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Original Article

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

Taiwo Olayemi Elufioye*, Tomayo Ireti Berida

ABSTRACT

Background: There are ongoing efforts to identify the chemical composition of plants used as food or medicines in other 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 vacou 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 IC_{50} of 6.20 ± 1.51µg/ml, better than the standard, ascorbic acid with IC_{50} 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%), (Z)-3-(Heptadec-10-en-1-yl) phenol (11.76%), n-Hexadecanoic acid (7.07%) and 13 compounds including 9,17-Octadecadienal (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.

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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.[3-5] The plant produces an oval, aromatic, smooth and often glossy fruits valued for it food.[6] 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.[7-8] 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,[9] antifungal,[10] larvicidal,[11] antioxidant, anticholinesterase[12] and antiulcer genic[13] 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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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 test.

Determination of Total Phenol Content (TPC)

The Folin-Ciocalteu's phenol reagent method of determining the total phenolic content.[14] 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 voltexed. After 5 min of standing, 1.0 ml of 7% (w/w) Na_2CO_3 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.-

$$X = q * \frac{V}{w}$$

X = Total content of flavonoid compound equivalent in Gallic acid.

q = concentration of quercetin established from the standard curve.

V = volume of extract (ml).

w = 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.[15] 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:

$$X = q * \frac{V}{w}$$

X = Total content of flavonoid compound equivalent in quercetin.

q = concentration of quercetin established from the standard curve.

V = volume of extract (ml).

w = weight of the crude methanol extract obtained.

DPPH radical scavenging assay

The radical scavenging ability of the various extracts was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate).[16] To 1 ml of different concentrations of the samples or standard (vitamin C) in a test tube was added 1 ml of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30 min after which the absorbance was read at 517 nm against a DPPH blank containing only 1 ml methanol in place of the extract.

The percent of inhibition was calculated using the following formula:

$$1\% = \frac{A_{blank} - A_{sample}}{A_{sample}} \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The concentration of sample providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against concentration of extract.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The n-hexane fractions of the whole fruit and stem bark were subjected to GC/MS analysis on an Agilent 5975C mass spectrometer coupled with model 7890A gas chromatograph under the following conditions: HP5MS column, 30 m x 0.32 mm (internal diameter) and thickness 0.25 µm with helium (99.9999% purity, flow rate = 1.4963 mL/min; average velocity = 45.618 cm/s) as carrier gas. 1 µL of 0.2 g/mL fraction was injected. A 7683B injector type was used in a split less mode; GC temperature program, 80 - 290°C at 10°C/ min (5 min. initial hold); Total run time of 28min. The mass spectra were recorded in electron ionization (EI) mode at 70 eV.

Identification of phytochemicals and interpretation of mass spectrum GC-MS was conducted by comparing retention time and mass spectra with those of authentic compounds using the National Institute of Standards and Technology (NIST) 14 Mass Spectral Library, Washington, DC, USA. The name, molecular weight, retention time and structure of the components of the test materials were ascertained and reported.

Statistical analysis

All determinations were done in triplicate, and the results reported as mean ± standard error of mean (S.E.M.). The calculation of IC_{50} values was done using Microsoft Excel 2010.

RESULTS

The total Phenolic and Total Flavonoid content of the Whole fruit and Stem bark extracts and partitioned fractions are as reported in Table 1 while Table 2 showed the DPPH Free Radical Scavenging Activities of the extracts.

The chemical analysis was also carried out. Table 3 and 4 shows the GC-MS analysis and mass spectral data of the n-hexane fraction of the whole fruit of the extracts showing molecular name, molecular weight, molecular formula, retention time, percentage content, and peak areas.

DISCUSSIONS

The total phenolic and total flavonoid content of the extracts and fractions of *S. purpurae* 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

Table 1: Total Phenolic and Total Flavonoid content of the Whole fruit and Stem bark extracts and partitioned fractions.

Partitioned fractions	Total Phenolic (mg GAE/g)	Total Flavonoid (mg QE/g)
Whole fruit extract 9.09 + 0.40 3.83 + 0.10		
Aqu	11.75 ± 0.91	1.83 ± 0.63
EtoAc	18.65 ± 0.8	3.99 ± 0.40
DCM	19.19 ± 1.36	2.72 ± 0.49
n-Hex	8.317 ± 0.25	3.15 ± 0.27
Stem bark extract 29.81 + 1.18 7.30 + 0.08		
Aqu	26.51 ± 2.49	3.35 ± 0.25
EtoAc	46.88 ± 1.23	14.97 ± 4.60
DCM	18.53 ± 0.77	5.89 ± 1.58
n-Hexane	4.029 ±0.092	7.42 ± 1.63

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 R^2 = 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, R^2 = 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.

