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Original Research Article

Antioxidant and Anti-inflammatory Activities of Extract and Fractions of *Spondias mombin* Leaf and Isolation of its Active Principles

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ABSTRACT

Spondias mombin L. (Anacardiaceae) leaves have shown remarkable efficacy in the ethnomedicinal management of inflammatory disorders. This study aims to examine the antioxidant and anti-inflammatory potentials of the plant material as well as identify its major phenolic components. The crude methanol extract (CME) and fractions [n-hexane (HF), dichloromethane (DF), ethyl acetate (EF), acetone (AF) and methanol (MF) fractions] of *S. mombin* were assessed for their antioxidant and anti-inflammatory effects using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method and egg-albumin induced rat paw edema model respectively. The total phenolic contents of the extract and fractions were quantified spectrophotometrically and some of the compounds were identified with the aid of HPLC, LC-ESI-MS/MS, and ^{1}H-NMR. CME, HF, DF, EF, AF, and MF exhibited dose-dependent DPPH scavenging property with IC_{50} values of 20, 350, 21, 78, 60, and 8 µg/mL, respectively. The activity of the methanol fraction was comparable to that of the regular drug, vitamin C (IC_{50} = 9.4 µg/mL). The crude extract and fractions, at the dose of 400 mg/kg showed poor anti-inflammatory activity in the rat model. EF exhibited the highest content of phenolic compounds with a percent gallic acid equivalent of 20.57%. The plant phenolic compounds identified were mainly gallocatechin, kaempferol, and quercetin glycosides and their methoxy products as well as gallic and ellagic acid derivatives. The outcomes show that the leaves of *S. mombin* contain antioxidant phenolic compounds which could explain the remarkable efficacy in the ethnomedicinal management of oxidative stress, but had poor anti-inflammatory activity.

Keywords: *Spondias mombin*, Anti-inflammatory, Medicinal plant, Phenolic compounds, Free radicals.

Introduction

Spondias mombin L. (Anacardiaceae), normally acknowledged as hog plum and locally called 'ijikara' in Igbo (Igbo is a language spoken by tens of millions in Nigeria), is a deciduous vertical tree that is 15-20 m tall with a stem size of 60 to 75 cm wide-range. The plant is found in tropical America, West Indies and has remained adapted in states of Africa, including Nigeria and some parts of Asia.[1,2] This is utilized traditionally for the management of several diseases. The leaves are helpful in the cure of bacterial contagions, prevention and inhibition of the progression of viral contagions, management of *Candida* contagions, and for expulsion of parasites like the abdominal worms. They are also utilized for the reduction of seizures and nervousness, demurring and discharging pain, subduing cough, aiding digestion and stimulating the uterus.[1] The bark is essential in the reduction of inflammation and spasms, healing of and wounds and rashes, stoppage of bleeding, relieving pains, eradicating bacteria and fungi. It also serves as a contraceptive.[1,3]
Aqueous ethanol leaf extract of *S. mombin* was reported to exhibit anti-conceptive property as a result of the extract activity on the uterus.[1,3]

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The leaves have also been reported to exhibit anthelmintic,[1,4] antibacterial, molluscicidal,[1,5] and haematinic activities.[6] The sedative, antiepileptic, anxiolytic, anthelmintic, hypoglycaemic, antioxidant, antimicrobial and antipsychotic effects have also been investigated and documented.[1,7] Nworu *et al.* (2011) demonstrated its association with the defeat of pro-inflammatory intermediaries like NO and TNF-α may be geared towards the imaginable mechanism underlying the observed anti-inflammatory action of the extracts from the leaf in the rat model. *S. mombin* has aromatic and astringent qualities.[3]
In a previous study, we have shown the larvicidal, adulticidal and repellent activities of the extract/fractions of the leaf of *S. mombin*.[8,9,10] In this study, we investigated the acute toxicity of the crude extract, antioxidant and anti-inflammatory properties of the crude methanol extract and fractions of the *S. mombin* leaves, identify the chemical constituents present in the fractions from *S. mombin* leaves.

Materials and Methods

Sample collection and preparation
Fresh leaves of *S. mombin* were collected from their natural habitat in Ezinano-Agulu, Anambra State of Nigeria, in June 2011. They were authenticated by a taxonomist, Mr. Alfred Ozioko of Bio-resources Development and Conservation Program (BDCP), Nsukka, Enugu State. A voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Anambra State, under the number PTM04/002. The leaves were cleaned and dried under room temperature to constant weight for two weeks. The dried leaves were pulverized into a fine powder using a mechanical grinder. Powdered materials were maintained at room temperature (25-27°C), protected from light until required for extraction and analysis.[10]

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Chemicals and reagents
Analytical grade of methanol, n-hexane, ethyl acetate, dichloromethane, acetone (Sigma Aldrich) were used. Other chemicals used are DPPH, silica gel, and Tween 80 (BDH). Pre-coated TLC plates (Silica Gel 60 F254, layer thickness 0.2mm) Merck, silica gel (70-230 mesh) and Sephadex LH-20 25−100 μm mesh size Merck, methanol was LiChroSolv HPLC (Merck), nano-pure water (distilled and heavy metals free water) was obtained by passing distilled water through nano- and ion exchange filter cells (Barnstead, France). Deuterated methanol and Dimethyl sulphoxide (Uvasol, Merck) were used for NMR measurements. Whatman No. 1 filter paper was also used. All laboratory reagents were newly prepared and newly distilled water were utilized.

