GC-MS Analysis and Antioxidant Activity of Spondias purpurea L (Anacardiaceae)

Taiwo Olayemi Elufioye¹, Tomayo Ireti Berida

ABSTRACT

Background: There are ongoing efforts to identify the chemical composition of plants used as food or medicines in order to correlate their components with the numerous claims of their medicinal usefulness in folklore. Objective: This work is aimed at profiling the phytochemical composition of *Spondias purpurea* using GC-MS, as well as to determine the total phenolic content, total flavonoid content and the antioxidant capacity by DPPH radical scavenging assay. Methods: Whole fruit and stem bark of *Spondias purpurea* were collected, dried, extracted with methanol and concentrated in vacuo before assessing them for their total phenolic content by Folin-Ciocalteu’s phenol reagent method; total flavonoid content and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities. The whole fruit and stem bark extracts were partitioned into n-hexane, dichloromethane, ethyl acetate and aqueous fractions. The n-hexane fraction of the stem bark and whole fruit were analyzed on GC-MS. Results: The stem bark had the highest phenolic content of 29.81 ± 1.18 GAE mg/g. Similarly, free radical scavenging activities assay showed the stem bark to be most active with IC₅₀ of 6.20 ± 1.51μg/ml, better than the standard, ascorbic acid with IC₅₀ of 11.51 ± 0.3μg/ml. The n-hexane partitioned fractions of the fruit and stem bark on GC-MS analysis showed 9 prominent compounds including 9,17-Octadecadienal (5.43%), 3-(4Z,7Z)-Heptadeca-4,7-dien-1-yl phenol(12%), (2Z,3)-Heptadec-10-en-1-yl) phenol (11.76%), n-Hexadecanoic acid (7.07%) and 13 compounds including 9,17-Octadecadienol (20.51%), trans-13-Octadecenoic acid (12.61%), Pentadecanoic acid (8.3%), n-Hexadecanoic acid(15.24%). Conclusions: This study provides justification for some of the folkloric use of *Spondias purpurea*. Key words: GC-MS, *Spondias purpurea*, Total Phenols, Total flavonoids, Antioxidant activity, DPPH.

INTRODUCTION

Traditional medicine has remains a vital alternative source of medicine world over especially in low income countries. It has been reported that 80% of the world population (about 4 billion people) are dependent primarily on herbal remedies and traditional medicinal practice for their health care.¹ The increasing incidence of resistance (especially to antibiotics), undesirable side effects, cost of available therapies and the better understanding of disease states has led to a renewed interest in the development of newer molecules from natural sources.² Consequently, aggressive screening of plants used in traditional medicine as well as attempt to identify and undertake chemical elucidation of the compounds responsible for their reported activities are been undertaken by scientists.

*Spondias purpurea* L. is a flowering plant in the Anacardiaceae family. It is indigenous to tropical South America but has now been naturalized in countries like Nigeria and Philippines.³,⁴ The plant produces an oval, aromatic, smooth and often glossy fruits valued for it food.⁴ In Nigeria, the plant is grown in the northern part of the country where it is referred to as Iyeye by the Okuns, Osinkara by the Ebira and Jinjere by the Nupe people. Report on this plant is scares in Nigeria and most studies on it are of South American origin. *S. purpurea* has been used to treat various diseases such as gastric disorders, diabetes and cholesterol disorder, skin infections and anemia in various traditional medicine systems.³,⁴ In Mexico, the fruits are considered to be diuretic and antispasmodic. In Nicaragua, a decoction made of the bark and leaves is used as an abortifacient and to treat malaria, fever and diarrhea. Similarly, in North Central Nigeria, a decoction of the leaves is used as diuretic and for inducing the expulsion of placenta in domestic animals like goats.

The plant has been reported to have antibacterial,⁶ antifungal,⁷ larvicidal,⁸ antioxidant, anticholines-terase¹⁰ and antiulcer genic¹¹ activities. Not much work has been done on the identification of the compounds responsible for these reported activities. Using GC-MS, we aimed to identify the phytochemicals present in the n-hexane fractions of the whole fruit and stem bark as well as determine the total phenolic content, total flavonoid content and

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Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, NIGERIA.

Correspondence
Taiwo Olayemi Elufioye
Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, NIGERIA.
Phone no : +234 80338 50773
E-mail: toonitaixo@yahoo.com

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the DPPH radical scavenging activity of the various extracts of *Spondias purpurea*.

**MATERIALS AND METHODS**

**Plant Collection**
The plant samples were collected from Oworo District of Lokoja Local Government Area, Kogi State, and North-Central Nigeria in the month of April 2016. The plant was authenticated at the Forest Herbarium Ibadan (FHI), where a voucher specimen (FHI 110431) was deposited.

