

Original Research Article

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

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 ($5.97 \pm 0.03\%$), Flavonoids ($3.00 \pm 0.01\%$), Tanins ($3.80 \pm 0.02\%$), Saponins ($7.50 \pm 0.1\%$), and Phenols ($0.9 \pm 0.04\%$).

Keywords: Antimicrobial activity, Zone of inhibition, *Spondias mombin*, Secondary metabolites, Extracts.

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

27 (Nascimento *et al*, 2000). In fact, around 80% of the population from developing
28 countries still use medicinal plants for their primary health care (Arokiyaraj *et al*,
29 2012). Therefore, there is a requirement to explore properties, safety and
30 efficiency of plants as an alternative to synthetic compounds.

31 The antimicrobial property of compounds extracted from medicinal plants is of
32 great significance for medical and food application. These compounds are
33 synthesized in the secondary metabolism of the plant such as flavonoids, tannin
34 and essential oils (Nascimento *et al*, 2000).

35  **Spondias mombin** linn, a medicinally important plant of Anacardiaceae family, is a
36 small tree that grows up to 20 m (60 ft.) high and 1.5 m (5 ft) in girth. It flowers
37 between January-May and fruits between July-September. The fruits have a
38 sharp, acid taste and are edible. The fruit pulp is either eaten fresh, or made into
39 juice, concentrate, jellies, and sherbets. The fruit juice is used as a febrifuge and
40 diuretic. The roots are also well-known febrifuge in Ivory Coast. The bark is used
41 as a purgative and in local application for leprosy. The bark decoction is used for
42 severe cough, causing relief through vomiting, serves as an emetic, a remedy for
43 diarrhea, dysentery, haemorrhoids and a treatment for gonorrhoea and
44 leucorrhea (Ayoka *et al*, 2008). In Mexico, the decoction of the astringent bark is
45 believed to expel calcification from the bladder. The bark is reported to contain a

certain amount of tannin and this reason the dry pulverized bark is used to treat wound. 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 (Rodrignes and Hesse, 2000; Rodrigne and Samuels, 1999). 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 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*, 2011). A tea made from the flowers and leaves is taken to relieve stomach ache, biliousness, urethritis, cystitis and eye and throat inflammations (Villegas *et al*, 1997; Nworu *et al*, 2011). A decoction of the root is used as purgative.

Materials and Methods

QUANTITATIVE PHYTOCHEMICAL SCREENING

Determination of Alkaloid (Harbone 1980).

The sample 5g of the sample was weighed and dispersed into 50ml of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stay for 4

65 hours before filtering. The filtrate was evaporated to a quarter of the original
 66 volume. Concentrated ammonium – hydroxide (conc. NH_4OH) was added drop
 67 wise to precipitate the alkaloid. The precipitate was filtered with a weighed filter
 68 paper and washed with 1% NH_4OH solution .The precipitate was then dried in at
 69 60°C for 30 minutes and reweighed.

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

72
 73 Where W = weight of sample

74 W_1 = weight of empty filter paper

75 W_2 = weight of the paper plus precipitate.

77 **Determination of Tannin (Pearson, 1976)**

78 A known weight 0.5g the sample was extracted with distilled water 1ml of
 79 the aqueous extract was mixed with 35ml of distilled water volumetric flask and
 80 agitated. This was left to stand for 30minutes at room temperature, being shaken
 81 every 5 minutes. At the end of the 30minutes, it was centrifuged and the extract
 82 gotten. 25mls of the supernatant (extract) was put into a 50ml volumetric flask.
 83 Similarly, 25ml of standard tannin acid solution was put into a separate 50ml
 84 flask. A 1.0ml Folin- Denis reagent was measure into each flask followed by 2.5ml

85 of standard Sodiumtrioxocarbonate(IV) (Na_2CO_3) solution. The mixture was
86 diluted to the 50ml mark and incubated at room temperature for 90mins. The
87 absorbance was measured at 250nm. Reading was taken with reagent blank at
88 zero.

89

90 **Phenol Determination: (Association of Official Analytical Chemist AOAC, 1990).**

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

95 Note: it formed a white precipitate which was filtered before the
96 spectrometer reading was taken.

97

98 Phenol content
$$\frac{A_u - A_b \times c}{A_s - A_b}$$

99

100

101 A_u = Absorbance of the test sample

102 A_b = Absorbance of blank

103 A_s = Absorbance of the standard phenol solution

104 C = Concentration of the standard phenol solution

105 **Determination of Saponin (Obadoni and Ochuko, 2001)**

106 The ground sample (10g) was dispersed in 100ml of 20% ethanol. The
 107 suspension was heated over a hot water bath for 4 hours with continuous stirring
 108 at about 55^o_c. The mixture was filtered and the residue re-extracted with another
 109 100ml of 20% ethanol. The combined extract was reduced to 20ml by heating
 110 over water bath at about 90^o_c the concentrate was transferred into a 250ml
 111 separating funnel and 10ml of diethyl ether was added and shaken vigorously.
 112 The aqueous layer was recovered while the ether layer was discarded 30ml of n-
 113 butanol was added and was washed twice with 5ml of 5% aqueous NaCl
 114 (sodium chloride). The remaining solution was heated on a water bath to
 115 evaporate it. After evaporation, the sample was dried in the oven to a constant
 116 weight.

$$\begin{aligned} 117 \quad \% \text{ saponin} &= \frac{\text{weight of the saponin}}{\text{Weight of the dried sample}} \times 100 \\ 118 \\ 119 \end{aligned}$$

120 **Determination of Flavonoid (Boham and Kocipal, 1994)**

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

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

$$\begin{aligned} 126 \quad \% \text{ flavonoid} &= \frac{\text{Weight of the dried extract}}{\text{Weight of the sample}} \\ 127 \end{aligned}$$

128 **Anti-Bacterial Evaluation Of *Spondias Mombin* Leaves Extract.**

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

133
134 **Micro-organisms:** The bacteria organisms used were *Staphylococcus aureus*,
135 *Samollena typhi*, and *Escherichia coli*. All the organisms were obtained from the
136 stock culture of the Federal Medical Center, Umuahia. Cultures were brought to
137 laboratory conditions by resuscitating the organisms in peptone water and
138 thereafter sub cultured into nutrient agar medium and incubated at 37^{0c} for 24
139 hours (Okigbo and Omodairo, 2006).

