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Antibacterial and Antifungal Efficacy of Partially Partitioned Fractions of *Spondias mombin* (*Linn*) Extracts (Root, Leaf and Stem Bark) against Clinical and Environmental Isolates

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Abstract

The purpose this research work is to determine the antibacterial and antifungal assay of partitioned fractions of *S. mombin* (*Linn)* extracts against clinical and environmental isolates**.** 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* (1 kg) 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 SMRE and SMREA were used to code for root part; SMLE and SMLEA for the leaf part; and SMSBE and SMSBEA for the stem-bark part, each was fractionated on column chromatography with silica as the stationary phase using *n*-hexane, ethyl acetate and ethanol as the eluting solvent to obtain *n*-hexane, ethyl acetate and ethanol fractions. Antimicrobial and antifungal screened was observed using agar well diffusion test. The result obtained showed that in partially purified ethyl acetate leaf extracts**,** Fraction (F_1 SMLEAH) showed significant inhibitory effect ($p \leq 0.05$) on all the test bacteria, except *Klebsiella pneumoniae* and *Salmonella typhi* at concentrations of 20.0-2.5 mg/ml, Fraction (F_2 SMLEAEA) was not effective against *Salmonella cholleraesuis, B. substilis, Citrobacter koseri* and *Salmonella typhi.* Fraction (F_3 SMLEAE) showed little or no inhibitory effect on most of the bacteria at all the concentration used. It can be deduced that in partially purified ethanolic leaf extracts, Fraction (F_1 SMLEH) showed inhibitory effect on *Burkholderia cepacia*. All the organisms were not susceptible to all the fractions except F_1 which had diameter of zones of growth inhibition ranging between 4.0-1.0 mm at 5 mg/ml-0.625 mg/ml on *Mycobacterium abscessus.* Partially purified ethanolic stem bark extracts, antifungal activity of the partially purified ethanolic extracts of *S. mombin*, Fractions (F_1 SMLEH, F_2 SMLEEA) and F_3 SMLEE), significant antifungal activity ($p \leq 0.05$) was observed at 20.0 mg/ml with most test fungi. *Trichoderma horizionum* was not susceptible to all the three fractions, while *Aspergillus niger* and *Syncephala strumracemosum* were susceptible to only fraction (F_2 SMLEEA). Fractions (F_1 SMSBEH, F_2 SMSBEEA), F_3 SMSBEE) on the test as the eluting solvent. Significant inhibitory effect ($p \leq 0.05$) was observed in all the fractions at 20 mg/ml against most of the test bacteria. While, zones of growth inhibition of the various fractions varied with the test bacteria with the highest diameter zone of 8.0 mm recorded in fraction F_1 against *Salmonella typhi.* Fractions (F_2 SMSBEEA and F_3 SMSBEE) possessed significant inhibitory effect ($p \leq 0.05$) at 20.0-5.0 mg/ml on the test fungi, except *Candida kruise* and *Rhizopus stonifer*. The plant part by solvent interactive effect was significant ($p<0.05$), suggesting that the MICs and MBCs of test bacteria were observed at 0.3125 and 0.1562 mg/ml and MICs and MFCs test fungal were observed at 0.3125 and 0.1562 mg/ml respectively, The various plants differ significantly according to extraction solvent, These findings demonstrate the possible effectiveness of the *S. mombin* plant, especially its stem bark extracts, in treating microbial infections.

Keywords: Antibacterial; Antifungal assay; Partitioned fractions; *Spondias mombin*; Environmental isolates; Agar well diffusion

Introduction

The plant *Spondias mombin* (Linn) also called yellow mombin in English, Igongo/Ichankla in Idoma, and Uchakuru in Igbo, is common in the forest and savanna regions of Nigeria. It is used in several countries of the world to treat various ailments including infectious diseases. *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; bark thick, corky, deeply fissured, slash pale pink, darkening rapidly, branches low, branchlets glabrous; leaves pinnate, leaflets 5-8 opposite pairs with a terminal leaflet. It belongs to the family Anacardiaceae. The roots are used as febrifuge in Ivory Coast and the bark is used as a purgative and in local applications for leprosy. The 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 bark is believed to expel calcifications from the bladder. A report showed that the bark contains a certain amount of tannin and this explains the reason why the dry pulverized bark is applied as a dressing to a wound [1].

