

ORIGINAL CONTRIBUTION

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

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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 aequinocalis* Welv, Brine shrimp, GC-MS

Background

Spondias mombin Linn. is a tree belonging to the family of Anacardiaceae and subfamily Spondiadoideae. It grows within the humid tropical climates, often in secondary vegetation derived from evergreen lowland forest or semi-deciduous forest areas of the continents of Africa (in countries like Nigeria, Congo, Central Africa Republic, etc), Asia (India) and South Americas (Brazil, Guatemala, Panama, Argentina, etc.) [1]. Its common

names include hog plum, yellow mombin, mombin and yellow Spanish plum. In Nigeria it is locally referred to as Ogheghe, Okighan in Edo, Tsáádàr Másàr in Hausa, Ijikara, Ngulungwu, Isikala in Igbo, Iyeye, Ekikan, Olosan in Yoruba, and Nsukakara in Ibibio [2]. It has been greatly exploited around the world for various purposes including ornamental, nutritional (as a beverage) and medicinal; anti-malarial [3], antiviral [4], antibacterial [5], wound-healing [6], enzyme inhibition [7], etc. The fruit hosts considerable amounts of vitamins A and C, while carotenoids are presumably present in reasonable concentrations [8]. Qualitative phytochemical screening of parts of the plant revealed the presence of flavonoids, alkaloids, tannins, phenolics, saponins and

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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 *Lemna aequinocitalis* Welv. containing a rosette 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), magnesium chloride (0.00362 g/L), ferric chloride (0.00540 g/L), zinc sulphate (0.00022 g/L), sodium molybdate and ethylene diamine 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:

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

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.

$$LD_{50} = \frac{\sqrt{D_0 \times D_{100}}}{2}$$

D₀ = Highest dose that gave no mortality

D₁₀₀ = 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 end mass of 650 amu for scan time of 200 ms. With Match Factor (MF) of ≥700 taken as satisfactory, the Spectra of the separated compounds were compared with the database of the National Institute of Standards and Technology (NIST) Reference Spectra Library using AMDIS V 2.69 (Automated mass spectral deconvolution and identification software). 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 fractions 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 Ethylacetate:methanol, 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

