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Original Research Article

Synergistic Antibacterial and Antifungal Activities of *Spondias mombin(Linn)* Extracts and Conventional Antibiotic and Antifungal Agents on Selected Clinical Microorganisms

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Abstract: *Spondias mombin* is a plant widely used in folkloric medicine. It has lots of potential as a medicinal plant. Most of these potentials are yet to be discovered and its anti-infective property could be maximized either by using it alone or combining it with other plants and conventional antibiotics as a means of combating the problem of antibiotic resistance. The aim of the present work is to investigate the synergistic antimicrobial activity of various concentration of extracts of *Spondias mombin* and ofloxacin investigate the synergistic antifungal activity of extract of *Spondias mombin* and fluconazole, compare the antimicrobial and antifungal activity of the various parts of the plant (leaf, bark and stem) and to compare the activity of the aqueous and crude ethyl acetate extracts of *Spondias mombim* and 30 organisms were used for the research. They include 10 (ten) typed bacterial, 10 (ten) locally isolated bacteria and 10 (ten) locally fungal isolates and the results obtained revealed a higher zone of inhibition against the pathogens tested. The findings from this work suggest that *Spondias mombin* contain some compounds that can enhance the effect of conventional antibiotics and antifungal agents in inhibiting the growth of pathogens.
Keywords: *Spondias mombin,* conventional antibiotics

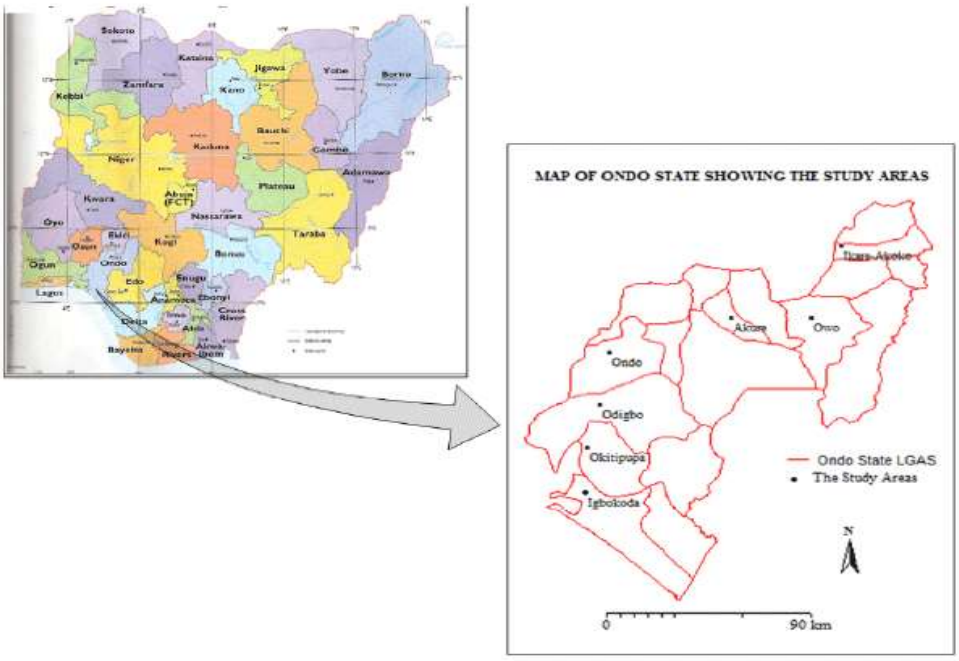
INTRODUCTION

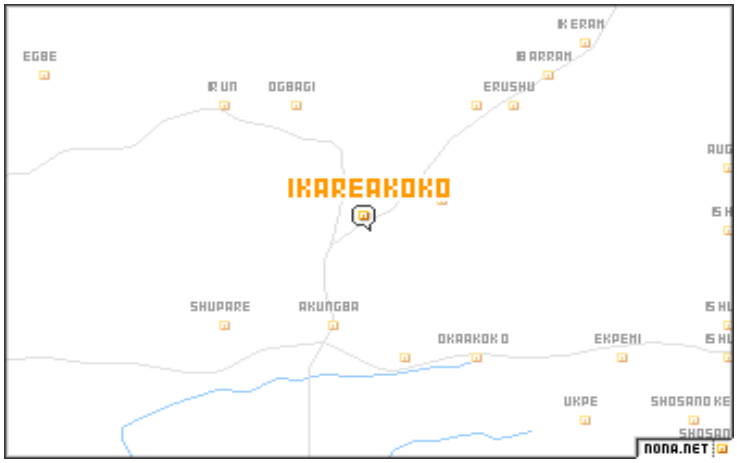
Spondias mombin Linn.is a widely cultivated economic plant that produces edible floral parts. It grows very easily from stakes to make live fences and enclosures. In West Nigeria the tree serves as shade and the stem is used for making fence. The fruits are commonly sold in West African markets. Ripe fruits are eaten out-of-hand, 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 B1 and C [1, 2].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 stomachache, 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 [3, 4]. The powdered bark is used for treating wounds. The gum is employed as an expectorant and anti-helminthic [5]. The plant is reported to have anti-bacterial, anti-fungal, and anti-viral effects [6, 7]. It also has sedative, anti-epileptic, anti-psychotic, anti-diarrheal, anti-inflammatory, cytotoxicity properties [8, 9, 5]. The leaf of the species has been reported to have α-amylase inhibitory effect [8]. 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 [9]. The plant accumulates phenolic compounds [7, 8]. 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 anti-microbial [5], anti-bacterial [10], anti-fungal [11] anti-viral properties [12, 13] have also reported the

abortifacient activity of the aqueous extract [13]. A novel beta-lactamase inhibitor was isolated from a hexane extract of the plant by [1]. Four new compounds mombirin, mombincone, mombinoate and mombinol with anti-tubercular properties [15] were isolated from the stem of *Spondias mombin*.