**Preparation of Plants**
The stem bark was dried in the oven at 60°C after which it was pulverized while the whole fruit was dried in the oven at 40°C. Weighed portions of the various parts were extracted with 80% methanol for 72 h with regular stirring. Extracts were filtered and concentrated using rotary evaporator. The extracts were packed in previously weighed glass containers and stored in the refrigerator until use.

**Partitioning**
The methanol extracts of the various parts were partitioned into n-hexane, dichloromethane, ethyl acetate and water. The fractions were concentrated *in vacuo* at 40°C and subsequently used for the various tests.

**Determination of Total Phenol Content (TPC)**
The Folin-Ciocalteu’s phenol reagent method of determining the total phenolic content. To a mixture of 0.1 ml of sample and 0.9 ml of water was added 0.2 ml of Folin-Ciocalteu’s phenol reagent and the resulting mixture vortexed. After 5 min of standing, 1.0 ml of 7% (w/w) Na₂CO₃ solution was added and the solution was then made up to 2.5 ml before incubation for 90 min at room temperature. The absorbance against a negative control containing 1 ml of water in place of the sample was then taken at 750 nm. The standard used was the Gallic acid at 0.1, 0.08, 0.06, 0.04, 0.02 mg/ml in order to determine Gallic acid Equivalent (GAE) of sample, after preparing a calibration curve.

\[ X = q \times \frac{V}{w} \]

\[ X = \text{Total content of flavonoid compound equivalent in Gallic acid.} \]
\[ q = \text{concentration of quercetin established from the standard curve.} \]
\[ V = \text{volume of extract (ml).} \]
\[ w = \text{weight of the crude methanol extract obtained.} \]

**Determination of Total Flavonoid Content (TFC)**
This was carried out based on the aluminum chloride colorimetric assay method. To 0.1 ml of extract/standard was added 0.4 ml of distilled water. This was followed by 0.1ml of 5% sodium nitrite. After 5min, 0.1ml of 10% Aluminum Chloride and 0.2 ml of sodium hydroxide was added and the volume was made up to 2.5 ml with distilled water. The absorbance was measured at 510nm against the blank. Standard quercetin with varying concentration 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml was used as standard in comparison to the sample extract. The total flavonoid content of the plant, expressed as mg quercetin equivalents per gram of the plant extract is calculated as:

\[ X = q \times \frac{V}{w} \]

\[ X = \text{Total content of flavonoid compound equivalent in quercetin.} \]
\[ q = \text{concentration of quercetin established from the standard curve.} \]
\[ V = \text{volume of extract (ml).} \]
\[ w = \text{weight of the crude methanol extract obtained.} \]

**DISCUSSIONS**
The total phenolic and total flavonoid content of the extracts and fractions of *S. purpurea* was evaluated. The results for the whole fruit and stem bark extracts and partitioned fractions are as presented in Table 1 below. Phenols are important class of secondary metabolite with good radical scavenging capability which they possess mainly due to the OH present in their structure. The Result of TPC is as shown in Table 1. The result of TPC is as shown in Table 1. The result of TPC is as shown in Table 1.
Table 1: Total Phenolic and Total Flavonoid content of the Whole fruit and Stem bark extracts and partitioned fractions.

<table>
<thead>
<tr>
<th>Partitioned fractions</th>
<th>Total Phenolic (mg GAE/g)</th>
<th>Total Flavonoid (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole fruit extract</td>
<td>29.81±1.18 7.30 0.08</td>
<td>18.83±0.63 0.40</td>
</tr>
<tr>
<td>Aqu</td>
<td>11.75±0.91 1.83 0.63</td>
<td>26.51±2.49 3.35 0.25</td>
</tr>
<tr>
<td>EtoAc</td>
<td>18.65±0.8 0.99 0.40</td>
<td>46.88±1.23 14.97 4.60</td>
</tr>
<tr>
<td>DCM</td>
<td>19.19±1.36 2.72 0.49</td>
<td>18.93±0.77 5.89 1.58</td>
</tr>
<tr>
<td>n-Hex</td>
<td>8.317±0.25 3.15 0.27</td>
<td>4.029±0.092 1.17 0.91</td>
</tr>
</tbody>
</table>

Key: Aqu: Aqueous, EtoAc: Ethyl acetate, DCM: Dichloromethane, n-Hex: n-Hexane

TPC was calculated as the total phenolic content equivalent of gallic acid using the equation of the curve y = 4.1503x - 0.0242 R² = 0.9841. The Total Flavonoid content was likewise calculated as the total Flavonoid equivalent of Quercetin using the equation of the curve, y = 1.035x + 0.0624, R² = 0.9661. Values are presented as mg GAE/g ± S.E.M and mg QE/g ± S.E.M respectively.

