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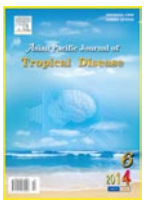

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Efficacy of fresh leaf extracts of *Spondias mombin* against some clinical bacterial isolates from typhoid patients

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PEER REVIEW

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Comments

The concept of the work is good, though similar studies have been carried out on the antimicrobial of the dry leaf extract against some bacteria, this work focused on the antibacterial efficacy of the fresh extract against bacteria of clinical origin.
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ABSTRACT

Objective: To evaluate the phytochemical properties and antibacterial activity of methanol, acetone, ethanol and aqueous extracts of fresh leaves of *Spondias mombin* (*S. mombin*) on some clinical bacterial isolates.
Methods: Clean and fresh leaves of *S. mombin* were collected in Ondo, Southwestern Nigeria. The leaves were blended, extracted with methanol, acetone, ethanol and water. The extracts were evaporated to dryness using rotary evaporator and tested for the presence of saponins, tannins, cardiac glycoside, terpenoids, flavonoids, reducing sugars, volatile oils, alkaloids and glycoside. The extract were tested against Gram-negative bacteria *Klebsiella pneumonia*, *Serratia marcescens*, *Salmonella typhi* and *Enterobacter aerogens*; Gram-positive bacteria *Staphylococcus aureus* by observing the zones of inhibition using agar well diffusion assay.
Results: The study showed that the leaves contained saponins, tannins, flavonoids, alkaloids and glycoside. All the solvent extracts showed activity against all the test bacteria. The methanol extract also showed the highest activity against *Enterobacter aerogens*, zone of diameter (15.00 ±1.89) mm, while the ethanol extract showed the highest activity against *Staphylococcus aureus* with zone of diameter (12.50±1.50) mm. The acetone extract showed the highest activity against *Salmonella typhi*, zone of diameter (17.50±0.29) mm followed by methanol extract showing zone of diameter (15.67±1.01) mm. The acetone extract showed the highest activity against *Klebsiella pneumonia* (15.17±0.67) mm, while the aqueous extract shows the highest activity against *Serratia marcescens* (14.67±2.68) mm. The minimum inhibitory concentration of the leaf extracts ranged between 10-90 mg/mL.
Conclusions: This study showed that the aqueous and organic solvents extract of fresh leaves of *S. mombin* has anti-microbial activity against all the tested organisms.

KEYWORDS

Phytochemicals, Antibacterial, *Spondias mombin*, Extracts

1. Introduction

Infectious diseases account for almost 50000 deaths every day and are the world's major threat to human health[1]. Pathogenic bacteria causing different kinds of life-threatening infections have increased and it is becoming an important cause of death in immune-compromised patients, especially in developing countries[2]. Conventional medicine is not cheap and a large number of people depend on medicinal plants for their health care needs. Medicinal plants are cheap, readily available and are known to contain certain substances that are toxic to bacteria[3]. They have played significant roles in maintaining human health and improving the quality of human life for many years. Medicinal and aromatic plants contain biologically active chemical substances and free radical scavenging molecules

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such as saponins, tannins, essential oils, flavonoids, alkaloids, anthocyanins, carotenoids, dietary glutathione and other chemical compounds that are rich in antioxidant activities[4-6], and they have demonstrated their contribution to the treatment of diseases such as HIV/AIDS[7], malaria, cataract[8], diabetes[9], skin disease[10], sickle-cell anaemia, mental disorders[11], and microbial infections[3,12,13].

Spondias mombin Linn (*S. mombin*) is a fructiferous tree that thrives in the rainforest and coastal areas of Africa. It belongs to the family Anacardiaceae. The plant is readily used in folk medicine and it is known by various names. The fruit is called iyeye or yeye in the Yoruba language, ngulungu in Igbo and isadaa in Hausa[14,15]. Other names may include hog plum, true yellow mombin, golden apple and java plum.