Pathogens are causative agents of diseases. They include: bacteria which are prokaryotes. They cause a wide range of diseases in man, animals and plants. Fungi are eukaryotes .They is known to cause some serious systemic infection called mycoses especially in immune-compromised patients. The incidence of this systemic fungal infection has increased with the advent of HIV. Management of these diseases require prolonged usage of antifungal agent which have their side effects on the human body hence there is a need for an alternative The diseases caused by these agents have serious financial, social and economic implications. Antibiotics are used to combat diseases caused by bacteria and they are either chemically synthesized or produced by bacteria and they inhibits the growth of these microorganisms at a concentration that is not harmful to man. The discovery of these antimicrobial agents in the 20th century revolutionized the treatment of infectious disease [16]. The ability to use these agents successfully to treat infectious disease suggested that the end has come to the burden of these diseases on mankind. This success was however short-lived because of the development of resistance by these microorganisms. The extraordinary genetic capacities of microbes have benefitted from man's overuse of antibiotics to exploit every form of resistance genes and every means of horizontal gene transmission to develop multiple mechanism of resistance for antibiotics [17]. Antimicrobial resistance is a major health challenge that has made it difficult to successfully manage infectious disease. It has therefore led to the search for another alternative therapy to manage infectious disease and one new approach is the use of medicinal plants screen for their possible antimicrobial properties which is novel, inexpensive and effective against pathogenic microorganisms [18]. Many naturally occurring compounds found in plants have shown to possess antifungal, antibacterial and anti-protozoan activities and serve as a source of antimicrobial agents that can be used either systemically or locally [19, 20].

Synergism is a positive interaction created when two agents combine and exert an inhibitory effect that is greater than the sum of their individual effects. Plants either contain antimicrobials that can operate in synergy with antibiotics or possess compounds that have no intrinsic antibacterial activity but are able to sensitize the pathogen to a previously ineffective antibiotic [21]. Ofloxacin is a synthetic antibiotic of the fluoroquinolone drug class considered to be a second generation fluoroquinolone [22, 23] and it is on the World Health Organization list of essential medicine [46] and it is a broad spectrum antibiotic that has a wide range of action against gram positive and gram negative organisms most of which were used in this experiment. It acts by inhibiting DNA gyrase, which is an enzyme that separates replicated DNA which is needed for bacterial cell division. It is available for topical, oral and intravenous administration.





MATERIALS AND METHODS

Collection of plant materials.

Leaves, bark and stem of *Spondias mombin* were collected at Ikare, Akoko, Ondo State, a tropical rain forest of Ondo State, Nigeria on the 1st of April 2014,with latitude (7.21692 North) and longitude (5.21561 East).The time of collection was 6.30 am in the morning, into a polythene bag for easy handling. The plant was authenticated at the herbarium of the Department of Pharmacy Obafemi Awolowo University, Ife, Ile -Ife, Osun State Nigeria. Synergistic, Antibacterial, Antifungal, Conventional antibiotic, Antifungal agents, Phytochemical

Preparation of the extracts.

The Leaf stem and bark was chopped into small pieces and air dried at room temperature for 21 days. The dry Leaf stem and bark were ground into fine powder using an electric grinder. The powdered plant material (1Kg) was sequentially extracted three times with 5 liters of ethyl-acetate and distilled water at room temperature for 48 hours. The extracts were filtered through cotton wool and Whatman No. 1 filter paper and concentrated with a rotary evaporator at 40^0C to dryness. The dried extracts were transferred to sample bottles which were placed in a desiccator containing anhydrous sodium sulphate to remove any traces of water that could have been present. The dry extracts were later kept in tightly stoppered bottles in a refrigerator for further analysis.

Preparation for Antibacterial assay of the *Spondias mombin* on selected organisms.

The activity of the plant extracts was tested against Ten American Type Culture Collection (ATCC) bacterial strains; ten clinically isolate bacterial and fungal strains. The bacterial and fungi strains were selected on the basis of the diseases against which *Spondias mombin* is used. The bark of *Spondias mombin* is used locally for the treatment of cough, commonly caused by *Klebsiella pneumoniae* and diarrhea, caused by E.coli. Testing of the plant extracts for antibacterial activity was done by the agar well diffusion method.

Standardization of microorganisms.

The test organisms are; *Mycobacterium fortuitum* ATCC 6841, *Mycobacterium smegmatis* ATCC 19420, *Mycobacterium abscessus* ATCC 19977, *Mycobacterium phlei* ATCC 19240, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 0157, *Salmonella typhi*, *Klebsiella pneumonia ATCC* 35659, *Candida albicans* ATCC 90029.etc.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 (18-24hrs). 0.1ml of the broth was introduced into 9.9ml of sterile distilled water to make a dilution of 1:1000 and also from the dilution another 0.1ml was pipetted into 9.9ml of sterile distilled water to make a dilution of 1:10,000.

