

## Phytochemical, FT-IR and Antimicrobial Screening of Stem-Bark Extract of *Spondias Mombin*

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### ABSTRACT

In the research work, phytochemical screening on the stem-bark extract of *spondias mombin* showed that the stem-bark extract contains flavonoids, tannins and saponins. Antimicrobial screening showed that the stem-bark extract is biologically act on against the tested microorganisms. The highest zone of the inhibition was observed against *Staphylococcus aureus* (20.20 ±0.08mm), followed by *Escherichia coli*(17.10 ±0.05mm), while the least activity was recorded against *Candida albicans* (13.08 ±0.04mm). This pattern suggest a broader antibacterial than antifungal potential of the extract. The FT-IR analysis was employed to identify the functional group in the stem-bark extract. The results revealed that stem-bark extract contains hydroxyl, alkyl, ester and carbonyl group, which confirmed the results of the phytochemical analysis.

**Keywords:** *Spondias mombin*, FT-IR, phytochemical, antimicrobial and Extraction.

### I. INTRODUCTION

*Spondias mombin*, commonly known as yellow mombin or hog plum, is a tropical tree species belongs to the mahogany family Anacardiaceae. The genus *spondias* is probably the most distinct of the family. Some species of *spondias* have been described such as *spondias mombin*, *spondias lutea* (which has similar fruits, most of the species of the small genus are cultivated for their edible plum likes fruits), *spondias mangifera*, which extend from west to East Africa [1]. The *ozorua*, *Anacardium* and *pseudospondias* belong to the same family with *spondias* which are the original source of whitish Grey Timber. It is native to various regions of Africa, Asia, and the Americas. The tree bears fruit, which is consumed both raw and processed for its culinary and medicinal uses. *Spondias mombin* has a long history of traditional use in folk medicine across its native range due to its various pharmacological properties (Okwu, [2]. The use of *Spondias mombin* as a medicinal plant has been documented for centuries among indigenous

populations in the regions where the plant is found. In traditional medicine, the bark, roots, fruits, and leaves of the plant are used for the treatment of various ailments such as diarrhea, stomachaches, inflammation, sore throat, fevers, and wounds. *Spondias mombin* is a medium-sized deciduous tree that can reach a height of up to 20-30 meters. It has a wide-spreading crown and a slender trunk covered in a grayish-brown bark. The leaves are pinnately compound, alternate, and cluster at the ends of branches. Each leaf is composed of 9-21 leaflets, which are oblong or lanceolate in shape and have a glossy green color. The tree produces small yellow or orange fruits, which are oval-shaped and have a tart or sweet taste depending on their ripeness. The various parts of *Spondias mombin*, including the leaves, fruits, and bark, have been used in traditional medicine for their medicinal properties. In indigenous communities, the tree is valued for its ability to treat a range of ailments [3]. There are several studies that have described a multitude of chemical compounds found within the *Spondias mombin* plant. [4], conducted a research study on the phenolic compounds present in *Spondias* species and discovered that *Spondias mombin* contained a variety of flavonoids, including quercetin, kaempferol, and myricetin. These compounds have been associated with anti-inflammatory, anti-oxidative, and anti-carcinogenic properties. In addition to flavonoids, various studies have reported the presence of saponins in *Spondias mombin*. They established a simple and reliable method to determine the quantity of flavonoids and saponins in plants [5]. The researchers determined that *S. mombin* exhibits both these compounds, and the same are thought to provide anti-microbial, antioxidant, and anti-inflammatory benefits. Also add light to the presence of tannins within the *Spondias mombin* plant [6] Tannins are known to have astringent properties and to contribute to wound healing, inhibition of inflammation, and reduction in blood pressure. These findings corroborate earlier studies that have revealed the role of *Spondias mombin* in traditional medicines

