Phytotoxicity, cytotoxicity and chemical composition of *Spondias mombin* Linn. Stem bark

Kissinger Obagie Orumwensodia, Patrick Ojeifo Uadia and Muhammed Iqbal Choudhary

**Abstract**

**Background:** *Spondias mombin* Linn. is a tropical climate plant with wide applications in ethnomedicinal practice. This study evaluates the phytotoxicity, cytotoxicity and chemical composition of the plant’s stem bark.

**Methods:** Dried stem bark sample of *Spondias mombin* Linn. was subjected to exhaustive extraction and partitioned into sub-fractions (hexane-ethylacetate, ethylacetate, ethylacetate-methanol and methanol) by graded polarity technique. The phytotoxicity and cytotoxicity indices of the crude hydro-ethanol extract and fractions were evaluated using *Lemna minor* and brine shrimp lethality assays, respectively, while chemical composition of the oily hexane:ethylacetate fraction was determined by gas chromatography-mass spectroscopy (GC-MS) technique.

**Results:** Phytotoxicity was dose-dependent which ranged from low (crude plant extract), moderate (hexane-ethylacetate and methanol fractions), high (ethylacetate-methanol fraction) to significant toxicity (ethylacetate fraction) at the highest dose. However, for brine shrimp lethality assay only hexane-ethylacetate (LD₅₀: 284.02 μg/mL) and ethylacetate (LD₅₀: 210.24 μg/mL) fractions were cytotoxic at the highest dose. The GC-MS profile of the oily hexane:ethylacetate fraction identified sixty-eight compounds comprising hydrocarbons, fatty acids, alcohols, steroids, nitrogen and fluoride-containing compounds, terpenes and esters.

**Conclusion:** This study concludes that fractions of *Spondias mombin* Lin. could be potentially toxic. While its phytotoxic potential can be useful in the agrochemical industry for the production of natural herbicides, its cytotoxic property can be cautiously harnessed for ethnomedicinal purposes.

**Keywords:** *Spondias mombin*, Phytotoxicity, Cytotoxicity, *Lemna aequinocitalis* Welv, Brine shrimp, GC-MS
proanthocyanins, which have been implicated in the healing potentials associated with medicinal plants like *Spondias mombin*. The use of these medicinal plants continues to gain grounds especially in low-income countries. A WHO report on traditional medicine strategy for 2014–2023, opined that a good number of the world’s population depend on medicinal plants for therapeutic remedies [9]. However, the ethno-pharmacological usage of medicinal plants including *Spondias mombin* has been overshadowed by toxicity concerns bothering on their safety. Phytotoxicity and cytotoxicity assays are two ready-to-use, less expensive and easy to apply laboratory tests used to determine the toxicity profile of plant samples including extracts/fractions/isolated compounds [10, 11]. For instance, brine shrimp of the brine shrimp lethality assay (an example of a cytotoxicity assay) is believed to have positive correlation with human nasopharyngeal carcinoma (KB cell line) [10, 11], therefore a plant material which shows toxicity towards it could be potentially relevant in anticancer drug formulation. On the other hand, phytotoxicity assay can serve the purpose of screening for plant materials with potential herbicidal activity [12], since some of these products are eco-friendly but toxic to weeds. Therefore, owing to the medicinal values associated with *S. mombin* locally and its wide applications, this study was designed to investigating the toxicity index and chemical profile of this plant species of Nigerian origin.

**Materials and methods**

**Chemicals**

All solvents (hexane, ethylacetate, methanol, and ethanol) were of analytical grade and products of Sigma-Aldrich, Germany. While Paraquat and Etoposide, the reference drugs, were products of ICN Biomedical Inc., California, USA.

**Plant materials**

Stem bark of *Spondias mombin* Linn. was harvested from its trees in the forest area of Southwest region of Nigeria within the month of November. The plant material was authenticated by Dr. H. A. Akinnibosun and Dr. J. Irabor of the Department of Plant Biology and Biotechnology, where voucher No. UBHa210 was assigned and herbarium samples deposited at the herbarium of Department of Plant Biology and Biotechnology, University of Benin. The plant part was washed with water to remove earthy materials, air dried and pulverized (<1 mm) to obtain the crude powdered sample.

**Extraction and fractionation**

Air-dried stem bark of *Spondias mombin* Linn. (750 g) was subjected to successive maceration (4 days × 3) using 70% ethanol/water (2.5 L) at room temperature. The concentrated hydro-ethanol extract (31.7 g) was fractionated in a stepwise gradient pattern of increasing solvent polarity of hexane (100%), hexane-ethylacetate (50:50), ethylacetate (100%), ethylacetate-methanol (50:50) and methanol (100%) to obtain hexane, hexane-ethylacetate, ethylacetate, ethylacetate-methanol and methanol soluble fractions under reduced pressure (20–200 mbar) using a rotavapor at 45 °C.