Standardization of extracts.

0.6g of the extract was weighed into a sterile bottle in which 2.5ml of DMSO (Dimethyl surfoxide) was used to reconstitute the extract after which 7.5ml of sterile distilled water was added to make up 10ml (60mg/ml) in total. 3ml of the reconstituted extract easy dispensed into another bottle carrying 3ml of sterile distilled water to make up 6ml (30mg/ml). The same procedure was done for 15mg/ml and 7.5mg/ml respectively.

Antibacterial assay of *Spondias mombin* plant extracts on selected organisms.

The susceptibility testing was investigated by the agar diffusion method, A 0.1 ml of 1:10,000 dilutions (equivalent to 10^6cfu/mL) of fresh overnight culture of the clinical species grown in Nutrient broth was seeded into 40 ml of molten Mueller-Hinton agar, and properly mixed in universal bottles. The mixture was aseptically poured into sterile Petri dishes and

allowed to set. Using a sterile cork borer of 6 mm diameter equidistant wells were made in the agar. Drops of the re-suspended extracts with concentrations between 60 to 7.5 mg/ml were introduced into the wells till it's filled plus Ofloxacin 2mg/ml was used for bacteria. The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation and the plates were incubated at 37^{o}C for 24 to 48 hours. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. All experiments were performed in duplicates.

Antifungal sensitivity testing of *Spondias mombin* extracts on selected organisms.

Spondias mombin extract was tested for antifungal activities. Using agar well diffusion method. A 5- day old fungal culture on potato dextrose agar was flooded with 2ml sterile distilled water containing 3% glycerol. The spores were harvested by scraping with a sterile inoculating loop. Sterile PDA plates were inoculated with 0.1ml of the fungal suspension using the spread plate technique. Four wells were bored on the PDA plates using a 6mm sterile cork borer. Drops of the re-suspended extracts with concentrations between 60 to 7.5 mg/ml were introduced into the wells till it's filled plus fluconazole 2mg/ml was used for fungi. The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation. The plates were allowed to stand on the bench for one hour before they were incubated at 25°c for 5 days. Diameter of zones of growth inhibition was then measured in millimeter with a vernier caliper. This experiment was done in duplicate.

RESULTS

Table-1- shows the synergistic antimicrobial activity of varying concentration of the crude aqueous extract of leaf, stem and bark of S*pondias mombin* with ofloxacin on typed organisms. All the typed strains were sensitive to the aqueous extracts of the leaf, bark and stem of S*pondias mombin* at higher concentration. The aqueous extracts of the leaf showed reduced activity to *Pseudomonas aeruginosa* ATCC 25619 of 19.0 mm at 60 mg / ml unlike that of the stem and bark that showed an activity of 28 mm each.

Table-2-shows the synergistic activity of crude aqueous extract of the leaf, stem and bark of *Spondias mombin* with ofloxacin against isolated organisms. Most of the organisms were susceptible to the combination of the extracts and ofloxacin with very high zone of inhibition.as high as 40mm.only a few were resistant and these occur at the lowest dose of the extracts.

Table-3-shows the synergetic activity of the crude aqueous extracts of the leaf, bark and stem of S*pondias mombin* with fluconazole against some fungi. Nearly all the organism were susceptible to the combination especially at higher doses of the extract except *Syncephalastrum racemosum* which showed the lowest result of 0.0mm to the combination with the aqueous extracts from the stem.

Table-4-shows the synergistic antimicrobial activity of varying concentration of the crude ethyl - acetate extracts of leaf, stem and bark of S*pondias mombin* with ofloxacin on typed organisms.And all the organisms responded well to the combination. Higher concentration of the extracts showed better results. The effect of the crude ethyl-acetate extract was higher when compared to the aqueous extract of the same plant.

Table-5-shows the synergistic antimicrobial activity of varying concentration of the crude ethyl - acetate extracts of leaf, stem and bark of S*pondias mombin* with ofloxacin on isolated organisms. Most of the organisms were susceptible to the combination of the extracts and ofloxacin with very high zone of inhibition as high as 40mm, only a few were resistant and these occur at the lowest dose of the extracts.

Table-6-shows the synergetic activity of the crude ethyl-acetate extract of the leaf, bark and stem of S*pondias mombin* with fluconazole against some fungi. *Aspergillus flavus* was not susceptible to any of the dose of the stem extracts while *Phytophera megakarya* had a zone of inhibition of 0.0mm to both the leaf and stem crude ethyl acetate extracts.

Table-7- shows the synergetic/antimicrobial activity of crude ethanolic extracts of S*pondias mombin* at concentration of 60, 30, 15 and 7.5mg/ml and ofloxacin2mg/ml on selected clinical microorganisms. It was observed that *Staphylococcus typhi* ATCC 35723 at 60mg/ml ha the highest inhibition ratio and *Mycobacterium fortuitum ATCC 6841* has the lowest ratio at 7.5mg/ml

Table-8-shows the synergetic/antibacterial activity of crude ethanolic extracts of *Spondias mombin* at concentration of 60, 30, 15 and 7.5mg/ml and ofloxacin 2mg/ml on selected clinical microorganisms. It was observed that *Shigella dysenteriae* has the highes*t* zone of inhibition at 60mg.ml.

