Original Research Article

Phytocompounds Profiling and Assessment of the Antioxidant and Anti-Inflammatory Efficacies of *Spondias mombin*(Linn)LeafExtracts

ABSTRACT

The medicinal properties of *Spondias mombin*Linnleaves have been recognized historically, particularly in cancer treatment, diarrhea, and diabetes management. The purpose of this studyis to investigate the chemical constituentsusing GC-MS and HPLC,assess the antioxidant and anti-inflammatory properties of *S. mombin*n-hexane and methanol leaf extracts in animal models. Antioxidant activity was assessed using DPPH, hydrogen peroxide and ABTS scavenging activity assays while the anti-inflammatory potential was assessed using acetic acid-induced vascular permeability and carrageenan-induced paw edema in rats. GC-MS analysis detected 10-octadecenoic acid methyl ester, 11,14-octadecadienoic acid methyl ester and phytol, as the main compounds in the methanol extract; while tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, phytol, and hexadecanoic acid methyl ester are present as key compounds in the n-hexane extract. The HPLC methanol extract profileidentifiedquercetin, kaempferol, naringenin, luteolin (flavonoids)along with cholesterol, saponin and maleic acid as the major bioactive compounds.Both leaf extractsdemonstrated a concentration-dependent increase in radical scavenging activities indicating a noteworthy antioxidant property. The methanol extract demonstrated significantly higher levels of total phenolics (185.194 ± 0.271 mg/g gallic acid), flavonoids (130.568 ± 0.744 mg/g rutin) and overall antioxidant capacity (58.389 ± 0.292 mg/g ascorbic acid) than the n-hexane extract.Furthermore, the methanol extract demonstrated promising anti-inflammatory effects, particularly at the 400 mg/kg dose, substantially inhibiting paw edema in the carrageenan-induced and acetic acid-induced vascular permeability models. Presence of various bioactive compounds suggest that *S. mombin*leaves could be valuable natural sources of antioxidants and anti-inflammatory agents, potentially useful in managing oxidative stress and inflammatory conditions.

Keywords: Antioxidant, anti-inflammatory, GC-MS, HPLC, phenolics, flavonoids, *Spondias mombin*

1. INTRODUCTION

Herbal plants have been utilized since historical eras to address human diseases. About 80% of the population in industrially emerging nations, continue to depend on plant resources such as medicinal plants for healthcare [1]. These plants, whether harvested from the wild or cultivated, are commonly used to treat bacterial infections, toxic infections, and other related disorders.

UNDER PEER REVIEW

*Spondias mombin*Linn(Anacardiaceae)alsorecognized as hog plum or yellow mombin is a fruit-bearing deciduous tree which is extensively found in Nigeria and various tropical forests worldwide [2]. In Nigeria and various other global regions, the leaves of *S. mombin* are extensively employed in treating reproductive tract infections. They are often utilized to promote pregnancy, lessen bleeding during and after childbirth, promote lactation, and act as a vaginal cleanser to manage infections occurring after childbirth in the uterus or vagina, particularly by midwives [2]. Moreover, in various African locales, the bark, leaves, or a mixture of both is regarded as effective treatments for a range of ailments including gonorrhea, cough, sore throat, fever, cholera, constipation, yaws,stomach issues and are also utilized as an intra-partum aid [3]. Different parts of *S. mombin* are known to be abundant in antioxidants and erstwhile beneficial phytocompounds [4]. However, there is no adequate documentation of these bioactive phytocompounds through the combined use of GC-MS and HPLC. Furthermore, comprehensive evaluations using both carrageenan-induced paw edema and acetic acid-induced vascular permeability inflammation models are also limited.

Therefore, this research is focused on identifying the phytocompounds existent in the methanol and n-hexane leaf extracts of *S. mombin* and also evaluate their *in vitro* antioxidant and *in vivo* anti-inflammatory efficacies.

2. MATERIALS AND METHODS

2.1Plant Collection and Identification

The plant material was sourced from the Ikolo community (latitude 4° 56′ 16″north and longitude 6° 13′ 48″ east)within the Yenagoa local government area of Bayelsa State, Nigeria. Prof. A. Oladele conducted the identification/authentication of the plant. A voucher sample with number NDUP/24/01 was deposited at the Herbarium of the Department of Pharmacognosy and Herbal

Medicine at Niger Delta University. The leaves were dried under a shady environment for a week before the extraction procedure.

2.2Extraction of Plant Material

500 g of pulverized leaves was extracted for 48 hours by cold maceration in 1800 mL methanol and 2000 mL n-hexane at ambient temperature with intermittent agitation every 7 hours for 3 days. Thereafter, it underwent filtration and the methanol was changed for maximum extraction. The resulting filtrate was concentrated to dryness utilizing a water bath maintained at 50□, the final residue after drying was damp and it was stored in a sample bottle in a refrigerator.

2.3Assessment of Total Phenolics

The total phenolic contents of the extracts was estimated using the Folin-Ciocalteau reagent, as reported by Katalinic*et al.* [5]. One milliliter portion of aliquots ofμg/mL gallic acid solutions were combined with 5.0 mL of diluted Folin-Ciocalteu reagent and 4.0 mL of sodium carbonate solution (75 g/L) to create the calibration curve. One milliliter (1 g/100 mL) of the extracts was individually mixed with the identical reagents employed in creating the calibration curve. After a duration of 30 minutes, absorbance was takenat 765 nm to calculate the total phenolic contents employing equation 1:

$TPC = (C \times V)/M$ ………………………………………………………….. Equation 1

Where, TPC = Total phenolic content in mg GAE per gram of extract.

C = Gallic acid concentration (mg/mL) by calibration curve,

V = total amount of extract utilized in the assay,

M = total weight of the dry extract (in grams) employed in the assay.

All measurements were carried out in triplicate and the results averaged.

UNDER PEER REVIEW

2.4Estimation of Total Flavonoids

The total flavonoids content was performed following the procedure outlined by Katalinic*et al.* [5]. An aliquot of 100 µLof the sample extracts in methanol (10 mg/mL) was combined with 100 µL of 10% aluminium trichloride in methanol and a small amount of acetic acid. Subsequently, the solution was diluted with methanol to a total volume of 5 mL. Absorbance value was measured at 417 nm after a 30-minute incubation period. Blank samples were created by combining 100 mL of sample extracts with a small addition of acetic acid, followed by dilution to 5 mL with methanol. The absorbance measurement of a standard rutin solution (0.5 mg/mL) in methanol was assessed under identical conditions. All measurements were conducted in triplicate. The total flavonoid content was quantified and expressed as milligrams of Rutin Equivalents per gram of extract (mg RE/g extract).

2.5Antioxidant Activity

2.5.1Total antioxidant capacity (TAC)

The total antioxidant capacity of the extracts was determined by the phosphomolybdate method [5]. 1 mL of a reagent solution received 0.1 mL of the extract (10 mg/mL concentration) dissolved in water. This solution was made by combining 0.6 M sulfuric acid (3.14 mL/100 mL), 28 mM sodium phosphate (397.49 mg/100 mL), and 4 mM ammonium molybdate (494.36 mg/100 mL). The mixture was heated to 95°C for 90 minutes before cooling to room temperature. The UV spectrophotometer (ThermospectronicBioMate 3, USA) measured the absorbance at 695 nm. All measurements were carried out in triplicate and the results averaged.

2.5.2. Determination of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

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DPPH was prepared by dissolving DPPH crystalline solid in analytical grade methanol and stored at 4°C. A 2 mL of DPPH solution was added to 2 mL of the extract (aliquots of five concentrations 10, 25, 50, 100 and 200 μg/mL). After 30 min of incubation in the dark, the absorbance at 517 nm using a UV-Vis Spectrophotometer (ThermospectronicBioMate 3, U.S.A) was recorded. Methanol was used as the blank. The antioxidant activity was expressed as:

% Inhibition = [(Acontrol-Asample)/Acontrol] × 100% ………………………… Equation 2

All measurements were carried out in triplicate and the results averaged.

2.5.3Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was evaluated employing the method outlined by Al-Amiery*et al.* [6]. The hydrogen peroxide scavenging activity of the extracts was evaluated by observing the decrease in H_2O_2 concentration. In a standard procedure, 0.4 mL of any solution was combined with 0.6 mL of 40 mM H_2O_2 solution, and the total volume was made up to 2 mL using 50 mM sodium phosphate buffer (pH 7.4). Absorbance reading was done at 230 nm afterincubation for 40 minutes at 30°C. All measurements were carried out in triplicate and the results averaged.