to treat wounds and inflammation. Moreover, the chemical analysis of *Spondias mombin* [7], provides a spectrum of constituents including alkaloids. The plant contains isoquinoline alkaloids, mombins A-D, which contribute to the plant's reported anti-plasmodial properties. Alkaloids are known for a wide range of pharmacological activities including anti-microbial, analgesic, anti-spasmodic, and anti-malarial actions. Other essential components of *Spondias mombin* include proteins, carbohydrates, fiber, and minerals. A study by confirms that the plant leaves are rich in protein and fiber, while the seeds contain significant amounts of carbohydrates. The fruit is also known as a rich source of vitamin C, making it an excellent nutritional supplement. A significant aspect of *Spondias mombin*, predominantly its bark and leaves, is its potent antimicrobial properties [8]. The plant extracts have been found effective against several microbial strains, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* [9]. This antimicrobial property perhaps justifies the plant's traditional use as a treatment for dysentery and diarrhoea in parts of West Africa. Furthermore, *Spondias mombin* has demonstrated remarkable anti-inflammatory properties. The leaf extract of *Spondias mombin* has been shown to reduce inflammation in tested animal models [10]. It thus suggests potential use in the treatment or management of inflammation-induced conditions such as arthritis. Another compelling feature of *Spondias mombin* is its antioxidative property. The plant's fruit is rich in antioxidants, particularly phenolic compounds, as confirmed by several studies [11]. Antioxidants play a crucial role in neutralizing destructive free radicals in the body, thus potentially reducing the risk of developing chronic diseases. Moreover, the plant also exhibits significant anticancer characteristics. An investigation revealed that its leaf extract could inhibit the growth of breast cancer cells [12]. While research in this area is limited, the findings suggest the possibility of developing the plant into an alternative cancer treatment in the future. Investigations on the therapeutic potential of *Spondias mombin* have also revealed excited anti-nociceptive attributes. The plant portrays considerable analgesic properties and can be leveraged as a natural pain reliever. This capability has been substantially reported in tested animal models, where the plant extract managed to exhibit a substantial reduction in pain [13]. The aim of the research is to investigate the phytochemical

screening, FT-IR and antimicrobial analysis of the stem bark extract of *Spondias mombin*.

## II. MATERIAL AND METHOD

### 2.1 Material

The chemical reagents used are n-Hexane, Methanol, chloroform (AR, Kermel, > 99%), Glacial acetic acid (36%, Loba Chemie), Hydrogen peroxide (AR, JHD, 99%), other's chemicals used were analytical grade, and distilled water.

#### 2.1.1 COLLECTION OF PLANT MATERIAL

*Spondia mombin* stem bark were collected from Federal Polytechnic, Ado-Ekiti (FPA) and identified at department of Science laboratory Technology (Microbiology unit), Federal Polytechnic, Ado-Ekiti, Ekiti state, Nigeria.

### 2.2 METHODS

#### 2.2.1 MATERIAL PREPARATION

After the stem-barks were collected in bulk, the stem barks are laid out on laboratory bench for several days, using air dry method dried to keep the colour.

#### 2.2.2 PULVERISATION OF THE PLANT MATERIAL

The dried stem-bark of *spondias mombin* was grinded into a fine power.

#### 2.2.3 EXTRACTION PROCEDURE

50g of the powdered sample was soxhlet extracted using three different solvents one after the other. The solvents used are Hexane, Chloroform and Methanol in that order. The extraction with a particular solvent is stopped when the extractant becomes colourless in the stem of the soxhlet extractor.

The extract obtained were concentrated by distillation. The extract percentage yield was calculated using the expression below:

$$\text{Yield} = \frac{\text{Extracted weight}}{\text{spondias mombin powder weight}} \times 100\%$$

The extract of the particular solvents was thereafter stored in an airtight container (glass) under cool condition before use.

### 2.3. DETERMINATION OF PHYTOCHEMICAL PROPERTIES OF STEM-BARK EXTRACT OF SPONDIAS MOMBIN

#### 2.3.1 Procedure

The standard procedures to determine Phytochemical properties of *Spondias mombin* were described and strictly followed according to

Association of official Analytical chemists [14], 15, 16]. The following tests were carried out on the extracts of the plant.