Plant parts	IC_{50} (µg/mL)
Whole fruit	280.0 ± 7.1
Stem bark	8.3 ± 1.51
Ascorbic Acid	11.5 ± 0.3

IC_{50} results are expressed as µg/mL ± SEM (Standard Error of Mean).

showed the whole fruit and the stem bark contain 9.01 ± 0.4 and 29.81± 1.18 mg GAE/g of phenol respectively. Different TPC values have been reported for various species of *Spondias*. Values of 55.0 mg and 44.6 mg of GAE/100 g for *S. purpurea* and *S. tuberosa* fruit pulp respectively have been reported.[17] A higher value of 112.2±13.2, 254.7±42.1 and 13.5±1.3 mg GAE/g for the peel kernel and pulp of *S. purpurea* has also been reported.[12] *S. mombin* was reported to contain 260.21±11.89 mg GAE/100g in the fruit pulp,[18] 573.32 mg of GAE/100g in the kernel,[19] and 213.50 ± 1.25 mg GAE/g polyphenol content in the leaves.[20] It is important to note that the Folin–Ciocalteu reagent assay is not specific for phenolic compounds since it can also be reduced by many non-phenolic compounds such as ascorbic acid.[12] The result for the TPC of

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

Serial No.	Molecular Name	Molecular weight (g/mol)	Molecular Formula	Retention Time (min)	Peak Area percent
1	Pentadecanoicacid[a]	242.403	$C_{15}H_{30}O_2$	18.725	3.85
2	n-hexadecanoicacid[b]	256.43	$C_{16}H_{32}O_2$	19.355	7.07
3	9,12-cctadecadienoic acid[c]	280.452	$C_{18}H_{32}O_2$	20.819	2.98
4	10-octadecenoic acid	282.468	$C_{18}H_{34}O_2$	20.894	2.99
5	Methyl stearate[d]	298.511	$C_{19}H_{38}O$	21.168	1.17
6	9,17-octadecadienal[h]	264.446	$C_{18}H_{32}O$	21.443	5.43
7	3-((4Z,7Z)-heptadeca-4,7-dien-1-yl) phenol[e]	328.5313	$C_{23}H_{36}O$	21.689	12.00
8	(Z)-3-(heptadec-10-en-1-yl) phenol	330.556	$C_{23}H_{38}O$	22.336	11.76
9	Phenol, 3-pentadecyl[f]	304.510	$C_{21}H_{36}O$	22.868	2.60

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.

Peak Compound	Molecular formular	Molecular weight	Molecular Formula	Retention Time (min)	Peak Area percent
1	Pentadecanoicacid[a]	242.403	$C_{15}H_{30}O_2$	18.742	8.30
2	n-hexadecanoicacid[b]	256.43	$C_{16}H_{32}O_2$	19.383	15.24
3	Ethyl 5-methylhexanoate	158.238	$C_9H_{18}O_2$	19.583	0.82
4	9,12-octadecadienoic acid[c]	280.452	$C_{18}H_{32}O_2$	20.836	4.95
5	trans-13-octadecenoic acid	282.468	$C_{18}H_{34}O_2$	20.934	12.61
6	13-octadecenoic acid	282.468	$C_{18}H_{34}O_2$	21.077	0.96
7	Methyl stearate	298.511	$C_{19}H_{38}O$	21.185	1.45
8	9,17-octadecadienal[h]	264.446	$C_{18}H_{32}O$	21.552	20.51
9	9,12,15-octadecatrienoic acid[g]	278.436	$C_{18}H_{30}O_2$	21.695	3.17
10	3-pentadecyl- phenol			23.091	1.48
11	Methyl 13-eicosenoate	324.549	$C_{21}H_{40}O_2$	23.531	2.27
12	(Z)-3-(pentadec-8-en-1-yl)phenol			26.095	4.59
13	Phenol, 3-tridecyl-	276.464	$C_{19}H_{32}O$	26.535	12.92

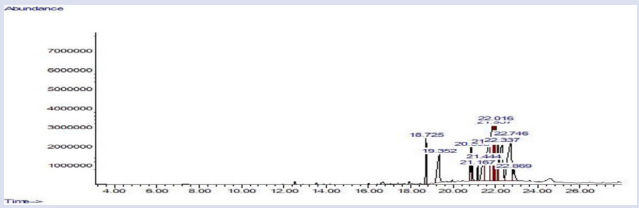

Figure 1: Chromatogram for the Gas Chromatography of n-hexane fraction of the whole fruit.