**Phytotoxicity assay**

The assay was done according to the modified methods of McLaughlin et al. [11]. Briefly, the extract/fractions were incorporated into sterilized conical flasks at varying concentrations of 10, 100, and 1000 μg/mL in methanol, and allowed to evaporate overnight. Each flask was inoculated with 20 mL of sterilized E-medium and 10 plants of *Lemma aequinocitalis* Welv. containing a roselle of two to three fronds. The E-medium was prepared by mixing several components, viz.; boric acid (0.00286 g/L), copper sulphate (0.00022 g/L), potassium dihydrogen phosphate (0.68 g/L), calcium nitrate (1.180 g/L), potassium nitrate (1.515 g/L), magnesium sulphate (0.492 g/L), magenous chloride (0.00362 g/L), ferric chloride (0.00540 g/L), zinc sulphate (0.00022 g/L), sodium molybdate and ethylene diamino tetracetic acid, in 1000 mL distilled water with the pH adjusted to between 5.5–6.0 by adding KOH pellets and autoclaved at 121 °C for 15 min. The negative control flasks were supplemented with methanol, while the reference inhibitor, paraquat, served as positive control. The experiment was done in triplicates and the flasks incubated at 30 °C for 7 days in a Fisons Fi-Totran 600H growth cabinet with experimental conditions set at 56 ± 10 rh (relative humidity), 12 h day length and 9000 lx light intensity. The growth of *L. aequinocitalis* in the treatment flasks was determined by counting the number of fronds per dose, while growth inhibition in percentage with reference to the negative control was determined as follows:

\[
\text{Growth regulation (GR)} = \frac{\text{Number of fronds in negative control} - \text{Number of fronds in test flasks}}{\text{Number of fronds in negative control}} \times 100
\]

**Brine shrimp lethality assay**

Brine shrimp lethality assay was performed according to the modified methods of Carballo et al. [12]. Briefly, the eggs of brine shrimp (*Artemia salina*), stored at 4 °C, were hatched and shrimp between 48 and 72 h after the initiation of hatching were used for the experiment. Test samples (extract/fractions of *Spondias mombin* Linn. stem bark) of concentrations 10, 100, and 1000 μg/mL.
dissolved in methanol were introduced into their respective vials and the solvent allowed to evaporate over night. Subsequently, ten larvae per vial (about 2 day old shrimp, nauplii) were placed into the vials with the aid of a Pasteur pipette and the vials filled with 5 mL sea water. The set up was incubated at 28–29°C for 24 h under illumination. Vials with solvent served as negative control, while the reference drug, Etoposide, was used as positive control. The experiment was performed in triplicate. Cytotoxicity of extract/fractions was evaluated by counting the numbers of live and dead larvae and LD50 value was determined according to the formula below. Data obtained were analyzed using Finney computer program and confidence level set at 95% confidence intervals.

\[ \text{LD}_{50} = \frac{\sqrt{D_0 \times D_{100}}}{2} \]

\[ D_0 = \text{Highest dose that gave no mortality} \]

\[ D_{100} = \text{Lowest dose that produced mortality} \]

Gas chromatography-mass spectrometry (GC-MS) analysis
The GC-MS analysis of the hexane:ethylacetate fraction (viscous oil) of Spondias mombin Linn. stem bark was performed in a GC-MS-TQQQ instrument equipped with Agilent USB39375HHP-5MS column and capillary dimensions 30 m × 250 μm × 0.25 μm. Helium was used as the carrier gas at a flow rate of 1.2 mL/min and pressure was maintained at 10.97 psi, while the injection volume was 1 μL. The oven equilibration was for 30 min and temperature was pre-set at 70 °C for 5 min, the 10 °C/min to 180 °C for 5 min, 10 °C/min to 280 °C for 10 min, and 5 °C/min to 290 °C for 30 min. While, the MS transfer line was sustained at a temperature of 325 °C, the total run time was 73 min. The ionization mode used was electron ionization at 70 eV with source temperature of 250 °C. Total Ion Count (TIC) was used for compound identification at start mass of 20 amu and retention time, peak area, and reverse match factor are presented in Table 2. The relative percentage compositions of the identified compounds were estimated from the GC peak area.

Statistical analysis
Data were expressed as percentage growth inhibition of three replicates. The data were subjected to one-way analysis of variance (ANOVA), and differences between means were determined by Duncan’s multiple range test using the Statistical Analysis System (SPSS Statistics 20.0) where applicable. Significance was set at \( P \) values ≤0.05.

Results
Phytotoxicity assay
At a dose of 10 μg/mL, all fractions and extract of Spondias mombin stem bark had zero inhibition growth effect on fronds of Lemna minor plant, while the methanol fraction had similar effect up to 100 μg/mL. Conversely, aside paraquat (the reference drug) only ethylacetate fraction at the highest dose of 1000 μg/mL had a 100% growth inhibition. However, other fractions displayed varying degrees of growth inhibition. Results are presented in Table 1.