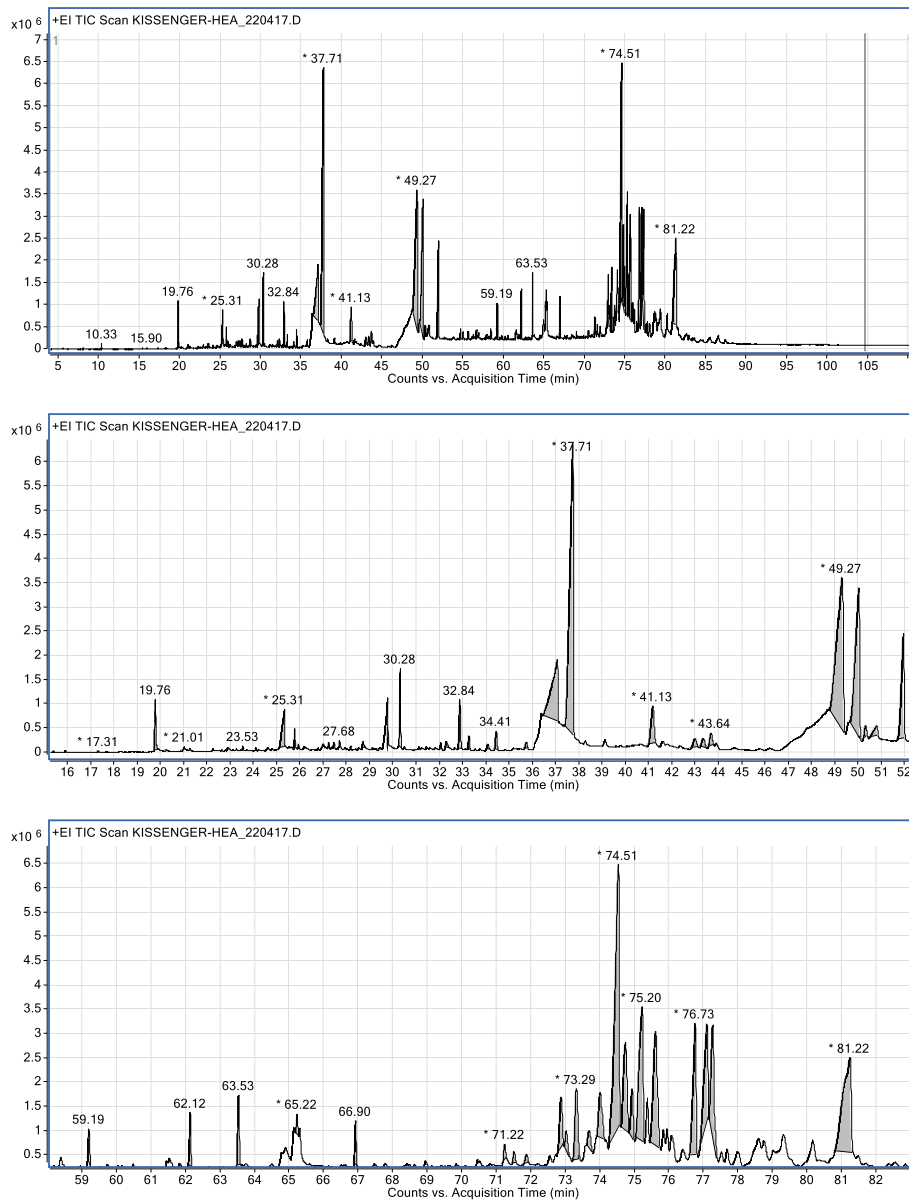


Fig. 1 Chromatogram of Phytoconstituents in *Spondias mombin* Linn. stem bark oil