#### 2.3.2 Test for Alkaloids

0.5g of the extract was stirred with 5ml of dilute hydrochloric acid on a steam bath and filter. The filtrate was then treated with Mayer's, Wagner's and Dragendorff's reagents. The test solutions were observed for turbidity or precipitate.

#### 2.3.3 Test for Flavonoids

- 2ml of sodium hydroxide was added to 2ml of each stem bark extracts. The mixture was observed for any colour change.
- 3ml of concentrated tetraoxosulphate (VI) acid was added to 2ml of each plant stem bark extracts. The mixture was observed for any colour change.
- 2ml of aqueous ammonia was added to 2ml of each stem bark extracts and the mixture was observed for any colour change.

#### 2.3.4 Test for Tannins

• Three matches' sticks were introduced into each portion of the stem bark extracts and few drop of hydrochloric acid was added. The mixture was observed for any colour change.

#### 2.3.5 Test for Saponins and Terpenes

- 2ml of each stem bark extract was shaken with 3ml of water in a test tube for about 2 minutes and the mixture was observed for any floating.
- 2ml of chloroform and 2ml of glacial acetic acid with few drops of concentrated H<sub>2</sub>SO<sub>4</sub> was added to each portion of the stem bark extract and the mixture was observed for any colour change.

#### 2.3.6 Test for Glycosides

- 2ml of methanol extract was mixed with 2.5ml of concentrated sulphuric acid and then boiled in a water bath for about 15 mins, cooled and neutralized with 20% potassium hydroxide and 5ml of the mixture of Fehling solution A and B was added and the mixture boiled again. The mixture was observed for change of colour.
- 5ml of 10% Ammonia solution was added to chloroform extract and the mixture was observed for colour change.

#### 2.3.7 Test for Cardiac Glycosides (Keller-Killian test)

- Chloroform extract was stirred with 1ml acetic acid containing trace of ferric chloride carefully

poured this solution into the surface of about 1ml of concentrated sulphuric acid in a test-tube to form two layers. The mixture was observed for any colour change in each layer.

#### 2.4 Antimicrobial screening of the stem bark extract.

##### 2.4.1 Test Microorganisms

The antimicrobial activity was evaluated against selected clinical isolates: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Candida albicans*. All test organisms were obtained from the Microbiology Laboratory, Federal Polytechnic Ado EKITI, and were maintained on nutrient agar and Sabouraud dextrose agar at 4 °C until use.

##### 2.4.2 Antimicrobial Susceptibility Test

The agar well diffusion method described by CLSI (2020) and Irobi et al. (1994) was employed. Briefly, 0.1 mL of standardized microbial suspension (0.5 McFarland standard ~10<sup>6</sup> CFU/mL) was evenly spread on the surface of Mueller-Hinton agar plates (for bacteria) and Sabouraud dextrose agar (for fungi). Wells of 6 mm diameter were punched and filled with 100 µL of extract (100 mg/mL). Plates were incubated at 37 °C for 24 hours (bacteria) and at 28 °C for 48 hours (fungi). Zones of inhibition were measured in millimeters.

##### 2.4.3 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of each extract was determined using the broth microdilution method in 96-well plates (Eloff, 1998). Two-fold serial dilutions ranging from 0.195 to 100 mg/mL were prepared. Each well received 100 µL of microorganism and was incubated at 37 °C for 24 hours. Resazurin (10 µL, 0.2 mg/mL) was added. A blue-to-pink color change indicated growth. MIC was the lowest concentration without a color change.

#### 2.5 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY Analysis

Fourier Transform Infrared Spectroscopy Spectrum of *Spondias mombin* stem-bark extract was obtained using FTIR Spectrophotometer (Agilent Technologies). FTIR used for chemical identification as each molecule and chemical structure creates a unique spectra. The IR spectra were accounted in % transmittance. The wave number region for analysis was 4000-650 Cm<sup>-1</sup> (mid infrared range) with resolution of 0.15cm<sup>-1</sup>.