A decoction of the young leaves is a remedy for diarrhea and dysentery and the juice of crushed leaves and the powder of dried leaves are used as poultices on wounds and inflammations. The gum is employed as an expectorant and to expel tapeworms [2]. A leaf infusion is a common cough remedy or used as a laxative for fever with constipation and leaf decoction is used in treatment of gonorrhea. The leaves are used for fresh wounds to prevent inflammation. A decoction of pounded leaves of *S. mombin* is used as an eye lotion and the juice

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pressed from young, warm leaves is given to children for stomach troubles. The extract has shown anti-inflammatory activity in Wister rats [3]. A tea made from the flowers and leaves is taken to relieve stomach ache, biliousness, and urethritis, cystitis and eye and throat inflammations. A decoction of the root is used as purgative [3,4].

Materials and Methods

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).

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.

Column fractionation of various crude extracts of the different plant parts

The SMRE and SMREA from root part; SMLE and SMLEA for the leaf part; and SMSBE and SMSBEA for the stem-bark part each was fractionated on column chromatography with silica as the stationary phase using *n*-hexane, ethyl acetate and ethanol as the eluting solvent to obtain *n*-hexane, ethyl acetate and ethanol fractions for each and coded as SMREH, SMREEA and SMREE for the ethanol extract of the root and SMREAH. SMREAEA and SMREAE represent the different fractions for the ethyl acetate extract of the root. On the other hand, SMLEH, SMLEEA and SMLEE for the ethanol extract of the leaf while SMLEAH, SMLEAEA and SMLEAE for the ethyl acetate extract of the leaf. SMSBEH, SMSBEEA and SMSBEE for the ethanol extract of the stem-bark and SMSBEAH. SMSBEAEA and SMSBEAE for the ethyl acetate extract of the stem-bark.

Preparation of different concentrations of extracts

Spondias mombin extracts (root, leaf and stem bark) (0.6 g) were weighed separately into a sterile bottle and reconstituted in 2.5 ml of Dimethyl surfoxide (DMSO) after which 7.5 ml of sterile distilled water was added to make up 10 ml (60 mg/ml) in total. Then 3 ml of the reconstituted extract was dispensed into another bottle containing 3 ml of sterile distilled water to make up 6 ml (30 mg/ml). The same procedure was repeated for 15 mg/ml and 7.5 mg/ml respectively [5].

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 repeated by the agar well diffusion method [6].

Antibacterial assay of partitioned fractions of *Spondias mombin* extracts (root, leaf, and bark): The antimicrobial assay of partitioned fractions of *Spondias mombin* extracts on the test bacteria was carried out by the agar diffusion method [6]. 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 corn 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. 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 (NCCLS).

Antifungal assay of partitioned fractions of *Spondias mombin* extracts (root, leaf, and stem bark): Antifungal assay of partitioned fractions of the 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 corn borer. The first, second, third and fourth well were filled with 60, 30, 15 and 7.5 mg/ml of the extracts, respectively, 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 [7].

Determination of minimum inhibitory concentration (MIC) partitioned fractions of *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 [8].

Determination of minimum bactericidal concentration (MBC) of partitioned fractions of *Spondias mombin* extracts (roots, 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 [8].

Results

The antibacterial activity of partially purified ethyl acetate leaf extracts of *Spondias mombin*