Equipment
The equipment used includes 6505 UV-VIS spectrophotometer (Jenway, UK), analytical HPLC (Dionex, Germany), LC-ESI-MS (ThermoFinningan LCQ-Deca mass spectrometer, Germany), AVANCE DMX-600 MHz, Bruker, Germany, rotary evaporator, RE300 (Stuart, Barloworld Scientific Ltd, Stone, Staffordshire, UK), fraction collector (Retriever II, ISCO, Germany).

Extraction and pre-chromatographic fractionation
This was "carried out as previously described, but with minor modifications."[10] The pulverized leaves (590 g) were extracted for 3 days by cold maceration in methanol with intermittent shaking. The maceration process was then repeated two times for exhaustive extraction. The combined methanol extracts were concentrated to dryness under vacuum at 40°C using a rotary evaporator. The crude methanol extract was screened for acute toxicity and acute anti-inflammatory activity. The crude methanol extract (CME) was thereafter absorbed on silica gel and sequentially extracted using n-hexane (HF), dichloromethane (DF), ethyl acetate (EF), acetone (AF) and methanol (MF). All the fractions were filtered using Whatman No. 1 filter paper and concentrated *in vacuo* using a rotary evaporator. The fractions were stored in the refrigerator at 4°C before use.

Chromatographic separation of ethyl acetate fraction
Preparation of Sephadex LH-20 gel
100 g of Sephadex LH-20 was dispersed in 200 mL of methanol and the mixture was sonicated for 30 min. The slurry was transferred into a column (50 × 2 cm, length × internal diameter) and the gel allowed to stabilize for 5 h before use.

Gel chromatographic separation of the ethyl acetate
150 mg of EF was reconstituted in 2 mL of methanol and the solution was sonicated for 5 min and then centrifuged (10 000 rpm) for 10 min to remove undissolved solid particles. The supernatant was pipetted and introduced into the Sephadex LH-20 column and eluted with 100% methanol with the flow rate adjusted to approximately 0.2 mL/min. 100 fractions of 2 mL each were collected with the aid of fraction collector (Retriever II, ISCO, Germany) and monitored with TLC on silica gel G_{254} developed with dichloromethane: methanol, 4:1. Similar fractions were combined and concentrated with a rotary evaporator to obtain fractions EF1 to EF7.

Quantification of phenolic compounds of crude extract and fractions
This was "carried out as previously described, but with minor modifications."[11]

HPLC analysis of the Sephadex fractions
This was carried out as formerly described.[9]

LC-ESIMS analysis of the Sephadex fractions
This was "carried out as previously described, but with minor modifications."[9]

Nuclear Magnetic Resonance Spectroscopy (NMR)
The ^{1}H-NMR spectrum was recorded at 300ºK on ARX 600 or NMR spectrometers. The sample was dissolved in a deuterated methanol, the selection was reliant on the solubility of the test sample. Tetramethylsilane (TMS) was utilized as the core reference signal. The experimental chemical shifts (δ) were noted in ppm and the coupling constants (*J*) were documented in Hz.

Pharmacological test animals
Wister rats and albino mice gotten from the laboratory animal facilities of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria were utilized for the assay. The animals were kept in usual conditions (25°C±1°C and 12 hr light/dark cycle). They were nourished with regular rodent pills (Vital Feed, Nigeria) and had unlimited contact to clean drinking water. All animal experiments were in agreement with the National Institute of Health Guide for Care and Used of Laboratory Animals (Pub. No. 85-23, revised 1985, as defined in procedures revised and approved by the Nnamdi Azikiwe University Institutional Animal Care and Use Committee (NAU/FPS/PHAT/017-21)

Acute toxicity tests
The LD_{50} of the crude methanol extract was evaluated as publicized previously.[12]

Acute-anti inflammation using egg albumin induces paw edema in rats
The test was evaluated as publicized previously.[13] The animals (n=5, per group) were fasted for 5 h and deprived of water only during the experiment. They were given intraperitoneal (i.p.) injection of the extracts at doses of 200 and 400 mg/kg. Control animals received 0.4 ml of 10% Tween 80 or 100 mg/kg aspirin. All the substances were administered i. p. 30 min before the subplantain injection of the phlogistic agent (0.1 ml of fresh undiluted egg albumin) in the rats. Paw volumes were measured by water displacement method at 0, 1, 2, 3 and 4 h after induction of edema. The anti-inflammatory effect was calculated at each time of observation as percent inhibition of edema in the animals treated with the substances under test in comparison with the vehicle-treated animals. The percent inhibition of edema was calculated using the formula

$$\% \text{ Inhibition} = \frac{(Vo - Vt)}{Vo} \; x \; 100$$

V_t is the volume of edema at the corresponding time, and
V_O is the volume of edema of vehicle-treated rats at the same time.