Table-9-shows the synergetic/antifungal activity of crude ethanolic extracts of *Spondias mombin* at concentration of 60, 30, 15 and 7.5mg/ml and fluconazole at 2mg/ml on selected clinical micro organisms locally isolated fungi. It was observed that *Fusarium solani* and *Yeast at* 60mg/ml has the highest zone of inhibition at 60mg/ml.

Table -1: Synergistic antimicrobial activity of crude aqueous extracts of leaf, bark and stem of *Spondias mombin* at concentration of 60, 30, 15 and 7.5mg/ml and 2 mg/ml ofloxacin on selected clinical microorganisms.

Clinical Organism	LEAF EXTRACT (Conc. in mg/ml)				BARK EXTRACT (Conc. in mg/ml)				STEM EXTRACT (Conc. in mg/ml)			
	60	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**
Mycobacterium fortuitum ATCC 6841	36.0	30.0	28.0	18.0	31.0	25.0	23.0	21.0	39.0	33.0	28.0	21.0
Mycobacterium smegmatis ATCC 19420	25.0	20.0	19.0	15.0	30.0	27.0	20.0	18.0	29.0	27.0	20.0	16.0
Mycobacterium abscessus ATCC 19977	32.0	30.0	28.0	20.0	33.0	25.0	21.0	19.0	31.0	30.0	22.0	18.0
Mycobacterium phlei ATCC 19240	27.0	25.0	18.0	15.0	31.0	29.0	18.0	14.0	34.0	22.0	19.0	14.0
Staphylococcus aureus ATCC 29213	27.0	25.0	23.0	17.0	25.0	23.0	20.0	16.0	28.0	24.0	20.0	16.0
Escherichia coli ATCC 25922	25.0	20.0	18.0	17.0	29.0	23.0	20.0	16.0	23.0	24.0	20.0	17.0
Klebsiella pneumoniae ATCC 35659	29.0	23.0	19.0	13.0	32.0	28.0	18.0	10.0	24.0	20.0	16.0	8.0
Candida albicans ATCC 90029	27.0	25.0	23.0	17.0	25.0	23.0	20.0	16.0	28.0	24.0	20.0	16.0
Staphylococcus typhi ATCC 35723	23.0	20.0	16.0	12.0	25.0	18.0	14.0	10.0	28.0	20.0	19.0	12.0
Pseudomonas aeruginosa ATCC 25619	19.0	15.0	10.0	4.0	28.0	25.0	19.0	10.0	28.0	20.0	15.0	11.0

Table -2: Synergistic antibacterial crude activity of aqueous extracts of the leaf, bark and stem of *Spondias mombin* at concentration of 60, 30, 15 and 7.5mg/ml and 2mg/ml ofloxacin on selected clinical microorganisms.

Clinical Organism	LEAF EXTRACT (Conc. in mg/ml)				BARK EXTRACT (Conc. in mg/ml)				STEM EXTRACT (Conc. in mg/ml)			
	60	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**
Salmonella choleraesuis	28.0	25.0	20.0	15.0	25.0	19.0	17.0	13.0	35.0	30.0	25.0	20.0
Salmonella arizonae	28.0	24.0	21.0	17.0	31.0	28.0	23.0	15.0	40.0	35.0	20.0	15.0
Proteus mirabilis	31.0	28.0	19.0	15.0	27.0	25.0	18.0	15.0	31.0	27.0	19.0	16.0
Aeromonas hydrophilia	25.0	21.0	16.0	13.0	27.0	20.0	17.0	14.0	26.0	23.0	18.0	12.0
Bacillus Subtilis	25.0	21.0	20.0	12.0	28.0	23.0	20.0	16.0	28.0	20.0	16.0	10.0
Salmonella Typhi	27.0	25.0	23.0	17.0	25.0	23.0	20.0	16.0	28.0	24.0	20.0	16.0
Shigella Dysenteriae	35.0	30.0	29.0	16.0	32.0	28.0	23.0	20.0	40.0	37.0	25.0	21.0
Burkholderia Cepacia	36.0	29.0	27.0	10.0	35.0	30.0	27.0	20.0	29.0	25.0	20.0	17.0
Citrobacter Koseri	39.0	36.0	30.0	28.0	32.0	27.0	21.0	19.0	31.0	29.0	25.0	21.0
Klebsiella ozaenae	33.0	31.0	27.0	19.0	32.0	25.0	22.0	20.0	30.0	26.0	21.0	18.0

Table -3: Synergistic antifungal activity of crude aqueous extracts of *Spondias mombin* at concentration of 60, 30, 15 and 7.5mg/ml and fluconazole at 2mg/ml on selected clinical microorganisms