Tables 1 and 2 shows the antibacterial activity of partially purified

	Fraction F_1 (SMLEAH)								Fraction F_2 (SMLEAEA)								Fraction F_3 (SMLEAE)							
	Diameter of zones of inhibition (mm)																							
Bacteria	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MBC 0.1562 mg/ml**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125**	**MBC 0.1562**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MBC 0.1562 mg/ml**
M. fortuitum	6	5	4	3	1	1	0	0	7	5	4	3	3	1	0	0	5	3	2	1	1	0	0	0
M. sinegmatis	5	4	2	2	1	1	0	0	8	5	3	0	0	0	0	0	7	5	5	3	2	0	0	0
M. abscessus	7	6	4	3	2	2	0	0	8	6	4	3	2	1	0	0	5	3	2	1	0	0	0	0
M. phlei	5	3	2	1	1	0	0	0	5	2	0	0	0	0	0	0	12	6	5	5	3	2	0	0
S. aureus	5	3	2	2	1	0	0	0	10	7	5	4	4	3	0	0	6	6	4	2	1	0	0	0
K. pneumoniae	1	0	0	0	0	0	0	0	9	6	5	3	3	2	0	0	8	6	3	2	1	1	0	0
E. coli	5	4	3	2	2.0	1	0	0	7	5	3	2	2	2	0	0	9	7	4	3	2	1	0	0
P. aeruginosa	7	5	3	3	3	2	0	0	10	7	6	5	3	1	0	0	9	8	5	3	3	1	0	0
S. typhi	6	3	3	2	1	0	0	0	9	7	4	3	1	1	0	0	8	5	4	2	1	1	0	0
S. choleraesuis	7	3	2	2	1	0	0	0	5	3	2	1	1	1	0	0	5	3	3	2	1	1	0	0
S. arizonae	7	5	4	2	1	0	0	0	6	4	3	2	2	2	0	0	2	2	2	0	0	0	0	0
P. mirabilis	2	0	0	0	0	0	0	0	8	5	4	3	2	2	0	0	5	2	2	1	0	0	0	0
A. hydrophilia	4	4	3	2	2	1	0	0	5	3	2	1	1	1	0	0	7	5	2	2	1	0	0	0
B. subtilis	11	7	5	4	4	3	0	0	7	5	3	2	1	1	0	0	2	0	0	0	0	0	0	0
S. typhi	6	5	3	3	2	1	0	0	6	4	3	2	0	0	0	0	3	2	1	0	0	0	0	0
S. dysenteriae	9	6	5	3	3	2	0	0	5	3	2	2	1	1	0	0	0	0	0	0	0	0	0	0
B. cepacia	6	5	3	2	2	1	0	0	9	7	5	3	2	2	0	0	3	2	2	1	0	0	0	0
C. Koseri	8	7	5	3	2	1	0	0	9	6	5	2	1	0	0	0	10	6	6	3	1	1	0	0
K. ozaenae	5	3	2	2	1	1	0	0	5	3	1	1	0	0	0	0	7	4	3	2	1	0	0	0

P value<0.0001; P value summary******; Significantly different standard deviations? (P<0.05) Yes**

Table 1: Diameter of zones of inhibition of various fractions of ethyl acetate leaf extracts of *Spondias mombin* on the test bacteria.

	Fraction F_1 (SMLEAH)								Fraction F_2 (SMLEAEA)								Fraction F_3 (SMLEAE)							
	Diameter of zones of inhibition (mm)																							
Fungi	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MFC 0.1562 mg/ml**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125**	**MFC 0.1562**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MFC 0.1562 mg/ml**
C. albicans	8	5	4	2	1	0	0	0	8	6	3	3	2	1	0	0	5	3	2	1	1	1	0	0
A. niger	6	5	4	2	1	1	0	0	5	4	2	2	1	1	0	0	5	5	4	3	3	2	0	0
F. solani	5	3	2	1	1	1	0	0	6	4	3	2	1	1	0	0	6	5	3	2	2	1	0	0
S. cerevisiae	8	6	3	2	1	1	0	0	6	4	3	2	0	0	0	0	4	4	3	2	0	0	0	0
A. flavus	9	7	4	3	2	1	0	0	4	2	2	2	0	0	0	0	4	3	3	3	0	0	0	0
P. megakarya	6	5		1	1	1	0	0	6	5	4	3	2	1	0	0	6	4	4	3	2	0	0	0
C. kruise	5	3	2	2	1	1	0	0	8	5	4	3	2	2	0	0	7	5	4	4	1	0	0	0
R. stonifer	0	0	0	0	0	0	0	0	8	5	3	2	1	1	0	0	8	6	4	3	2	2	0	0
T. horizionum	7	4	3	1	1	1	0	0	7	5	4	3	2	1	0	0	6	4	3	3	3	1	0	0
F. vortercelium	7	5	3	1	1	1	0	0	4	3	1	0	0	0	0	0	8	5	5	1	0	0	0	0
S. racemosum	9	7	6	3	2	1	0	0	6	4	2	0	0	0	0	0.)	6	3	3	2	1	0	0	0

P value<0.0001; P value summary******; Significantly different standard deviations? (P<0.05)**

Table 2: Diameter of zones of inhibition of partially purified ethyl acetate leaf extracts of *Spondias mombin* on the test fungi.

(various fractions-F_1 (SMLEAH), F_2 (SMLEAEA) and F_3 (SMLEAE) of ethyl acetate leaf extracts of *S. mombin* using *n*-hexane, ethyl acetate **a**nd ethanol as the eluting solvents. There was no significant inhibitory activity observed against the entire test bacteria at all the various concentrations of the extracts tested, except *B. subtilis* at 20 mg/ml concentration where the highest zone of inhibition of 11.0 mm was recorded. Similarly, there was no significant antifungal activity observed in fractions F_1 (SMLEAH), F_2 (SMLEAEA) and F_3 (SMLEAE) at concentrations of 2.5-0.625 mg/ml.