140
141 **Antibacterial Assay:** The antibacterial activity was performed by filter paper disc
142 diffusion technique. Filter paper disc (whatman No.1, 6mm diameter) were placed
143 in glass Petri dishes and sterilized in hot air oven (Ekundayo and Ezeogu 2006).

144 The media (10g nutrient agar in 200ml distilled water, auto-claved at 115^{0c} for 30
 145 minutes) was cooled to 50^{0c}. The sterile nutrient agar media were poured into the
 146 sterile Petri dishes and allowed to solidify. The bacteria were swabbed with a
 147 sterile wire loop. Each disc was impregnated with 0.2ml of plant extracts and
 148 standard-Ciprofloxacin. Discs with DMSO (100mg/ml) served as a control.
 149 The discs were used after drying them in an incubator at 40^{0c} to remove any trace
 150 of solvent (Anishmon and Toji, 2005). Discs were introduced onto the surface of
 151 the medium. The plates were incubated at 37^{0c} for 24 hours to obtain zones of
 152 inhibition. The experiments were repeated three times for each extract and twice
 153 for reference antibiotic to minimize error and the average of these values were
 154 tabulated.

155

156 **Minimum Inhibitory Concentration (MIC):**

157 The minimum inhibitory concentration of the extracts was determined by
 158 incorporating constant volumes (0.2ml) of each dilution of the extract into
 159 perforated discs on a seeded nutrient agar plate as described in the antimicrobial
 160 susceptibility testing section (Okigbo and Omodamiro, 2006).

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

RESULTS AND DISCUSSIONS

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

Photochemical Composition of *Spondias Mombin*

Table 1 shows the 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

Result based on mean ± standard deviation of triplicate sampling measurements.

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

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

174 of tannins in the leaves of *spondias mombin* can attest to its use for healing of
175 wounds, hemorrhoids and burn in herbal medicine (Deferraya, 1981).

176 **Alkaloids** – Alkaloids are the most efficient phytochemical plant chemical
177 compound. True isolated alkaloids and the synthetic derivatives are used as the
178 basic medicinal compounds because of their analgesic, anti-spasmodic and
179 bacterial properties (Corthout *et al*, 1994).

180 **Flavonoids** – The leaves of *spondias mombin* contain an appreciable amount of
181 flavonoids up to 3.0%. The biological functions of flavonoids include protection
182 against allergies inflammation, platelets aggregation, and microbes. Flavonoids
183 through their free radical and scavenging property tend to lower cholesterol level
184 and reduce the risk of heart attack (Salah *et al*, 1995). Flavonoids have multiple
185 biological activities that include estrogenic effect as well as inhibiting the action of
186 some enzymes (Middleton and Kandaswani, 1992, Waladkhani and Clemen,
187 2001). The relatively high percentage of saponin explains the foaming nature of
188 the organic extract during rotary evaporator concentration, causing bumping. The
189 high saponin content (7.50%) shows that *spondias mombin* has cytotoxic effects
190 such as permeabilization of the intestine.

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

193 **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

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

195 replicates.

196 **Table 3 MIC of Ethanol Extract**

Organism	Concentration of extract			
	6.25ml	12.5ml	25.0ml	50.0ml
<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

197

198 **Table 4 MIC of Chloroform extract**

Organism	Concentration of extract			
	6.25ml	12.5ml	25.0ml	50.0ml
<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
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199

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

203 The results showed that extract possesses antibacterial activity against the
 204 pathogens. The chloroform extracts showed more antibacterial efficacy against
 205 the tested organisms. The minimum inhibitory concentration of the leaf extracts
 206 of the plant against the tested organisms at different concentration 6.25ml,
 207 12,5ml, 25.0ml, 50.0ml are presented in table 3 and 4. At 6.25-12.5ml the
 208 extracts recorded 0.00mm inhibition zone for the bacteria *E.coli* and *Staph.*
 209 *aureus*. The extracts gave an inhibition for *salmonella* at 12.5ml with chloroform
 210 2.2mm at 12.5ml. The inhibitory effects of the extracts on the tested organisms
 211 increase with increase in the concentrations. The non-inhibitory effect of the
 212 extracts at 6.25ml on the pathogens implies that they cannot serve as good
 213 antibacterial at that concentration in the treatment of diseases associated with
 214 the test organisms.

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

218 CONCLUSION

219 The inhibitory effect of the plant extracts at various concentration against the
220 tested gram positive bacteria *S. aureus* and gram negative *E.coli* has established
221 antibacterial potential of the leaf. Findings based on this study with respect to the
222 phytochemical constituent of the leaves of *spondias mombins* support it uses as
223 herbal remedies. The use of *spondias mombin* to treat inflammation, diarrhea,
224 haemorrhoids, and dysentery, suggest that further work be carried out on the
225 plant.

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