Percentage of H_2O_2 inhibition was then determined according to Equation 3

Percentage (%) of H_2O_2 radical scavenging assay =

[(Acontrol - Asample)/Acontrol] × 100% ………………………………………Equation 3

2.5.4Determination of 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS+) free radical scavenging activity

The ABTS scavenging activity of *S. mombin* methanol leaf extract was evaluated utilizing the ABTS assay as outlined by Re *et al.* [7]. Various concentrations of the extract (10, 25, 50, 100 and 200 μg/mL) were prepared, with ascorbic acid serving as a standard antioxidant for

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comparison. After incubation, absorbance was measured at 734 nm, and the scavenging activity was assessed as the percentage inhibition using Equation 4.

% inhibition of ABTS scavenging activity =

[(Acontrol - Asample)/Acontrol] × 100% ……………………………………….Equation 4

All measurements were carried out in triplicate and the results averaged.

2.6Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The GC-MS examination of *S. mombin* leaf extracts was done using a GC-MS instrument; Model, QP 2010 PLUS, Shimadzu, Japan. The interpretation of the mass spectrum was done using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 patterns. The spectra of the unknown compounds were compared with those of known compounds stored in the NIST library. This comparison facilitated the identification of the names, molecular formulas, molar masses, and chemical structures of the compounds detected in the samples.

2.7HPLC Equipment Conditions for Flavonoid Analysis

Chromatographic studies were carried out on an Agilent 1200 Series outfitted with AGILENT 1260 Diode Array Detector (DAD). To obtain a composition profile of the studied matrices, a C_{18} HPLC column with dimensions of 250×4.6 mm I.D, at a flow rate of 1.0 mL/min was employed. Flavonoid compounds from various classes were selected for separation. The mobile phase consisted of a gradient elution using a mixture of solvent A (acetonitrile) and solvent B (0.1% phosphoric acid in de-ionized water). The flow rate was set at 1.0 mL/min, and 20 µL of samples and standards were injected. During the analysis, the column temperature remained constant at 25°C, and the spectra were recorded at a wavelength of 280 nm

2.8 HPLC Chromatographic Conditions for Phenolics Analysis

UNDER PEER REVIEW

Chromatographic studies were carried out on an Agilent HPLC system 1260 series (Agilent technology USA) outfitted with G1300A LC pump, G1315A ultraviolet detector set at 257 nm and 7725 Rhedyne injector. Reverse phase LC was performed isocratically at 28°C using column Poroshell 120 EC C18 150 mm X 4.6 mm, 5 µm at a flow rate of 0.700 mL/min. Acetonitrile and 0.1% formic acid (70:30) formed the mobile phase. Chemstation acquisition software was used to process data obtained from the instrument.

2.9Anti-Inflammatory Activity

2.9.1 Experimental Animals

Thirty-two (32) male Wistar rats were acquired from the animal house of the Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria. The animals were kept in standard plastic cages and acclimatised for one week in the animal house in the Department of Pharmacology, College of Health Sciences, Niger Delta University. They were maintained under a 12-hour light/ dark cycle, controlled temperature (20-25°C), free ventilation, and were fed conventional animal food and water as often as necessary.

2.9.2Assessment of acetic acid-induced rat vascular permeability

Sixteen (16) rats were categorized into four groups, **I - IV**comprisingfour rats per group and treatments were administered as outlined in Table 9. The anti-inflammatory effect was assessed following the established guidelines outlined by Wang *et al.* [8], with minor adjustments. One hour after administering the *S.mombin* extract, the animals received an intravenous injection of 0.5 mL of 0.25% Evans blue. They were also given an abdominal injection of 0.6% acetic acid (10 mL/kg). Thereafter, theywere euthanized under anesthesia 30 minutes after receiving the acetic acid injection, and their abdominal cavities were rinsed with normal saline. The rinsed solution was subjected to centrifugation, and the optical density (OD) of the supernatant was

UNDER PEER REVIEW

assessed at 590 nm using a microplate reader. All treatmentswere carried out in triplicate and the results averaged.Equation 5was utilized to calculate the percentage inhibition of vascular permeability

$$\% \text{ inhibition} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100 \quad \text{.. Equation 5}$$

where $OD_{control}$is the optical density of the control group,

OD_{test} is the optical density of the test group.

2.9.3Carrageenan-induced paw edema

The anti-inflammatory potential of *S. mombin* extract was also evaluated by administering carrageenan (0.1 mL) as the inflammation-inducing agent and using aspirin as the reference drug. Experimental units were separated into four groups**I - IV**, and treatments were administered as detailed in Table 10. Assessment of the anti-inflammatory effect followed the methodology outlined by Winter *et al.* [9]. The rats' paw diameter was quantified in millimeters using a Vernier caliper and documented prior to inducing inflammation. Subsequently, one hour after treatment administration, the inflammation-inducing agent was injected into the sub-plantar tissue of the left hind paw. Thereafter, the paw diameter was measured at one-hour intervals following the induction of inflammation, up to the fourth hour. Alteration in paw volume at 1, 2, 3, and 4 hours after carrageenan injection was considered the parameter for inflammation measurement. All treatments were carried out in quadruplet and the results averaged. Equation 6 was employed to calculate the percentage inhibition of inflammation:

$$\% \text{ inhibition of inflammation} = \frac{V_c - V_t}{V_c} \times 100 \quad \text{..Equation 6}$$

Where,

Vc represents the mean value of the paw volume of the control group,

UNDER PEER REVIEW

Vt represents the mean paw volume of the test group.

2.10Statistical Analysis

The data were examined with SPSS version 21.0. Descriptive statistics were used and findings were given as mean ± SD. A one-way analysis of variance (ANOVA) was utilized to discover significant differences at $P<0.05$. Values sharing the same superscripts were regarded as not significantly different statistically.

3. RESULTS

3.1 Quantitative Phytochemical Analysis

Table 1 details the quantitative phytochemical analysis results fortotal phenolics, flavonoids and antioxidant capacity of *S. mombin*leaf extracts.

Table 1.Quantitative phytochemical analysisof *S. mombin* leaf extracts

Component	n-Hexane	Methanol
Total phenolics (mg/g gallic acid)	153.884 ± 0.327	185.194 ± 0.271
Total flavonoids (mg/g rutin)	57.508 ± 0.722	130.568 ± 0.744
Total antioxidant capacity (mg/g Ascorbic acid)	49.999±0.331	58.389 ± 0.292

Values are presented as MEAN ± SEM (n=3)

Methanol extract yielded notably higher levels of total phenolics (185.194 ± 0.271 mg/g gallic acid) and TAC (58.389 ± 0.292 mg/g ascorbic acid) compared to n-hexane extract (153.884 ± 0.327 mg/g gallic acid and 49.999 ± 0.331 mg/g ascorbic acid) respectively as presented in Table 1. Total flavonoid values for the methanol and n-hexane leaf extractsare 130.568 ± 0.744 mg/g rutin equivalent and 57.508 ± 0.722 mg/g rutin respectively (Table 1).

3.2Antioxidant Activities

3.2.1 DPPHradical scavenging activity

Table 2 presents DPPH radical scavenging activity of S. *mombin*leaf extractsand ascorbic acid at different concentrations. Antioxidant effect of n-hexane leaf extract was moderate, as evidenced

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by the increase in inhibition percentage from 12.49±0.23% at 10 µg/mL to 83.27±0.17% at 200 µg/mL. The methanol leaf extract showed the lowest DPPH radical scavenging activity, with % inhibition ranging from 9.79±0.23% at 10 µg/mL to 72.69±0.17% at 200 µg/mL. By contrast, ascorbic acid exhibited the highest % inhibition across all concentrations, with values ranging from 66.50±0.06% at 10 µg/mL to 94.34±0.07% at 200 µg/mL, indicating its superior DPPH radical scavenging activity.

Table 2.DPPH radical scavenging activity (% inhibition) of *S. mombin* leaf extracts

Concentration (µg/mL)	Ascorbic	n-Hexane	Methanol
10	66.50±0.06[b]	12.49±0.23[a]	9.79±0.23[a]
25	90.32±0.11[b]	20.93±0.28[a]	13.09±0.29[a]
50	91.82±0.13[b]	56.53±0.45[a]	36.16±0.32[a]
100	93.32±0.07[b]	75.47±0.20[a]	53.64±0.34[a]
200	94.34±0.07[b]	83.27±0.17[a]	72.69±0.17 [a]

Data is presented as mean ± standard deviation (n = 3); significant differences ($p<0.05$) were detected between values in the same row with different superscripts.

3.2.2 Hydrogenperoxide scavenging activity

Hydrogen peroxide scavenging activity assessment provides critical insights into the antioxidant potential of natural compounds. From the result in Table 3, both leaf extracts of *S. mombin* showed a concentration-dependent increase in % inhibition, but no statistically significant difference in their H_2O_2 scavenging activities. Comparatively, the methanol extract exhibited slightly higher H_2O_2 scavenging activity than the n-hexane extract, particularly at 50 µg/mL and above. However, ascorbic acid consistently exhibited the highest % inhibition across all concentrations, ranging from 59.24±0.28% at 10 µg/mL to 92.77±0.57% at 200 µg/mL, confirming its strong H_2O_2 scavenging activity.

