

## Original Research Article

# Bioactive Constituents and Assessment of the Antioxidant and Anti-Inflammatory Efficacies of *Spondias mombin* Leaves

### Abstract

The medicinal properties of *Spondias mombin* leaves have been recognized historically, particularly in cancer treatment, diarrhea, and diabetes management. The purpose of this study is to examine the chemical constituents using GC-MS and HPLC, assess the *in vitro* antioxidant and *in vivo* anti-inflammatory properties of *S. mombin*-hexane and methanol leaf extracts. 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 analysis detected quercetin, kaempferol, naringenin, luteolin (flavonoids) and cholesterol, saponin and maleic acid as the major bioactive phenolic compounds. Both leaf extracts exhibited a concentration-dependent increase in radical scavenging activity in all assays indicating a significant antioxidant activity. The methanol extract demonstrated higher total phenolics ( $185.194 \pm 0.271$  mg/g gallic acid), total flavonoids ( $130.568 \pm 0.744$  mg/g rutin) and total antioxidant capacity ( $58.389 \pm 0.292$  mg/g ascorbic acid). Furthermore, the methanol extract demonstrated promising anti-inflammatory effects, particularly at the 400 mg/kg dose, significantly inhibiting paw edema in the carrageenan-induced and acetic acid-induced vascular permeability models. The presence of the 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*

### INTRODUCTION

Herbal plants have been utilized since historical eras to address human diseases. Approximately 80% of the populace in developing nations, continue to rely 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.

*Spondias mombin*, also referred to as the hog plum or yellow mombin, belongs to the Anacardiaceae family. The fruit-bearing deciduous tree is extensively found in Nigeria, particularly in the southwestern areas, and various tropical forests worldwide [2]. In Nigeria and various other global regions, the leaves of *S. mombin* are widely 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 gonorrhoea, cough, sore throat, fever, cholera, constipation, yaws, and stomach issues, and are also utilized as an intra-partum aid [3]. The leaves, fruits, and bark of *S. mombin* are known to be abundant in antioxidants and other beneficial phytochemicals [4]. However, there is no adequate documentation of these bioactive phytochemicals 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 phytochemicals present in the methanol and n-hexane leaf extracts of *S. mombin* and to also evaluate their *in vitro* antioxidant and *in vivo* anti-inflammatory efficacies.

## **MATERIALS AND METHODS**

### **Plant collection and identification**

The plant material was sourced from the Ikolo community within the Yenagoa local government area of Bayelsa State, Nigeria. Dr. Gideon Alade, from the Department of Pharmacognosy and

Herbal Medicine at Niger Delta University, conducted the identification of the plant. The leaves were shade dried for a week before the extraction procedure.

### **Extraction of plant material**

With the use of a blender, the dried leaves were ground into powder. About 500 g of the pulverized leaves were 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. Afterward, it underwent filtration utilizing Whatman No. 1 filter paper; then the solvent was changed for maximum extraction. The resulting filtrate was concentrated to dryness utilizing a water bath maintained at 50°C, the final residue after drying was damp and it was stored in a refrigerator.

### **Assessment of total phenolics**

The total phenolic contents of the extracts was estimated using the Folin-Ciocalteu reagent, as reported by Katalinic *et al.* [5]. One milliliter portion of 10, 20, 50, 100, and 200 µg/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. Absorbance readings were taken after 30 minutes at 765 nm. 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, the absorbance value was assessed to calculate the total phenolic contents employing equation 1:

$$\text{TPC} = (\text{C} \times \text{V})/\text{M} \dots\dots\dots \text{Equation 1}$$

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.

The assay was carried out in triplicate and the mean of results was calculated.

### **Estimation of total flavonoids**

The total flavonoids content was performed following the procedure outlined by Katalin *et al.* [5]. The procedure relies on the creation of a flavonoid-aluminum complex, exhibiting maximum absorbance at 417 nm. A 100  $\mu$ L aliquot of the sample extracts in methanol (10 mg/mL) was combined with 100  $\mu$ L of 10% aluminum 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  $\mu$ L 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).

### **Gas 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 spectrum of the unknown compound was 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.

### **HPLC chromatographic condition for the assay of phenolics in plant extract**

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

### **HPLC equipment conditions for flavonoid analysis**

It was carried out on an Agilent 1200 Series equipped with AGILENT 1260 Diode Array Detector (DAD). To obtain a composition profile of the studied matrices, a C<sub>18</sub> 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.

### **Antioxidant Activity**

#### **Total 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 mean of results was calculated.

**Determination of antioxidant activity using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method**

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:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\% \dots\dots\dots \text{Equation 2}$$

The assay was carried out in triplicate and the results were averaged.

**Hydrogen 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<sub>2</sub>O<sub>2</sub> concentration. In a standard procedure, 0.4 mL of any solution was combined with 0.6 mL of 40 mM H<sub>2</sub>O<sub>2</sub> solution, and the total volume was made up to 2 mL using 50 mM sodium phosphate buffer (pH 7.4). Following incubation for 40 minutes at 30°C, the absorbance reading was done at 230 nm. The assay was carried out in triplicate and the mean of results was calculated.

. The percentage of H<sub>2</sub>O<sub>2</sub> inhibition was then determined according to Equation 3

Percentage (%) of H<sub>2</sub>O<sub>2</sub> radical scavenging assay =

$$[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\% \dots\dots\dots \text{Equation 3}$$

**Determination 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 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 =  
[(A<sub>control</sub> - A<sub>sample</sub>)/A<sub>control</sub>] × 100% .....Equation 4

All measurements were carried out in triplicate and the mean of results was calculated.

**Evaluation of Anti-Inflammatory Activity**

**Assessment of acetic acid-induced rat vascular permeability**

Sixteen (16) rats were categorized into four groups, **I - IV** consisting of four 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 rats 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). The rats were 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 assessed at 590 nm using a microplate reader. The assay was carried out in triplicate and the results were averaged. Equation 5 was utilized to calculate the percentage inhibition of vascular permeability

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

where  $OD_{\text{control}}$  is the optical density of the control group, and  $OD_{\text{test}}$  is the optical density of the test group.