DPPH Free radical scavenging activity test on the extract and fractions
The free radical scavenging activity of the extract and fractions of *S. mombin* was assessed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) model.[14,15] DPPH solution (0.6 mM) was freshly prepared in methanol and 0.5 ml of this solution was mixed with 0.5 ml of different dilutions of the extract and fractions. The volume of the solution was adjusted with methanol to a final volume of 5 ml. After incubation in the dark for 30 min at room temperature, the absorbances of the mixtures were measured at 520 nm against methanol as blank using UV-spectrophotometer. Ascorbic acid was used as a standard. Mean absorbances were calculated from triplicate measurements.
The antioxidant activities of the extract and fractions were evaluated by comparing their absorbances with that of the control (containing 0.5 ml of DPPH solution and 4.5 ml of methanol). The free radical scavenging activity was quantified using the relationship shown below.

DPPH scavenging activity = 100 x [(AC – AS)/ AC]
AC = Absorbance of control
AS = Absorbance of Sample

The concentrations that produce 50% inhibition (IC_{50}) of free radicals were also deduced from the dose-response curve.

Statistical analysis
The values obtained in triplicates were represented as the mean ± standard error of the mean (SEM). The data analyzed by ANOVA using SPSS 20, the significance level was set at $p < 0.05$.

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Results and Discussion

Quantification of total phenolic compounds

S. mombin leaves are part of the feed-stocks fed to the native animals in South Eastern Nigeria. The fresh leaves can be cooked green vegetables.[16] Pilot phytochemical study as publicized previously shown the existence of saponins, alkaloids, and flavonoids. These phytochemicals have been shown in previous reports to be responsible for the various biochemical and pharmacological activities of the leaves when consumed by animals.[17] The total phenolic contents of the extract and fractions as quantified by gallic acid equivalent are shown in Figure 1. The CME showed high content of phenolic compounds with a percent gallic acid equivalent of 69.5%. Of all the fractions, EF showed the highest content of phenolic compounds (20.57±2.50% gallic acid equivalent) while the HF showed the least content of phenolic compounds (0.57±1.07% gallic acid equivalent).

Detected compounds and structures

The extract and the fractions were thus investigated for their phenolic compound contents. As shown in Figure 1, the CME showed a very high phenolic compound content. Amid the fractions, the EF showed the highest phenolic compound content followed by DF. We further subjected the EF to Sephadex LH-20 gel separation and the separated fractions were subsequently analyzed by LC-ESI-MS/MS. The MF, which showed the highest antioxidant activity, was also analyzed by LC-ESI-MS/MS. We identified a total of 12 phenolic compounds (Table 1) with the help of their MS fragments and in association with literature standards. Their chemical structures are displayed in Figure 2.

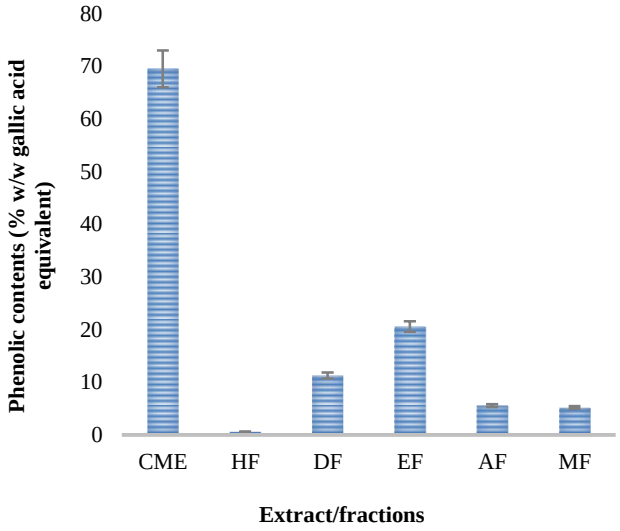

Figure 1: Total phenolic contents of the extract and fractions. CME: Crude methanol extract, HF: hexane fraction, DF: dichloromethane fraction, EF: ethyl acetate fraction, AF: acetone fraction and MF: methanol fraction.