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Table 3.Hydrogen peroxide scavenging activity (% inhibition) of *S. mombin* leaf extracts

Concentration (µg/mL)	Ascorbic	n-Hexane	Methanol
10	59.24±0.28[b]	17.28±0.74[a]	18.53±0.41[a]
25	84.57±0.25[b]	22.77±0.57[a]	24.67±0.33[a]
50	88.10±0.33[b]	39.84±0.49[a]	45.82±0.34[a]
100	91.90±0.34[b]	61.36±0.43[a]	68.53±0.33[a]
200	92.77±0.57[b]	79.67±0.41[a]	85.60±0.47[a]

The results are given as mean ± standard deviation (n = 3), and there is a significant difference ($p<0.05$) among values in the same row with different superscripts.

3.2.3 ABTS radical scavenging assay

Table 4displays the findings of ABTS scavenging activities of ascorbic acid and *S. mombin* leaf extracts at different concentrations.Both leaf extracts showed a concentration-dependent increase in ABTS scavenging activity.By contrast, ascorbic acid consistently exhibited the highest % inhibition across all concentrations, from 65.73±0.07% at 10 µg/mL to 96.35±0.13% at 200 µg/mL, demonstrating its superior antioxidant capacity. Again no statistically significant difference is observed in their ABTS scavenging activities.

Table 4. ABTS scavenging activity (% inhibition) of *S. mombin* leaf extracts

Concentration (µg/mL)	Ascorbic	n-Hexane	Methanol
10	65.73±0.07[b]	13.91±0.12[a]	7.72±0.07[a]
25	92.70±0.07[b]	22.67±0.12[a]	12.14±0.07[a]
50	93.97±0.07[b]	59.09±0.12[a]	56.51±0.07[a]
100	94.89±0.07[b]	79.87±0.07[a]	80.87±0.07[a]
200	96.35±0.13[b]	87.90±0.12[a]	87.02±0.07[a]

The values are displayed as mean ± standard deviation (n = 3); there is a significant difference ($p<0.05$) between values in the same row with different superscripts.

3.3 GC-MS Examination of Methanol Leaf Extract of *Spondias mombin*

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GC-MS data identified 28 compounds in the methanol leaf extract (Fig. 1 and Table 5). The main compounds detected based on the relative contents were hexadecanoic acid methyl ester (24.59%), 10-octadecenoic acid methyl ester (20.9%), phytol (14.11%), 11,14-octadecadienoic acid methyl ester(11.04%) and methyl stearate (8.53%).

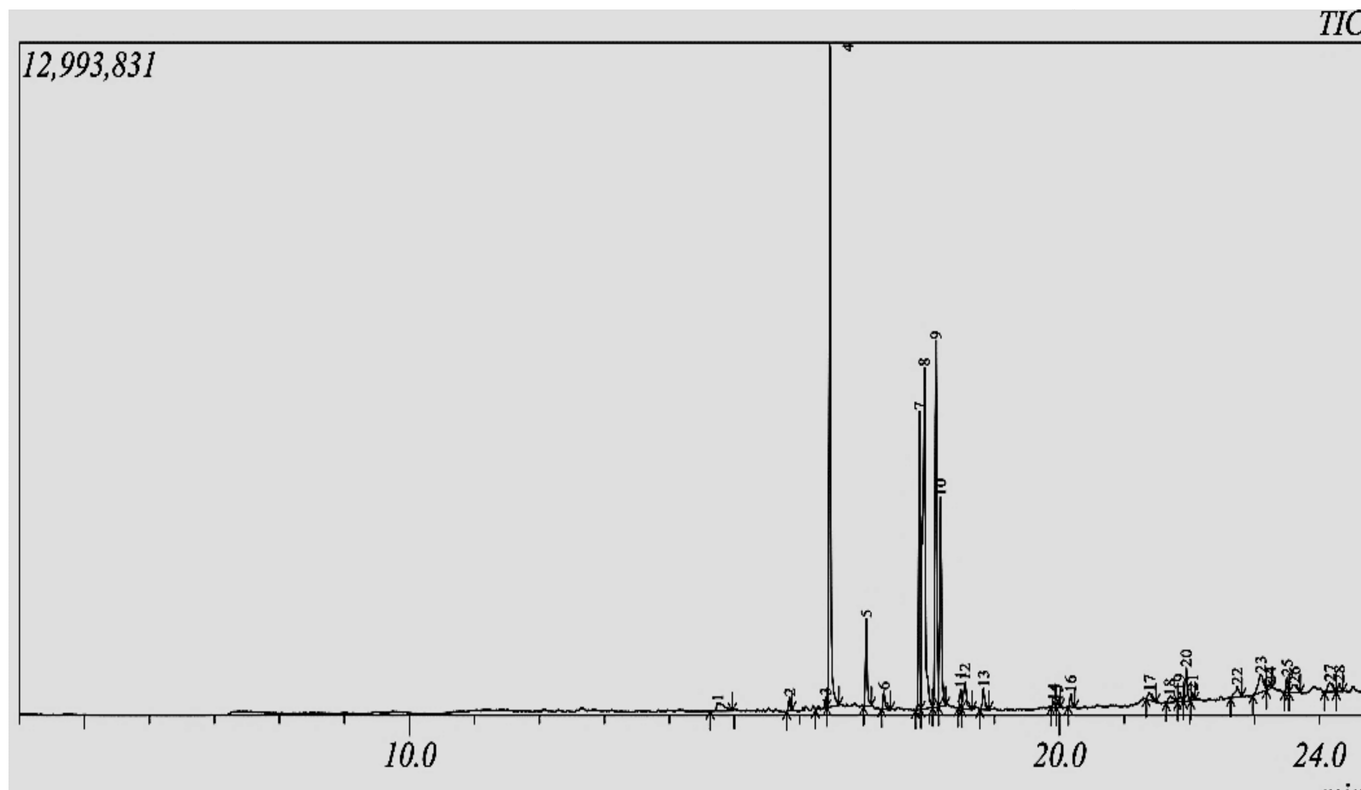

Fig 1.GC-MS chromatogram of methanol leaf extract of *S. mombin*

Table 5.GC-MS analysis of methanol extract of *S. mombin*

S/N	Retention Time (min)	Compound Name	Molecular formula	Peak area (%)
1	14.739	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	1.37
2	15.848	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl)methyl]	$C_{21}H_{38}O_2$	0.47
3	16.402	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	0.42
4	16.471	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	24.59
5	17.027	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	3.27

6	17.300	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	0.77
7	17.848	11,14-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	11.04
8	17.922	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	20.92
9	18.100	Phytol	$C_{20}H_{40}O$	14.11
10	18.171	Methyl stearate	$C_{19}H_{38}O_2$	8.53
11	18.476	n-Propyl 9,12-octadecadienoate	$C_{21}H_{38}O_2$	0.77
12	18.549	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	1.77
13	18.829	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	1.02
14	19.909	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	0.25
15	19.965	Longifolene	$C_{15}H_{24}$	0.17
16	20.174	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	0.51
17	21.383	4,22-Stigmastadiene-3-one	$C_{29}H_{46}O$	0.58
18	21.692	Phenol, 3-pentadecyl-	$C_{21}H_{36}O$	0.75
19	21.845	Kauren-18-ol, acetate, (4.beta.)-	$C_{22}H34O_2$	0.56
20	21.949	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	1.45
21	22.043	Androsta-4,16-dien-3-one	$C_{19}H_{26}O$	0.29
22	22.736	Methyl 21-methyldocosanoate	$C_{24}H_{48}O_2$	0.91
23	23.096	Testosterone cypionate	$C_{27}H_{40}O_3$	2.28
24	23.242	Dimethylmalonic acid, 2-fluoro-3-trifluoromethylphenyl heptadecyl ester	$C_{29}H_{44}F_4O_4$	0.31
25	23.499	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	0.60
26	23.618	Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester	$C_{17}H_{26}0_3$	0.89

27	24.158	5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro-3,88,11a-tetramethyl-[3S-(3.alpha.,7a.alpha11a.alpha,11a.beta.,11b .alpha)]	$C_{18}H_{30}O_2$	0.98
28	24.303	Squalene	$C_{30}H_{50}$	0.42

3.4 GC-MS Examination of n-hexane *Spondias mombin*Leaf Extract

GC-MS examination of n-hexane extractuncovered the presence of 19 phytochemicals, as depicted in Table 6 and Fig2. Among these, 13 compounds were notably abundant, with their respective areas in parentheses: Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-trans-1,10 (12.07%), phytol (9.09%), hexadecanoic acid, methyl ester (7.47%), n-tetracosanol-1 (4.55%), heneicosane (4.46%), bis(2-ethylhexyl) phthalate (4.06%), 2-methyltetracosane (3.39%), methyl stearidonate (2.92%), behenic alcohol (2.78%), 11,14-octadecadienoic acid, methyl ester (2.77%), methyl stearate (2.56%), 9-octadecenoic acid (Z)-, methyl ester (2.48%), and 9-eicosene, (E)- (2.20%). The remaining compounds had a composition of less than 2% by peak area. Some of the detected phytochemicals are reported to have biological activities.