Brine shrimp (Artemia salina) lethality assay
Only Hexane:ethylacetate and ethylacetate frations had cytotoxic effect at the highest dose of 1000 μg/mL. Other fractions including the crude hydro-ethanol extract demonstrated no cytotoxic effect. Results are presented in Table 2.

Gas chromatography-mass spectrometry (GC-MS)
The GC-MS chromatograms in Fig. 1a, b and c, revealed sixty-eight (68) peaks matching phytoconstituents in the class of hydrocarbons, fatty acids, alcohols, steroids, nitrogen and fluoride-containing compounds, terpenes and esters. Their molecular formula, molecular weight, retention time, peak area, and reverse match factor are presented in Table 3.

Discussion
The use of herbal preparations as potent therapeutic interventions predates modern medicine. Plants have been found to contain several bioactive principles with significant value in the drug formulation process. These bioactive principles otherwise referred to as phytochemicals

### Table 1 Phytotoxic effect of Spondias mombin stem bark and Paraquat at various concentrations against fronds of Lemna minor

| Test Samples   | % Growth regulation at different doses |
|---------------|--------------------------------------|
|               | 10 μg/mL  | 100 μg/mL | 1000 μg/mL |
| Hex:EA        | 0.0       | 28.5 ± 0.41 | 59.5 ± 0.33 |
| EA            | 0.0       | 14.0 ± 0.20* | 100.0 |
| EA:Met        | 0.0       | 37.5 ± 0.11 | 65.6 ± 0.20 |
| Met           | 0.0       | 0.0        | 52.4 ± 0.10 |
| Cpe           | 0.0       | 30.9 ± 0.10 | 38.1 ± 0.25 |
| Paraquat (0.015 μg/mL) | 100.0 | 100.0 | 100.0 |

Values are mean ± S.E.M (n=3), *p < 0.05. Hex:EA Hexane:ethylacetate, EA Ethylacetate, EA:Met Ethylacetatemethanol, Met Methanol and Cpe Crude plant extract. Paraquat: reference drug
Table 2  Cytotoxic effect of *Spondias mombin* stem bark and Etoposide at various concentrations against shrimps of *Artemia salina*

| Test Samples | No. of survivals out of 30 shrimps at different doses | LD_{50} (μg/mL) |
|--------------|------------------------------------------------------|-----------------|
|              | 10 (μg/mL) | 100 (μg/mL) | 1000 (μg/mL) |                     |
| Hex:EA       | 29        | 22          | 07           | 284.0 ± 0.20        |
| EA           | 28        | 27          | 01           | 210.2 ± 0.15        |
| EA:Met       | 28        | 26          | 24           | –                   |
| Met          | 29        | 28          | 27           | –                   |
| CpE          | 30        | 30          | 22           | –                   |
| Etoposide    | 00        | 00          | 00           | 7.5                 |

Values are mean ± S.E.M (n = 3), *p < 0.05. Hex:EA Hexane:ethylacetate, EA Ethylacetate, EA:Met Ethylacetate:methanol, Met Methanol and CpE Crude plant extract. Etoposide: reference drug.