Table 3 Compounds identified in *Spondias mombin* stem bark oil

| Compound name | Molecular formula | MW | RT (min) | Peak Area % | RMF (DB) |
|--|---|-----|----------|-------------|----------|
| 2,3-Dimethyl-1-pentanol | C ₇ H ₁₆ O | 116 | 8.10 | 0.01 | 849 |
| 2-Ethylhexan-1-ol | C ₈ H ₁₈ O | 130 | 10.33 | 0.06 | 943 |
| 2-Propyl-1-heptanol | C ₁₀ H ₂₂ O | 158 | 15.90 | 0.01 | 835 |
| (2E)-2-Tridecenal | C ₁₃ H ₂₄ O | 196 | 17.31 | 0.02 | 777 |
| Eugenol | C ₁₀ H ₁₂ O ₂ | 164 | 19.76 | 0.01 | 943 |
| d-Mannose | C ₆ H ₁₂ O ₆ | 180 | 20.25 | 0.72 | 741 |
| Vanillin lactoside | C ₂₀ H ₂₈ O ₁₃ | 476 | 21.01 | 0.02 | 778 |
| (Z)-7-Hexadecenal | C ₁₆ H ₃₀ O | 238 | 21.26 | 0.06 | 870 |
| 6-Pentyl-5,6-dihydro-2H-pyran-2-one (Massoia lactone) | C ₁₀ H ₁₆ O ₂ | 168 | 22.88 | 0.02 | 842 |
| Tetradecane, 2,6,10-trimethyl- | C ₁₇ H ₃₆ | 240 | 23.53 | 0.05 | 827 |
| Undecanoic acid, 10-methyl-, methyl ester | C ₁₃ H ₂₆ O ₂ | 214 | 24.08 | 0.03 | 866 |
| Dodecanoic acid (Lauric Acid) | C ₁₂ H ₂₄ O ₂ | 200 | 25.31 | 0.02 | 908 |
| Dodecanoic acid, ethyl ester (Ethyl laurate) | C ₁₄ H ₂₈ O ₂ | 228 | 25.75 | 0.04 | 932 |
| Nonadecane | C ₁₉ H ₄₀ | 268 | 25.99 | 1.24 | 927 |
| 3,4,5-Trimethoxyphenol | C ₉ H ₁₂ O ₄ | 184 | 26.17 | 0.21 | 827 |
| Octatriacontyl pentafluoropropionate | C ₄₁ H ₇₇ F ₅ O ₂ | 696 | 26.43 | 0.06 | 799 |
| 2,2',5,5'-Tetramethyl-1,1'-biphenyl | C ₁₆ H ₁₈ | 210 | 27.17 | 0.06 | 847 |
| 1,4-Methanoazulen-3-ol, decahydro-1,5,5,8a-tetramethyl-, [1S-(1α,3β,3aβ,4α,8aβ)]- (Longiborneol) | C ₁₅ H ₂₆ O | 222 | 26.76 | 0.02 | 746 |
| 2-(2-Nitro-2-propenyl) cyclohexanone | C ₉ H ₁₃ NO ₃ | 183 | 26.98 | 0.05 | 746 |
| Epiglobulol | C ₁₅ H ₂₆ O | 222 | 27.21 | 0.09 | 789 |
| Globulol | C ₁₅ H ₂₆ O | 222 | 27.44 | 0.02 | 845 |
| 1-Hexadecanol (Cetyl Alcohol) | C ₁₆ H ₃₄ O | 242 | 27.68 | 0.06 | 900 |
| Tetradecyl trifluoroacetate | C ₁₆ H ₂₉ F ₃ O ₂ | 310 | 27.68 | 0.07 | 887 |
| 2-Methyl-1-hexadecanol | C ₁₇ H ₃₆ O | 256 | 28.17 | 0.03 | 770 |
| 3-Hydroxydodecanoic acid | C ₁₂ H ₂₄ O ₃ | 216 | 28.51 | 0.03 | 755 |
| Tetradecanoic acid (Myristic acid) | C ₁₄ H ₂₈ O ₂ | 228 | 29.76 | 0.14 | 901 |
| Tetradecanoic acid, ethyl ester (Myristic acid, ethyl ester) | C ₁₆ H ₃₂ O ₂ | 256 | 30.28 | 0.02 | 911 |
| Hexadecanoic acid, ethyl ester (Palmitic acid, ethyl ester) | C ₁₈ H ₃₆ O ₂ | 284 | 32.02 | 0.98 | 785 |
| Ethyl 13-methyl-tetradecanoate | C ₁₇ H ₃₄ O ₂ | 270 | 32.02 | 0.09 | 845 |
| Oleic Acid (9-Octadecenoic acid (Z)-) | C ₁₈ H ₃₄ O ₂ | 282 | 32.27 | 0.18 | 763 |
| 1-Hexadecanol | C ₁₆ H ₃₄ O | 242 | 32.84 | 0.96 | 946 |
| Pentadecanoic acid, ethyl ester | C ₁₇ H ₃₄ O ₂ | 270 | 33.24 | 0.23 | 918 |
| Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (Ethyl ferulate) | C ₁₂ H ₁₄ O ₄ | 222 | 34.05 | 0.14 | 884 |
| Docosanoic acid, ethyl ester | C ₂₄ H ₄₈ O ₂ | 368 | 35.71 | 0.37 | 754 |
| n-Hexadecanoic acid | C ₁₆ H ₃₂ O ₂ | 256 | 37.03 | 0.16 | 929 |
| Undecanoic acid, ethyl ester | C ₁₃ H ₂₆ O ₂ | 214 | 37.71 | 4.85 | 837 |
| Oleyl Alcohol | C ₁₈ H ₃₆ O | 268 | 41.13 | 13.3 | 900 |
| 11-Hexadecen-1-ol, (Z)- (Virelure) | C ₁₆ H ₃₂ O | 240 | 41.13 | 1.49 | 943 |
| 1-Eicosanol | C ₂₀ H ₄₂ O | 298 | 42.95 | 0.42 | 908 |
| Isopropyl Palmitate | C ₁₉ H ₃₈ O ₂ | 298 | 43.30 | 0.34 | 793 |
| Heptadecanoic acid, ethyl ester | C ₁₉ H ₃₈ O ₂ | 298 | 43.64 | 0.49 | 826 |
| 9,12-Octadecadienoic acid, ethyl ester | C ₂₀ H ₃₆ O ₂ | 308 | 49.27 | 12.47 | 910 |
| 9-Octadecenoic acid, ethyl ester, (E)- | C ₂₀ H ₃₈ O ₂ | 310 | 50.28 | 7.87 | 864 |