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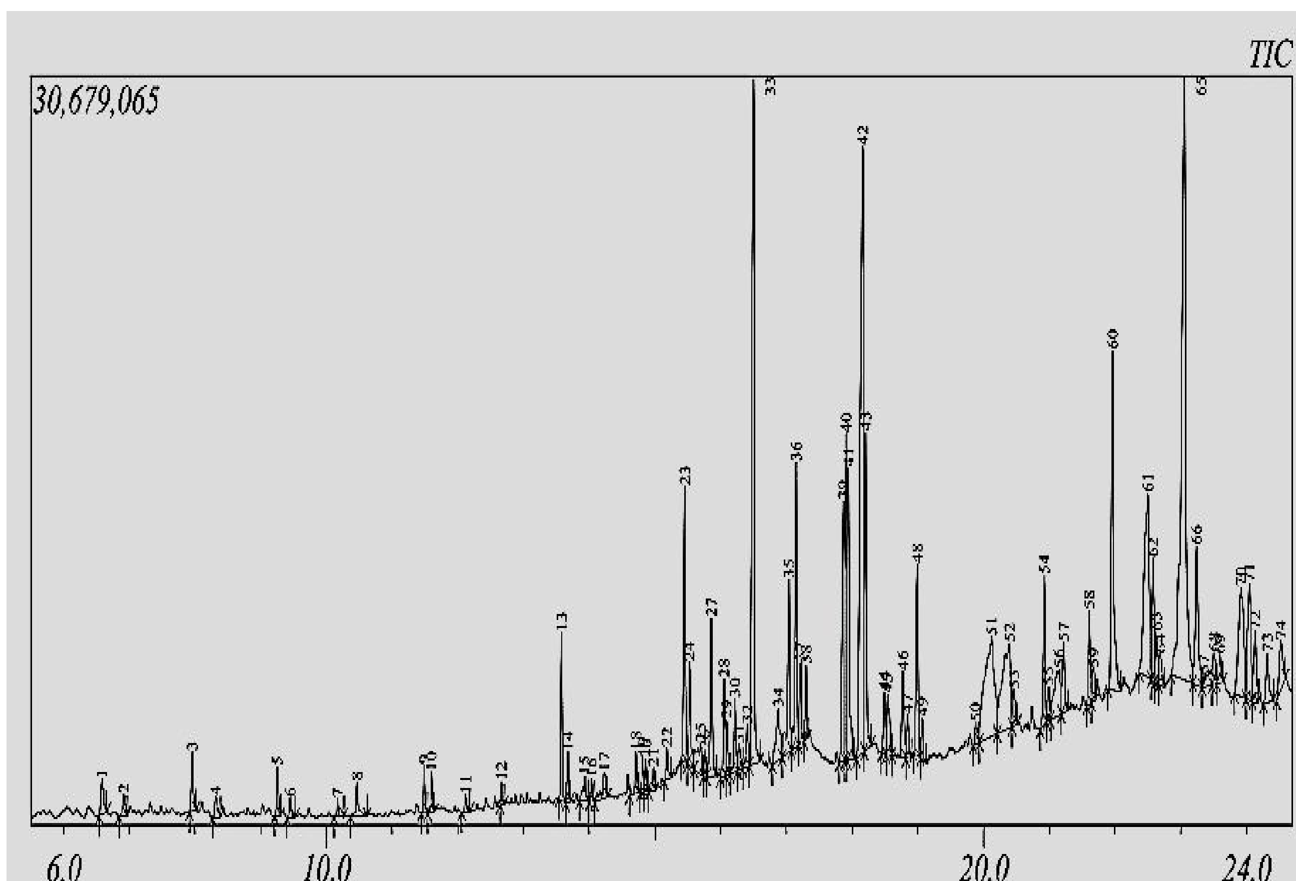

Fig 2.GC-MS chromatogram of n-hexane *S. mombin* leaf extract

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Table 6.GC-MS analysis of n-hexane leaf extract of *S. mombin*

S/N	Retention Time (min)	Compound Name	Molecular formula	Peak area (%)
1	13.578	Cetene	$C_{16}H_{32}$	1.18
2	15.453	9-Eicosene, (E)-	$C_{20}H_{40}$	2.20
3	15.858	Phytol, acetate	$C_{22}H_{42}O_2$	1.37
4	16.501	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	7.47
5	16.876	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1.08
6	17.038	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	1.87
7	17.867	11,14-Octadecadienoic acid, methyl ester		2.77
8	17.911	Methyl Stearidonate	$C_{19}H_{30}O_2$	2.92
9	17.944	9-Octadecenoic acid (Z)-, methyl ester		2.48
10	18.162	Phytol	$C_{20}H_{40}O$	9.09
11	18.207	Methyl stearate	$C_{19}H_{38}0_2$	2.56

12	20.123	n-Tetracosanol-1		4.55
13	20.392	2-methyltetracosane		3.39
14	21.963	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	4.06
15	22.499	Heneicosane	$C_{21}H_{44}$	4.46
16	22.577	1-Heneicosanol	$C_{21}H_{44}O$	1.10
17	23.056	Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-trans-1,10	$C_{16}H_{24}$	12.07
18	23.240	Phenol, 3-pentadecyl-	$C_{21}H_{36}O$	1.74
19	23.915	Behenic alcohol		2.78

3.5 HPLC Analysis of Flavonoids in *Spondias mombin*Methanol Leaf Extract

Thirty (30) different flavonoid compounds were obtained from the HPLC data (Table 7 and Fig 3). Among the identified flavonoids, quercetin exhibited the highest concentration at 44.00 mg/100g, followed by kaempferol (12.36 mg/100g), naringenin (10.33 mg/100g), luteolin (10.22 mg/100g), myricetin (7.69 mg/100g), isorhamnetin (7.63 mg/100g), apigenin (3.30 mg/100g) and (+) – catechin (1.77 mg/100g) while others were detected in trace amounts.

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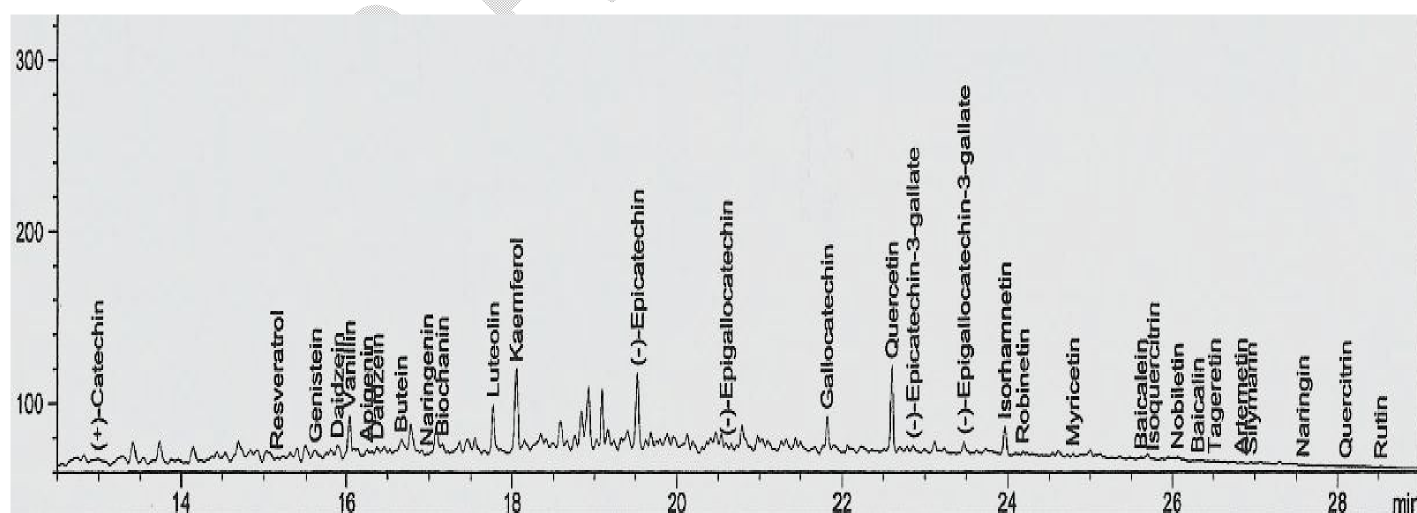