Fig. 1 Chromatogram of Phytoconstituents in *Spondias mombin* Linn. stem bark oil.
| Compound name                                       | Molecular formula | MW  | RT  (min) | Peak Area % | RMF  (DB) |
|-----------------------------------------------------|------------------|-----|-----------|-------------|----------|
| 2,3-Dimethyl-1-pentanol                              | C₇H₁₄O           | 116 | 8.10      | 0.01        | 849      |
| 2-Ethylhexan-1-ol                                    | C₈H₁₈O           | 130 | 10.33     | 0.06        | 943      |
| 2-Propyl-1-heptanol                                  | C₁₀H₂₀O          | 158 | 15.90     | 0.01        | 835      |
| (2E)-2-Tridecenal                                    | C₁₃H₂₄O₂         | 196 | 17.31     | 0.02        | 777      |
| Eugenol                                             | C₁₀H₁₂O₂         | 164 | 19.76     | 0.01        | 943      |
| d-Mannose                                           | C₆H₁₂O₆          | 180 | 20.25     | 0.72        | 741      |
| Vanillin lactoside                                   | C₁₂H₂₂O₁₃        | 476 | 21.01     | 0.02        | 778      |
| (Z)-7-Hexadecenal                                    | C₁₆H₃₂O           | 238 | 21.26     | 0.06        | 870      |
| 6-Pentyl-5,6-dihydro-2H-pyran-2-one (Massoa lactone)| C₁₀H₁₆O₂         | 168 | 22.88     | 0.02        | 842      |
| Tetradecane, 2,6,10-trimethyl-                        | C₁₃H₂₆          | 240 | 23.53     | 0.05        | 827      |
| Undecanoic acid, 10-methyl-, methyl ester            | C₁₃H₂₈O₂        | 214 | 24.08     | 0.03        | 866      |
| Dodecanoic acid (Lauris Acid)                        | C₁₂H₂₄O₂        | 200 | 25.31     | 0.02        | 908      |
| Dodecanoic acid, ethyl ester (Ethyl laurate)         | C₁₄H₂₈O₂        | 228 | 25.75     | 0.04        | 932      |
| Nonadecane                                          | C₁₉H₃₈O           | 268 | 25.99     | 1.24        | 927      |
| 3,4,5-Trimethoxyphenol                               | C₆H₁₂O₄          | 184 | 26.17     | 0.21        | 827      |
| Octatriacontyl pentafluoroproprionate                | C₄₁H₇₇F₅O₂      | 696 | 26.43     | 0.06        | 799      |
| 2,2',5,5'-Tetramethyl-1,1'-biphenyl                  | C₁₆H₁₈          | 210 | 27.17     | 0.06        | 847      |
| 1,4-Methanazulen-3-ol, decahydro-1,5,5,8a-tetramethyl-| C₁₅H₂₆O         | 222 | 26.76     | 0.02        | 746      |
| 2-(2-Nitro-2-propenyl) cyclohexanone                 | C₁₅H₁₉N₂O        | 183 | 26.98     | 0.05        | 746      |
| Epiglobulol                                          | C₁₃H₂₈O₂        | 222 | 27.21     | 0.09        | 789      |
| Globulol                                             | C₁₃H₂₈O₂        | 222 | 27.44     | 0.02        | 845      |
| 1-Hexadecanol (Cetyl Alcohol)                        | C₁₆H₃₄O           | 242 | 27.68     | 0.06        | 900      |
| Tetradecyl trifluoroacetate                          | C₁₆H₃₂F₂O₂      | 310 | 27.68     | 0.07        | 887      |
| 2-Methyl-1-hexadecanol                               | C₁₇H₃₄O          | 256 | 28.17     | 0.03        | 770      |
| 3-Hydroxydodecanoic acid                             | C₁₂H₂₆O₃        | 216 | 28.51     | 0.03        | 755      |
| Tetradecanoic acid (Myristic acid)                   | C₁₄H₂₈O₂        | 228 | 29.76     | 0.14        | 901      |
| Tetradecanoic acid, ethyl ester (Myristic acid, ethyl ester) | C₁₆H₃₂O₂   | 256 | 30.28     | 0.02        | 911      |
| Hexadecanoic acid, ethyl ester (Palmitic acid, ethyl ester) | C₁₈H₃₆O₂   | 284 | 32.02     | 0.98        | 785      |
| Ethyl 13-methyl-tetradecanoate                       | C₁₇H₃₈O₂        | 270 | 32.02     | 0.09        | 845      |
| Oleic Acid (9-Octadecenoic acid (Z)-)                | C₁₈H₃₆O₂        | 282 | 32.27     | 0.18        | 763      |
| 1-Hexadecanol                                        | C₁₆H₃₄O           | 242 | 32.84     | 0.96        | 946      |
| Pentadecanoic acid, ethyl ester                      | C₁₇H₃₈O₂        | 270 | 33.24     | 0.23        | 918      |
| Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (Ethyl ferulate) | C₁₂H₁₄O₄ | 222 | 34.05     | 0.14        | 884      |
| Docosanoic acid, ethyl ester                         | C₂₄H₄₈O₂        | 368 | 35.71     | 0.37        | 754      |
| n-Hexadecanoic acid                                  | C₁₆H₃₄O₂        | 256 | 37.03     | 0.16        | 929      |
| Undecanoic acid, ethyl ester                         | C₁₃H₂₆O₂        | 214 | 37.71     | 4.85        | 837      |
| Oleyl Alcohol                                        | C₁₈H₃₆O           | 268 | 41.13     | 13.3        | 900      |
| 11-Hexadecen-1-ol, (Z)- (Virelure)                   | C₁₈H₃₆O           | 240 | 41.13     | 1.49        | 943      |
| 1-Eicosanol                                          | C₂₀H₄₀O          | 298 | 42.95     | 0.42        | 908      |
| Isopropyl Palmitate                                  | C₁₉H₃₈O₂        | 298 | 43.30     | 0.34        | 793      |
| Heptadecanoic acid, ethyl ester                      | C₁₉H₃₈O₂        | 298 | 43.64     | 0.49        | 826      |
| 9,12-Octadecadienoic acid, ethyl ester              | C₂₀H₄₀O₂        | 308 | 49.27     | 12.47       | 910      |
| 9-Octadecenoic acid, ethyl ester, (E)-               | C₂₀H₄₀O₂        | 310 | 50.28     | 7.87        | 864      |
are classed into saponins, tannins, flavonoids, phenolics, glycosides, organic acids, essential oils etc., and are believed to play a key role in the plant defense mechanism against invading pathogens. More so, several biological activities including antioxidant, anti-inflammatory, antibacterial, antifungal, enzyme modulation, as well as inhibition of cell proliferation amongst others have also been associated with these phytoconstituents [13]. Functioning as a sole molecule or in synergistic fashion, these potential drug candidates have helped to arrest several ailments [14–16]. Despite these seeming advantages, consumption of herbal formulations has been dabbed in controversies around safety issues. Therefore, scientific approaches that test the safety or otherwise of these products are required to resolve this conundrum. The result of phytotoxicity study of stem bark of *Spondias mombin* against *L. aequinoctialis* Welv. (*Lemna minor*) (Table 1) indicates a possible phytotoxic effect at the highest tested dose of 1000 μg/mL, relative to the reference drug, Paraquat. The ethylacetate fraction was significantly phytotoxic against fronds of *Lemna minor* plant at the highest dose tested. This was followed by ethylacetate:methanol fraction with high phytotoxic activity. Hexane:ethylacetate and methanol fractions both had moderate activity, while the crude hydro-ethanol extract showed weak phytotoxicity. Plants with phytotoxic activity have been exploited for use as natural herbicides [17]. Thus, the phytotoxic potential of *Spondias mombin* stem bark can be harnessed by agrochemical industries for the formulation of natural herbicides. Similarly, the result of brine shrimps lethality test (Table 2) shows some fractions had cytotoxic effect against *Artemia salina* at the highest dose of 1000 μg/mL. Although, the crude hydro-ethanol extract, ethylacetate:methanol and