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

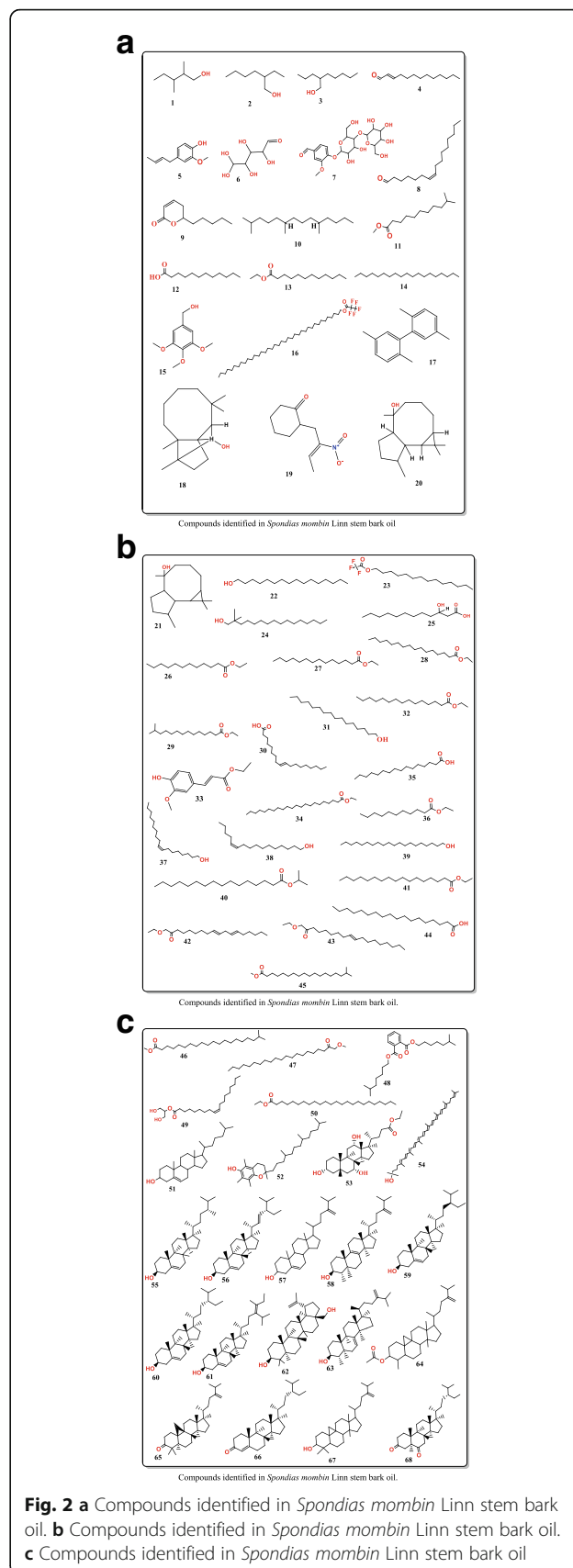
| Compound name | Molecular formula | MW | RT (min) | Peak Area % | RMF (DB) |
|--|--|-----|----------|-------------|----------|
| Octadecanoic acid (Stearic acid) | C ₁₈ H ₃₆ O ₂ | 284 | 50.76 | 0.37 | 891 |
| Methyl 17-methyl-octadecanoate | C ₂₀ H ₄₀ O ₂ | 312 | 51.90 | 0.59 | 869 |
| Methyl 19-methyl-eicosanoate | C ₂₂ H ₄₄ O ₂ | 340 | 59.19 | 3.78 | 871 |
| Eicosanoic acid, ethyl ester | C ₂₂ H ₄₄ O ₂ | 340 | 59.19 | 0.43 | 896 |
| 1,2-Benzenedicarboxylic acid, diisooctyl ester (Isooctyl phthalate) | C ₂₄ H ₃₈ O ₄ | 390 | 62.12 | 0.68 | 951 |
| 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Glyceryl 2-oleate) | C ₂₁ H ₄₀ O ₄ | 356 | 65.22 | 0.95 | 872 |
| Ethyl tetracosanoate | C ₂₆ H ₅₂ O ₂ | 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 | C ₂₇ H ₄₆ O | 386 | 71.22 | 0.5 | 867 |
| Vitamin E | C ₂₉ H ₅₀ O ₂ | 430 | 71.49 | 0.28 | 827 |
| Ethyl iso-allocholate | C ₂₆ H ₄₄ O ₅ | 436 | 71.86 | 0.23 | 801 |
| Rhodopin | C ₄₀ H ₅₈ O | 554 | 71.86 | 0.23 | 733 |
| Campesterol | C ₂₈ H ₄₈ O | 400 | 72.86 | 1.28 | 841 |
| Stigmasterol | C ₂₉ H ₄₈ O | 412 | 73.29 | 0.35 | 918 |
| Ergosta-5,24(28)-dien-3 β -ol (Chalinasterol) | C ₂₈ H ₄₆ O | 398 | 73.66 | 1.94 | 845 |
| 4,14-Dimethylergosta-8,24(28)-dien-3-ol (Obtusifolol) | C ₃₀ H ₅₀ O | 426 | 73.99 | 0.4 | 882 |
| γ -Sitosterol | C ₂₉ H ₅₀ O | 414 | 74.51 | 1.39 | 927 |
| β -Sitosterol | C ₂₉ H ₅₀ O | 414 | 74.51 | 10.01 | 916 |
| Cholest-5-en-3-ol, 24-propylidene-, (3 β)- ((E)-24-Propylidenecholesterol or 29-Methylisofucosterol) | C ₃₀ H ₅₀ O | 426 | 74.72 | 2.76 | 812 |
| Betulin | C ₃₀ H ₅₀ O ₂ | 442 | 74.72 | 0.92 | 747 |
| Ergosta-7,24(28)-dien-3-ol, 4-methyl-, (3 β ,4 α ,5 α)- (Gramisterol) | C ₂₉ H ₄₈ O | 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, (3 β ,4 α ,5 α)-) | C ₃₂ H ₅₂ O ₂ | 468 | 75.59 | 4.15 | 815 |
| 24-Methylenecycloartan-3-one | C ₃₁ H ₅₀ O | 438 | 76.73 | 3.47 | 858 |
| Stigmast-4-en-3-one (Sitostenone) | C ₂₉ H ₄₈ O | 412 | 77.08 | 3.61 | 908 |
| 19-Cyclolanostan-3-ol, 24-methylene-, (3 β)- | C ₃₁ H ₅₂ O | 440 | 77.24 | 2.57 | 919 |
| Stigmastane-3,6-dione, (5 α)- | C ₂₉ H ₄₈ O ₂ | 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