Fig 3.HPLC chromatogram of the flavonoids in methanol leaf extract of *S. mombin*

Table 7.Distribution of flavonoids in methanol leaf extract of *S. mombin*

S/N	Retention	Concentration	Flavonoid Compound

	Time (min)	(mg/100g)	
1	12.985	1.771	(+) – Catechin
2	15.155	3.878×10^{-6}	Resveratrol
3	15.617	1.278×10^{-5}	Genistein
4	15.891	1.124×10^{-5}	Daidzein
5	16.037	2.347×10^{-3}	Vanillin
6	16.244	3.303	Apigenin
7	16.669	2.236×10^{-5}	Butein
8	16.976	10.331	Naringenin
9	17.159	8.639×10^{-6}	Biochanin
10	17.769	10.222	Luteolin
11	18.052	12.357	Kaempferol
12	19.519	4.576×10^{-6}	(-) – Epicatechin
13	20.592	5.718×10^{-4}	(-) – Epigallocatechin
14	21.821	1.264×10^{-4}	Gallocatechin
15	22.602	44.004	Quercetin
16	22.852	3.955×10^{-4}	(-) – Epicatechin-3-gallate
17	23.471	3.510×10^{-4}	(-) – Epigallocatechin-3-gallate
18	23.968	7.633	Isorhamnetin
19	24.186	6.316×10^{-6}	Robinetin
20	24.792	7.699	Myricetin
21	25.616	5.856×10^{-6}	Baicalein
22	25.761	5.980×10^{-1}	Isoquercitrin
23	26.063	1.003×10^{-5}	Nobiletin
24	26.300	2.513×10^{-6}	Baicalin

UNDER PEER REVIEW

25	26.503	3.088×10^{-6}	Tageretin
26	26.829	4.601×10^{-6}	Artemetin
27	26.950	5.309×10^{-6}	Silymarin
28	27.575	1.801×10^{-6}	Naringin
39	28.097	1.342×10^{-1}	Quercitrin
30	28.527	2.398×10^{-1}	Rutin

3.6 HPLC Analysis of Phenolics in *Spondias mombin*Methanol Leaf Extract

The HPLC data obtained from *S. mombin* methanol leaf extract disclosed eleven (11) bioactive phytocompounds with distinct retention times and concentrations (Table 8 and Fig4); while the main phenolics are displayed in Fig8. These bioactive compounds varied in abundance, with some compounds present in higher concentrations than others. Among the bioactive compounds detected, quercetin (12.74804 mg/L) emerged as the most abundant, followed by cholesterol (4.69111 mg/L), saponin (3.32492 mg/L), and maleic acid (1.92208 mg/L).

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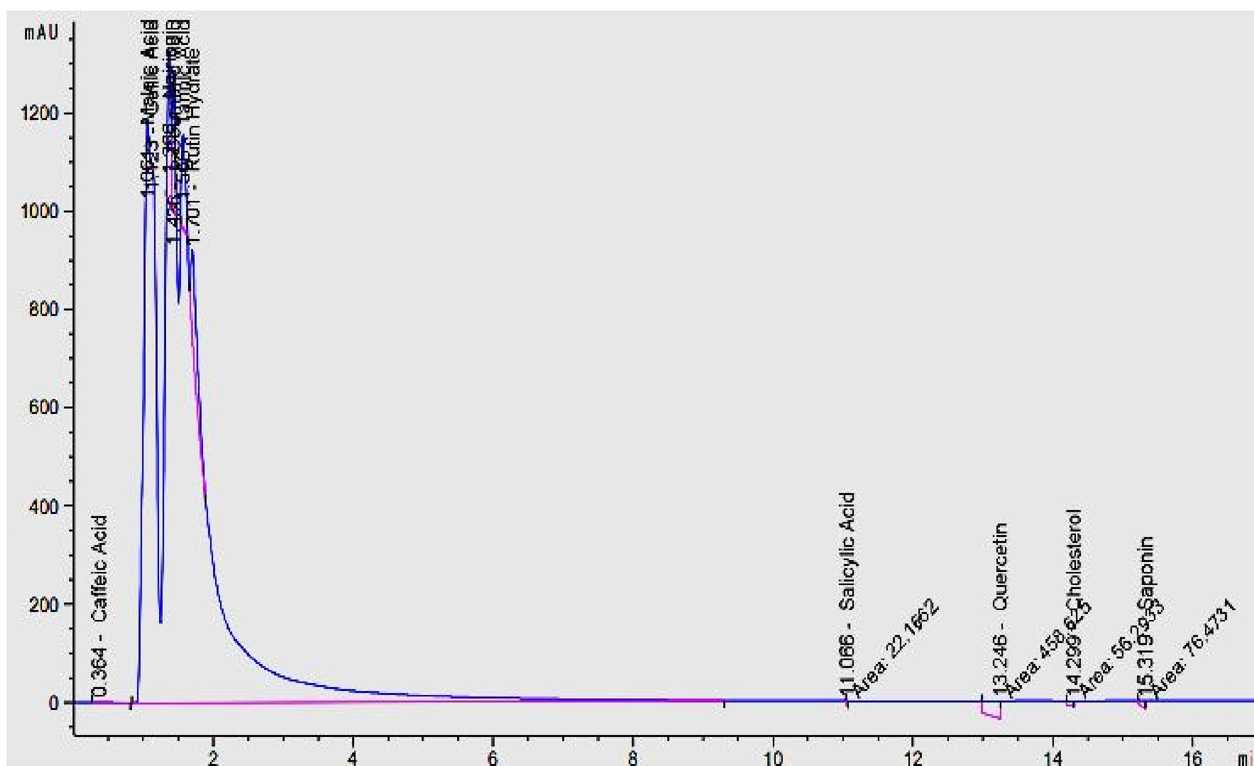

Fig 4.HPLC Chromatogram of some bioactive compounds in methanol leaf extract of *S. mombin*

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Table 8.Bioactive compounds in methanol leaf extract of *S. mombin*

S/N	Retention Time (Min)	Concentration (mg/L)	Bioactive Compound
1	0.364	0.03992	Caffeic acid
2	1.061	1.92208	Maleic Acid
3	1.123	0.01542	Gallic Acid
4	1.366	0.27297	Naringin
5	1.436	0.20133	p-Coumaric Acid
6	1.566	0.11279	Tannic acid
7	1.701	0.072719	Rutin hydrate
8	11.066	0.04151	Salicylic Acid
9	13.246	12.74804	Quercetin

10	14.299	4.69111	Cholesterol
11	15.319	3.32492	Saponin

3.7 Anti-Inflammatory Activities

3.7.1 Acetic acid-induced vascular permeability in rat

Table 9 presents anti-inflammatory property of the methanol *S. mombin* extract, evaluated through its effect on acetic acid-induced vascular permeability in rats. The control group, treated with normal saline, exhibited the highest optical density (OD) of 0.195 ± 0.007, establishing a baseline for maximum vascular permeability and inflammation. This indicates a successful induction of inflammatory response using acetic acid and serves as a baseline for comparison with the treatment groups[10].

Table 9.Anti-inflammatory property of methanol extract of *S. mombin* by acetic acid-induced increase of vascular permeability in rat

Group	Dosage	Optical Density	% Inhibition
I	Aspirin (100 mg/kg)	0.079 ± 0.011^{a}	59.49%
II	*S. mombin* extract (200 mg/kg)	0.141 ± 0.014^{b}	27.69%
III	*S. mombin* extract (400 mg/kg)	0.121 ± 0.008^{c}	37.95
1V (Control)	Normal saline (10 mL/kg)	0.195 ± 0.007^{d}	-

Data shown as mean ± standard deviation (n=4); significant differences (P<0.05) from control group marked by unique superscript letters in the same column.

3.7.2 Carrageenan-induced paw edema

Table 10 indicates notable variations in paw size measurements among the treatment groups at different time points following carrageenan induction. The control group, which received distilled water, showed significant increases in paw thickness over time, confirming the inflammatory response induced by carrageenan and the lack of inherent anti-inflammatory effects from the treatment. This pattern suggests that the acute inflammatory response was

UNDER PEER REVIEW

effectively induced. There was no significant variance observed among the treatment and control groupsat the first hour after induction, indicating that early reaction to inflammation was identical. However, the doses of 200 mg/kg and 400 mg/kg of the extract demonstrated appreciable effects ($P<0.05$) at 2 hr, 3 hr, and 4 hr post-induction compared to the control group, indicating notable anti-inflammatory effects. Notably, after two, three, and four hours after induction, the higher dosage (400 mg/kg) of *S. mombin* extract reduced paw thickness more significantly than the lower dosage (200 mg/kg). Higher dosage of *S. mombin* extract (400 mg/kg) caused better anti-inflammatory effects, with a maximum inhibition of 57.35% at 3 hours and 46.15% at 4 hours, compared to the lower dose, which peaked at 36.76% inhibition at 3 hours and 30.77% at 4 hours.