### **Carrageenan-induced paw edema**

The anti-inflammatory potential of *S. mombin* extract was examined 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. The paw diameter was then measured at one-hour intervals following the induction of inflammation, up to the fourth hour. The alteration in paw volume at 1, 2, 3, and 4 hours after carrageenan injection was considered the parameter for inflammation measurement. The assay was carried out in quadruplet and the results were averaged. Equation 6 was employed to calculate the percentage inhibition of inflammation:

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

Where,

$V_c$  represents the mean value of the paw volume of the control group,

$V_t$  represents the mean paw volume of the test group.

### **Statistical Analysis**

The data were examined with SPSS version 21.0. Descriptive statistics were used and findings were given as mean  $\pm$  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.

## RESULTS AND DISCUSSION

### Quantitative Phytochemical Analysis

Table 1 details the quantitative phytochemical analysis derived from the solvent leaf extracts of *Spondias mombin*.

**Table 1:** Quantitative phytochemicals in *Spondias 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)

### Total phenolics and total antioxidant capacity (TAC)

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. These findings suggest that methanol extraction is more efficient in extracting phenolic compounds and conferring a higher antioxidant capacity than n-hexane extraction. The higher values of total antioxidant capacity 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 involving the amount of phenolics and antioxidant activity in plant extracts [10]. The presence of phenolics in the extracts may contribute to its antioxidant capacity and potential health-promoting effects.

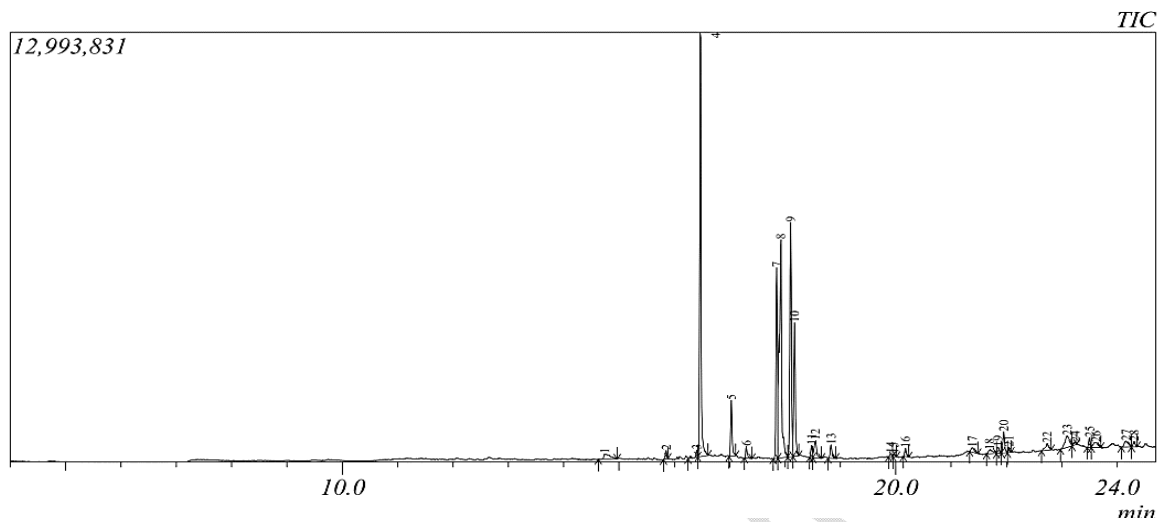
### Total Flavonoids

Total flavonoid contents in the methanol and n-hexane leaf extracts of *S. mombin* was obtained are  $130.568 \pm 0.744$  mg/g rutin equivalent and  $57.508 \pm 0.722$  mg/g rutin respectively (Table 1). 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 [11]. 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 [12]. Flavonoids have also been observed to remove free radicals and reduce oxidative strain, sparing cells from harm [13]. Thus, the methanol extract's flavonoid content is expected to contribute to its total antioxidant activity and possible therapeutic benefits.

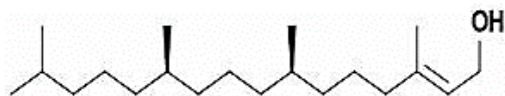
#### **GC-MS examination of methanol leaf extract of *Spondias mombin***

The GC-MS data of methanol leaf extract from *S. mombin* displayed in Fig. 1 and Table 2 identified 28 compounds. The main compounds (Figures 2-7) identified 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%). Most of the compounds extracted with methanol were fatty acid methyl esters commonly found in plant oils. These phytochemicals 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 [14]. Phytol, a diterpene alcohol, has shown anticonvulsant, antioxidant and antinociceptive effects [15, 16]. Methyl stearate, another fatty acid methyl ester detected in the extract, has demonstrated antimicrobial properties [17], anti-diarrheal, cytotoxic and anti-proliferative activities [18]; while 10-octadecenoic acid methyl ester has been reported to exhibit both antimicrobial and antioxidant

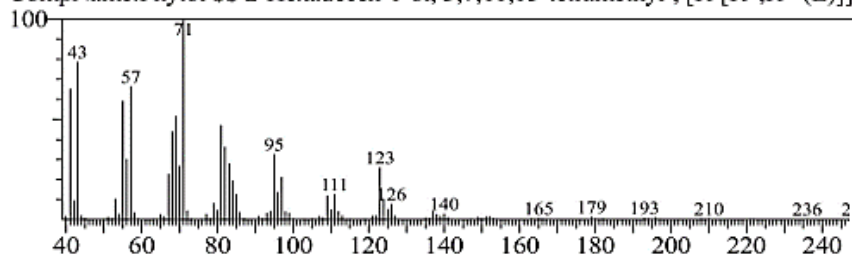
effects [19]. Testosterone cypionate, a synthetic derivative of testosterone, is used medically to remedy disorders related to testosterone deficiency and several types of breast cancer [20]. Also, squalene is stated to have antioxidant and wound healing property [21].