All the parts of the tree are important in traditional medicine. The fruits are popular for eating and the extracted juice is used to prepare ice cream, cool beverages, jam and wine like Viro de Taperiba[16]. The fruit juice is drunk as a diuretic and febrifuge. The decoction of the astringent bark serves as an emetic, a remedy for diarrhoea, dysentery, haemorrhoids and a treatment for gonorrhoea and leucorrhoea[17]. All the parts of the plant are reported to be medicinally useful and their traditional uses in reproduction have been reported. It has also been reported that the leaves serves as an antidiarrheal, antimicrobial, anti-viral and also contains vitamin C[17,18]. Infusion of its leaves has been used for a long time without any report of collateral effects due to its activity. Its uses show that it is a medicinal plant with a lot of potential, valuable, untapped resource of active drugs for treating diseases. This present study aimed to investigate the phytochemical properties and antimicrobial potential of fresh leaf extracts of *S. mombin* against some clinical bacteria isolates from typhoid patients.

2. Materials and methods

2.1. Plant material collection and identification

Fresh and healthy leaves of *S. mombin* were collected around the vicinity of Wesley University of Science and Technology, Ondo. The identification and authentication was carried out at the Department of Botany, Obafemi Awolowo University, Ile-Ife with herbarium number 16856.

2.2. Plants extract preparation

The fresh leaves of *S. mombin* were extracted with four solvents: ethanol, methanol, acetone and aqueous solvent using modified methods[19-21].

Healthy and fresh plant leaves of *S. mombin* collected were grounded into fine texture using a sterilized electric blender. The fresh water extract was prepared by suspending 200 g of the fine blended fresh leaves into 300 mL of distilled water. The ethanol extract was also prepared by suspending 200 g of the blended leaves into 200 mL of 98% ethanol. The methanol extract was prepared by suspending 300 g into 500 mL of 98% methanol and the acetone extract was prepared by suspending 80 g into 90 mL of 98% acetone for 24 h. Thereafter, the suspensions of the fresh leaves were filtered into sterile beakers separately using sterile muslin cloth.

The extracts were subsequently concentrated to dryness in vacuum at 40 °C for the organic solvents and 80 °C for the aqueous solvent using a rotary evaporator. They were then placed in a sterile container, labelled and stored in the desiccator for phytochemical screening and antimicrobial analysis.

2.3. Collection and identification of test organisms

The test organisms used for this study were bacterial isolates from typhoid patients at the Medical Microbiology Laboratory, Federal Medical Centre, Owo, Ondo State, Nigeria. They are four Gram-negative and one Gram-positive bacteria. The Gram-negative isolates are *Klebsiella pneumoniae* (*K. pneumoniae*), *Serratia marcescens* (*S. marcescens*), *Salmonella typhi* (*S. typhi*) and *Enterobacter aerogens* (*E. aerogens*). *Staphylococcus aureus* (*S. aureus*) served as the Gram-positive isolate.

2.4. Standardization of test organisms

Pure colonies of the identified isolates were inoculated into sterile nutrient agar slants, incubated for 24 h at 37 °C and kept at 4 °C till further use.

All inoculums were standardized using the Mcfarland nephelometer method. Test-tubes of varying concentrations of barium chloride and sulphuric acid were used. The standard that was used for this work corresponds to 15×10^{8}/ bacterial suspension per millimetre.

2.5. Antibacterial susceptibility screening

The agar well diffusion technique was used to determine the antibacterial activity of the plant extracts[21]. About 0.1 mL of the different standardized organisms were introduced separately and thoroughly mixed with 20 mm of Mueller Hinton agar each in a sterile Petri dish and allowed to set. A sterile 5 mm cork borer was then used to punch holes in the seeded agar. The wells were filled with each of the extracts and antibiotic (streptomycin) serves as the positive control which were labelled accordingly while two wells contained the extractant (*i.e.* the solvent used for the extraction) to serve as negative control. These were then left on the bench for 2 h for adequate diffusion of the extracts and incubated at 37 °C for 24 h. After incubation, the diameters of the zones

of inhibition around each well were measured to the nearest mm and the mean of the reading were calculated. All experiments were done in triplicates.

2.6. Antibiotic sensitive test

Antibiotic susceptibility test was carried out on the test bacteria using multi-disc diffusion method as described[22]. The test organism was introduced on the surface sterile nutrient agar in Petri dish. The multi-disc antibiotic was then placed on the culture plates aseptically, incubated at 37 °C for 24 h. After incubation, the diameter of the zone of inhibition around each well was measured.

