



## Phytochemical Screening and Antibacterial Activity of *Spondias mombin* Leaves

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### Authors' contributions

This work was carried out in collaboration between all authors. Author AAA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AIO, CSE and POU managed the analyses of the study. Authors OJC and POU worked on the literature search and review. Author OJC reviewed the study and approved the final manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

Phytochemical investigation was carried out on the crude ethanol extracts of the leaves of *Spondias mombin*. The antibacterial activity of the ethanolic and the Chloroform leaf extract was tested against four bacteria; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* using disc diffusion method. The results indicated that both extracts showed inhibitory activity against clinical isolates of the gram negative bacteria *Staphylococcus aureus*, *P. aeruginosa* and *E. coli* as well as gram positive bacteria *S. aureus*. These also revealed that chloroform extract was more potent than the ethanol extract. Phytochemical constituents present in the extract were found to include Alkaloids (5.97±0.03%), Flavonoids (3.00±0.01%), Tanins (3.80±0.02%), Saponins (7.50±0.1%), and Phenols (0.9±0.04%).

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## 1. INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health. Especially, in recent years, plants with various biological properties such as antioxidants, antimicrobial, and anti-inflammatory have been introduced and investigated increasingly in pharmaceutical and food industries since some reports showed that medicine derived from plant sources is free from side effects on human health compared to synthetic substances (Nascimento et al. [1]).

In fact, around 80% of the population from developing countries still use medicinal plants for their primary health care (Arokiyaraj et al. [2]). Therefore, there is a requirement to explore properties, safety and efficiency of plants as an alternative to synthetic compounds.

The antimicrobial properties of compounds extracted from medicinal plants are of great significance for medical and food application. These compounds are synthesized in the secondary metabolism of the plant such as flavonoids, tannins and essential oils Nascimento et al. [1].

*Spondias mombin* linn, a medicinal important plant of *Anacardiaceae* family, is a small tree that grows up to 20 m (60 ft.) high and 1.5 m (5 ft) in girth. It flowers between January-May and fruits between July-September. The fruits have a sharp-acid taste, and are edible. 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 well-known febrifuge in Ivory Coast. The bark is used as a purgative, and in local application, for leprosy. The bark decoction is used for severe cough, causing relief through vomiting, serves as an emetic, a remedy for diarrhea, dysentery, haemorrhoids and a treatment for gonorrhoea and leucorrhoea. Ayoka et al. [3] in Mexico, the decoction of the astringent bark is believed to expel calcification from the bladder. The bark is reported to contain a certain amount of tannin, for this reason, the dry pulverized bark is used to treat wounds. In Belize, a decoction of the young leaves is a remedy for diarrhea and dysentery. The juice of crushed leaves and the powder of dried leaves

are also used for the treatment of wounds and inflammations. The gum is employed as an expectorant and to expel tapeworms. Rodrigues and Hesse, [4]; Rodrigne and Samuels, [5]. A decoction of the mashed leaves is used by the Igbos (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 the treatment of gonorrhoea. A decoction of pounded leaves of *S. mombin* is used as an eye lotion, and the juice pressed from young, warm leaves is given to children for stomach troubles. The extract has shown anti-inflammatory activity in Wistar rats Nworu et al. [6]. A tea made from the flowers and leaves is taken to relieve stomach ache, biliousness, urethritis, cystitis, eye and throat inflammations (Villegas et al. [7], Nworu et al. [8]). A decoction of the root is used as purgative.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Leaves of *Spondias mombin* were harvested from Ovom (1) in Obingwa Local Government Area of Abia State in Nigeria, on the 15<sup>th</sup> of October 2016. The plant was identified and authenticated by Mr. I. Ndukwe in plant taxonomy section, Forestry Department of Michael Okpara University of Agriculture Umudike, Nigeria.

### 2.2 Quantitative Phytochemical Screening

#### 2.2.1 Determination of alkaloid (Harbone 1980)

The 5 g of the sample was weighed and dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for 4 hours before filtering. The filtrate was evaporated to a quarter of the original volume. Concentrated ammonium hydroxide (conc. NH<sub>4</sub>OH) was added drop wise to precipitate the alkaloid. The precipitate was filtered with a weighed filter paper and washed with 1% NH<sub>4</sub>OH solution. The precipitate was then dried at 60°C for 30 minutes and reweighed.

$$\% \text{Alkaloid} = \frac{W_2 - W_1}{W} \times 100$$

Where

W = weight of sample  
 $W_1$  = weight of empty filter paper  
 $W_2$  = weight of the paper plus precipitate.