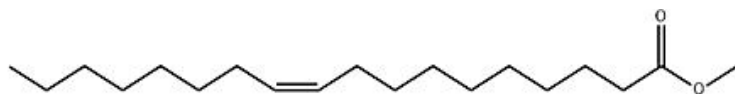
**Fig 1:** GC-MS chromatogram of methanol leaf extract of *Spondias mombin*



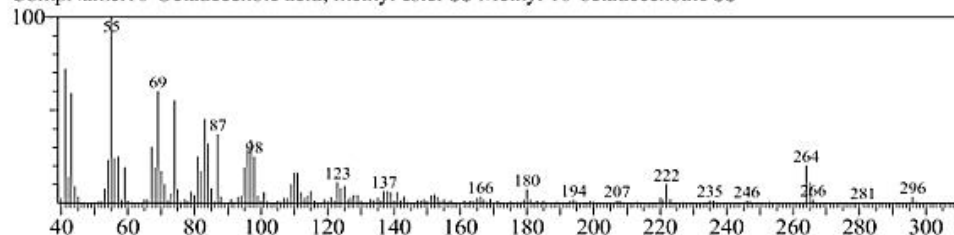
Hit#:4 Entry:25944 Library:NIST11s.lib  
 SI:76 Formula:C<sub>20</sub>H<sub>40</sub>O CAS:150-86-7 MolWeight:296 RetIndex:2045  
 CompName:Phytol \$S 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R\*,R\*-(E)]]



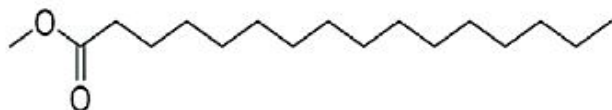
**Fig 2:** Mass spectra for phytol



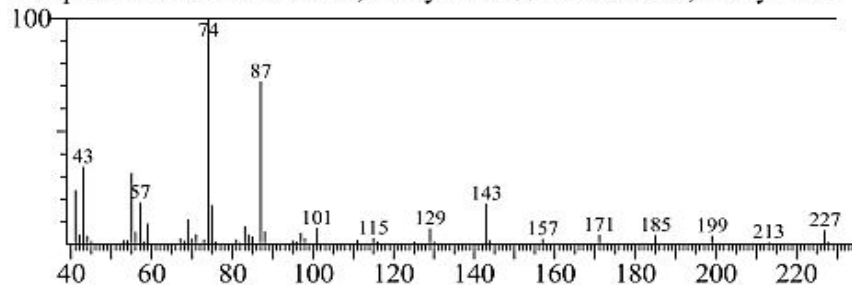
Hit#:1 Entry:115408 Library:NIST11s.lib  
 SI:94 Formula:C19H36O2 CAS:13481-95-3 MolWeight:296 RetIndex:2085  
 CompName:10-Octadecenoic acid, methyl ester \$\$ Methyl 10-octadecenoate \$\$



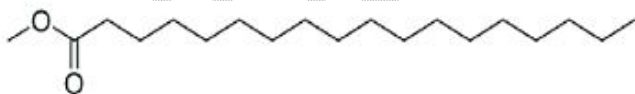
**Fig 3:** Mass spectra of 10-octadecenoic acid, methyl ester



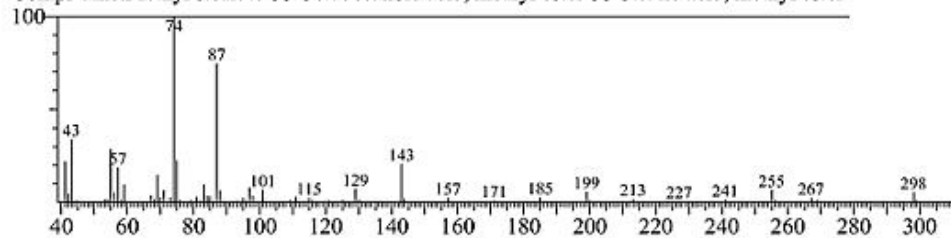
Hit#:1 Entry:24299 Library:NIST11s.lib  
 SI:96 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878  
 CompName:Hexadecanoic acid, methyl ester \$\$ Palmitic acid, methyl ester



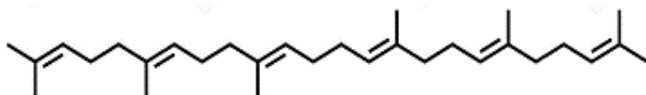
**Fig 4:** Mass spectra of hexadecanoic acid, methyl ester



Hit#:1 Entry:26033 Library:NIST11s.lib  
 SI:96 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077  
 CompName:Methyl stearate \$\$ Octadecanoic acid, methyl ester \$\$ Stearic acid, methyl ester



**Fig 5:** Mass spectra of methyl stearate



Hit#:1 Entry:29859 Library:NIST11s.lib  
 SI:73 Formula:C30H50 CAS:111-02-4 MolWeight:410 RetIndex:2914  
 CompName:Squalene SS 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- SS all-trans-Squalene

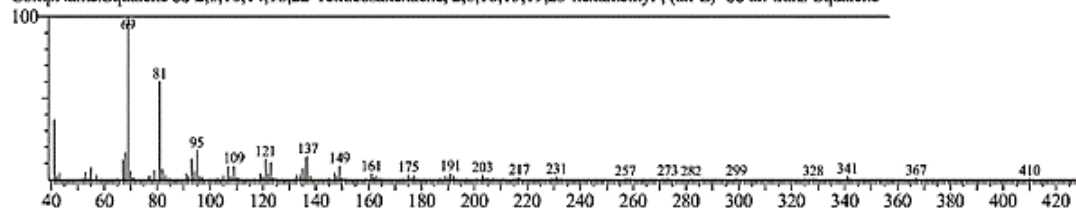
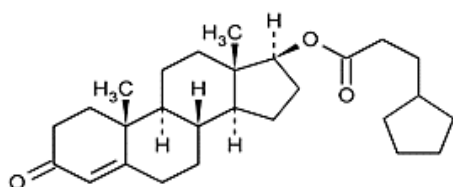