2.7. Determination of minimum inhibitory concentration (MIC)

The minimum bacterial growth inhibition of the various extracts was determined using the broth dilution technique. Different increasing concentration of the extracts was used. About 2 mL of Mueller Hinton broth was prepared into test tubes for each extract, and 0.5 mL of 10-90 mg/mL of each extract was added to the different test tubes containing the Mueller Hinton broth. This was prepared for each organism. A colony of 24 h cultured-organism was inoculated into test tubes containing 1 mL of sterile distilled water to form turbidity of 0.5 McFarland standards and was thereafter dispensed into the test tubes containing the suspension of the Mueller Hinton broth and the different extracts. This was done for all the organisms at the tested concentrations. All test tubes were properly corked and incubation at 37 °C for 24 h, after which they were observed for the absence or present of visible growth. The lowest concentration without visible growth (turbidity) of organisms was regarded as the MIC.

2.8. Phytochemical screening

The extracts were analysed to test for the presence of the alkaloids, saponins, tannins, terpenoids, flavonoids, cardiac glycosides, volatile oils and reducing sugars[23].

2.9. Statistical analysis

The data obtained from the study were analysed by the use of Two-way analysis of variance with replicates (ANOVA) to determine the significance antimicrobial activity of *S. mombin* leaves. The values were expressed in mean±SEM.

3. Results

The phytochemical screening of the fresh leaves of *S. mombin* showed the presence of saponins, tannins, glycosides, alkaloids and flavonoids, while terpenoids, reducing sugars, volatile oils and cardiac glycosides were not detected (Table 1).

Table 1
Phytochemical screening of *S. mombin* leaves.

Phytochemicals	Ethanol extract	Methanol extract	Acetone extract	Aqueous extract
Saponins	+	+	+	+
Tannins	+	+	+	+
Cardiac glycosides	−	−	−	−
Terpenoids	−	−	−	−
Reducing sugars	−	−	−	−
Volatile oils	−	−	−	−
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Glycosides	+	+	+	+

+: present, −: absent.

All the examined extracts showed varying degrees of antibacterial activities against the pathogens. Table 2 shows the results of the antibacterial susceptibility test of the extracts against the test isolates. From the results, the methanol extract also showed the highest activity against *E. aerogens*, zone of diameter (15.00±1.89) mm, while the ethanol extract showed the highest activity against *S. aureus* with zone of diameter (12.50±1.50) mm. The acetone extract showed the highest activity against *S. typhi*, zone of diameter (17.50±0.29) mm followed by methanol extract showing zone of diameter (15.67±1.01) mm. The acetone extract showed the highest activity against *K. pneumonia* (15.17±0.67) mm, while the aqueous extract shows the highest activity against *S. marcescens* (14.67±2.68) mm.

Table 2
Antibacterial activity of extracts of *S. mombin* leaves against some clinical isolates.

Organisms	Zone of diameter (mm)				
	Aqueous extract	Ethanol extract	Acetone extract	Methanol extract	Streptomycin
K. pneumoniae	12.83±3.42	11.50±1.15	15.17±0.67	12.83±2.46	12.33±1.09
S. aureus	10.67±2.62	12.50±1.50	11.17±3.44	11.83±3.18	08.67±3.98
S. marcescens	14.67±2.68	11.50±3.21	11.33±2.89	10.17±0.88	11.00±4.31
S. typhi	12.17±0.33	12.00±1.00	17.50±0.29	15.67±1.01	12.67±1.42
E. aerogenes	13.17±1.17	13.50±0.87	13.33±0.88	15.00±1.89	12.33±3.22

Data are expressed as mean±SEM.

Table 3 shows the susceptibility of the test organisms to different antibiotics. All the test bacteria were inhibited by at least one antibiotic except for *S. marcescens* and *S. aureus* which were resistant to all the antibiotics. *S. typhi* and *E. aerogens* were susceptible to ofloxacin, ciprofloxacin and pefloxacin.

The methanol and ethanol extracts showed the lowest MIC of 10 mg/mL against all the tested isolates (Table 4). There was no inhibition at all the tested concentration for the aqueous extract except with MIC of 50 mg/mL on *K. pneumoniae*.

Table 3
Antibiotic susceptibility of the test organisms.