### **2.2.2 Determination of tannin Pearson, [9]**

The method described by Pearson [9] was adopted. A known weight (0.5 g) of the sample was extracted with distilled water. One ml of the aqueous extract was mixed with 35 ml of distilled water in the volumetric flask and agitated. This was left to stand for 30 minutes at room temperature, being shaken every 5 minutes. At the end of the 30 minutes, it was centrifuged and the extract gotten. 25 ml of the supernatant (extract) was put into a 50 ml volumetric flask. Similarly, 25 ml of standard tannin acid solution was put into a separate 50 ml flask. A 1.0 ml Folin-Denis reagent was measured into each flask followed by 2.5 ml of standard sodium trioxocarbonate (1V) solution ( $\text{Na}_2\text{CO}_3$ ). The mixture was diluted to the 50 ml mark and incubated at room temperature for 90 min. The absorbance was measured at 250 nm. Readings were taken with reagent blank at zero.

### **2.2.3 Phenol determination: Association of official analytical chemist AOAC, [10]**

A known weight 0.2 g of the sample was treated with methanol by soaking and filtering to extract phenol. 1 ml of solution was added. The intensity of the developed color was measured using spectrophotometer at 560 nm. The standard phenol was treated in the same way.

**Note:** It formed a white precipitate which was filtered before the spectrometer reading was taken.

$$\text{Phenol content} = \frac{\text{Au} - \text{Ab} \times c}{\text{As} - \text{Ab}}$$

Au = Absorbance of the test sample

Ab = Absorbance of blank

As = Absorbance of the standard phenol solution

C = Concentration of the standard phenol solution

### **2.2.4 Determination of saponin Obadoni and Ochuko, [11]**

The ground sample (10 g) was dispersed in 100 ml of 20% ethanol. The suspension was

heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 100 ml of 20% ethanol. The combined extract was reduced to 20 ml by heating over water bath at about 90°C the concentrate was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded 30 ml of n-butanol was added and was washed twice with 5 ml of 5 ml of 5% aqueous NaCl (sodium chloride). The remaining solution was heated on a water bath to evaporate it. After evaporation, the sample was dried in the oven to a constant weight.

$$\% \text{ saponin} = \frac{\text{weight of the saponin}}{\text{Weight of the dried sample}} \times 100$$

### **2.2.5 Determination of flavonoid Boham and Kocipal, [12]**

The sample (100 g) was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm).

The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed.

$$\% \text{ flavonoid} = \frac{\text{Weight of the dried extract}}{\text{Weight of the sample}}$$

## **2.3 Anti-bacterial Evaluation of *Spondias mombin* Leaves Extract**

### **2.3.1 Preparation of extracts**

The test solution of each extract was prepared by dissolving 0.1 g of the plant extracts in 1.0 ml of dimethylsulphoxide (DMSO) to get a concentration of 100 mg/ml.

### **2.3.2 Micro-organisms**

The bacteria organisms used were *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. All the organisms were obtained from the stock culture of the Federal Medical Center, Umuahia. Cultures were brought to laboratory conditions by resuscitating the organisms in peptone water and thereafter subcultured into nutrient agar medium and incubated at 37°C for 24 hours (Okigbo and Nmeke EC, [13]).

### 2.3.3 Antibacterial assay

The antibacterial activity was performed by filter paper disc diffusion technique. Filter paper disc (whatman No.1, 6 mm diameter) were placed in glass Petri dishes and sterilized in hot air oven (Ekundayo and Ezeogu [14]). The media (10 g nutrient agar in 200 ml distilled water, autoclaved at 115°C for 30 minutes) was cooled to 50°C. The sterile nutrient agar media were poured into the sterile Petri dishes and allowed to solidify. The bacteria were swabbed with a sterile wire loop. Each disc was impregnated with 0.2ml of plant extracts and standard-Ciprofloxacin. Discs with DMSO (100 mg/ml) served as a control.

The discs were used after drying them in an incubator at 40°C to remove any trace of solvent (Anishmon and Toji, [15]). Discs were introduced onto the surface of the medium. The plates were incubated at 37°C for 24 hours to obtain zones of inhibition. The experiments were repeated three times for each extract and twice for reference antibiotic to minimize error and the average of these values were tabulated.

### 2.3.4 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the extracts was determined by incorporating constant volumes (0.2 ml) of each dilution of the extract into perforated discs on a seeded nutrient agar plate as described in the antimicrobial susceptibility testing section (Okigbo and Nmeko EC, [16]).

0.1 g of each extract was dissolved in 1 ml of DMSO to obtain 100 mg/ml. This 100 mg/ml concentration was then doubly diluted in DMSO to obtain concentration of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml.

## 3. RESULTS AND DISCUSSION

The results of the experiments are stated and discussed as follows;

### 3.1 Photochemical Composition of *Spondias mombin*

Table 1 shows the result of the photochemical contents of the leaf of *Spondias mombin*.

*Spondias mombin* leaves are rich in phytochemicals such as tannins, alkaloids, flavonoids saponins and phenols.