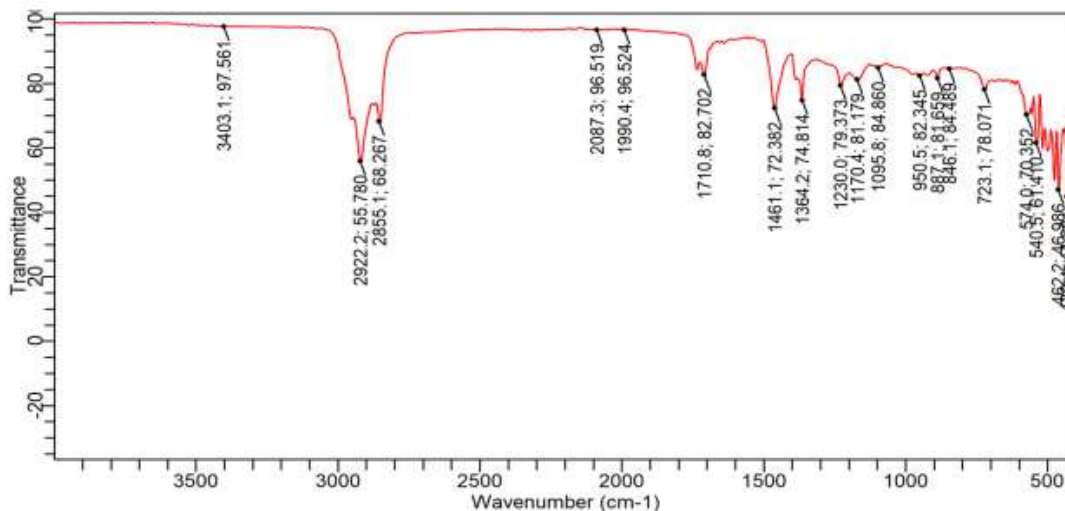


Figure 1: FT-IR spectra for stem-bark of spondias mombin

**Table 1: Test for flavonoids**

SN	Test	Observation	Inference
Hexane Extract			
1.	Sample + aq NaOH	yellow colouration was observed	Suggest the presence of flavonoids. Leucanthocyanins, Isoflavones, and Isoflavonones
2.	Sample + Conc. H <sub>2</sub> SO <sub>4</sub>	crimson colouration was observed	Suggest the presence of flavones, and Leucanthocyanins
3.	sample + NH <sub>3</sub>	Yellow colouration observed	Suggest the Presence of flavones, flavonols, xanthenes, and flavonones.
Chlorofoam Extract			
1	Sample + aq NaOH	A dirty brown colouration was observed	Suggest the presence of flavonoids, Isoflavones, and Isoflavonones.
2	Sample + Conc. H <sub>2</sub> SO <sub>4</sub>	crimson colouration was observed	Suggest the presence of flavones, and Leucanthocyanins
3	Sample + Bromine water	No precipitate was formed, a dirty yellow colouration was observed	Suggest the presence of Leucanthocyanins
4	sample + NH <sub>3</sub> solution	Yellow colouration observed	Suggest the Presence of flavones, flavonols, and flavonones.
Methanol Extract			
1	Sample + aq NaOH	A yellow colouration was not observed, but brown colouration	Suggest the presence of flavones, flavonol, and Leucanthocyanins
2	Sample + Conc. H <sub>2</sub> SO <sub>4</sub>	crimson colouration was observed	Suggest the presence of flavones, and Leucanthocyanins
3	Sample + Bromine water	A brown colouration was observed	Suggest the presence of flavones, Leucanthocyanins
4	sample + NH <sub>3</sub> solution	A dark brown colouration observed	Suggest the Presence of flavones, flavonols, and flavonones.