The antibacterial activity of partially purified ethyl acetate leaf extracts of *Spondias mombin* using ethyl acetate

Tables 3 and 4 show the diameter of zones of inhibition of partially purified ethyl acetate leaf extracts of *Spondias mombin* using ethyl acetate as the eluting solvent. In Table 3, Fraction F_1 (SMLEAH) showed significant inhibitory effect ($p \leq 0.05$) on all the test bacteria, except *Klebsiella pneumoniae* and *Salmonella typhi* at concentrations of 20.0-2.5 mg/ml. Meanwhile, fraction F_2 (SMLEAEA) was not effective against *Salmonella cholleraesuis, B. substilis, Citrobacter koseri and Salmonella typhi. Similarly,* fraction F_3 (SMLEAE) showed little or no inhibitory effect on most of the bacteria at all the concentration used (Table 3). Only fraction F_1 (SMLEAH) showed significant antifungal effect ($p \leq 0.05$) on some of the test fungi (Table 4).

The antibacterial activity of partially purified ethanolic leaf extracts of *Spondias mombin*

Tables 5 and 6 shows the various zones of growth inhibition of partially purified ethanolic leaf extracts of *Spondias mombin* fractions F_1 (SMLEH), F_2 (SMLEEA), F_3 (SMLEE) on the test bacteria using N hexane, ethyl acetate and ethanol as the eluting solvents. The diameter of zones of inhibition varied with the test bacteria. Meanwhile, there was no significant ($p \leq 0.05$) antibacterial activity observed at concentrations of 1.25 mg/ml and 0.625 mg/ml of all the fractions. None of the shows no inhibitory activity against *Proteus mirabilis* at all the concentrations used, neither did fraction F_1 (SMLEH) show inhibitory effect on *Burkholderia cepacia*. Similarly, all the organisms were not susceptible to all the fractions except F_1 which had diameter of zones of growth inhibition ranging from 4.0-1.0 mm at 5 mg/ml-0.625 mg/ml on *Mycobacterium abscessus* (Table 5). The antifungal activity of the partially purified ethanolic extracts of *Spondias mombin* fractions F_1 (SMLEH), F_2 (SMLEEA) and F_3 (SMLEE) on the test fungi is presented in Table 6. Significant antifungal activity ($p \leq 0.05$) was observed at 20.0 mg/ml with most test fungi. However, *Trichoderma horizionum* was not susceptible to all the three fractions, while *Aspergillus niger* and *Syncephala strumracemosum* were susceptible to only fraction F_2 (SMLEEA).

The antibacterial activity of partially purified ethanolic stem bark extracts of *Spondias mombin* using ethanol as the eluting solvent

Tables 7 and 8 shows the diameter of zones of growth inhibition of the partially purified ethanolic stem bark extracts {Fractions F_1 (SMSBEH), F_2 (SMSBEEA), F_3 (SMSBEE)} on the test as the eluting solvent. Significant inhibitory effect ($p \leq 0.05$) was observed in all the fractions at 20 mg/ml against most of the test bacteria. Meanwhile, zones of growth inhibition of the various fractions varied with the test bacteria with the highest diameter zone of 8.0 mm recorded in fraction F_1 against *Salmonella typhi.* In Table 7, fraction F_1 (SMSBEH) showed a significant antifungal activity ($p \leq 0.05$) on all the test fungi, except, *Candida albicans*, and *Rhizopus stonifer* that were not susceptible at all the various concentrations used. Similarly, fractions F_2 (SMSBEEA) and F_3 (SMSBEE) possessed significant inhibitory effect ($p \leq 0.05$) at 20.0-5.0 mglml on the test fungi, except, *Candida kruise* and *Rhizopus stonifer* (Table 8).

Discussion

The increasing trend of resistance to the antibiotics in current use has drawn the attention of researchers to natural alternative treatments of bacterial infections as potential sources of new, novel antimicrobial agents. This study indicated that *Spondias mombin* could be used as medicinal plants in various regions of the world are active against a broad spectrum of clinical and environmental Isolates In this study, all the plant parts (leaf, root, and stem bark) assayed possessed varying degree of antimicrobial activities. The partially purified extracts possessed antimicrobial activity with pronounced activity recorded in the stem bark of the plant extract of *Spondias mombin*. Ndukwe [9] and other researchers reported that partially purified fractions of *Spondias mombin* have impressive antimicrobial effect on *E. coli* and *S. typhi* [10-12].