Clinical Organism	LEAF EXTRACT (Conc. in mg/ml)				BARK EXTRACT (Conc. in mg/ml)				STEM EXTRACT (Conc. in mg/ml)			
	60	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**
Aspergillus Niger	26.0	20.0	15.0	8.0	29.0	25.0	20.0	14.0	32.0	28.0	23.0	19.0
Fusarium Solani	31.0	28.0	25.0	23.0	40.0	36.0	27.0	15.0	31.0	25.0	23.0	19.0
Yeast	31.0	29.0	25.0	20.0	30.0	25.0	20.0	10.0	32.0	30.0	29.0	21.0
Aspergillus Flavus	40.0	36.0	30.0	29.0	29.0	20.0	16.0	8.0	33.0	28.0	25.0	20.0
Phytophera *megakarya*	27.0	22.0	16.0	9.0	29.0	25.0	22.0	14.0	32.0	28.0	23.0	19.0
Candida Kruise	32.0	27.0	24.0	24.0	40.0	36.0	26.0	15.0	31.0	25.0	23.0	19.0
Rhizopus Stonifer	30.0	28.0	26.0	21.0	30.0	25.0	22.0	10.0	32.0	30.0	29.0	21.0
Trichodermahorizionum	39.0	35.0	29.0	30.0	29.0	20.0	17.0	8.0	33.0	28.0	25.0	20.0
Fusarium vortercelium	15.0	10.0	5.0	2.0	18.0	15.0	10.0	7.0	21.0	15.0	12.0	8.0
Syncephalastrum racemosum	20.0	14.0	5.0	2.0	13.0	10.0	8.0	4.0	0.0	0.0	0.0	0.0

Table -4: Synergistic antimicrobial activity of crude ethyl -acetate extracts of *Spondias mombin* at concentration of 60, 30, 15 and 7.5mg/ml and 2mg/ml ofloxacin on selected clinical microorganisms.

Clinical Organism	LEAF EXTRACT (Conc. in mg/ml)				BARK EXTRACT (Conc. in mg/ml)				STEM EXTRACT (Conc. in mg/ml)			
	60	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**
Mycobacterium fortuitum ATCC 6841	30.0	29.0	20.0	16.0	36.0	31.0	28.0	15.0	29.0	24.0	18.0	13.0
Mycobacterium smegmatis ATCC 19420	29.0	26.0	21.0	17.0	32.0	29.0	27.0	20.0	31.0	25.0	20.0	16.0
Mycobacterium abscessus ATCC 19977	31.0	26.0	21.0	14.0	31.0	27.0	21.0	20.0	28.0	23.0	19.0	12.0
Mycobacterium phlei ATCC 19240	31.0	27.0	22.0	19.0	30.0	27.0	22.0	18.0	32.0	28.0	23.0	18.0
Staphylococcus aureus ATCC 29213	35.0	28.0	24.0	22.0	31.0	27.0	21.0	17.0	28.0	20.0	16.0	7.0
Escherichia coli ATCC 25922	31.0	28.0	21.0	19.0	28.0	26.0	19.0	16.0	31.0	28.0	20.0	16.0
Klebsiella pneumoniae ATCC 35659	23.0	20.0	17.0	10.0	30.0	29.0	26.0	22.0	32.0	30.0	23.0	16.0
Candida albicans ATCC 90029	26.0	23.0	19.0	12.0	28.0	27.0	25.0	20.0	29.0	26,0	21.0	18.0
Salmonella typhi ATCC 35723	28.0	25.0	17.0	14.0	29.0	26.0	21.0	20.0	26.0	26.0	23.0	17.0
Pseudomonas aeruginosa ATCC 25619	29.0	24.0	19.0	17.0	25.0	22.0	18.0	16.0	29.0	25.0	17.0	15.0

Table -5: Synergistic antibacterial activity of crude ethyl-acetate extracts of the leaf, bark and stem of *Spondias mombin* at concentration of 60, 30, 15 and 7.5mg/ml and ofloxacin 2mg/ml onselected clinical microorganisms.

Clinical Organism	LEAF EXTRACT (Conc. in mg/ml)				BARK EXTRACT (Conc. in mg/ml)				STEM EXTRACT (Conc. in mg/ml)			
	60	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**
Salmonella choleraesuis	19.0	17.0	14.0	12.0	23.0	21.0	17.0	15.0	25.0	20.0	20.0	15.0
Salmonella arizonae	27.0	25.0	19.0	17.0	25.0	19.0	18.0	16.0	35.0	29.0	20.0	18.0
Proteus mirabilis	31.0	29.0	18.0	16.0	26.0	25.0	19.0	16.0	30.0	25.0	20.0	15.0
Aeromonas hydrophilia	26.0	23.0	18.0	12.0	25.0	21.0	17.0	12.0	28.0	25.0	16.0	13.0
Bacillus Subtilis	36.0	27.0	21.0	18.0	35.0	29.0	21.0	17.0	27.0	24.0	20.0	18.0
Salmonella Typhi	26.0	20.0	15.0	8.0	29.0	25.0	20.0	14.0	32.0	28.0	23.0	19.0
Shigella dysenteriae	31.0	28.0	25.0	23.0	40.0	36.0	27.0	15.0	31.0	25.0	23.0	19.0
Burkholderia cepacia	31.0	29.0	25.0	20.0	30.0	25.0	20.0	10.0	32.0	30.0	29.0	21.0
Citrobacter Koseri	40.0	36.0	30.0	29.0	29.0	20.0	16.0	8.0	33.0	28.0	25.0	20.0
Klebsiella ozaenae	36.0	31.0	27.0	25.0	32.0	26.0	21.0	18.0	32.0	29.0	21.0	20.0

Table-6: Synergistic antifungal activity of crude ethyl -acetate extracts of *Spondias mombin* at concentration of 60, 30, 15 and 7.5 mg/ml and fluconazole at 2 mg/ml on selected clinical microorganisms.