Fig 6: Mass spectra of Squalene



Hit#:1 Entry:186802 Library:NIST11.lib  
 SI:70 Formula:C27H40O3 CAS:58-20-8 MolWeight:412 RetIndex:2918  
 CompName:Testosterone cypionate SS Androst-4-en-3-one, 17-(3-cyclopentyl-1-oxopropoxy)-, (17.β.)-

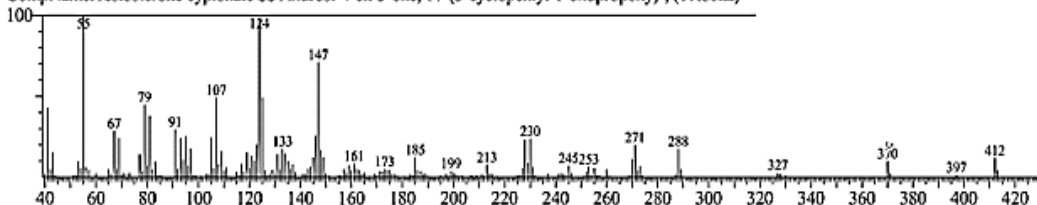


Fig 7: Mass spectra of testosterone cypionate

Table 2: GC-MS analysis of methanol extract of *Spondias mombin*

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

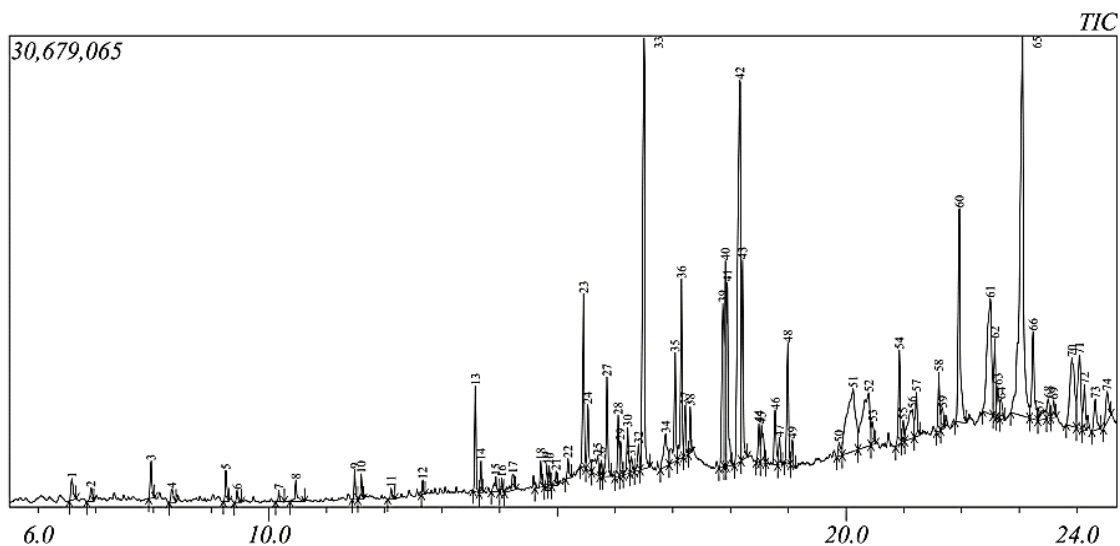
|           |        |  |                      |       |
|-----------|--------|--|----------------------|-------|
| <b>6</b>  | 17.300 | Heptadecanoic acid, methyl ester   | $C_{18}H_{36}O_2$    | 0.77  |
| <b>7</b>  | 17.848 | 11,14-Octadecadienoic acid, methyl ester   | $C_{19}H_{34}O_2$    | 11.04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

|    |        |   |  |      |
|----|--------|---|--|------|
| 27 | 24.158 | 5H-3,5a-Epoxy-naphth[2,1-c]oxepin, dodecahydro-3,8,8,11a-tetramethyl-[3S-(3.alpha.,7a.alpha,11a.alpha,11a.beta.,11b.alpha)] | C <sub>18</sub> H <sub>30</sub> O <sub>2</sub> | 0.98 |
| 28 | 24.303 | Squalene  | C <sub>30</sub> H <sub>50</sub>                | 0.42 |

### GC-MS examination of n-hexane *Spondias mombin* leaf extract

The GC-MS examination of the n-hexane *Spondias mombin* leaf extract uncovered the existence of 19 phytochemicals, as depicted in Table 3 and Figure 8. Among these, 13 compounds (Figures 9-12) 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.

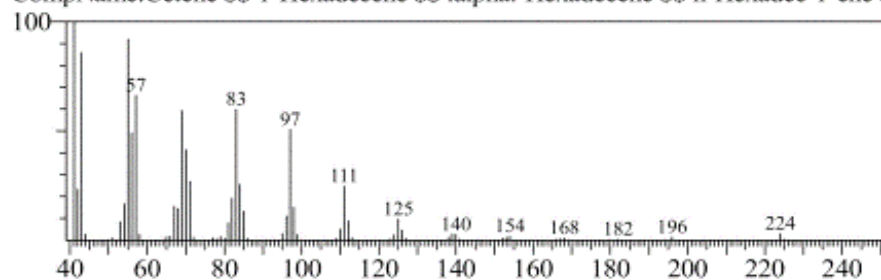
Hexadecanoic acid, ethyl ester has the ability to destroy nematodes, pests, and reduce 5-alpha inhibitors [22]. Cetene an unsaturated aliphatic hydrocarbon has been reported to have antimicrobial and antioxidant effect, also had highest value of antifungal activity [23]. The identification of n-hexadecanoic acids in the extract has been involved in the synthesis of prostaglandins [24]. Heneicosane possesses bactericidal actions [25]. 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 hepatoprotective, antiasthmatic, diuretic, anticancer functions [26].