The result obtained from flux fractionating of extract revealed that the fractions of stem bark and leaf of *Spondias mombin* using ethyl acetate as the eluting solvent showed better antimicrobial activity compared to *n*-hexane and ethanol which has been earlier reported. Ethyl acetate fractions of S*pondias 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 [13].

Other active eluting solvents in the study include ethanol and *n*-hexane [14]. Gram negative bacteria are reported to be resistant against most antibacterial agents as a result of the more complicated nature of their cell wall compared to Gram positive bacteria [9,15,16]. However, *Spondias mombin* was found to be active against the two groups of Clinical and Environmental Isolates underlining their ethnomedicinal use for treatment of various infectious diseases [17-20].

Moreover, the ethyl acetate extract was highly effective against Gram positive and negative bacteria (clinical and environmental Isolates) as well as all fungal species while the ethyl acetate extract only had high antimicrobial activities against all test bacteria species but not against fungal species except on *C. kruise, F. vortercelium* and *S. racemosum*. This finding established active principles of most species of S*pondias mombin*. Aliyu et al. [20] found the ethyl acetate and n-butanol extracts as the most active extracts of S*pondias mombin* while Das et al. [19] established the antimicrobial activity of ethyl acetate extract of S*pondias mombin* (Linn).

Overall the test organisms were most sensitive to the ethyl acetate extract followed by the ethanol extract. The other extracts *n*-hexane only exhibited limited activities, This result shows that ethyl acetate are the most efficient extracting solvents for active principles of these plants. The ethyl acetate extract also contain novel bioactive compounds which might be present in low concentrations but which on further purification might demonstrate equally high antimicrobial activities Osuntokun et al. [21].

In conclusion, the broad spectrum activity exhibited by S*pondias mombin* extracts establishes the scientific basis for their use as ethno medicine and their potential for use for development of novel antibacterial and antifungal agents effective for treatment of microbial

	Fraction F_1 (SMLEAH)								Fraction F_2 (SMLEAEA)								Fraction F_3 (SMLEAE)							
	Diameter of zone of inhibition (mm)																							
Fungi	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MFC 0.1562 mg/ml**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125**	**MFC 0.1562**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MFC 0.1562 mg/ml**
Candida albicans	5	3	2	2	1	0	0	0	3	2	1	1	0	0	0	0	6	4	3	2	0	0	0	0
Aspergillus niger	4	3	1	1	1	0	0	0	3	2	1	1	0	0	0	0	5	4	2	1	1	0	0	0
Fusarium solani	6	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5	3	2	1	1	0	0	0
Saccharomyces cerevisiae	4	2	1	0	0	0	0	0	3	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Aspergillus flavus	0	0	0	0	0	0	0	0	4	3	2	1.0	0	0	0	0	3	2	1	1	1	1	0	0
Phytophera megakarya	3	2	1	0	0	0	0	0.0	0	0	0	0	0	0	0	0	6	4	2	1	0	0	0	0
Candida kruise	2	1	0	0	0	0	0	0	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Rhizopus stonifer	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	3	2	1	0	0	0	0	0
Trichoderma horizionum	5	3	1	0	0	0	0	0	4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium vortercelium	5	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0	0	0	0
Syncephalastrum racemosum	2	1	1	1	0	0	0	0	2	1	1	0	0	0	0	0.0	0	0	0	0	0	0	0	0

P value<0.0001; P value summary****; Significantly different standard deviations? (P<0.05)

Table 3: Diameter of zone of inhibition of partially purified ethyl acetate leaf extracts of *Spondias mombin* on the test fungi using ethyl acetate as the eluting solvent.