Table 1: Summary of the LC-MS data of the detected compounds

S/N	Molar mass	Name	MS Diagnostic peaks	Mode of identification
1	306	Gallocathechin	307 $[M+H]^+$; 306 $[M-H]^-$	UV, HPLC, LC-ESI/MS
2	580	Kaempferol-3-O-β-D-xylopyranosyl(1→ 6) β-D-glucopyranoside	580.9 $[M+H]^+$; 604 $[M+Na]^+$; 579.3 $[M-H]^-$, 448.9 [M-Xylose $+H]^+$ 287.3 [M-Xylose-Glucose$+H]^+$	UV, HPLC, LC-ESI/MS
3	462	3',4'-Dimethoxy- 5, 7-dihydroxyflavone-3-O-β-D –xylopyranoside	463 $[M+H]^+$; 485 $[M+Na]^+$; 461.2 $[M-H]^-$; 331.2 [M-xylose $+H]^+$	UV, HPLC, LC-ESI/MS
4	301	Ellagic acid	605 $[2M-H]^-$, 301 $[M+H]^+$	UV, HPLC, LC-ESI/MS, ^{1}H NMR
5	448	Kaempferol-3-O β-D-glucopyranoside	448.9 $[M+H]^+$; 447.3 $[M-H]^-$; 287.3 [M-Glucose $+H]^+$	UV, HPLC, LC-ESI/MS
6	478	4',5,7-Trimethoxyflavone-3-O-α-L-rhamnopyranoside	478.8 $[M+H]^+$; 477.2 $[M-H]^-$; 333.2 [M-Rhamnose$+H]^+$; 287.2 [M-Rhamnose $-3CH_3]^+$	UV, HPLC, LC-ESI/MS
7	464	Quercetin-3-O- β-D-glucopyranoside	464.9; $[M+H]^+$; 463.2 $[M-H]^-$; 303.3 [M-Glucose + $H]^+$	UV, HPLC, LC-ESI/MS
8	434	Quercetin-3-O- β-D-xyloopyranoside	434.9 $[M+H]^+$; 433.2 $[M-H]^-$; 303 [M-Xylose$+H]^+$	UV, HPLC, LC-ESI/MS
9	434	Quercetin-3-O- β-D-xyloopyranoside	434.9 $[M+H]^+$; 433.2 $[M-H]^-$; 303 [M-Xylose$+H]^+$	UV, HPLC, LC-ESI/MS, ^{1}H NMR
10	418	Kaempferol-3-O-β-D-xylopyranoside	418.9 $[M+H]^+$; 417.3 $[M-H]^-$	UV, HPLC, LC-ESI/MS
11	434	Ellagic acid -3- O-β-D-xylopyranoside	434.9 $[M+H]^+$; 457 $[M+Na]^+$; 433.2 $[M-H]^-$ 303.3 [M-Xylose$+H]^+$	UV, HPLC, LC-ESI/MS
12	634	A glucosylated ellagic acid and gallic acid derivative	635.2 $[M+H]^+$, 633.2 $[M-H]^-$ 464.9 [M-gallic acid$+H]^+$ 301[M-gallic acid-glucose -H]	UV, HPLC, LC-ESI/MS

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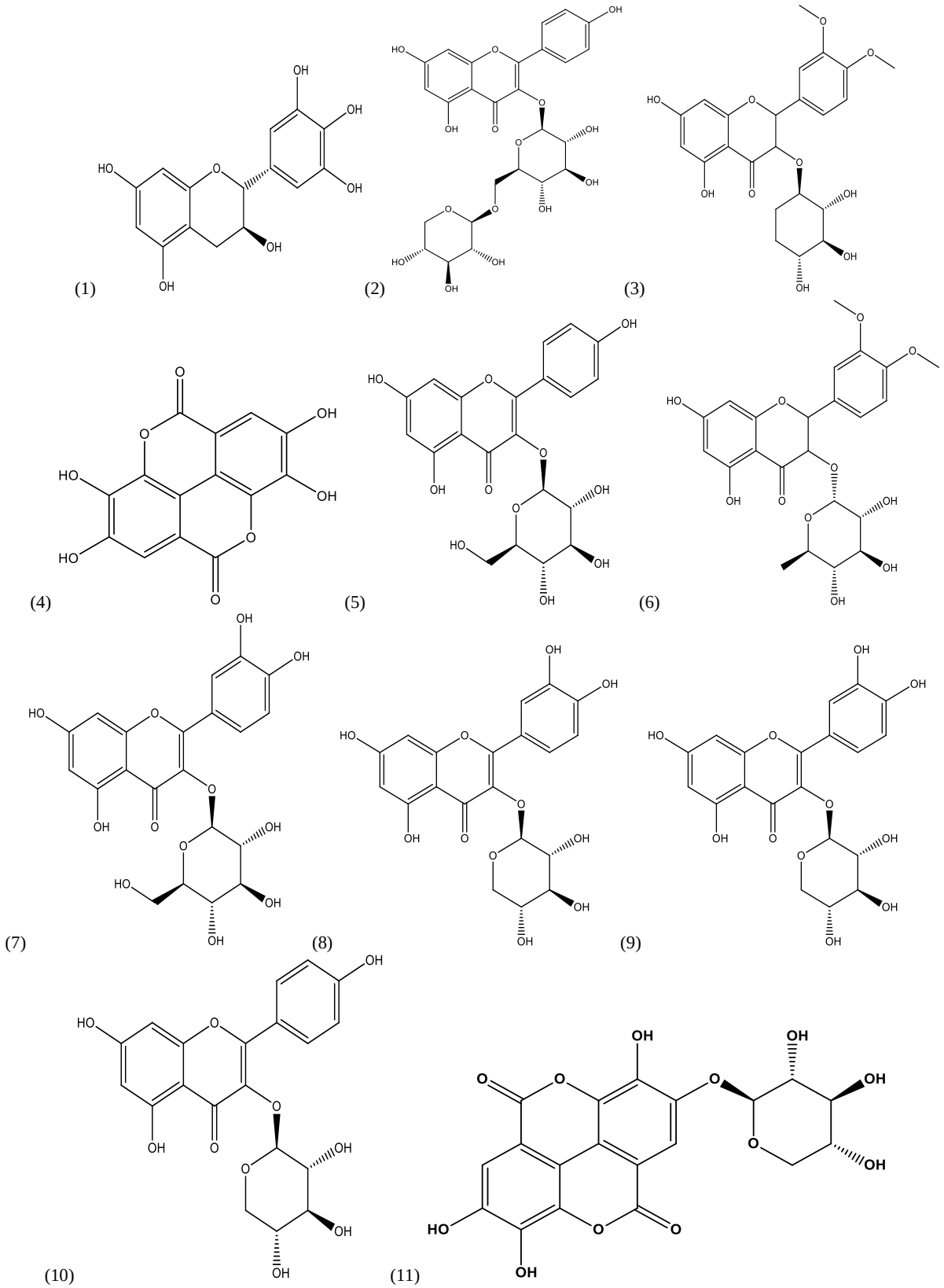