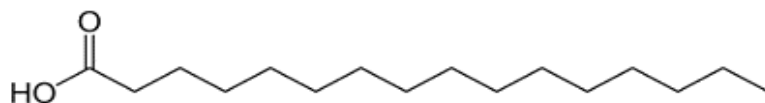
**Fig 8:** GC-MS chromatogram of n-hexane *Spondias mombin* leaf extract



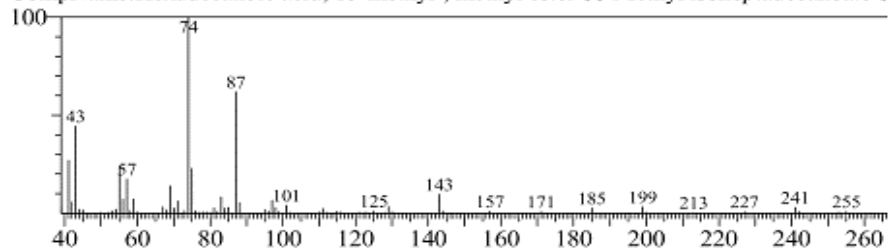
Hit#:5 Entry:20515 Library:NIST11s.lib  
 SI:94 Formula:C16H32 CAS:629-73-2 MolWeight:224 RetIndex:1602  
 CompName:Cetene \$\$ 1-Hexadecene \$\$ .alpha.-Hexadecene \$\$ n-Hexadec-1-ene !



**Fig 9:** Mass spectra of Cetene



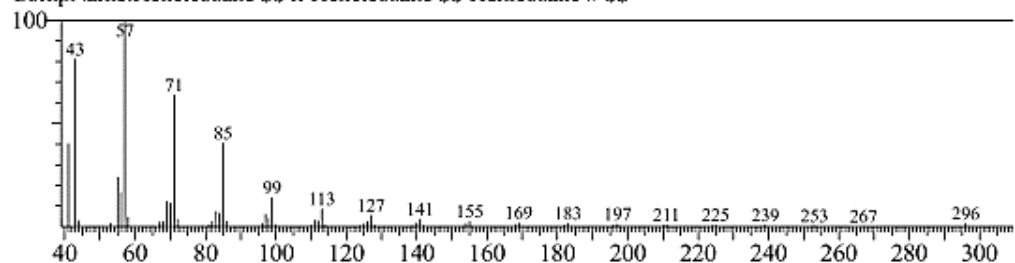
Hit#:5 Entry:106180 Library:NIST11.lib  
 SI:87 Formula:C18H36O2 CAS:6929-04-0 MolWeight:284 RetIndex:1914  
 CompName:Hexadecanoic acid, 15-methyl-, methyl ester \$\$ Methyl isoheptadecanoate \$



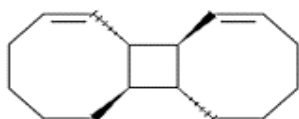
**Fig 10:** Mass spectra of n-Hexadecanoic acid



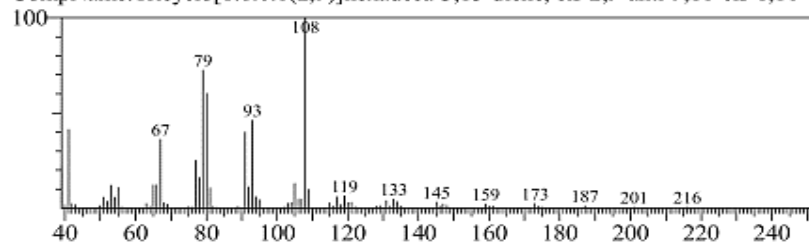
Hit#:3 Entry:25950 Library:NIST11s.lib  
 SI:85 Formula:C<sub>21</sub>H<sub>44</sub> CAS:629-94-7 MolWeight:296 RetIndex:2109  
 CompName:Heneicosane \$\$ n-Heneicosane \$\$ Heneicosane # \$\$



**Fig 11:** Mass spectra of Heneicosane



Hit#:2 Entry:54987 Library:NIST11.lib  
 SI:81 Formula:C<sub>16</sub>H<sub>24</sub> CAS:0-00-0 MolWeight:216 RetIndex:1687  
 CompName:Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, cis-2,9-anti-9,10-cis-1,10-



**Fig 12:** Mass spectra of Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-trans-1,10

**Table 3:** GC-MS analysis of n-hexane leaf extract of *Spondias mombin*

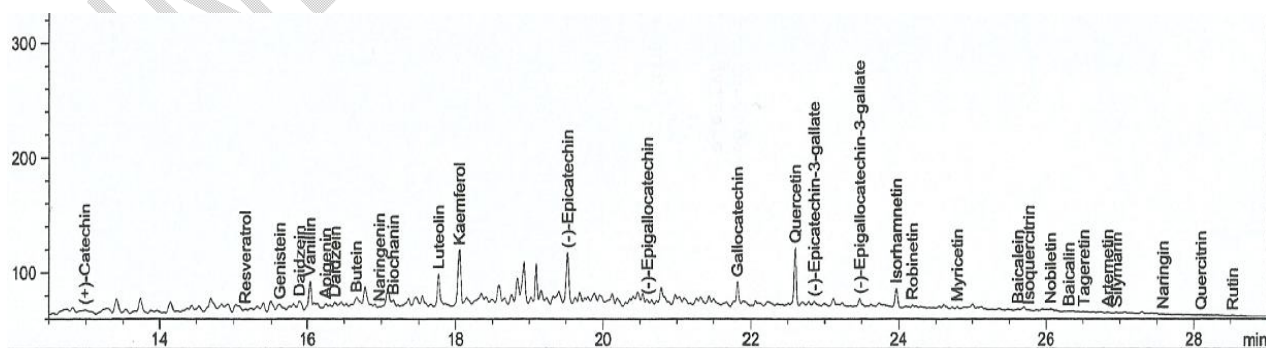
| S/N | Retention Time (min) | Compound Name                            | Molecular formula                              | Peak area (%) |
|-----|----------------------|--|--|---------------|
| 1   | 13.578               | Cetene                                   | C <sub>16</sub> H <sub>32</sub>                | 1.18          |
| 2   | 15.453               | 9-Eicosene, (E)-                         | C <sub>20</sub> H <sub>40</sub>                | 2.20          |
| 3   | 15.858               | Phytol, acetate                          | C <sub>22</sub> H <sub>42</sub> O <sub>2</sub> | 1.37          |
| 4   | 16.501               | Hexadecanoic acid, methyl ester          | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> | 7.47          |
| 5   | 16.876               | n-Hexadecanoic acid                      | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> | 1.08          |
| 6   | 17.038               | Hexadecanoic acid, ethyl ester           | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> | 1.87          |
| 7   | 17.867               | 11,14-Octadecadienoic acid, methyl ester |  | 2.77          |
| 8   | 17.911               | Methyl Stearidonate                      | C <sub>19</sub> H <sub>30</sub> O <sub>2</sub> | 2.92          |