Table 2: DPPH Free Radical Scavenging Activities.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole fruit</td>
<td>280.0 ± 7.1</td>
</tr>
<tr>
<td>Stem bark</td>
<td>8.3 ± 1.51</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>11.5 ± 0.3</td>
</tr>
</tbody>
</table>

IC₅₀ results are expressed as µg/mL ± SEM (Standard Error of Mean).

Table 3: GC-MS analysis and mass spectral data of the whole fruit of *Spondias purpurea* showing molecular name, molecular weight, molecular formula, retention time, percentage content, and peak areas.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Molecular Name</th>
<th>Molecular weight (g/mol)</th>
<th>Molecular Formula</th>
<th>Retention Time (min)</th>
<th>Peak Area percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pentadecanoic acid</td>
<td>242.403</td>
<td>C₁₅H₂₆O₂</td>
<td>18.725</td>
<td>3.85</td>
</tr>
<tr>
<td>2</td>
<td>n-hexadecanoic acid</td>
<td>256.43</td>
<td>C₁₆H₃₂O₂</td>
<td>19.383</td>
<td>7.07</td>
</tr>
<tr>
<td>3</td>
<td>9,12-octadecadienonic acid</td>
<td>280.452</td>
<td>C₁₆H₂₆O₂</td>
<td>20.819</td>
<td>2.98</td>
</tr>
<tr>
<td>4</td>
<td>10-octadecenoic acid</td>
<td>282.468</td>
<td>C₁₆H₂₈O₂</td>
<td>20.894</td>
<td>2.99</td>
</tr>
<tr>
<td>5</td>
<td>Methyl stearate</td>
<td>298.511</td>
<td>C₁₇H₃₅O2</td>
<td>21.168</td>
<td>1.17</td>
</tr>
<tr>
<td>6</td>
<td>9,17-octadecadienonic acid</td>
<td>264.446</td>
<td>C₁₇H₃₂O₂</td>
<td>21.443</td>
<td>5.43</td>
</tr>
<tr>
<td>7</td>
<td>3-((4Z,7Z)-heptadeca-4,7-dien-1-yl) phenol</td>
<td>328.5313</td>
<td>C₁₇H₃₅O2</td>
<td>21.689</td>
<td>12.00</td>
</tr>
<tr>
<td>8</td>
<td>(Z)-3-(heptadec-10-en-1-yl) phenol</td>
<td>330.556</td>
<td>C₁₇H₃₅O2</td>
<td>22.336</td>
<td>11.76</td>
</tr>
<tr>
<td>9</td>
<td>Phenol, 3-pentadecyl</td>
<td>304.510</td>
<td>C₁₅H₂₆O₂</td>
<td>22.868</td>
<td>2.60</td>
</tr>
</tbody>
</table>

Table 4: GC-MS analysis and mass spectral data of the n-hexane fraction of the stem bark of *Spondias purpurea* showing molecular name, molecular weight, molecular formula, retention time, percentage content, and peak areas.

<table>
<thead>
<tr>
<th>Peak Compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Molecular Formula</th>
<th>Retention Time (min)</th>
<th>Peak Area percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pentadecanoic acid</td>
<td>242.403</td>
<td>C₁₅H₂₆O₂</td>
<td>18.742</td>
<td>8.30</td>
</tr>
<tr>
<td>2</td>
<td>n-hexadecanoic acid</td>
<td>256.43</td>
<td>C₁₆H₃₂O₂</td>
<td>19.383</td>
<td>15.24</td>
</tr>
<tr>
<td>3</td>
<td>9,12-octadecadienonic acid</td>
<td>280.452</td>
<td>C₁₆H₂₆O₂</td>
<td>20.836</td>
<td>4.95</td>
</tr>
<tr>
<td>4</td>
<td>10-octadecenoic acid</td>
<td>282.468</td>
<td>C₁₆H₂₈O₂</td>
<td>20.934</td>
<td>12.61</td>
</tr>
<tr>
<td>5</td>
<td>Methyl stearate</td>
<td>298.511</td>
<td>C₁₇H₃₅O2</td>
<td>21.077</td>
<td>0.96</td>
</tr>
<tr>
<td>6</td>
<td>9,17-octadecadienonic acid</td>
<td>264.446</td>
<td>C₁₇H₃₂O₂</td>
<td>21.185</td>
<td>1.45</td>
</tr>
<tr>
<td>7</td>
<td>3-((4Z,7Z)-heptadeca-4,7-dien-1-yl) phenol</td>
<td>328.5313</td>
<td>C₁₇H₃₅O2</td>
<td>21.552</td>
<td>20.51</td>
</tr>
<tr>
<td>8</td>
<td>(Z)-3-(heptadec-10-en-1-yl) phenol</td>
<td>330.556</td>
<td>C₁₇H₃₅O2</td>
<td>21.695</td>
<td>3.17</td>
</tr>
<tr>
<td>9</td>
<td>Phenol, 3-pentadecyl</td>
<td>304.510</td>
<td>C₁₅H₂₆O₂</td>
<td>22.868</td>
<td>2.60</td>
</tr>
</tbody>
</table>
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The fractions showed the ethyl acetate fractions for both the whole fruit and stem bark as having the highest amount of 18.65407 ± 0.8 mg GAE/g and 46.88 ± 1.23 mg GAE/g respectively, higher than those of the aqueous, dichloromethane and n-hexane fractions. Flavonoids are a group of phenolic secondary metabolites with the basic structural skeleton C₆-C₅-C₆, and known to have several health benefits due to their good antioxidant activities. The total flavonoid content of the various extracts of the plant was estimated as the total flavonoid equivalent of Quercetin (Table 1). In a similar trend to that of TPC, the TFC results showed the stem bark to contained the highest flavonoids content with a value of 7.39 ± 0.08 mg QE/g while the ethyl acetate fractions also had the highest value of 14.97 ± 4.60.