**Table 3** Compounds identified in *Spondias mombin* stem bark oil (Continued)

| Compound name | Molecular formula | MW | RT (min) | Peak Area % | RMF (DB) |
|---------------|-------------------|----|----------|-------------|----------|
| Octadecanoic acid (Stearic acid) | C₁₈H₃₆O₂ | 284 | 50.76 | 0.37 | 891 |
| Methyl 17-methyl-octadecanoate | C₂₀H₄₀O₂ | 312 | 51.90 | 0.59 | 869 |
| Methyl 19-methyl-eicosanate | C₂₂H₄₄O₂ | 340 | 59.19 | 3.78 | 871 |
| Eicosanoic acid, ethyl ester | C₂₂H₄₂O₂ | 340 | 59.19 | 0.43 | 896 |
| 1,2-Benzenedicarboxylic acid, diisooctyl ester (Isocetyl phthalate) | C₃₄H₅₆O₄ | 390 | 62.12 | 0.68 | 951 |
| 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Glyceryl 2-olate) | C₂₁H₄₀O₄ | 356 | 65.22 | 0.95 | 872 |
| Ethyl tetracosanate | C₂₉H₅₀O₂ | 396 | 66.90 | 0.25 | 814 |
| 17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol | C₂₇H₄₆O | 386 | 71.22 | 0.5 | 867 |
| Vitamin E | C₂₀H₄₀O₂ | 430 | 71.49 | 0.28 | 827 |
| Ethyl iso-allocholate | C₂₀H₄₀O₂ | 436 | 71.86 | 0.23 | 801 |
| Rhodopin | C₂₁H₄₂O₂ | 554 | 71.86 | 0.23 | 733 |
| Campesterol | C₂₈H₄₈O | 400 | 72.86 | 1.28 | 841 |
| Stigmasterol | C₂₉H₄₈O | 412 | 73.29 | 0.35 | 918 |
| Ergosta-5,24(28)-dien-3β-ol (Chalinasterol) | C₂₉H₄₈O | 398 | 73.66 | 1.94 | 845 |
| 4,14-Dimethylergosta-8,24(28)-dien-3-ol (Obtusifoliol) | C₃₀H₅₀O | 426 | 73.99 | 0.4 | 882 |
| γ-Sitosterol | C₂₉H₄₈O | 414 | 74.51 | 1.39 | 927 |
| β-Sitosterol | C₂₉H₄₈O | 414 | 74.51 | 10.01 | 916 |
| Cholest-5-en-3-ol, 24-propyli dine-, (3β)- (E)-24-Propyli denec cholesterol or 29-Methylisofuco sterol) | C₃₀H₅₀O | 426 | 74.72 | 2.76 | 812 |
| Betulin | C₃₀H₅₀O₂ | 442 | 74.72 | 0.92 | 747 |
| Ergosta-7,24(28)-dien-3-ol, 4-methyl-, (3β,4α,5α) - (Granisterol) | C₂₉H₄₈O | 412 | 74.91 | 4.5 | 860 |
| 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate (9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α) -) | C₃₂H₅₂O₂ | 468 | 75.59 | 4.15 | 815 |
| 24-Methylenecycloarten-3-one | C₁₇H₂₈O | 438 | 76.73 | 3.47 | 858 |
| Stigmast-4-en-3-one (Sitostenone) | C₂₉H₄₈O | 412 | 77.08 | 3.61 | 908 |
| 19-Cyclolanostan-3-ol, 24-methylene-, (3β)- | C₁₉H₃₂O | 440 | 77.24 | 2.57 | 919 |
| Stigmastane-3,6-dione, (5α)- | C₂₀H₃₀O₂ | 428 | 81.22 | 6.88 | 866 |