|    |        |  |  |       |
|----|--------|--|--|-------|
| 9  | 17.944 | 9-Octadecenoic acid (Z)-, methyl ester                                       |  | 2.48  |
| 10 | 18.162 | Phytol   | C <sub>20</sub> H <sub>40</sub> O              | 9.09  |
| 11 | 18.207 | Methyl stearate  | C <sub>19</sub> H <sub>38</sub> O <sub>2</sub> | 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 <sub>24</sub> H <sub>38</sub> O <sub>4</sub> | 4.06  |
| 15 | 22.499 | Heneicosane  | C <sub>21</sub> H <sub>44</sub>                | 4.46  |
| 16 | 22.577 | 1-Heneicosanol   | C <sub>21</sub> H <sub>44</sub> O              | 1.10  |
| 17 | 23.056 | Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene,<br>trans-2,9-anti-9,10-trans-1,10 | C <sub>16</sub> H <sub>24</sub>                | 12.07 |
| 18 | 23.240 | Phenol, 3-pentadecyl-  | C <sub>21</sub> H <sub>36</sub> O              | 1.74  |
| 19 | 23.915 | Behenic alcohol  |  | 2.78  |

### HPLC analysis of flavonoids in *Spondias mombin* methanol leaf extract

Thirty (30) different flavonoid compounds were obtained from the HPLC data (Table 4 and Fig 13). The major flavonoids found in the extract of *S. mombin* are displayed in Figure 14.

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 (Table 3).



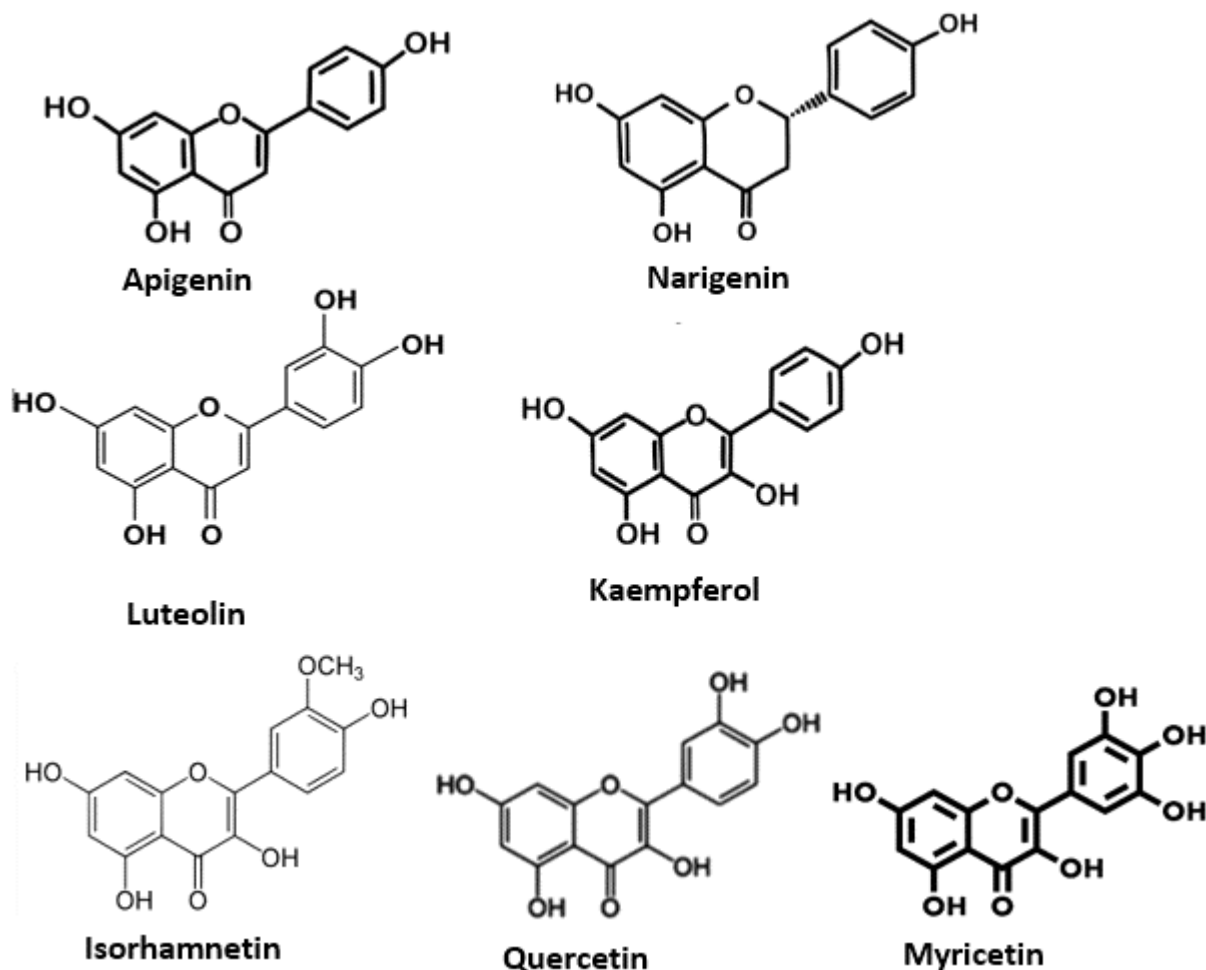
**Fig 13:** HPLC chromatogram of the flavonoids in methanol leaf extract of *Spondias mombin*

Some of these flavonoids perform different biological activities, especially antioxidant properties. The presence of quercetin, kaempferol, naringenin, luteolin, myricetin, isorhamnetin, and apigenin in the methanol leaf extract of *S. mombin* highlights its potential pharmacological significance. Quercetin ameliorates inflammatory conditions such as arthritis and inflammatory bowel disease [11, 27]. Moreover, quercetin has demonstrated anticancer potential by inducing apoptosis, inhibiting proliferation, and suppressing angiogenesis in various cancer cell lines [28]. Kaempferol exhibits neuroprotective activity and protects human cells from damage due to the actions of free radicals [29].