Clinical Organism	LEAF EXTRACT (Conc. in mg/ml)				BARK EXTRACT (Conc. in mg/ml)				STEM EXTRACT (Conc. in mg/ml)			
	60	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**
Aspergillus Niger	31.0	28.0	19.0	15.0	25.0	23.0	19.0	5.0	30.0	28.0	25.0	18.0
Fusarium Solani	31.0	29.0	24.0	20.0	30.0	25.0	20.0	16.0	25.0	20.0	17.0	8.0
Yeast	31.0	25.0	20.0	15.0	29.0	27.0	20.0	15.0	31.0	28.0	25.0	18.0
Aspergillus Flavus	26.0	20.0	12.0	8.0	30.0	20.0	10.0	9.0	0.0	0.0	0.0	0.0
Phytophera *megakarya*	0.0	0.0	0.0	0.0	25.0	20.0	18.0	9.0	0.0	0.0	0.0	0.0
Candida kruise	24.0	20.0	16.0	10.0	23.0	20.0	16.0	10.0	20.0	15.0	11.0	9.0
Rhizopus stonifer	15.0	8.0	3.0	2.0	18.0	12.0	10.0	8.0	17.0	12.0	8.0	3.0
Trichoderma horizionum	10.0	7.0	3.0	0.0	19.0	10.0	8.0	6.0	12.0	7.0	5.0	2.0
Fusarium vortercelium	20.0	15.0	10.0	8.0	19.0	12.0	8.0	3.0	15.0	10.0	5.0	2.0
Syncephalastrum racemosum	18.0	10.0	7.0	4.0	19.0	15.0	10.0	8.0	12.0	6.0	2.0	0.0

Table -7: Synergistic antimicrobial activity of crude ethanolic leaf, bark and stem extracts of *Spondias mombin* at concentration of 60, 30, 15 and 7.5 mg/ml and ofloxacin 2 mg/ml on selected clinical microorganisms.

Clinical Organism	LEAF EXTRACT (Conc. in mg/ml)				BARK EXTRACT (Conc. in mg/ml)				STEM EXTRACT (Conc. in mg/ml)			
	60	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**
Mycobacterium fortuitum ATCC 6841	19.0	16.0	10.0	9.0	26.0	21.0	18.0	12.0	21.0	19.0	16.0	11.0
Mycobacterium smegmatis ATCC19420	24.0	21.0	20.0	14.0	20.0	17.0	15.0	10.0	29.0	26.0	20.0	16.0
Mycobacterium abscessus ATCC 19977	30.0	26.0	20.0	17.0	30.0	24.0	20	17.0	29.0	24.0	20	14.0
Mycobacterium phlei ATCC 19240	34.0	26.0	22.0	14.0	24.0	20.0	17.0	14.0	30.0	25.0	21.0	16.0
Staphylococcusaureus ATCC 29213	25.0	20.0	19.0	13.0	27.0	20.0	16.0	13.0	24.0	20.0	15.0	12.0
Escherichia coli ATCC 25922	27.0	23.0	17.0	13.0	32.0	28.0	20.0	12.0	27.0	18.0	15.0	10.0
Klebsiella pneumonia ATCC 35659	33.0	28.0	20.0	17.0	33.0	28.0	24.0	20.0	31.0	28.0	25.0	19.0
Candida albicans ATCC 90029	28.0	18.0	16.0	10.0	31.0	28.0	21.0	18.0	26.0	20.0	19.0	13.0
Salmonella typhi ATCC 35723	37.0	31.0	26.0	20.0	27.0	24.0	21.0	16.0	29.0	23.0	21.0	18.0
Pseudomonas aeruginosa ATCC 25619	22.0	22.0	19.0	13.0	34.0	28.0	21.0	16.0	27.0	23.0	20.0	14.0

Table-8: Synergistic antibacterial activity of crude ethanolic extracts of *Spondias mombin* at concentration of 60, 30, 15 and 7.5 mg/ml and ofloxacin 2 mg/ml on selected clinical microorganisms

Clinical Organism	LEAF EXTRACT (Conc. in mg/ml)				BARK EXTRACT (Conc. in mg/ml)				STEM EXTRACT (Conc. in mg/ml)			
	60	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**
Salmonella choleraesuis	30.0	26.0	17.0	13.0	26.0	23.0	20.0	18.0	25.0	20.0	17.0	13.0
Salmonella arizonae	35.0	26.0	20.0	18.0	30.0	27.0	21.0	18.0	30.0	27.0	22.0	18.0
Proteus mirabilis	26.0	22.0	19.0	16.0	31.0	28.0	23.0	16.0	26.0	24.0	19.0	16.0
Aeromonas hydrophilia	25.0	21.0	17.0	12.0	28.0	25.0	16.0	13.0	30.0	27.0	18.0	14.0
Bacillus subtilis	26.0	21.0	18.0	12.0	30.0	26.0	20.0	13.0	29.0	24.0	20.0	11.0
Salmonella typhi	30.0	26.0	23.0	18.0	29.0	26.0	21.0	19.0	31.0	29.0	22.0	17.0
Shigella dysenteriae	36.0	29.0	25.0	21.0	32.0	26.0	20.0	16.0	28.0	20.0	16.0	7.0
Burkholderia cepacia	32.0	29.0	20.0	18.0	27.0	25.0	18.0	15.0	31.0	28.0	20.0	16.0
Citrobacter koseri	36.0	30.0	28.0	18.0	31.0	25.0	23.0	21.0	39.0	33.0	28.0	21.0