**Table 1. Result of the photochemical contents of the leaf of *Spondias mombin***

Constituent	Quantity
Tannins	3.80 ± 0.02
Alkaloids	5.97 ± 0.03
Flavonoid	3.00 ± 0.01
Saponins	7.50 ± 0.1
Phenols	0.96 ± 0.04

*Results based on mean ± standard deviation of triplicate sampling measurements*

The high tannin content 3.80% can be attributed to the bitter taste of the leaves. Tannins has astringent properties, quickens the healing of wounds. The presence of tannins in the leaves of *Spondias mombin* can attest to its use for healing of wounds, hemorrhoids and burn in herbal medicine (Deferraya, [17]).

#### 3.1.1 Alkaloids

Alkaloids are the most efficient phytochemical plant chemical compound. True isolated alkaloids and the synthetic derivatives are used as the basic medicinal compounds because of their analgesic, anti-spasmodic and bacterial properties (Corthout et al. [18]).

#### 3.1.2 Flavonoids

The leaves of *Spondias mombin* contain an appreciable amount of flavonoids up to 3.0%. The biological functions of flavonoids include protection against allergies inflammation, platelets aggregation, and microbes. Flavonoids through their free radical and scavenging property tend to lower cholesterol level and reduce the risk of heart attack Salah et al. [19]. Flavonoids have multiple biological activities that include estrogenic effect as well as inhibiting the action of some enzymes (Middleton and Kandaswani, [19], Waladkhani and Clemen, [20]). The relatively high percentage of saponin explains the foaming nature of the organic extract during rotary evaporator concentration, causing bumping. The high saponin content (7.50%) shows that *Spondias mombin* has cytotoxic effects such as permeabilization of the intestine.

Antibacterial Activity of Extracts of *Spondias mombin* Leaves against some bacterial Isolates.

The results of the antibacterial activity of the ethanolic and chloroform extracts of *Spondias mombin* leaves against some bacteria isolates *S. aureus*, *E. coli* and *Salmonella typhi* are presented in Table 2.

**Table 2. Zone of inhibition**

Organisms	Ethanol extract	Chloroform extract	Ciprofloxacin
<i>S. aureus</i>	10.7±1.5	11.6±1.7	10.2±0.4
<i>S. typhi</i>	11.6±1.2	12.7±1.0	9.9±0.1
<i>E. coli</i>	10.0±1.2	11.0±1.0	9.7±0.2
<i>P. aeruginosa</i>	9.7±1.3	13.2±1.2	10.0±0.1

Values are mean zones of inhibition (mm) ± standard deviation of three replicates

**Table 3. MIC of ethanol extract**

Organism	Concentration of extract			
	6.25 ml	12.5 ml	25.0 ml	50.0 ml
<i>E. coli</i>	0.00±0.00	0.00±0.00	3.2±0.3	5.6±0.5
<i>Staph. aureus</i>	0.00±0.00	0.00±0.00	4.8±0.4	5.2±0.3
<i>S. typhi</i>	0.00±0.00	1.5±0.2	5.3±0.4	6.0±0.4
<i>P. aeruginosa</i>	0.00±0.00	1.3±0.1	4.7±0.2	5.4±0.5

**Table 4. MIC of chloroform extract**

Organism	Concentration of extract			
	6.25 ml	12.5 ml	25.0 ml	50.0 ml
<i>E. coli</i>	0.00±0.00	0.00±0.00	4.0±0.2	7.2±0.3
<i>Staph. Aureus</i>	0.00±0.00	0.00±0.00	4.9±0.4	8.5±0.2
<i>S. typhi</i>	0.00±0.00	2.2±0.3	5.5±0.4	7.4±0.2
<i>P. aeruginosa</i>	0.00±0.00	2.8±0.2	5.2±0.3	6.0±0.1

The results showed that extract possesses antibacterial activity against the pathogens. The chloroform extracts showed more antibacterial efficacy against the tested organisms. The minimum inhibitory concentration of the leaf extracts of the plant against the tested organisms at different concentrations, 6.25 ml, 12.5 ml, 25.0 ml, 50.0 ml are presented in Tables 3 and 4. At 6.25-12.5 ml the extracts recorded 0.00 mm inhibition zone for the bacteria *E. coli* and *Staph. aureus*. The extracts gave an inhibition for *salmonella* at 12.5 ml with chloroform 2.2 mm at 12.5 ml. The inhibitory effects of the extracts on the tested organisms increase with increase in the concentrations. The non-inhibitory effect of the extracts at 6.25 ml on the pathogens implies that they cannot serve as good antibacterial at that concentration in the treatment of diseases associated with the tested organisms.

From the results, the observed antibacterial properties could be attributed to the presence of bioactive compounds present in them. These include tanins, flavonoids, saponins, alkaloids and phenolic compounds.

#### 4. CONCLUSION

The inhibitory effect of the plant extracts at various concentration against the tested gram positive bacteria *S. aureus* and gram negative

*E. coli* has established antibacterial potential of the leaf. Findings based on this study with respect to the phytochemical constituent of the leaves of *Spondias mombins* support its uses as herbal remedies. The use of *Spondias mombin* to treat inflammation, diarrhea, haemorrhoids, and dysentery, suggest that further work should be carried out on the plant.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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