GC-MS was done using Agilent GC-MS triple quad USB39375HHP-5MS. The identification of compounds was based on a mass spectral survey performed using NIST library for spectral comparison and identification.
methanol fractions demonstrated no cytotoxic activity relative to the reference drug, Etoposide, the hexane: ethylacetate and ethylacetate fractions had cytotoxic effect against Artemia salina. These findings, though on the stem bark of the plant, are in agreement with in vivo studies conducted on the aqueous and ethanolic leaf extracts of S. mombin, which revealed that prolonged usage of this plant at high doses could be potentially cytotoxic [18, 19]. The cytotoxic property of some fractions of Spondias mombin stem bark at high concentration underscores the need for cautious use of the plant in ethno-medicinal practice. Nonetheless, phytoconstituents contained in the plant as revealed in this study via the GC-MS profiling of the oily hexane:ethylacetate fraction (Table 3 and Figs. 1, 2a, b, c) indicates a rich array of compounds, some of which have diverse pharmacological potentials. Sixty-eight compounds comprising hydrocarbons, fatty acids, alcohols, steroids, nitrogen and fluoride-containing compounds, terpenes and esters were identified (Figs. 1, 2a, b, c). These compounds include 2, 3-Dimethyl-1-pentanol (1); 2-Ethylhexan-1-ol (2); 2-Propyl-1-heptanol (3); (2E)-2-Tridecenal (4); Eugenol (5); d-Mannose (6); Vanillín lactoside (7); (Z)-7-Hexadecenal (8); Massoa lactone (9); Tetradecane, 2,6,10-trimethyl- (10); Undecanoic acid, 10-methyl-, methyl ester (11); Dodecanoic acid (Lauris Acid) (12); Dodecanoic acid, ethyl ester (Ethyl laurate) (13); Nonadecane (14); 3,4,5-Trimethoxyphenol (15); Octatriacontyl pentafluoropropionate (16); 2,2′,5,5′-Tetramethyl-1,1′-biphenyl (17); Longiborneol (18); 2-(2-Nitro-2-propenyl) cyclohexanone (19); Epiglobulol (20); Globulol (21); Cetyl Alcohol (22); Tetradecyl trifluoroacetate (23); 2-Methyl-1-hexadecanol (24); 3-Hydroxydodecanoic acid (25); Myristic acid (26); Myristic acid, ethyl ester (27); Palmitic acid, ethyl ester (28); Ethyl 13-methyltetradecanoate (29); Oleic Acid (30); I-Hexadecanol (31); Pentadecanoic acid, ethyl ester (32); Ethyl ferulate (33); Docosanoic acid, ethyl ester (34); n-Hexadecanoic acid (35); Undecanoic acid, ethyl ester (36); Oleyl Alcohol (37); Vire lure (38); 1-Eicosanol (39); Isopropyl Palmitate (40); Heptadecanoic acid, ethyl ester (41); 9,12-Octadecadienoic acid, ethyl ester (42); 9-Octadecenoic acid, ethyl ester, (E)- (43); Stearic acid (44); Methyl 17-methyl-octadecanoate (45); Methyl 19-methyl-eicosanoic acid (46); Eicosanoic acid, ethyl ester (47); Isooctyl phthalate (48); Glyceryl 2-oleate (49); Ethyl tetraocanoate (50); 17-(1,5-Dimethylyxyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol (51); Vitamin E (52); Ethyl iso-allocholate (53); Rhodopin (54); Campest erol (55); Stigmasterol (56); Chalinen sterol (57); Obtusifoliol (58); γ-Sitosterol (59); β-Sitosterol (60); Cholest-5-en-3-ol, 24-propylidene-, (3β)- (61); Betulin (62); Gramisterol (63); 9,19-Cycloergost-24(28)-en-3-ol, 4,14-