	Fraction F_1 (SMLEH)								Fraction F_2 (SMLEEA)								Fraction F_3 (SMLEE)							
	Diameter of zones of inhibition (mm)																							
Bacteria	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MBC 0.1562 mg/ml**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125**	**MBC 0.1562**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MBC 0.1562 mg/ml**
M. fortuitum	5	3	2	1	0	0	0	0	6	4	3	3	1	0	0	0	5	4	3	3	1	0	0	0
M. sinegmatis	7	5	3	1	1	0	0	0	4	3	3	3	2	1	0	0	7	5	3	2	1	0	0	0
M. abscessus	0	0	4	2	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
M. phlei	7	5	3	2	1	1	0	0	5	2	2	1	1	0	0	0	6	4	1	1	0	0	0	0
S. aureus	5	3	2	2	1	1	0	0	8.0	5	3	2	1	1	0	0	5	3	3	2	1	1	0	0
K. pneumoniae	4	2	2	2	0	0	0	0	8.0	5	4	3	2	2	0	0	7	5	2.0	2	1	0	0	0
E. coli	6	4	3	2	1	1	0	0	7	5	4	3	2	1	0	0	2	2	2	0	0	0	0	0
P. aeruginosa	7	5	3	3	0	0	0	0	6	5	3	2	1	0	0	0	9	8	7	4	2	1	0	0
S. typhi	4	2	2	1	0	0	0	0	4	3	2	2	1	1	0	0	6	3	3	2	1	0	0	0
S. choleraesuis	4	3	1	0	0	0	0	1	5	4	3	3	1	0	0	0	5	4	3	3	0	0	0	0
S. arizonae	6	4	2	1	1	1	0	0	3	3	3	3	1	1	0	0	4	3	3	2	2	1	0	0
P. mirabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. hydrophilia	3	2	1	0	0	0	0	0	3	2	2	1	1	0	0	0	5	3	2	1	1	0	0	0
B. subtilis	4	3	1	0	0	0	0	0	6	4	4	3	1	1	0	0	7	5	4	3	1	1	0	0
S. typhi	5	3	1	0	0	0	0	0	6	5	4	3.0	2	2	0	0	6	5	4	3	2	1	0	0
S. dysenteriae	3	2	1	0	0	0	0	0	5	3	3	2	1	1	0	0	8	5	3	2	1	0	0	0
B. cepacia	0	0	0	0	0	0	0	0	9	5	4	3	2	1	0	0	6	5	4	3	2	1	0	0
C. koseri	4	3	2	1	0	0	0	0	5	3	3	3	1	0	0	0	6	5	3	2	1	1	0	0
K. ozaenae	2	4	3	2	4	3	0	0	4	3	2	4	3	2	4	3	4	3	2	2	1	0	0	0

P value<0.0001; P value summary** Significantly different standard deviations? (P<0.0)

Table 4: Diameter of zones of inhibition of partially purified ethanolic leaf extracts of *Spondias mombin* on the test bacteria.

	Fraction F_1 (SMLEH)								Fraction F_2 (SMLEEA)								Fraction F_3 (SMLEE)							
	Diameter of zones of inhibition																							
Fungi	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MFC 0.1562 mg/ml**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125**	**MFC 0.1562**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MFC 0.1562 mg/ml**
C. albicans	6	4	3	2	0	0	0	0	6	5	3	3	1	0	0	0	5	2	2	1	0	0	0	0
A. niger	0	0	0	0	0	0	0	0	5	3	2	1	1	0	0	0	0	0	0	0	0	0	0	0
F. solani	0	0	0	0	0	0	0	0	6	4	3	1	0	0	1	0	3	2	1	1	0	0	0	0
S. cerevisiae	5	3	2	1	1	1	0	0	3	2	2	1	0	0	0	0	7	5	2	2	1	1	0	0
A. flavus	3	2	1	1	0	0	0	0	4	3	2	1	1	0	0	0	0	0	0	0	0	0	0	0
P. megakarya	4	3	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0	0	0	0
C. kruise	5	4	2	1	1	0	0	0	6	3	2	0	0	0	0	0	5	4	4	3	2	1	0	0
R. stonifer	7	4	3	2	1	0	0	0	2	1	1	1	1	1	0	0	3	2	1	0	0	0	0	2
T. horizionum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. vortercelium	4	2	2	1	1	0	0	0	5	4	3	2	1	0	0	0	6	3	3	2	1	0	0	2
S. racemosum	0	0	0	0	0	0	0	0	4	3	1	1	1	1	0	0	0	0	0	0	0	0	0	0

P value<0.0001; P value summary****; **Significantly different standard deviations? (P<0.05)**

Table 5: Diameter of zones of inhibition of partially purified ethanolic leaf extracts of *Spondias mombin* on the test fungi.