Table 10. Anti-inflammatory property of S. *mombin* on carrageenan-induced paw edema in Wistar rats

Group	Dosage	Initial paw volume (mm)	Paw thickness (mm) after induction (% Inhibition)			
			1 hour	2 hours	3 hours	4 hours
I Plant extract	200 mg/kg	0.10±0.00	$0.22±0.07^{a}$ (0.00%)	$0.39±0.01^{c}$ (-30.00%)	$0.43±0.06^{c}$ (36.76%)	$0.27±0.05^{c}$ (30.77%)
II Plant extract	400 mg/kg	0.10±0.00	$0.17±0.05^{a}$ (22.73%)	$0.25±0.02^{a}$ (16.67%)	$0.29±0.01^{b}$ (57.35%)	$0.21±0.01^{b}$ (46.15%)
III Distilled H_2O	0.2 mL/kg	0.10±0.00	$0.22±0.03^{a}$	$0.30±0.02^{b}$	$0.68±0.03^{d}$	$0.39±0.08^{d}$
IV Aspirin	200 mg/kg	0.10±0.00	$0.17±0.05^{a}$ (22.73%)	$0.23±0.02^{a}$ (23.33%)	$0.18±0.08^{a}$ (73.53%	$0.14±0.02^{a}$ (64.10%)

The data are expressed as means ± standard deviation (n=3); significant differences ($P<0.05$) compared to the control group are denoted by different superscript alphabets in the same column

4. DISCUSSION

UNDER PEER REVIEW

4.1 Total Phenolics, Flavonoids and Total Antioxidant Capacity

Results obtained from total flavonoids and phenolics analysis suggest that methanol extraction ismore efficient in extracting phenolic compounds thanwith n-hexane thereby conferring a higher antioxidant capacity. The higher values achieved for TAC and total phenolics in methanol extract suggest an increased concentration of biologically active compounds with antioxidant properties, which may contribute to its greater antioxidant potential.Previous research has demonstrated the correlation between the amount of phenolics compounds and antioxidant activity in plant extracts [11].Presence of phenolics in the extracts may contribute to their antioxidant capacity and potential health-promoting effects.

The n-methanol leaf extract exhibited superior total flavonoid content compared to the n-hexane leaf extract. Flavonoids, a type of phenolic molecule, are renowned for their antioxidant and anti-inflammatory properties [12]. The extract's high flavonoid concentration implies its potential as a natural origin of bioactive chemicals with strong antioxidant property, which is in agreement with past research highlighting the antioxidant effect of flavonoid-rich plant extracts [13]. Flavonoids have also been observed to remove free radicals and reduce oxidative strain, sparing cells from harm [14]. Thus, the methanol extract's flavonoid content is expected to contribute to its total antioxidant activity and possible therapeutic benefits.

4.2 Antioxidant Activities

4.2.1 DPPH radical scavenging activity

The hydrogen releasing ability of antioxidants is believed to have tremendous effect on the action of DPPH. Radical foraging actions of antioxidants are noteworthy to inhibit the harmful function of free radicals in several malaises, including tumor [15].

UNDER PEER REVIEW

All samples displayed a clear concentration-dependent increase in DPPH radical scavenging activity, consistent with typical antioxidant behavior (Table 2). Appreciable increase in the concentration of the extracts resulted in increased suppression of DPPH radicals, indicating high radical scavenging activity [16].This dose-dependent response is consistent with the principle that higher concentrations of active compounds provide more effective radical scavenging [17]. Among the *S. mombin* extracts, the n-hexane extract exhibited superior scavenging activity compared to the methanol extract whichimplies the n-hexane extract contains potent antioxidant compounds that are more effectively extracted using this solvent. Previous studies of *S. mombin* extract have shown good antioxidant activity using the DPPH scavenging model, which aligns with the current findings [18].

4.2.2 Hydrogen peroxide scavenging activity

Both leaf extracts of *S. mombin* showed a concentration-dependent increase in % inhibition, but no statistically significant difference in their H_2O_2 scavenging activities (Table 3). Comparatively, the methanol extract exhibited slightly higher H_2O_2 scavenging activity than the n-hexane extract at most concentrations, particularly at 50 µg/mL and above. The scavenging of $H_2O_2$by the extracts can be ascribed to active phytochemical, particularly phenolic compounds, which defuse$H_2O_2$by releasing electrons and thereby converting it into H_2O[19].

4.2.3 ABTS radical scavenging activity

Both leaf extracts showed a concentration-dependent increase in ABTS scavenging activity (Table 3). Again, no statistically significant difference is observed in their ABTS scavenging activities. The methanol extract shows a lower scavenging activity at lower concentrations (10 and 25 µg/mL) compared to the n-hexane extract, suggesting that non-polar compounds in the n-

UNDER PEER REVIEW

hexane extract might be more effective at these concentrations. The significant ABTS scavenging activity of *S. mombin* leaf extracts highlights their potential as natural antioxidants.

4.3 GC-MS Examination of Methanol Leaf Extract of *Spondias mombin*

Most of the phytocompounds extracted with methanol were fatty acid methyl esters (Figure 5) commonly found in plant oils. These phytocompounds have been depicted to display numerous biological functions. The most abundant hexadecanoic acid methyl ester has been found to decrease blood cholesterol and fight against inflammatory actions [20]. Phytol, a diterpene alcohol, has shown anticonvulsant, antioxidant and antinociceptive effects [21, 22]. Methyl stearate, another fatty acid methyl ester detected in the extract, has demonstrated antimicrobial properties [23], anti-diarrheal, cytotoxic and anti-proliferative activities [24]; while 10-octadecenoic acid methyl ester has been reported to display both antimicrobial and antioxidant effects [25]. Testosterone cypionate, a synthetic derivative of testosterone, is used medically to remedy disorders related to testosterone deficiency and several types of breast cancer [26]. Also, squalene is stated to have antioxidant and wound healing property [27].

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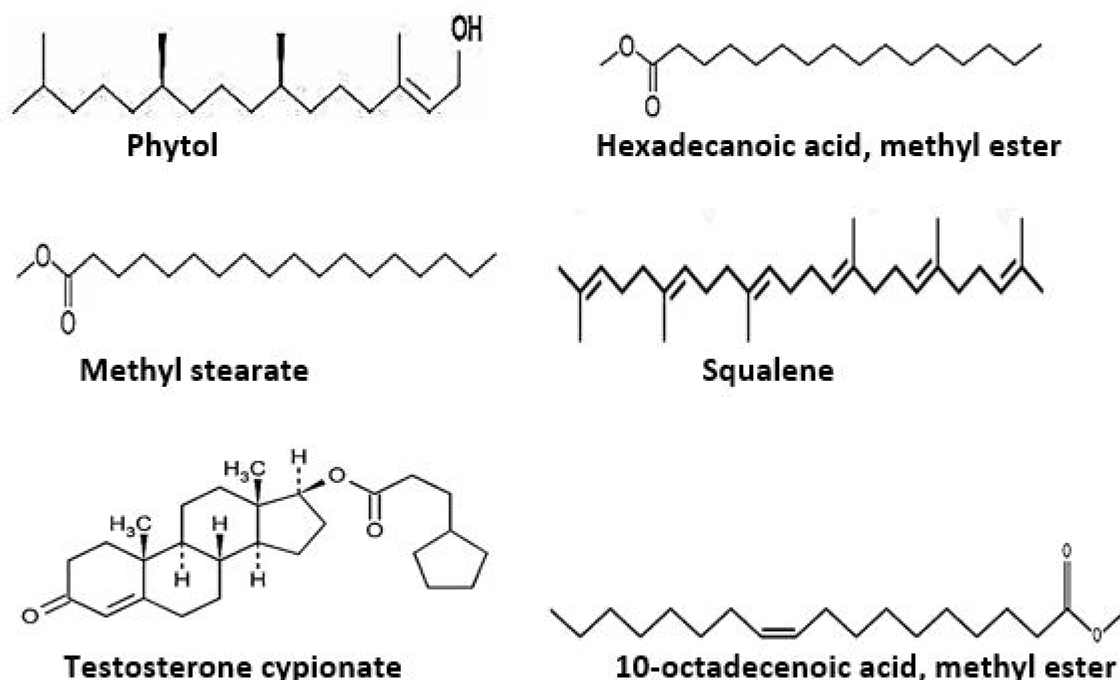

Fig. 5.Major phytocompounds detected by GC-MS in methanol leaf extract of *S. mombin*

4.4 GC-MS Examination of n-hexane *Spondias mombin*Leaf Extract

The major phytocompounds found in n-hexane extract of *S. mombin* are displayed on Fig. 6. Hexadecanoic acid, ethyl ester has the ability to destroy nematodes, pests, and reduce 5-alpha inhibitors [28]. Cetene an unsaturated aliphatic hydrocarbon has been reported to have antimicrobial and antioxidant effect, also had highest value of antifungal activity [29].The identification of n-hexadecanoic acids in the extract has been involved in the synthesis of prostaglandins [30]. Heneicosane possesses bactericidal actions [31]. The steroid, tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-trans-1,10 has been found to have haepatoprotective,antiasthmatic, diuretic, anticancer functions [32].