Fig. 2 a Compounds identified in Spondias mombin Linn stem bark oil. b Compounds identified in Spondias mombin Linn stem bark oil. c Compounds identified in Spondias mombin Linn stem bark oil.
dimethyl-, acetate (9,19-Cycloergost-24(28)-en-3-ol, 4, 14-dimethyl-, acetate, (3β,4α,5α)-) (64); 24-Methylenecycloarten-3-one (65); Sitostenone (66); 19-Cyclolanostan-3-ol, 24-methylene-, (3β) (67) and Stigmasterane-3,6-dione, (5α)- (68) (Fig. 2a, b, c). Some of these compounds as earlier mentioned have been found to possess profound biological activities. For instance, the long chain fatty acid alcohol, (2E)-2-Tridecanal, is known for its antibacterial activity [20]. Eugenol, which belongs to the class of allylbenzene and a naturally occurring phenolic molecule has anti-inflammatory, neuroprotective, antipyretic, antioxidant, antifungal and analgesic properties [21–23], antiproliferative and proapoptotic activity [24] and antimicrobial property [25]. Aside its pharmacological importance [26], reported the herbicidal role of eugenol in commercially available herbicide, clove oil (a herbicide formulation of Burnout II weed and grass killer). Therefore, its phytotoxic effect could be due to the presence of compounds like eugenol. Fatty acids such as oleic acid enhances membrane function [27], while stearic acid regulates mitofusin activity, ditto mitochondrial morphology and function, reduces blood pressure, improves heart function, and reduces cancer risk [28]. Some phytosterols such as campsterol, graminoster and stigmasterol were found to promote WEHI-3 cell anti-proliferative activity, anti-inflammatory effect and cytotoxicity against some cancer cell lines [29]. Thus, the cytotoxic effect of this plant could be linked to in part, its fatty acid and phytosterol contents amongst other molecules. Several terpenoids (mono-, di-, and tri-) have been observed to have antiurease activity [30], however, betuline and betulinic acid as pentacyclic triterpenes possess anti-HIV-1, antitumoural, anti-inflammatory and in vitro antimalarial effects [31]. Therefore, the activities of these compounds either singly or in concerted manner could be responsible for the observed biological effects.

Conclusion
In this study it was observed that the stem bark extract of *Spondias mombin* Linn is rich in the various compounds identified using GS-MS. The stem bark extract of this plant was found to have potential phytotoxic effect which can be further studied as an effective agent against parasitic plants. Though at high dose it could exert some lethal effect, but its medicinal potential can be cautiously harnessed for therapeutic gains.

Acknowledgements
We thank Natural Products Research and Disease Control Laboratory, University of Benin, Benin City, Nigeria, for the plant material and technical assistance during the preliminary stage of this study. Also, we appreciate the International Centre for Chemical and Biological Sciences (ICCBS) for the laboratory space, equipment and technical assistance.

Authors’ contributions
KOO conceptualized, designed, carried out the study and prepared the manuscript. POU and MIC conceptualized, designed and supervised the study. The authors read and approved the final manuscript.

Funding
This work was supported by The World Academy of Science, TWAS-ICCBS Postgraduate Fellowship (FR Number: 3240287191) and Tertiary Education Trust Fund Research Grant awarded to Kissing Obaogie Orumwensodia. The authors are immensely grateful.

Availability of data and materials
All data relating to this study have been included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interest.

Received: 16 January 2021 Accepted: 23 June 2021

Published online: 30 June 2021

References
1. Njoku PC, Akumefula MI. Phytochemical and nutrient evaluation of *Spondias mombin* leaves. Pak J Nutr. 2007;6(6):613–5. https://doi.org/10.3923/pjn.2007.613.615.

2. Agbokhan EJ. Annotated checklist of vascular plants of southern Nigeria, a quick reference guide to the vascular plants of southern Nigeria: a systematic approach. Benin: Uniben Press; 2014.

3. Caraballo A, Caraballo B, Rodríguez-Acosta A. Preliminary assessment of medicinal plants used as antimalarials in the south-eastern Venezuelan Amazon. Rev Soc Bras Med Trop. 2004;37(2):186–8. https://doi.org/10.1590/S0037-86822004000200016.

4. Corthout J, Pieters LA, Vanden Berge VH, Viletinck AJ. Antiviral Caffeoyl Ester from *Spondias mombin*. Phytochemistry. 1992;31:79.

5. Corthout J, Pieters LA, Claeyse M, Vandenberghe DA, Viletinck AJ. Antimicrobial activity of *Spondias mombin* Linn., *Spondias* spp. and *Spondias* leaf extracts in vitro. Toxicol Ind Health. 2013;29(2):181–200. https://doi.org/10.1177/0748233712439512.

6. Villegas LF, Fernández TD, Maldonado H, Torres R, Zavaleta A, Vaisberg AJ, et al. Evaluation of wounds healing of selected plants from Peru. Peru J Ethnopharmacol. 1997;55(3):193–200. https://doi.org/10.1016/S0378-8717(96)01500-0.

7. Coates NJ, Gilpin ML, Gwynn MN, Lewis DE, Milner PH, Spear SR, et al. SB-20742 a novel beta-lactamase inhibitor isolated from *Spondias mombin* Linn. J Nat Prod. 1994;57(5):654–7. https://doi.org/10.1021/np900107a016.

8. Orwa C, Mutua A, Kihika R, Jamnadass R, Anthony S. *Spondias mombin* Linn., a tree reference and selection guide version 4.0. Agroforestry Database. 2009. http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp.

9. WHO. WHO traditional medicine strategy: 2014–2023. World Health Organization; 2013. https://apps.who.int/iris/handle/10665/92455. Accessed 14 Jan 2021.