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 dabbled 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* Welw. (*Lemna minor*)

(Table 1) indicates a possible phytotoxic effect at the highest tested dose of 1000 μ g/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 μ g/mL. Although, the crude hydro-ethanol extract, ethylacetate:methanol and

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); Vanillin lactoside (7); (Z)-7-Hexadecenal (8); Massoia lactone (9); Tetradecane, 2,6,10-trimethyl- (10); Undecanoic acid, 10-methyl-, methyl ester (11); Dodecanoic acid (Lauric 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-methyl-tetradecanoate (29); Oleic Acid (30); 1-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); Virelure (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-eicosanoate (46); Eicosanoic acid, ethyl ester (47); Isooctyl phthalate (48); Glyceryl 2-oleate (49); Ethyl tetracosanoate (50); 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 (51); Vitamin E (52); Ethyl iso-allochololate (53); Rhodopin (54); Campesterol (55); Stigmasterol (56); Chalinasterol (57); Obtusifoliol (58); γ -Sitosterol (59); β -Sitosterol (60); Cholest-5-en-3-ol, 24-propylidene-, (3 β)- (61); Betulin (62); Gramisterol (63); 9,19-Cycloergost-24(28)-en-3-ol, 4,14-



dimethyl-, acetate (9,19-Cycloergost-24(28)-en-3-ol, 4, 14-dimethyl-, acetate, (3 β ,4 α ,5 α -) (64); 24-Methylenecycloartan-3-one (65); Sitostenone (66); 19-Cyclolanostan-3-ol, 24-methylene-, (3 β -) (67) and Stigmastane-3,6-dione, (5 α -) (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-Tridecenal, is known for its antibacterial activity [20]. Eugenol, which belongs to the class of allylbenzene and a naturally occurring phenolic molecule has anti-inflammatory, neuro-protective, 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 campesterol, gramisterol 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 anti-urease 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 *Spondium 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.

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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.

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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.

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