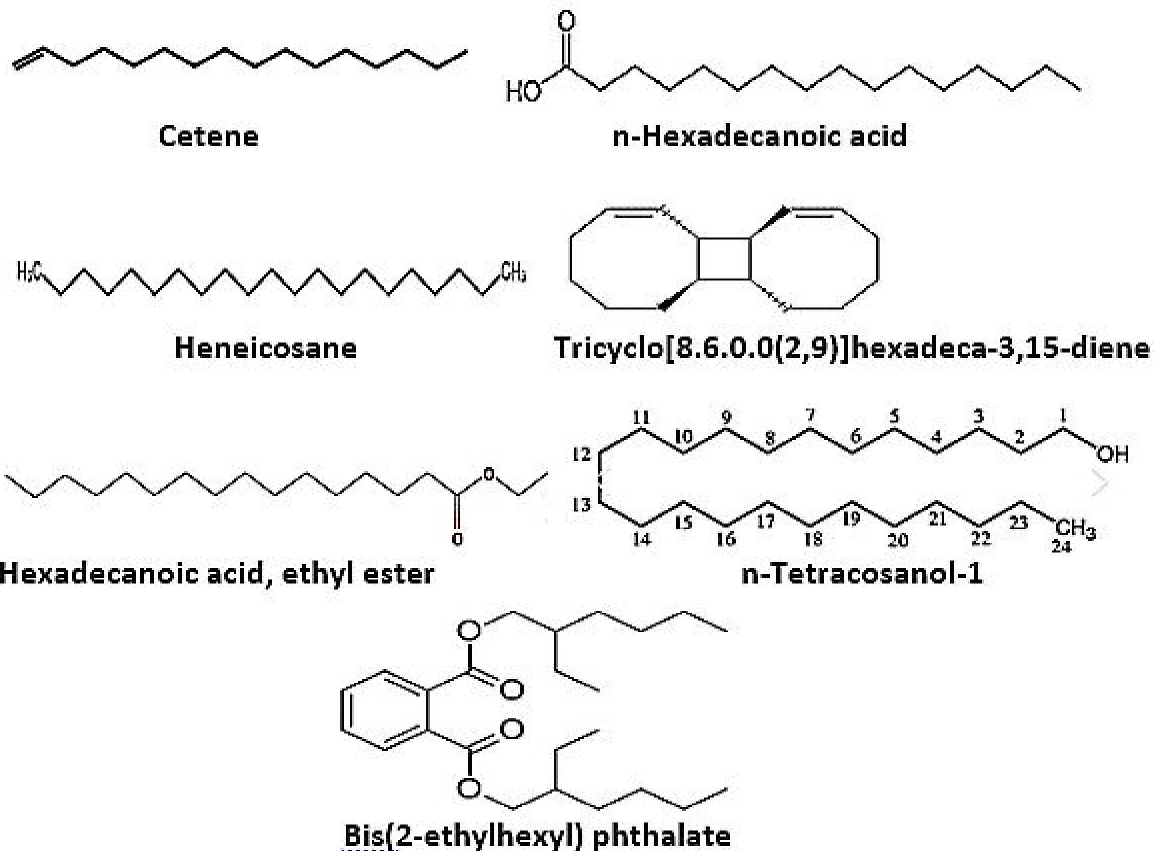

Fig. 6.Major phytocompounds detected by GC-MS in hexane leaf extract of *S. mombin*

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4.5 HPLC Analysis of Flavonoids

Some of the flavonoids detected in methanol leaf extract of *S. mombin*perform different biological activities, especially antioxidant properties. The presence of quercetin, kaempferol, naringenin, luteolin, myricetin, isorhamnetin, and apigenin (Fig. 7) in*S. mombin* highlights its potential pharmacological significance. Quercetin ameliorates inflammatory conditions such as arthritis and inflammatory bowel disease [12, 33]. Moreover, quercetin has demonstrated anticancer potential by inducing apoptosis, inhibiting proliferation, and suppressing angiogenesis in various cancer cell lines [34].Kaempferol exhibits neuroprotective activity and protects human cells from damage due to the actions of free radicals [35].

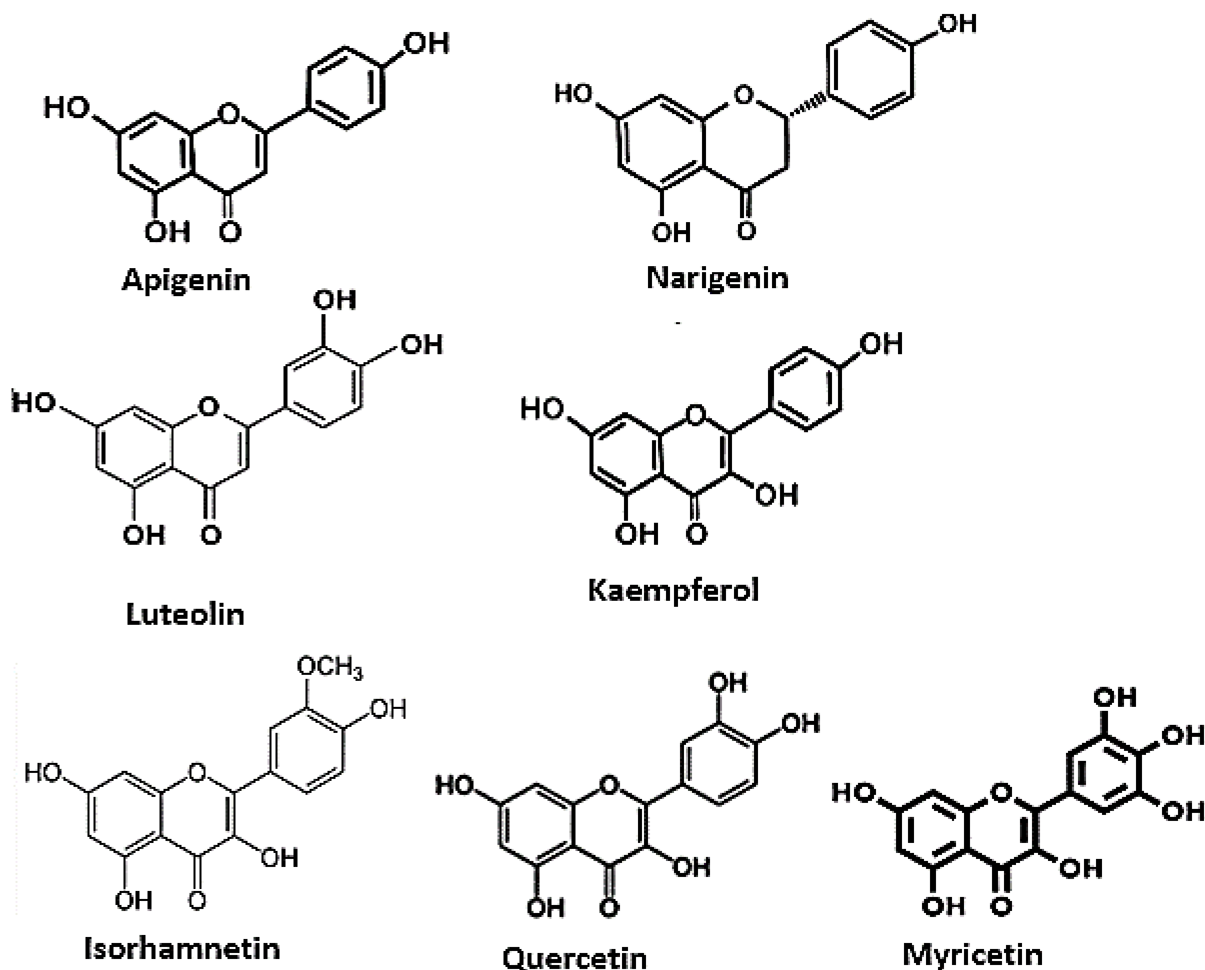

Fig 7.Structures of major flavonoids detected in *S. mombin* leaves

4.6 HPLC Analysis of Phenolics

Phenols are renowned for their antioxidant abilities, which aid to forage free radicals and reduce oxidative strain, potentially protecting the body against chronic illnesses [36].The major bioactive compounds mostly phenolics identified by HPLC in *S. mombin* leaf extract are displayed in Fig 8.These phytochemicals encompass a diverse array of pharmacological properties, indicating *S. mombin* as a potential medicinal plant. Quercetin, a flavonoid with well-established antioxidant properties was detected in relatively high concentrations in the extract [37]. Studies have demonstrated quercetin's ability to mitigate oxidative stress and inflammation,

thereby conferring cardiovascular protection and immune modulation [38]. Saponins have both antifungal and antimicrobial activities and they are also recognized to help boost the immune system [39]. Maleic acid, a dicarboxylic acid has been implicated in the production of certain drugs and chemicals [40].