**Table 4:** Distribution of flavonoids in methanol leaf extract of *Spondias mombin*

| S/N | Retention Time (min) | Concentration (mg/100g) | Flavonoid Compound     |
|-----|----------------------|-------------------------|------------------------|
| 1   | 12.985               | 1.771                   | (+) – Catechin         |
| 2   | 15.155               | $3.878 \times 10^{-6}$  | Resveratrol            |
| 3   | 15.617               | $1.278 \times 10^{-5}$  | Genistein              |
| 4   | 15.891               | $1.124 \times 10^{-5}$  | Daidzein               |
| 5   | 16.037               | $2.347 \times 10^{-3}$  | Vanillin               |
| 6   | 16.244               | 3.303                   | Apigenin               |
| 7   | 16.669               | $2.236 \times 10^{-5}$  | Butein                 |
| 8   | 16.976               | 10.331                  | Naringenin             |
| 9   | 17.159               | $8.639 \times 10^{-6}$  | Biochanin              |
| 10  | 17.769               | 10.222                  | Luteolin               |
| 11  | 18.052               | 12.357                  | Kaempferol             |
| 12  | 19.519               | $4.576 \times 10^{-6}$  | (-) – Epicatechin      |
| 13  | 20.592               | $5.718 \times 10^{-4}$  | (-) – Epigallocatechin |

|    |        |                        |                                  |
|----|--------|------------------------|----------------------------------|
| 14 | 21.821 | $1.264 \times 10^{-4}$ | Gallocatechin                    |
| 15 | 22.602 | 44.004                 | Quercetin                        |
| 16 | 22.852 | $3.955 \times 10^{-4}$ | (-) – Epicatechin-3-gallate      |
| 17 | 23.471 | $3.510 \times 10^{-4}$ | (-) – Epigallocatechin-3-gallate |
| 18 | 23.968 | 7.633                  | Isorhamnetin                     |
| 19 | 24.186 | $6.316 \times 10^{-6}$ | Robinetin                        |
| 20 | 24.792 | 7.699                  | Myricetin                        |
| 21 | 25.616 | $5.856 \times 10^{-6}$ | Baicalein                        |
| 22 | 25.761 | $5.980 \times 10^{-1}$ | Isoquercitrin                    |
| 23 | 26.063 | $1.003 \times 10^{-5}$ | Nobiletin                        |
| 24 | 26.300 | $2.513 \times 10^{-6}$ | Baicalin                         |
| 25 | 26.503 | $3.088 \times 10^{-6}$ | Tageretin                        |
| 26 | 26.829 | $4.601 \times 10^{-6}$ | Artemetin                        |
| 27 | 26.950 | $5.309 \times 10^{-6}$ | Silymarin                        |
| 28 | 27.575 | $1.801 \times 10^{-6}$ | Naringin                         |
| 39 | 28.097 | $1.342 \times 10^{-1}$ | Quercitrin                       |
| 30 | 28.527 | $2.398 \times 10^{-1}$ | Rutin                            |

