *Spondias mombin* both showed inhibition against test microbes indicating that the plant possesses antimicrobial properties. This could be attributed to the presence of chemical compounds in the extracts.

The test Gram-positive bacteria were more susceptible than Gram-negative bacteria to the extracts. This could be ascribed to the differences in their cell wall constituents and their arrangement [14]. The Gram-positive bacteria contains a peptidoglycan layer, which is an ineffective permeability barrier while Gram-negative bacteria are surrounded by an additional outer membrane carrying the structural lipopolysaccharide components, which makes it impermeable to lipophilic solutes and porins, hence constituting a selective barrier to the hydrophilic solutes [15,16].

Ethyl acetate fractions of *Spondias mombin* extract were more potent in activity against the entire test organisms than other solvent fractions. The difference in polarity among the various solvents are perhaps responsible for the differences in solubility of plant active compounds, hence variation in degree of activity [17]. Although aqueous (water) extraction is commonly used by the traditional healers, it has been shown that plant extracts obtained using organic solvents give more potent and consistent antimicrobial activity result than aqueous extract [17,18] this can be deduced from the result obtained in Tables 1-6.

The crude *Spondias mombin* extract tested in this study showed antimicrobial activities against all the test bacterial and fungal isolates. However, differences were observed between their antimicrobial activities. These differences could be attributed to the differences in their chemical composition and amount of the bioactive compounds extracted by the solvent. These compounds usually accumulate in different parts of *Spondias mombin* [19], and such secondary metabolites have been found to produce many effects including antibacterial and antiviral properties, this is in line with the observation of Hassan HS et al. [20].

The inhibition of bacterial strains *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* suggests that the *Spondias mombin* possesses broad spectrum of antibacterial properties which could be used in the treatment of skin diseases of which the microbes are commonly implicated. The inhibition of the fungal strain (*Candida albicans*) suggests also that the *Spondias mombin* possesses antifungal property and could be used for the treatment of refractory candidacies (oral) that has a global challenge with HIV/AIDS patients [21].

Stem bark extract of *Spondias mombin* showed higher diameter of zones of inhibition against the microbes than the leaf and root extracts. This according to Kudi AC et al. [22] could be attributed to presence of higher bioactive compounds in Stem bark extracts of *Spondias mombin*. Furthermore, the sensitivity and susceptibility of the microbes to the *Spondias mombin* extracts varied. The fungal strains were highly sensitive and susceptible to the plant extracts. The difference according to Udgire MS et al. [23] is due to the fact that gram positive bacteria such as *Escherichia coli* develop resistant to inhibition caused by plant extract except when the extracts are used at higher concentration.

Kudi et al. [22] reported that plant extracts exhibiting IZD of 6 mm and above against a selected pathogen are considered to possess some antimicrobial activity while Adeleye IA et al. [24] suggested IZD of 10 mm and above. Because, many organisms are now exhibiting high resistance to most antimicrobials, this study proposes that plant extract exhibiting IZD greater than or equal to 10 mm against selected organisms should be considered to possess antimicrobial activity whereas those showing IZD $\geq$ 20 mm against selected organisms are considered noteworthy. Some papers on Nigerian plant extract reported IZD $\geq$ 20 mm [25].

Generally, the ethyl acetate extracts are more effective than the ethanol extract though the reverse was the case at higher (60 mg/ml) concentrations. The findings conform to the study of Lifongo LL et al. [17] who observed higher microbial activity of ethyl acetate extract of lemon grass against human pathogen at higher concentration of plant extracts, [26] reported that at increasing concentration of extract, differences in interaction between phytochemicals and solvent do exist and that this may account for differences in microbial activity of extracts of different solvents. Made et al. [27], reported that antimicrobial activity is solvent dependent with ethanol extract being most potent than aqueous extract.

Conclusion

Many medicinal plants like *Spondias mombin* emerged as plot with potentially significant therapeutic application against human pathogens. It is only through research efforts that these potentials could be discovered for eradication of these resistant strains of microbes, this study revealed that the plant extracts possessed antibacterial and antifungal activities against some highly infectious clinical and environmental pathogens which justified their use in ethno-medicine for treatment of infectious diseases. The ethyl acetate extract showed significantly higher inhibition than the ethanol and aqueous extract in all concentrations except at 60 mg/ml. The data obtained from the study indicated that the plant possessed antimicrobial properties.

Recommendation

It is thereby recommended to explore and total purification of medicinal plants such as the one studied, *Spondias mombin*, to fight against public health problems.

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