Figure 2: Structures of some of the detected compounds.

The structures were elucidated based on their UV, ^{1}H NMR, and mass spectrometric data. The identification of the compounds by HPLC-ESI/MS provided additional supporting evidence for the compounds. Compound **4** was obtained as a brownish powder. The mass spectral data (retention time 29.77 min) show molecular ion peak at m/z 301, in agreement with the molecular formula of $C_{14}H_5O_8$. Its UV spectrum (λmax 248, 320, 334, 358, 364) was similar to that of ellagic acid[18] suggesting that compound **4** is ellagic acid. The ^{1}H-NMR spectrum exposed two protons as singlets at δ 7.44 and 7.44, assignable to H-2 and H-2', respectively as shown in Table 2.

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Table 2: ^{1}H-NMR assignment for compound 4

Position	δH	Literature value
1	-	-
2	7.44 s	7.46 s
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
1'	-	-
2'	7.44 s	7.46 s
3'	-	-
4'	-	-
5'	-	-
6'	-	-
7'	-	-

Table 3: ^{1}H-NMR assignment for compound 9

Position	δH	Literature value
2		
3		
4		
5		
6	6.18 s	6.19 d (1.8)
7		
8	6.37 s	6.40 d (1.8)
9		
10		
1'		
2'	7.74 s	7.58 m
3'		
4'		
5'	6.87 d (8.5)	6.84 d (9.0)
6'	7.58 dd (8.5)	7.58 m
1"	5.14 d (7.8)	5.46 d (7.2)
2"	3.91	3.09 – 3.58 (m)
3"	3.64	3.09 – 3.58 (m)
4"	3.81	3.09 – 3.58 (m)
5"	3.47 Ha	3.09 – 3.58 (m)
	3.85 Hb	3.09 – 3.58 (m)

Compound **9** was obtained as a yellowish powder. The diagnostic mass fragments achieved by HPLC-ESI/MS in the negative mode at 301 characterized the aglycone as quercetin. The neutral loss of 132 mass units allowed the identification of xylose (a pentose). The results of HPLC analysis of the compound show chromatogram peaks with bands at 240-285 nm and 350-380 nm which are characteristic of flavonoids. The ^{1}H NMR spectrum for compound **9** is unwavering with the derived data from MS. ^{1}H-NMR data of compound **9** come to an agreement with the usual quercetin chemical shifts. The anomeric proton at δ 5.14 (J = 7.8 Hz) indicated the presence of ß-D-xylopyranoside[19] as shown in Table 3. The isolated compounds were identified as ellagic acid (compound **4**) and quercetin 3-*O*-ß-D-xylopyranoside (compound **9**) respectively. Flavonoids containing xylose in the sugar part are rare in nature.

Acute toxicity study

The acute toxicity for the crude extract is 2449.5 mg/kg. The crude extract can be classified as slightly toxic.[20] The CME was also orally administered at different doses for acute toxicity and no mortality was observed up to the fourteenth day of monitoring at 2000 mg/kg. Further observation of animals for another 14 days did not reveal any sign of late toxicity of the extract.

Inhibition of egg albumin induced paw edema in rats

The results of the outcome of the extract and fractions on egg albumin induced paw edema in rats are shown in Table 4.

The EF, however, exhibited a dose-dependent inhibition of inflammation at 4 h. The crude methanol extract and the fractions showed poor inhibition of egg-albumen induced paw edema at 200 and 400 mg/kg. Investigation of the acute anti-inflammatory effect in rodents displayed that the extract and the fractions exhibited very poor anti-inflammatory effect at the doses of 200 and 400 mg/kg when compared with the positive control - aspirin and also the negative control – 10% Tween 80. This result somewhat contradicts an initial report of the strong anti-inflammation activity of the methanol extract in carrageanan induced paw edema in rats.[7]

Human cells are constantly exposed to the destructive outcomes of reactive oxygen types, which include superoxide, hydroxyl radicals, singlet oxygen, peroxynitrite and peroxyl radicals. These result in oxidative pressure, which is connected to ischemic injury, atherosclerosis, aging, inflammation, neurodegenerative diseases and cancer.[11,25,26] Flavonoids and phenolics, in general, may help protect against these diseases by donating, alongside with antioxidant enzymes and vitamins, to the antioxidant defensive mechanism of the human frame.[27] The beneficial effect of S. mombin leaves in the managing of disease states linked with oxidative stress may be related to their possible make-up of antioxidant flavonoids and other phenolic compounds.