	Fraction F_1 (SMSBEH)								Fraction F_2 (SMSBEEA)								Fraction F_3 (SMSBEE)							
	Diameter of zones of inhibition																							
Bacteria	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MBC 0.1562 mg/ml**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125**	**MBC 0.1562**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg**	**MBC 0.1562 mg/ml**
M. fortuitum	4	3	3	2.0	2	0	0	0	2	1	1	0	0	0	0	0	4	3	2	1	0	0	0	0
M. sinegmatis	4	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	3	2	1	0	0	0	0	0
M. abscessus	3	3	2	1	1	0	0	0	1	0	0	0	0	0	0	0	4	3	2	1	0	0	0	0
M. phlei	3	2	1	0	0	0	0	0	1	1	0	0	0	0	0	0	4	2	2	0	0	0	0	0
S. aureus	2	1	0	0	0	0	0	0	7	5	4	3	3	2	0	0	6	4	4	3	2	1	0	0
K pneumoniae	4	3	2	1	0	0	0	0	5	3	3	3	3	0	0	0	6	4	3	3	3	1	0	0
E. coli	5	3	1	0	0	0	0	0	8	5	3	3	2. 0	1	0	0	7	5	4	4	1	0	0	0
P. aeruginosa	8	6	6	3	0	0	0	0	7	5	3	3	2	1	0	0	1	1	1	1	0	0	0	0
S. typhi	6	4	2	1	1	0	0	0	5	3	2	1	1	0	0	0	5	3	2	1	1	0	0	0
S. choleraesuis	5	4	4	3	1	0	0	0	4	3	2	1	1	0	0	0	0	0	0	0	0	0	0	0
S. arizonae	5	3	2	2	1	1	0	0	9	7	5	3	2	1	0	0	5	4	2	2	1	1	0	0
P. mirabilis	6	5	4	3	2	2	0	0	6	5	3	2	1	0	0	0	6	5	4	3	2	2	0	0
A. hydrophilia	4	3	2	1	1	0	0	0	7	5	4	2	1	0	0	0	0	0	0	0	0	0	0	0
B/subtilis	5	2	2	1	0	0	0	0	8	5	4	3	1	1	0	0	5	3	2	2	1	0	0	0
S. typhi	7	4	4	2	1	0	0	0	6	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0
S. dysenteriae	2	1	0	0	0	0	0	0	2	2	1	1	1	1	0	0	1	0	0	0	0	0	0	0
B. cepacia	4	5	3	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. koseri	8	6	3	3	2	1	0	0	7	6	5	3	1	0	0	0	7	5	3	3	2	2	0	0
K. ozaenae	5	4	3	2	1	0	0	0	4	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0

P value<0.0001; P value summary*** **Significantly different standard deviations? (P<0.05)**

Table 6: Diameter of zones of inhibition of partially purified ethanolic leaf extracts of *Spondias mombin* on the test bacteria.

	Fraction F_1 (SMSBEH)								Fraction F_2 (SMSBEEA)								Fraction F_3 (SMSBEE)							
	Diameter of zones of inhibition																							
Bacteria	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MBC 0.1562 mg/ml**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125**	**MBC 0.1562**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg**	**MBC 0.1562 mg/ml**
M. fortuitum	3	3	3	2.0	2	0	0	0	2	1	1	0	0	0	0	0	4	3	2	1	0	0	0	0
M. sinegmatis	3	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	3	2	1	0	0	0	0	0
M. abscessus	3	3	2	1	1	0	0	0	1	0	0	0	0	0	0	0	4	3	2	1	0	0	0	0
M. phlei	3	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	4	2	2	0	0	0	0	0
S. aureus	2	0	0	0	0	0	0	0	7	5	4	3	3	2	0	0	6	4	4	3	2	1	0	0
K pneumoniae	3	2	2	1	0	0	0	0	5	3	3	3	3	0	0	0	6	4	3	3	3	1	0	0
E. coli	3	2	1	0	0	0	0	0	8	5	3	3	2. 0	1	0	0	7	5	4	4	1	0	0	0
P. aeruginosa	9	7	6	3	0	0	0	0	7	5	3	3	2	1	0	0	1	1	1	1	0	0	0	0
S. typhi	5	3	2	1	1	0	0	0	5	3	2	1	1	0	0	0	5	3	2	1	1	0	0	0
S. choleraesuis	6	5	4	3	1	0	0	0	4	3	2	1	1	0	0	0	0	0	0	0	0	0	0	0
S arizonae.	5	4	2	2	1	1	0	0	9	7	5	3	2	1	0	0	5	4	2	2	1	1	0	0
P. mirabilis	7	6	4	3	2	2	0	0	6	5	3	2	1	0	0	0	6	5	4	3	2	2	0	0
A. hydrophilia	5	3	2	1	1	0	0	0	7	5	4	2	1	0	0	0	0	0	0	0	0	0	0	0
B/subtilis	5	3	2	1	0	0	0	0	8	5	4	3	1	1	0	0	5	3	2	2	1	0	0	0
S. typhi	8	5	4	2	1	0	0	0	6	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0
S. dysenteriae	1	0	0	0	0	0	0	0	2	2	1	1	1	1	0	0	1	0	0	0	0	0	0	0
B. cepacia	5	4	3	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. koseri	7	5	3	3	2	1	0	0	7	6	5	3	1	0	0	0	7	5	3	3	2	2	0	0
K. ozaenae	6	3	3	2	1	0	0	0	4	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0

P value<0.0001; P value summary*** Significantly different standard deviations? (P<0.05)

Table 7: Diameter of zones of inhibition of partially purified ethanolic stem bark extracts of *Spondias mombin* on the test bacteria.