**Table 2:** Test for Tannins

SN	Test	Observation	Inference
Hexane Extract			
1	Sample + Bromine water	Red colouration was not observed	Suggest the absence of either pseudo or hydrolysable tannins
2	Sample + 4ml water + few drops of FeCl <sub>3</sub> solution	A yellowish green colouration was observed	Suggest the absence of tannins
3	Sample + matches stick + HCl drops	No change in colour	Suggest the absence of tannins
Chlorofoam Extract			
1	Sample + Bromine water	A Precipitate was formed with yellow green cour	Suggest the presence of tannins
2	Sample + 4ml water + few drops of FeCl <sub>3</sub> solution	A dark brown precipitate was formed	Suggest the absence of tannins
3	Sample + matches stick + HCl drops	No change in colour	Suggest the absence of tannins

**Table 3:** Test for Saponins and Terpenes.

SN	Test	Observation	Inference
Hexane Extract			
1.	Sample + 4ml of chlorofoam + 1ml of glacial acetic acid + few drops of conc H <sub>2</sub> SO <sub>4</sub>	Reddish colouration was not observed. A brown colouration was observed	Suggest the absence of Saponin and terpenes
2.	2ml of extract was shaken in test tube for about 2mins.	No frothing was observed	Suggest the absence of saponins
3	2.5ml of sample + 2.5ml of a mixture of fehling solution A and B then boiled	Brick red colouration was not observed, a brown colouration was observed.	Suggest the absence of saponins
Methanol Extract			
1	Sample + 4ml of chlorofoam + 1ml of glacial acetic acid + few drops of conc H <sub>2</sub> SO <sub>4</sub>	Reddish colouration was observed.	Suggest the absence of Saponins.
2	2ml of extract was shaken in test tube for about 2mins.	frothing was observed	Suggest the presence of saponins
3	2.5ml of sample + 2.5ml of a mixture of fehling solution A and B then boiled	A dirty brown colouration was observed.	Suggest the absence of saponins glycosides.

**Table 4:** Test for Alkaloids

SN	Test	Observation	Inference
Hexane Extract			
1.	Sample + 5ml of 1% HCl + water's reagent	No ppt was formed	Suggest the absence of alkaloids
2.	Sample + mayer's reagent	Cream colouration ppt was not observed	Suggest the absence of alkaloids
3.	Sample + wagner's reagent	No ppt was observed	Suggest the absence of alkaloids
4.	Sample + dragnedroff's reagent	Red ppt was not observed	Suggest the absence of alkanloids
Methanol Extract			
1	Sample + 5ml of 1% HCl + water's reagent	No ppt was formed	Suggest the absence of alkaloids

2	Sample + mayer's reagent	Cream colouration ppt was not observed	Suggest the absence of alkaloids
3	Sample + wagner's reagent	No ppt was observed	Suggest the absence of alkaloids
4	Sample + dragnedroff's reagent	Red ppt was not observed	Suggest the absence of alkanloids

**Table 5: Test for Glycosides**

SN	Test	Observation	Inference
Hexane Extract			
1.	2ml of sample + 2.5ml of H <sub>2</sub> SO <sub>4</sub> then boiled in water bath for about 15mins cooled and neutralized with 20% KOH + 5ml of a mixture of fehling solution A and B and boiled again.	A dirty brown colouration was observed with no precipitate formed.	Suggest the absence of glycosides

**Table 6: summary table for phytochemical analysis of stem-bark extract of spondias mombin results**

	Alkaloids	Flavonoids	tannins	saponins	Glycosides
<b>Hexane Extract</b>	-	++	-	-	-
<b>Chlorofoam Extract</b>	-	++	++	-	-
<b>Methanol Extract</b>	-	++	-	++	-