Research indicates that naringin supplementation can ameliorate myocardial injury and improve vascular function, highlighting its potential role in cardiovascular health [41].Research suggests that tannic acid supplementation can improve endothelial function and attenuate cardiovascular risk factors, emphasizing its potential cardioprotective effects [42].

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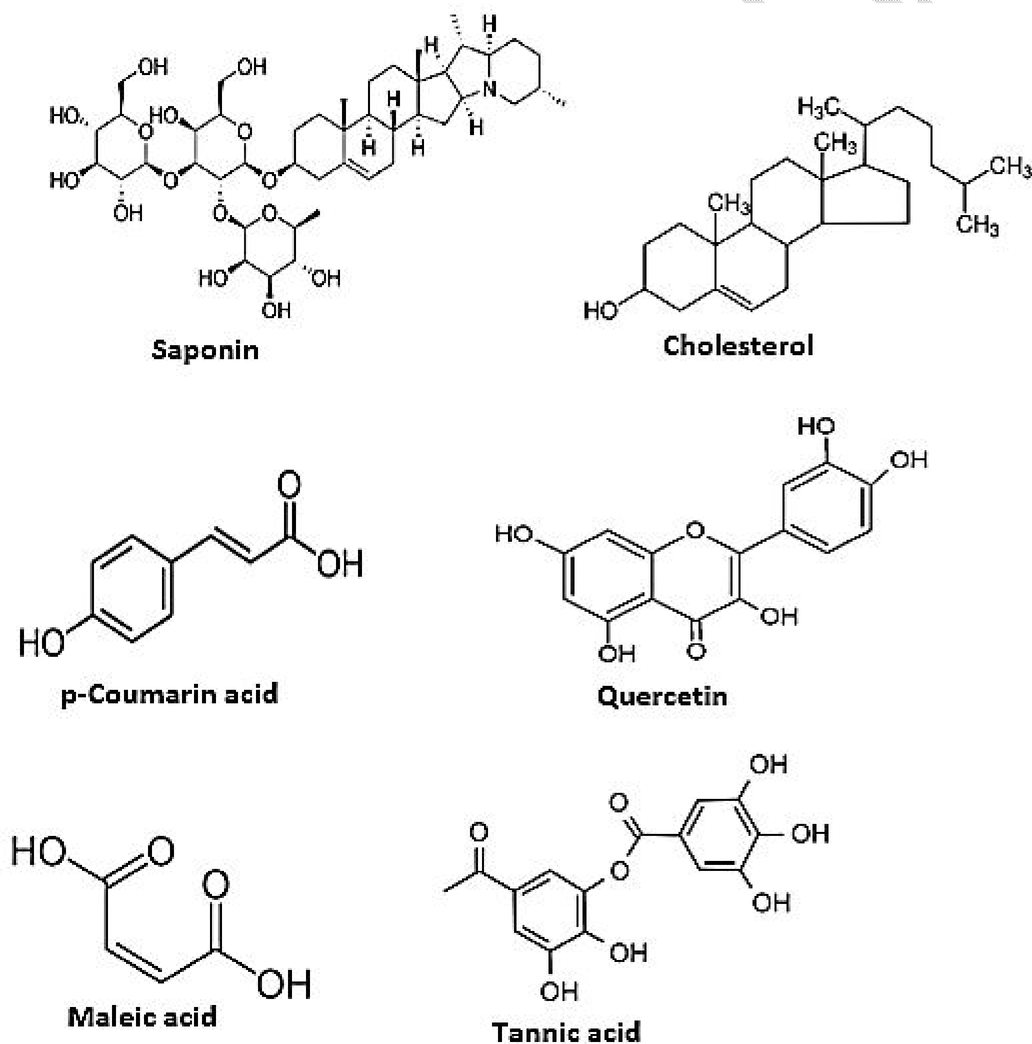

Fig. 8. Major bioactive compounds found in *S. mombin* leaf extract

4.7 Anti-inflammatory Activities

4.7.1 Acetic acid-induced vascular permeability in rat

The *S. mombin* leaf extract at 200 mg/kg dosageled to an OD of 0.141 ± 0.014, reflecting a 27.69% inhibition of vascular permeability. This moderate inhibition suggests the existence of bioactive compounds in the extract that enhance its anti-inflammatory effects. Phytochemicals like flavonoids and phenols, recognized for their anti-inflammatory and antioxidant effects, are likely responsible for this effect [11, 12, 36].

At a higher dose of 400 mg/kg, the extract further reduced the OD to 0.121 ± 0.008, yielding a 37.95% inhibition of vascular permeability. This dose-dependent increase in anti-inflammatory activity indicates that the efficacy of *S. mombin* extract is correlated with the concentration of its bioactive constituents. The increased presence of bioactive components such as flavonoids at greater dosages may likely boost the extract's potency.

The group administered with aspirin (100 mg/kg) demonstrated a significant reduction in OD to 0.079 ± 0.011, corresponding to a 59.49% inhibition of vascular permeability. Aspirin exercises its anti-inflammatory actions by blocking cyclooxygenase enzymes, thereby reducing the synthesis of pro-inflammatory prostaglandins [43]. This substantial inhibition confirms the validity of the experimental model used to assess anti-inflammatory effect.

4.7.2 Carrageenan-induced paw edema

This pattern suggests that the acute inflammatory response was effectively induced. The methanol leaf extract of *S. mombin*demonstratedremarkable anti-inflammatory action basedon a dose-dependent pattern in the experimental rats with carrageenan-induced paw edema, as evidenced by the reduction of paw size (Table 10).The dose of 400 mg/kg displayed greater

UNDER PEER REVIEW

inhibition of paw edema compared to the 200 mg/kg dose, particularly evident at 3- and 4-hours post-induction. This phenomenon could be ascribed to the swift metabolism and removal of the active principle(s), which may have been available in inadequate concentrations at the lower dose level of the extract [44].These outcomes are in harmony with the research done by Nworu*et al.*[45], which illustrated that administering methanol extract from *S. mombin* leaves at 100, 200, and 400 mg/kg doses hindered carrageenan-induced paw edema in a dose-dependent way. Aspirin (200 mg/kg) consistently displayed higher anti-inflammatory activity than both doses of *S. mombin* extract. It showed the highest % inhibition at 3 hours (73.53%) and 4 hours (64.10%), indicating its superior effectiveness in reducing inflammation.

These outcomes indicate that methanol leaf extract of *S. mombin* has strong anti-inflammatory effect that rises with increase in dosage. The extract's effectiveness, while not as high as aspirin, is notable and supports the traditional utilization of *S. mombin* for treating inflammation.

The existence of numerous groups of phytcompounds in *S. mombin*leaves might be accountable for its anti-inflammatory effects. Research has noted that within the diverse bioactive constituents sourced from plants, flavonoids and phenolics are primarily recognized for their efficacy as anti-inflammatory agents [11, 12, 36]. These flavonoids prevent the formation of proinflammatory cytokines like interleukin-6 (IL-6) and enhance the secretion of interleukin-10 (IL-10) under different conditions [12]. Additionally, some flavonoids function as phospholipase inhibitors, thereby blocking the cyclooxygenase and lipoxygenase pathways [46].

5. CONCLUSION

The methanol and n-hexane leaf extracts of *Spondias mombin*were found to contain several phyto compounds as theyalso exhibit noteworthy antioxidant and anti-inflammatory properties. Existence of these compounds validates the traditional exploitation of *S. mombin* for the healing

UNDER PEER REVIEW

of several ailments. The n-hexane extract demonstrates substantially better antioxidant activity at lower dosages, whereas the methanol extract shows stronger dose-dependent anti-inflammatory effects. These findings indicate that *S. mombin* extracts could be excellent natural sources of antioxidants and anti-inflammatory compounds, with potential applications in treating oxidative stress and inflammatory conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing ofthis manuscript.

ETHICAL APPROVAL

Certificate of ethical approval issued by the Niger Delta University Ethical Committee for this researchwas obtained by the authors.

CONFLICT OF INTEREST

Authors declareno competing interests exist that are germane to the content of this paper.

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