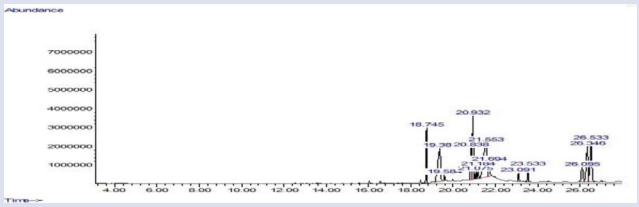

Figure 2: Chromatogram for the Gas Chromatography of n-hexane fraction of the stem bark.

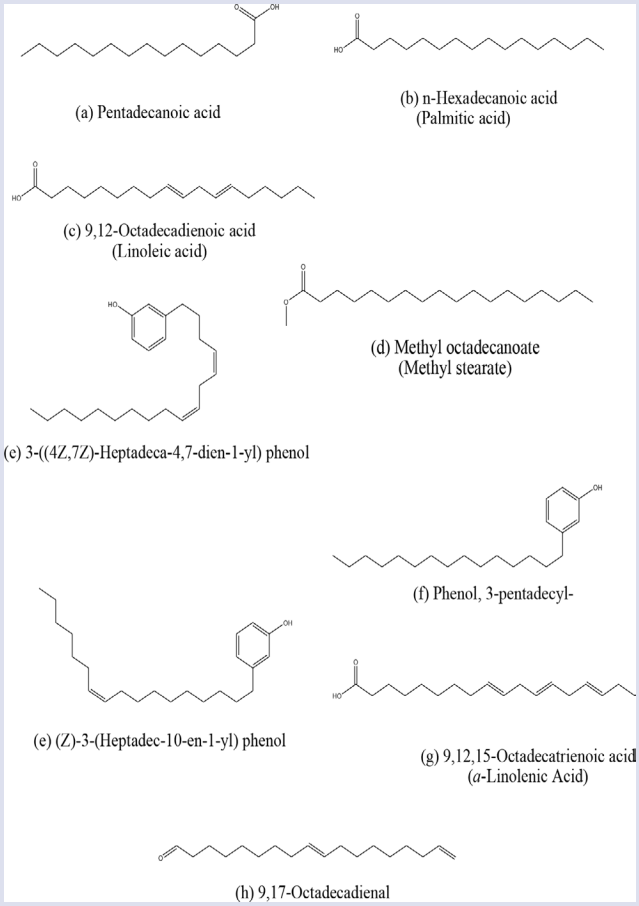

Figure 3: Structures of some compounds identified in *Spondias purpurea* through CG-MS.

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 C_6-C_5-C_6, 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-picryl-hydrazyl (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.[21] 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 IC_{50} of 8.3± 1.5 µg/mL, a value actually lower than that of the standard drug ascorbic acid (IC_{50} of 11.5 ± 0.3µg/mL). However, the whole fruit showed the low antioxidant capacity with an IC_{50} of 280.0 ± 7.1 µg/mL An IC_{50} value of 36.37µg/mL and 173.37µg/mL was reported for the seeds of *S. purpurea* and *S. tuberosa* respectively.[12]

The phytoconstituents in the fractions of *S. purpurae* 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.[5] Reported biological activities for the identified compounds showed n-hexadecanoic (palmitic acid) acid and pentadecanoic to possess antioxidants property and cyclooxygenase.[23-25] Also, linoleic and linolenic acid were reported to possess anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective and antihistaminic properties.[26-28] The essential fatty acid, 9,12,15-octadecatrienoic acid (α-linoleic acid) is reported to have neuroprotective property against soma-induced neuropathology.[29] Perez-Pinzon and Lin[30] 2017 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.[31] Moreover, previous researchers have reported that phenolic and fatty acids possesses high antioxidant activity.[25,32-33]

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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.

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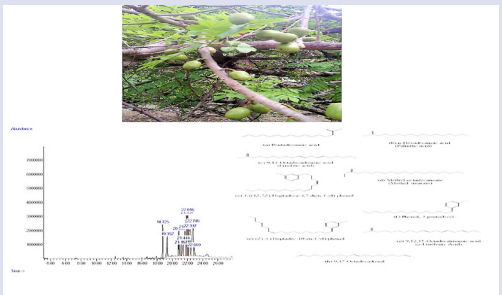
GRAPHICAL ABSTRACT



SUMMARY

Spondias purpurae possesses antioxidant properties that can be justified by the chemical compounds identified in its various fractions.

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