	Fraction F_1 (SMSBEH)								Fraction F_2 (SMSBEEA)								Fraction F_3 (SMSBEE)							
	Diameter of zones of inhibition																							
Fungi	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MFC 0.1562 mg/ml**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125**	**MFC 0.1562**	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**MIC 0.3125 mg/ml**	**MFC 0.1562 mg/ml**
C. albicans	0	0	0	0	0	0	0	0	4	4	3.0	1	0	0	0	0	8	6	4	3	2.0	2	0	0
A. niger	6	4	4	3	1	1	0	0	0	0	0	0	0	0	0	0	5	3	2.0	1	0	0	0	0
F. solani	5	4	3	2	1	1	0	0	5	4	2	0	0	0	0	0	8	5	3	2	1	1	0	0
S. cerevisiae	7	5	4	3	2	1	0	0	7	6	4	3	1	1	0	0	2	1	1	1		0	0	0
A. flavu	5	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	4	2	1	1	0	0	0	0
P megakarya	5	4	3	2	1	1	0	0	5	3	2	2	1	0	0	0	7	5	3	1	1	0	0	0
C. kruise	7	5	4	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R. stonifer	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	2	1	1	0	0	0	0.0
T. horizionum	5	4	3	2	1	1	0	0	5	4	3	2	2	1	0	0	0	0	0	0	0	0	0	0
F. vortercelium	7	5	3	3	2	1	0	0	5	4	3	3	3	2	0	0	4	2	1	0	0	0	0	0
S. racemosum	5	3	3	2	1	0	0	0	5	3	3	2	1	0	0	0	3	2	1	0	0	0	0	0

P value<0.0001; P value summary**** Significantly different standard deviations? (P<0.05)

Table 8: Diameter of zones of inhibition of partially purified ethanolic stem bark extracts of *Spondias mombin* on the test fungi.

infectious diseases. Their usefulness in the formulation of antibiotics is also recommended if the active principles can be isolated and purified. Further investigations of the S*pondias mombin* plants for the isolation, purification and characterization of the active principles are ongoing.

Recommendations

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

Nomenclature

Code	Fractions of Different Extracts
SMREH	*N*-hexane fraction of the ethanol extract of the *S. mombin* root
SMREEA	Ethyl acetate fraction of the ethanol extract of the *S. mombin* root
SMREE	Ethanol fraction of the ethanol extract of the *S. mombin* root
SMREAH	*N*-hexane fraction of the ethyl acetate extract of the *S. mombin* root
SMREAEA	Ethyl acetate fraction of the ethyl acetate extract of the *S. mombin* root
SMREAE	Ethanol fraction of the ethyl acetate extract of the *S. mombin* root
SMLEH	*N*-hexane fraction of the ethanol extract of the *S. mombin* leaf
SMLEEA	Ethyl acetate fraction of the ethanol extract of the *S. mombin* leaf
SMLEE	Ethanol fraction of the ethanol extract of the *S. mombin* leaf
SMLEAH	*N*-hexane fraction of the ethyl acetate extract of the *S. mombin* leaf
SMLEAEA	Ethyl acetate fraction of the ethyl acetate extract of the *S. mombin* leaf
SMLEAE	Ethanol fraction of the ethyl acetate extract of the *S. mombin* leaf
SMSBEH	*N*-hexane fraction of the ethanol extract of the *S. mombin* stem-bark
SMSBEEA	Ethyl acetate fraction of the ethanol extract of the *S. mombin* stem-bark
SMSBEE	Ethanol fraction of the ethanol extract of the *S. mombin* stem-bark
SMSBEAH	*N*-hexane fraction of the ethyl acetate extract of the *S. mombin* stem-bark
SMSBEAEA	Ethyl acetate fraction of the ethyl acetate extract of the *S. mombin* stem-bark
SMSBEAE	Ethanol fraction of the ethyl acetate extract of the *S. mombin* stem-bark

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