DPPH free radical scavenging activity

The antioxidant activity of the extract and fractions was carried out using the DPPH scavenging model. DPPH is a steady organic nitrogen radical, which has a deep purple color in methanol solution.[21] The free radical scavenging evaluation determines the reducing capability of antioxidants toward DPPH. Once reduced, the color of the DPPH solution diminishes. As a result, test samples with elevated antioxidant capacity result in a quick drop in the absorbance of the solution.[22,23]

The effect of antioxidants on DPPH may be attributed to their hydrogen donating ability. Previous studies of *S. mombin* showed good antioxidant activity using DPPH scavenging model. According to the studies, the hydroethanol extract and butanol fraction of *S. mombin* leaves exhibited a potential donation of electrons or H^{+} ions with values within the range of 66% to 76%.[24]

The results of the antioxidant screening are shown in Figure 3. The CME showed high DPPH radical scavenging activity with an IC_{50} value of 20 µg/mL. All the fractions indicated variable amounts of DPPH scavenging property with the activity of MF (IC_{50} = 8 µg/mL) equivalent to that of regular drug, ascorbic acid (IC_{50} = 9.4 µg/mL), which is the positive control. The IC_{50} values calculated from the dose-response curve of the pure elucidated compounds are shown in Figure 4. The activity of compound 9 (IC_{50} = 11.8 µg/mL) was similar to that of the regular drug, ascorbic acid (IC_{50} = 9.4 µg/mL). This is in agreement with a study carried out[28] using the ethanol leaf extract of *S. mombin*, where the DPPH scavenging property was comparable with the regular drug – ascorbic acid. A positive result was also observed for the extracts/fractions of the leaf of *S. mombin* using in vitro DPPH scavenging assay.[27] There was a connection between the phenolic content with the antioxidant property of the extract and/or fractions. In this study, the high quantity of the phenolic compounds gave rise to high antioxidant properties.

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Table 4: Egg albumin induced paw edema in rats

Treatment	Dose (mg/kg)	Mean edema (ml, mean ± SEM)			
		1hr	2hr	3hr	4hr
CME	400	1.12 ± 0.09 (0)^{c}	1.12 ± 0.09 (0)^{c}	1.12 ± 0.09 (0)^{f}	0.96 ± 0.10 (14.2)^{e}
HF	400	1.12 ± 0.04 (3.4)^{c}	1.0 ± 0.06(13.8)^{c}	0.88 ± 0.04(24.1)^{b}	0.88 ± 0.04 (24.1)^{c}
DF	200	1.36 ± 0.09 (0)^{b}	1.2 ± 0.10(11.8)^{d}	1.08 ± 0.07(20.6)^{e}	1.08 ± 0.07 (20.6)^{g}
DF	400	1.2 ± 0.06 (0)^{d}	1.0 ± 0.06 (16.7)^{c}	0.92 ± 0.04 (23.3)^{c}	0.84 ± 0.07 (30)^{b}
EF	200	1.4 ± 0.06 (0)^{f}	1.32 ± 0.04 (5.7)^{e}	1.16 ± 0.07 (17.1)^{g}	1.12 ± 0.07 (20)^{h}
EF	400	1.16 ± 0.07 (9.4)$^{c, d}$	1.0 ± 0.06 (21.9)^{c}	0.88 ± 0.04 (31.3)^{b}	0.88 ±0.04 (31.3)^{c}
AF	400	1.52 ± 0.07 (7.3)^{g}	1.24 ± 0.04 (24.4)^{d}	1.16 ± 0.07 (29.3)^{g}	1.12 ± 0.07 (31.7)^{h}
MF	200	1.04 ± 0.07 (10.3)^{b}	0.92 ± 0.04 (20.7)^{b}	0.88 ± 0.04 (24.1)^{b}	0.84 ± 0.004 (27.6)^{b}
MF	400	1.2 ± 0.10 (6.3)^{d}	0.96 ± 0.04 (25.0)^{b}	0.92 ± 0.04 (28.1)^{c}	0.92 ± 0.04 (28.1)^{d}
Aspirin	100	0.78 ± 0.10 (11.36)^{a}	0.55 ± 0.12 (38.89)^{a}	0.40 ± 0.12 (45.95)^{a}	0.30 ± 0.10 (53.13)^{a}
10% Tween 80		1.28 ± 0.04^{e}	1.12 ± 0.07^{d}	1.04 ± 0.04^{d}	1.04 ± 0.04^{f}

CME = crude methanolic extract, HF = hexane fraction, DF= dichloromethane fraction, EF= ethyl acetate fraction, AF = acetone fraction, MF = methanol fraction; Aspirin = standard drug for the study. Values in parentheses represent percent inhibition of edema. Means within a product followed by the same letter do not differ significantly at p = 0.05 (Student-Newman-Keuls's test); *: p < 0.05; **: p < 0.01; ***: p < 0.001. Number of replicates: 3.