Table-9: Synergistic antifungal activity of crude ethanolic extracts of *Spondias mombin* at concentration of 60, 30, 15 and 7.5mg/ml and fluconazole at 2mg/ml on selected clinical microorganisms

Clinical Organism	LEAF EXTRACT (Conc. in mg/ml)				BARK EXTRACT (Conc. in mg/ml)				STEM EXTRACT (Conc. in mg/ml)			
	60	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**	**60**	**30**	**15**	**7.5**
Aspergillus Niger	29.0	25.0	20.0	15.0	36.0	28.0	25.0	20.0	28.0	25.0	23.0	20.0
Fusarium Solani	36.0	28.0	23.0	18.0	29.0	26.0	18.0	10.0	28.0	23.0	18.0	10.0
Yeast	36.0	25.0	20	18.0	30.0	28.0	23.0	20.0	29.0	25.0	20.0	17.0
Aspergillus Flavus	23.0	18.0	15.0	10.0	23.0	20.0	15.0	8.0	24.0	20.0	16.0	10.0
Phytophera *Megakarya*	0.0	0.0	0.0	0.0	2.0	16.0	10.0	8.0	20.0	15.0	9.0	3.0
Candida kruise	26.0	20.0	13.0	10.0	30.0	26.0	21.0	17.0	20.0	16.0	8.0	6.0
Rhizopus Stonifer	12.0	5.0	4.0	1.0	13.0	8.0	5.0	2.0	17.0	13.0	10.0	8.0
Trichoderma Horizionum	17.0	10.0	5.0	2.0	19.0	12.0	10.0	8.0	17.0	9.0	3.0	0.0
Fusarium Vortercelium	16.0	10.0	5.0	2.0	23.0	20.0	15.0	9.0	10.0	7.0	5.0	2.0
Syncephalastrum Racemosum	18.0	10.0	5.0	3.0	23.0	21.0	15.0	9.0	20.0	10.0	6.0	2.0

DISCUSSION

The antimicrobial activities of *Spondias mombin* were demonstrated in the above result. The zones of inhibition were larger when compared with the assay done with the chemically synthesized antibiotics and antifungal drugs. The typed strains of *mycobacteria* species tested in this experiment were susceptible to the combination of *Spondias mombin* and ofloxacin. They are generally called Non tubercular *Mycobacterium* (NTM) and are usually saprophytes which can be opportunistic and sometimes deadly pathogens [26]. They are known to cause localized or disseminated disease and the extent of this disease is determined by the level of spread and the immune status of the individual. They cause infection in the lungs, lymph glands, bone, joints, bursa, skin and sepsis [26]. They are usually treated successfully with Anti TB drugs like Rifampicin, isoniacid and ethambutol. *Spondias mombin* alone was reported to have anti-tubercular activity [15]. The extracts of the stem bark was discovered to contain 4 compounds which are mombirin, mombincone, mombinoate and mombinol which are antitubercular in nature. At a concentration of 40 µg/ml, the four compounds exhibited significant inhibitions ($P < 0.05$) against *M. tuberculosis*. These inhibitions are comparable to the 99.8 % inhibition exhibited by the reference drug (rifampicin).

The synergistic effect of the aqueous extracts of the leaf of *Spondias mombin* at varied concentration with 15mg of ofloxacin is demonstrated with the larger zone of inhibition noticed on the plates as shown in the table 3.3 above. With CLSI standard of antimicrobial interpretation, All bacteria except *Pseudomonas aeruginosa* were susceptible to this combination at aqueous extracts of 30mg/ml and 60mg/ml. *Pseudomonas aeruginosa* had a zone of inhibition of 19.0mm at 60mg/ml and 15.0mm at 30mg/ml respectively.

Psedomonas aeruginosa is a highly virulent aerobic gram negative bacterium. It is a well-known etiologic agent in a variety of infection. Its ability to colonise and invade its host cells has made it a culprit of much systemic infection. Its ability to colonise surgery wound sites has made it a popular isolate in Nosocomial infection and it has multiple drug resistance. The indiscriminate use of broad spectrum antibiotics has further increased the incidence of Infection by *Pseudomonas aeruginosa*. The sensitivity profile of *P aeruginosa* with 5ug of ofloxacin done by [27] revealed a mean zone of inhibition of 14.0mm while the result of the assay done by [28] on the effect of the leaf extract of *Spondias mombin* on *Pseudomonas aeruginosa* revealed that it was resistant to the 100mg/ml of the aqueous leaf extracts but had 40% sensitivity to the ethanol extract of the leaf of the same plant. The larger zone of inhibition observed in this research further corroborate the positive effect *Spondias mombin* has on the minimum inhibitory concentration of ofloxacin with *P. aeruginosa*.