## 2.2 Antimicrobial analysis of stem bark extract of Spondias mombin

**Table 7: Antimicrobial Activities of Spondias mombin Extract Against Selected Microorganisms**

Microorganism	Zone of Inhibition (mm) ± SD	MIC (mg/mL) ± SD
Escherichia coli	17.1 ± 0.05	6.25 ± 0.2
Staphylococcus aureus	20.2 ± 0.08	3.12 ± 0.1
Pseudomonas aeruginosa	13.10 ± 0.03	12.5 ± 0.3
Candida albicans	13.08 ± 0.04	25.0 ± 0.5
Bacillus subtilis	18.3 ± 0.09	6.25 ± 0.2
Positive Control (Ciprofloxacin)	24.7 ± 0.5	0.25 ± 0.0
Negative Control (DMSO)	0.0 ± 0.0	ND

ND: Not Detected; SD: Standard Deviation; MIC: Minimum Inhibitory Concentration

## III. RESULTS AND DISCUSSION

### 3.1 Phytochemical analysis of stem-bark extract of Spondias mombin

The phytochemical screening for stem-bark extract of spondias mombin were summary in table 6. It was observed that the stem-bark of spondias mombin showed that it contains flavonoids which are present in Hexane, chlorofoam, and methanol Extract of the plant. Saponins was present in methanol extract of the

plant. The chlorofoam extract of the plant showed the present of Tannins.

### 3.2 Antimicrobial screening of stem-bark extract of Spondias mombin

The aqueous extract of Spondias mombin demonstrated varying degrees of antimicrobial activity against the tested microorganisms. The highest zone of inhibition was observed against Staphylococcus aureus (14.8 ± 0.4 mm), followed by Escherichia coli (13.6 ± 0.5 mm), while the least

activity was recorded against *Candida albicans* ( $9.4 \pm 0.5$  mm). This pattern suggests a broader antibacterial than antifungal potential of the extract. The minimum inhibitory concentration (MIC) values further supported the observed activity. For instance, *Staphylococcus aureus* had the lowest MIC (12.5 mg/mL), implying a higher sensitivity compared to *Candida albicans* which had an MIC of 100.0 mg/mL. These findings are consistent with previous reports by [18, 20], who noted the potential of *Spondias mombin* extracts in managing bacterial infections. Comparatively, the aqueous extract showed lower activity than the standard antibiotics (ciprofloxacin and fluconazole), as expected. This aligns with studies by [19, 21], which demonstrated that plant extracts, although effective, often require higher concentrations to match the efficacy of synthetic drugs.

The observed antimicrobial activity supports the ethnomedicinal use of *Spondias mombin* in treating infections, particularly those caused by Gram-positive bacteria.

### 3.3 FT-IR RESULT

FT-IR analysis was employed to identify surface functional groups on stem-bark extract of *spondias mombin*. Results are shown in figure 2. An absorption band between  $3403.1\text{Cm}^{-1}$  show the present of O-H stretching vibration, an hydroxyl group which indicate Phenolic compound in the plant. While  $2922$  and  $2855.1\text{cm}^{-1}$  are C-H stretching of methylene group which indicate alkyl chain in the plant extract. The band at  $1710.8$  due to C=O stretching vibration, this indicate the presence of carboxylic acids or esters from the plant extract of *spondias mombin*.

## IV. CONCLUSION

In the present study, we investigated the phytochemical and antimicrobial screening of stem-bark of *spondias mombin* and TTIR was used to identify the functional group. The phytochemical test carried out on the stem-bark extracts showed that it contains flavonoids, tannins and saponins.

The antimicrobial studies showed that the stem-bark extract are active against tested microorganisms. The zone of the inhibition was observed against *Staphylococcus aureus* with the highest, followed by *Escherichia coli*, and *Candida albicans* with the least. This pattern suggest a broader antibacterial than antifungal potential of the extract.

FTIR spectra indicates the presence of hydroxyl, alkyl, ester and carboxyl group, which

confirmed the functional group in the results of the phytochemical properties tested of the stem-bark extract of *spondias mombin*.

These results has confirmed the use of this plant for the treatment of disease caused by *Staphylococcus aureus* and *Escherichia coli* such fever, food poisoning, Urinary tracts infections, infant meningitis, wound infection, septicemia, baby infections, Osteomyelitis wound infection, and pneumonic abscesses.

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