10. Karadeniz B, Ulker Z, Alpsoy L. Genotoxic and cytotoxic effects of storax products Chemistry. Amsterdam: Elsevier; 1991. p. 383–409.

11. McLaughlin JL, Chang CJ, Smith DL. Bench Top Bioassays for the Discovery of Bioactive Natural Products. In: Atta-Uri-Rehman, editor. Studies in Natural Products Chemistry. Amsterdam: Elsevier; 2012. p. 181–6. https://doi.org/10.1016/B978-0-444-53237-1423642.

12. Orumwensodia KO, Osemwenkhae PO, Orumwensodia K. Screening of isolated bacterial strains of *Spondias mombin* seed. Nig J Life Sci. 2015;5:195–202.
14. Rahman S, Parvez AK, Islam R, Khan MH. Antibacterial activity of natural spices on multiple drug resistant Escherichia coli isolated from drinking water. Bangladesh Ann Clin Microbiol Antimicrob. 2011;10(1):10. https://doi.org/10.1186/1476-0711-10-10.

15. Hafidh RR, Abdulamir AS, Vern LS, Bakar FA, Abas F, Jahanshiri F, et al. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. Open Microbiol J. 2011;5(1):96–106. https://doi.org/10.2174/1874285801105010096.

16. Oriakhi K, Orumwensodia KO. Combinatorial effect of Gallic acid and Catechin on some biochemical and pro-inflammatory markers in CCl4-mediated hepatic damage in rats. Phytomedicine Plus. 2021;1(1):100017. https://doi.org/10.1016/j.phyplu.2020.100017.

17. Azizuddin, Choudhary MI. Antibacterial, phytotoxic, insecticidal and cytotoxic potential of Vitis agnus-castus. J Med Plant Res. 2011;5:5642–5.

18. Asuquo OR, Ekanem TB, Eluwa MA, Oko OO, Ikpi DE. Evaluation of Toxicological Effects of Spondias mombin in Adult Male Wistar Rats. J Nat Sci Res. 2012;2:144–51.

19. Nwaogwugwu J, Uhegbu F, Okeereke S, Egege A, Atasi O. Toxicological evaluation of aqueous leaf extract of Spondias mombin using albino rat. J Med Herbs Ethnomed. 2018;4:23–30.

20. Orhan I, Sener B. Lead compounds and drug candidates from some Turkish plants for human health: advances in Phytomedicine. 2nd ed. Oxford: Elsevier; 2006.

21. Park SH, Sim YB, Lee JK, Kim SM, Kang YJ, Jung JS, et al. The analgesic effects and mechanisms of orally administered eugenol. Arch Pharm Res. 2011;34(3):501–7. https://doi.org/10.1007/s12272-011-0320-z.

22. Dal Bo W, Luiz AP, Martins DF, Mazzardo-Martins L, Santos AR. Eugenol reduces acute pain in mice by modulating the glutamatergic and tumor necrosis factor alpha (TNF-alpha) pathways. Fundam Clin Pharmacol. 2013;27(5):17–25. https://doi.org/10.1111/fcp.12052x.

23. Fonseca DV, Salgado PR, Aragão Neto Hde C, Golio AM, Caldas Filho MR, Melo CG, et al. Ortho-eugenol exhibits anti-nociceptive and anti-inflammatory activities. Int Immunopharmacol. 2016;38:402–8. https://doi.org/10.1016/j.intimp.2016.06.005.

24. Pisano M, Pagnan G, Loi M, Mura ME, Tilocca MG, Palmieri G, et al. Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. Mol Cancer. 2007;6(1):8. https://doi.org/10.1186/1476-4598-6-8.

25. Marchese A, Barbieri R, Coppo E, Orhan IE, Daglia M, Nabavi SF, et al. Antimicrobial activity of eugenol and essential oils containing eugenol: a mechanistic viewpoint. Crit Rev Microbiol. 2017;43(6):668–89. https://doi.org/10.1080/1040841X.2017.1295225.

26. Ahuja N, Batish DR, Singh HP, Kohli RK. Herbicidal activity of eugenol towards some grassy and broad-leaved weeds. J Pest Sci. 2013;88:209–18.

27. Fontana A, Spolaore B, de Lauroe PP. The biological activities of protein/oleic acid complexes reside in the fatty acid. Biochim Biophys Acta, Proteins Proteomics. 1834;2013:1125–43.

28. Venkateswarlu B, Rao TKB, Sivaprasad BAB, Pushpender. Insecticidal activity of eugenol towards stored products pests. J Sci Ind Res. 1987;46(4):168–71.

29. Khandelwal KR. Pharmacognosy. 6th ed. New Delhi: Banarsidas Bhanot; 2014.

30. Oyewole AO, Oyewole AU. Antibacterial activity of eugenol against multiple drug-resistant bacteria. J Med Microbiol. 2011;60(12):1544–9.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.