At concentration of 15mg/ml of the aqueous extracts from the leaf of *Spondias mombin*, *Aeromonas hydrophillus, E coli, Klebsiella,Salmonella typhi and Proteus mirabilis* were in the intermediate zone of

inhibition. These are common aetiogical agent of major infections and the work done by [28] also revealed resistance by *E coli and K pneumonia* and 20% sensitivity by *Staphylococcus aureus* to 100mg/ml of aqueous extracts of *Spondias mombin* leaf whereas they were sensitive even at 30mg/ml of the same aqueous extracts of *Spondias mombin* when used with ofloxacin. *E coli,* which a major aetiological agent in diarrhoea and dysentery, these are major killer diseases in human beings. The ability of this organism to invade the intestinal cells causes large amount of fluid loss and weakness in individuals infected by this organism. The cost of intravenous fluid and Oral Rehydration Solution coupled with loss of working hours due to hospital admission have added to the socio-economic burden of diarrhoeal disease. The large zone of inhibition demonstrated on this organism by the aqueous and crude ethyl acetate extract of *Spondias mombin* justifies its use in folkloric medicine in the treatment of diarrhoea and dysentery. The activity of *Spondias mombin* along with other medicinal plants was also tested by [29] against some serotypes of *Vibrio cholera*, the causative agent of Cholera whose outbreak has led to lots of death as a result of the fluid and electrolyte loss. The aqueous and ethanol extract of *Spondias mombin* showed zone of inhibition ranging from 22 to 25mm against the sero-groups tested [29].

The activity of *Spondias monbin* and fluconazole was tested against some isolated fungal agents like *Aspergillus niger ,Candida cruise, Trichoderma horizonum* and others .Fungal are known causative agent of Cutaneous and systemic infection in human and animals. They are transmitted by spores or hyphae and may enter the body through lungs or skin. Most fungi are not so virulent they are usually opportunistic pathogens. Their invasion is usually as a result of suppression of the immune system like in prolonged use of corticosteroids, in malignancy, HIV infection, Alteration of mucosa flora, antibiotic treatment and pregnancy.

Fungal infections are usually chronic and often difficult to treat some have no effective treatment yet while some require prolonged chemotherapy. The high zone of inhibition shown by most of these agents to the combination of the extract from *Spondias mombin* and fluconazole further highlights the antifungal properties of *Spondias mombin*.

Syncephalastrum racemosum showed the lowest result of 0.0mm to the combination with the aqueous extracts from the stem even at the highest concentration. It was susceptible to the aqueous extract from the leaf and bark of the plant. The aqueous extract from the stem showed no antifungal activity against *Syncephalastrum racemosum* and even inhibited fluconazole. It is a filamentous fungus [30-32]. Azygomycete which is widely found in the environment [32]. It has a low pathogenic potential and usually cause disease as an opportunistic pathogen. It causes nail diseases onychomycosis [33] and a case of intra-abdominal zygomycoses [34]. It was said to have complicated an abdominal surgical wound done in a patient with abdominal trauma. It was successfully managed with debridement and Amphotericin b lipid complex was used for a total of 29 days [32].The susceptibility of this organism to *Spondias mombin* leaf extracts (aqueous and ethyl acetate) at 60mg/ml in synergism with fluconazole is an indication that *Spondias mombin* is a medicinal plant whose antifungal properties if properly harnessed could signify the end to the chronicity of fungal infection and the prolonged use of antifungal agents.

Phytophera megakarya was resistant to the crude ethyl acetate extract of the leaf and stem extracts of *Spondias mombin*. Cocoa is a very important cash crop and a good source of foreign exchange. Of all cocoa diseases in the world, black pod disease, which *Phytophthora* fungus is the causative agent, causes the largest loss of pods [35]. *Phytophthora megakarya*, one of the four fungus strains, is present only in Central and West African cocoa producing countries, and is the most damaging of the cocoa diseases in this region [35]. *Spondias mombin* tree has been identified as good on farmers 'fields in part of Ashanti [37]. It is said to be good when planted alongside cocoa because of its good shade and deep rooting [36]. The antifungal properties of this plant against the major disease of cocoa pods could also be of economic benefit as it could reduce the cost of fungicides thereby increasing the farmer's returns.

The variation in the antimicrobial and antifungal activities of the leaf, stem and bark extract of the plant wasn't constant. Some organisms were more susceptible to either the leaf, bark and stem extract than others. Obvious difference was noticed in the bark and stem extracts of *Pseudomonas aeruginosa* which had a higher zone of inhibition when compared with the zone of inhibition of the leaf extracts. The crude ethyl acetate extract yielded a higher result. *Syncephalastrum racemosum* was also resistant to all the doses of the aqueous stem extracts of the plant but was sensitive to the leaf and stem extracts but the crude ethyl acetate extract yielded a higher zone of inhibition.

The crude ethyl acetate extract yielded higher zone of inhibition when comparing the results obtained from table 1,2,3 with 4,5 and 6 except for *Aspergillus flavus* and *Phytophera megakarya as* it appeared that the antifungal properties of the plant on the 2 organisms was affected by the ethyl acetate solvent used.

CONCLUSION

This research has further corroborated that the use of medicinal plants and chemically synthesized antibiotics and antifungal produce much more effect than the individual effect.

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