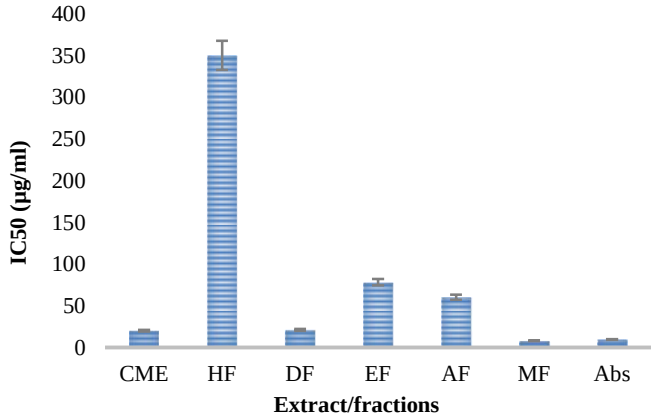

Figure 3: DPPH activity (IC_{50}) of the extract/fractions from the leaves of *S. mombin*.
CME: Crude methanol extract, HF: hexane fraction, DF: dichloromethane fraction, EF: ethyl acetate fraction, AF: acetone fraction, MF: methanol fraction, and Abs: ascorbic acid.

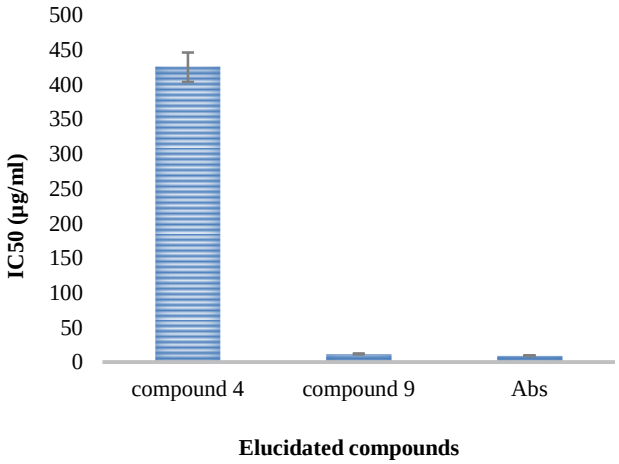

Figure 4: DPPH activity (IC_{50}) of elucidated compounds from the leaves of *S. mombin*. Abs: ascorbic acid.

Gallocathechin, quercetin, gallic acid and ellagic acid derivatives have been previously reported as strong antioxidants.[29] Gallic and ellagic acids are standard commercially available antioxidants and the presence of their derivatives in the MF may explain the high antioxidant activity exhibited by this fraction. All the compounds detected and/or isolated from the fractions of the leaf of *S. mombin* are all phenolic compounds, this correlates well with the total phenolic content of the extract/fractions and with the high antioxidant properties.