The DPPH free Radical Scavenging Activities (RSA) of the extracts and fractions were also determined. The result of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities of the extracts and fractions are as presented in Table 2. Oxidative stress has been linked to several chronic diseases such as Alzheimer's disease, cancer, hypertension, atherosclerosis, diabetics, rheumatoid arthritis etc and natural antioxidant are now being considered as capable of preventing or ameliorating these diseases. The result of the DPPH free RSA assay revealed that all the parts of the plant have some antioxidant property (Table 2) with the stem bark being the highest with IC₅₀ of 8.3± 1.5 µg/mL, a value actually lower than that of the standard drug ascorbic acid (IC₅₀ of 11.5 ± 0.3µg/mL). However, the whole fruit showed the low antioxidant capacity with an IC₅₀ of 280.0 ± 7.1 µg/mL. An IC₅₀ value of 36.37µg/mL and 173.37µg/mL was reported for the seeds of S. purpurea and S. tuberosa respectively.

The phytoconstituents in the fractions of S. purpurea were analysed by GC-MS with the chromatograms as reported in Figures 1 and 2. Many compounds were identified and their chemical names, formula, molecular weight and peak areas (%) are listed as in Tables 3 and 4 with representative structures Figure 3. Nine compounds including 9, 17-octadecadienal (5.43%), 3-((4Z, 7Z)-heptadeca-4,7-dien-1-yl) phenol (12%), (Z)-3-(heptadec-10-en-1-yl) phenol (11.76%), n-hexadecanoic acid (7.07%) were identified in the n-hexane fractions of the whole fruit. Similarly, 13 compounds were detected in the stem bark n-Hexane fraction. These include 9, 17-octadecadienal (20.51%), trans-13-octadecenoic acid (12.61%), pentadecanoic acid (8.3%), n-hexadecanoic acid (15.24%). These compounds are long chain fatty acid esters, alkanal and phenols having between 9-23 carbons, generally longer and of higher molecular weight than compounds such as hexanal and 2-hexen-1-ol detected in the volatile oil of the fruit. Reported biological activities for the identified compounds showed n-hexadecanoic (palmitic acid) and pentadecanoic to possess antioxidants property and cyclooxygenase. Also, linoleic and linolenic acid were reported to possess anti-inflammatory, insecticite, hypcholesterolemic, cancer preventive, hepatoprotective and antiasthmatic properties. The essential fatty acid, 9,12,15-octadecatrienoic acid (α-linolenic acid) is reported to have neuroprotective property against soma-induced neuropathology. Perez-Pinzon and Lin reported that polyunsaturated or saturated fatty acids such as palmitic acid methyl ester and α-linolenic acid are capable of offering neuroprotection after ischemia.

**CONCLUSION**

Based on these results, the high phenolic and flavonoid contents of the stem bark extract could be responsible for its greater antioxidant activity as reflected in the DPPH free radical scavenging effect. Moreover, previous researchers have reported that phenolic and fatty acids possesses high antioxidant activity.
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The authors acknowledge the contributions of the National Institute of Technology in the identification of the chemical constituents of the plant.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATIONS

DPPH: 2, 2-diphenyl-1-picrylhydrazyl; GAE: Gallic acid Equivalent; GC-MS: Gas Chromatography-Mass Spectroscopy; NIST: National Institute of Standards and Technology; TFC: Total Flavonoid Content; TPC: Total Phenol Content.

REFERENCES