Organisms	Antibiotic susceptibility	Resistant
K. pneumoniae	NIT, GEN, COT,OFL, AMX, CPX, TET, PFX	CRO, AUG
S. aureus	No susceptibility	CRO, NIT, GEN, COT, OFL, AMX, CPX, TET, PFX, AUG
S. marcescens	No susceptibility	CRO, NIT, GEN, COT, OFL, AMX, CPX, TET, PFX, AUG
S. typhi	OFL, CPX, PFX	CRO, NIT, GEN, COT, AMX, TET, AUG
E. aerogens	OFL, CPX, PFX	CRO, NIT, GEN, COT, AMX, TET, AUG

CRO: Ceftriazone (30 μg), NIT: Nitrofurantoin (200 μg), GEN: Gentamicin (10 μg), COT: Co- trimoxazole (25 μg), OFL: Ofloxacin (5 μg), AUG: Augumentin (30 μg), AMX: Amoxicilin (25 μg), PFX: Pefloxacin (5 μg), TET: Tetracycline (30 μg), CPX: Ciprofloxacin (10 μg).

Table 4
MIC of various extracts of fresh leaves of *S. mombin* (mg/mL).

Organisms	Aqueous extract			Methanol extract			Acetone extract			Ethanol extract		
	10	50	90	10	50	90	10	50	90	10	50	90
K. pneumonia	+	−	−	−	−	−	+	−	−	−	−	−
S. aureus	+	+	+	−	−	−	−	−	−	−	−	−
S. marcescens	+	+	+	−	−	−	+	−	−	−	−	−
S. typhi	+	+	+	−	−	−	−	−	−	−	−	−
E. aerogens	+	+	+	−	−	−	−	−	−	−	−	−

+: growth, −: no growth.

4. Discussion

The therapeutic value of medicinal plants lies in the various chemical constituents it contains. The bioactivity of plant extracts is attributed to phytochemical constituents. Plants rich in tannins have antibacterial potential due to their character that allow them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane. In a similar study, it was reported that *S. mombin* contains tannins, saponins and anthraquinone glycosides that showed significant antimicrobial activity[24,25]. Most of the effects observed with extract of *S. mombin* may be attributed to the constituent compounds of phenols, tannins, anthraquinones and flavonoids presence in the plant.

There may be several factors that will predispose bacteria to antibacterial agents such as previous encounters with the agent or the nature of the medium used which may affect the ability of the agent to diffuse[22]. The fact that the extracts were active against both Gram-negative and Gram-positive bacteria indicated a broad spectrum of activity.

The presences of bioactive substance in plant extracts are responsible for the antibacterial activities and the difference in the efficacy of the organic extracts may have different polarities for different solvents[18,21,26].

This study demonstrated that folk medicine can be as effective as modern medicine to combat infectious diseases. The use of this plant in folk medicine suggests that they represent an economic alternative to treat diseases. The antimicrobial activity of the plants may be due to the presence of various active constituents in the leaves. Further studies are needed to isolate and characterize the properties to develop new antimicrobial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The study evaluated the phytochemical properties and antibacterial activities of methanol, acetone, ethanol and aqueous extracts of fresh leaves of *S. mombin* on some clinical bacterial isolates *viz. K. pneumonia, S. marcescens, S. typhi*, *E. aerogens*, and *S. aureus* by observing the zones of inhibition using agar well diffusion assay. This study showed that the aqueous and organic solvents extract of fresh leaves of *S. mombin* has anti-microbial activity against all the tested organisms.

Research frontiers

S. mombin contains a number of phytochemicals such as tannins, saponins and anthraquinone glycosides that showed significant antimicrobial activity. Studies are in the area of isolating and characterizing new antimicrobial agents from such plants in order to develop novel therapeutic agents.

Related reports

This study investigated the use of different organic solvents (aqueous, ethanol, methanol and acetone) of fresh leaf extracts of *S. mombin* against clinical isolates instead of only the ethanol and aqueous dry leaf extract in previous report of Ajao *et al.* (1985) against some bacterial isolates. The report of this study confirms the antimicrobial ability of the leaf.

Innovations & breakthroughs

Enteric disease is common in the developing countries. This study confirms the antibacterial potential of different organic extracts of the fresh leaf extract of *S. mombin* against clinical bacterial isolates.

Applications

This research will be useful to develop new antibacterial agents with potential for use as chemotherapeutic ability in the pharmaceutical industry especially against enteric diseases.

Peer review

The concept of the work is good, though similar studies have been carried out on the antimicrobial of the dry leaf extract against some bacteria, this work focused on the antibacterial efficacy of the fresh extract against bacteria of clinical origin.

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