Conclusion

The present study reveals the high phenolic content of the extract and fractions of S. mombin leaves. However, the plant extract was moderately toxicity and had poor anti-inflammatory activity. Some of the fractions (MF, EF, AF and DF) identified in *S. mombin* leaf extract showed higher antioxidant activity. This suggests that *S. mombin* leaf could be a source of new antioxidant molecules or drugs. This could enlighten us on the efficacy of the extracts in the ethnomedicinal managing of disease conditions related with oxidative stress. The phenolic compounds identified focused more on the ethyl acetate fraction and these compounds are mainly gallocatechin, quercetin and kaempferol glycosides and their methoxy derivatives. Gallic acid and ellagic acid derivatives were also recognized in the most active fraction. Since there is high efficacy of these antioxidant compounds, *S. mombin* leaf can serve as a therapeutic agent for the prevention of different diseases and complications. Hence, these bioactive compounds have great potentials in pharmaceutical use.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Nusrat AH. Hepatoprotective toxicological assessment of *Spondias mombin* L. (Anacardiaceae) in rodents [dissertation]. Kwame Nkrumah University of Science and Technology; 2010. 22-26 p. http://ir.knust.edu.gh/handle/123456789/806
2. Esua OJ, Makinde OO, Arueya GL, Chin NL. Antioxidant potential, phytochemical and nutrient compositions of Nigerian hog plum (*Spondias mombin*) seed kernel as a new food source. Intl Food Res J. 2016; 23(Suppl):S179-85.
3. Uchendu CN and Isek T. Antifertility activity of aqueous ethanol leaf extract of *Spondias mombin* (Anacardiaceae) in rats. Afr Health Sci. 2008; 8(3):163-167.
4. Gbolade AA and Adeyemi AA. Anthelmintic activities of three medicinal plants from Nigeria. Phytother. 2008; 79(3):223-225.
5. Olugbuyiro JAO, Moody JO, Hamann MT. AntiMtb activity of triterpenoid-rich fractions from *Spondias mombin* L. Afr J Biotech. 2009; 8(9):1807-1809.
6. Asuquo RO, Ekanem BT, Udoh BP, Mesembe EO, Ebong EP. Haematinic potential of *Spondias mombin* leaf extract in wistar rats. Adv Biores. 2013; 4(2):53-56.
7. Nworu CS, Akah PA, Okoye FBC, Toukam DT, Udeh J, Esimone CO. The leaf extract of *Spondias mombin* L. displays an anti-inflammatory effect and suppresses inducible formation of tumor necrosis factor-α and nitric oxide (NO). J Immunotoxicol. 2011; 8(1):10-16.
8. Ajaegbu EE, Danga YSP, Okoye FBC. Larvicidal activity of the leaf extracts of *Spondias mombin* Linn. (Anacardiaceae) from various solvents against malarial, dengue and filarial vector mosquitoes (Diptera: Culicidae). J Vector Borne Dis. 2014; 51(4):300-306.
9. Ajaegbu EE, Lame Y, Danga YSP, Uzochukwu IC, Okoye FBC. Mosquito adulticidal activity of the leaf extracts of *Spondias mombin* L. against *Aedes aegypti* L. and isolation of active principles. J Vector Borne Dis. 2016; 53(1):17-22.
10. Ajaegbu EE, Lame Y, Danga YSP, Uzochukwu IC, Okoye FBC. Mosquito repellent activity of *Spondias mombin* L. (Family Anacardiaceae) crude methanol extract and fractions against *Aedes aegypti* (L.). Intl J Nat Pro Res. 2016; 7(3):240-244.
11. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol. 2004; 25(1):29-38.
12. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983; 53(4):275-289.
13. Okoye FBC and Osadebe PO. Studies of the mechanisms of anti-inflammatory activity of the extracts and fractions of *Alchornea floribunda* leaves. Asian Pac J Trop Med. 2009; 2(3):7-14.
14. Sajjad KM, Khanam S, Deepak M, Shivananda BG. Antioxidant activity of a new diary heptanoid from *Zingiber officinale.* Pharmacogn Mag. 2006; 2(8):254-257.
15. Patel A, Patel A, Patel A, Patel NM. Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* Linn. Leaves (Leguminosae). Pharmacog Res. 2010; 2(3):152-158.
16. Ayoka AO, Akomolafe RO, Akinsomisoye OS, Ukponmwan OE. Medicinal and Economic value of *Spondias mombin*. Afri J Biomed Res. 2008; 11(2):129-136.
17. Igwe CU, Onyeze GOC, Onwuliri VA, Osuagwu CG, Ojiako AO. Evaluation of the chemical composition of the leaf of *Spondias mombin* Linn from Nigeria. Aust J Basic Appl Sci. 2010; 4(5):706-710.
18. Saadullah M, Asif M, Sattar A, Rehman K, Shah S, Saleem M, Shah A, Wajid M, Rasool A, Uzair M, Afzal S, Afzal K. Cytotoxic and antioxidant potentials of ellagic acid derivatives from *Conocarpus lancifolius* (Combretaceae). Trop J Pharm Res. 2020; 19(5):1073-1080.
19. Zhu Y, Liu Y, Zhan Y, Liu L, Xu Y, Xu T, Liu T. Preparative Isolation and Purification of Five Flavonoid Glycosides and One Benzophenone Galloyl Glycoside from *Psidium guajava* by High-Speed Counter-Current Chromatography (HSCCC). Molecules 2013, 18(12):15648-15661.
20. Udobi CE and Onaolapo JA. Cell kill pattern and acute toxicity studies of the aqueous fraction of the methanol extract of parts of *Parkia biglobosa*. Afr J Biotech. 2010; 8(31):4993-4998.
21. Prior R, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005; 53(10):4290-4302.
22. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem. 2004; 84(4):551-562.
23. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005; 53(6):1841-1856.
24. Cabrala B, Siqueiraa EMS, Bitencourtb MAO, Limab MCJS, Limac AK, Ortmannd CF, Zucolottoa SM. Phytochemical study and anti-inflammatory and antioxidant potential of Spondias mombin leaves. Braz J Pharmacogn. 2016; 26(3):304-311.
25. Stocker R and Keaney JF Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev. 2004; 84(4):1381-1478.
26. Grassi D, Desideri G, Croce G, Tiberti S, Aggio A, Ferri C. Flavonoids, vascular function and cardiovascular protection. Curr Pharm Des. 2009; 15(10):1072-1084.
27. Grassi D, Desideri G, Tiberti S, Ferri C. Oxidative stress, endothelial dysfunction and prevention of cardiovascular diseases. Agro Food Industry Ii-tech. 2009; 20(4):76-79.
28. Bhandarkar AP, Bhat RA, Vinodraj K, Shetty MS, Shenoy GK. In vitro evaluation of antioxidant activity of S. mombin leaf extract: discovering future avenues for an affordable and efficient antioxidant. Int Res J Pharm. 2015; 6(2):164-168.
29. Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Scavenging effects of tea cathechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Rad Biol Med. 1996; 21(6):895-902.

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