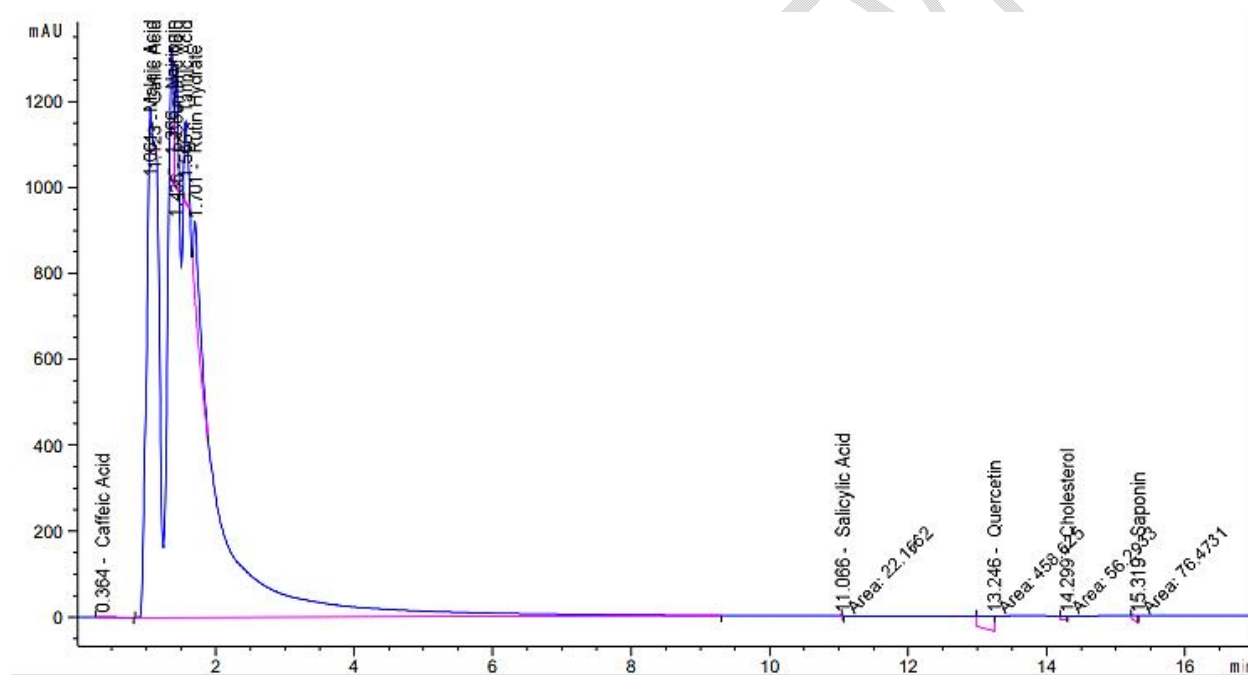


**Fig 14:** Structures of major flavonoids found in *Spondias mombin* leaves

#### HPLC analysis of phenolics in *Spondias mombin* methanol leaf extract

Phenols are well-known for their antioxidant qualities, which aid to scavenge free radicals and reduce oxidative stress, potentially protecting the human body against chronic illnesses [30]. The HPLC data obtained from *S. mombin* methanol leaf extract disclosed eleven (11) bioactive phenolic phytochemicals with distinct retention times and concentrations (Table 5 and Figure 15); while the main phenolics are displayed in Figure 16. 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). 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 [31]. Studies have demonstrated quercetin's ability to mitigate oxidative stress and inflammation, thereby conferring cardiovascular protection and immune modulation [32]. Saponins have both antifungal and antimicrobial activities and they are also known to help boost the immune system [33]. Maleic acid has been implicated in the production of certain drugs and chemicals [34]

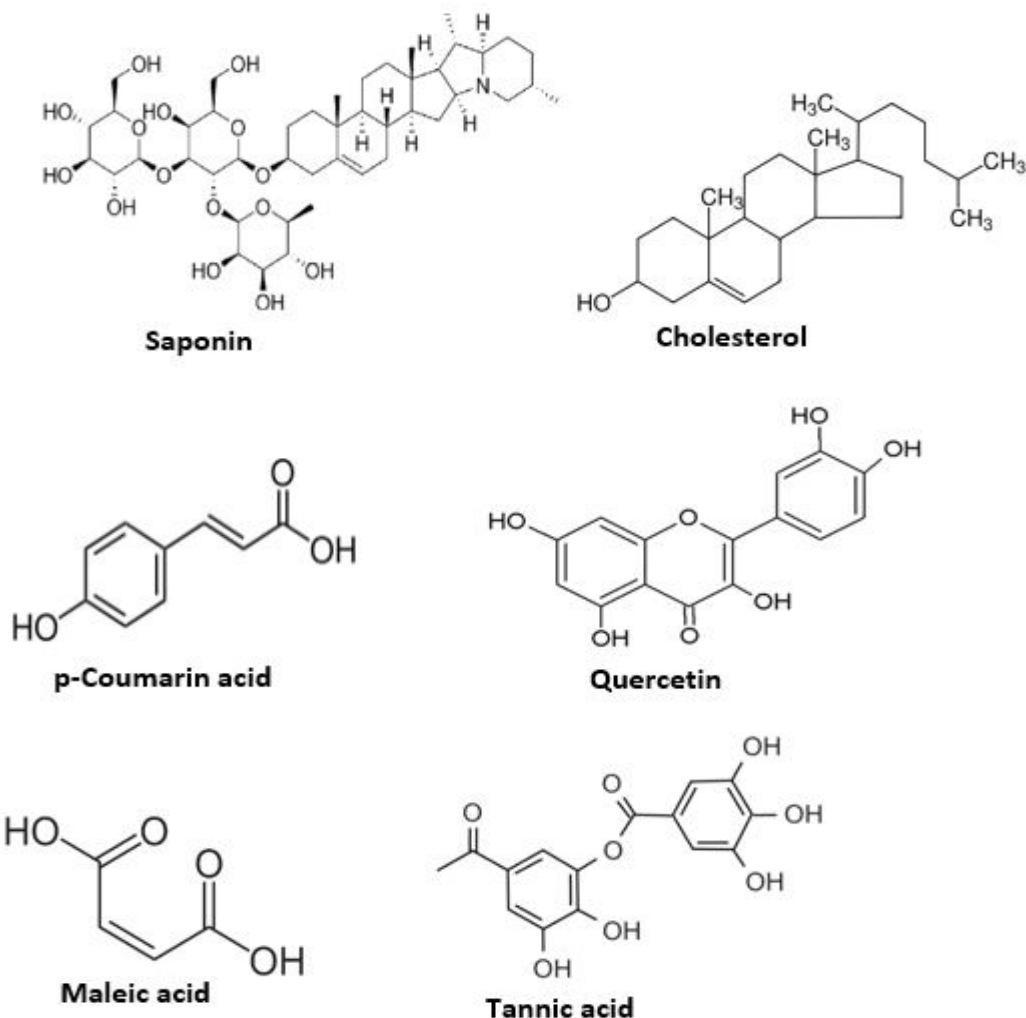


**Fig 15:** HPLC Chromatogram of some bioactive compounds in methanol leaf extract of *Spondias mombin*

Research indicates that naringin supplementation can ameliorate myocardial injury and improve vascular function, highlighting its potential role in cardiovascular health [35]. Research suggests that tannic acid supplementation can improve endothelial function and attenuate cardiovascular risk factors, emphasizing its potential cardioprotective effects [36].

**Table 5:** Phenolic compounds in methanol leaf extract of *S. mombin*

| <b>S/N</b> | <b>Retention Time (Min)</b> | <b>Concentration (mg/L)</b> | <b>Bioactive Compound</b> |
|------------|-----------------------------|-----------------------------|---------------------------|
| 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                   |



**Fig 16:** Major phenolic compounds found in *S. mombin* leaf extract

## Antioxidant Activities

### DPPH Radical Scavenging Activity

Table 6 presents the results of the DPPH radical scavenging activity of methanol leaf extract of *S. mombin* and ascorbic acid at different concentrations. From the results ascorbic acid exhibited the highest % inhibition across all concentrations, with values ranging from  $66.50 \pm 0.06\%$  at  $10 \mu\text{g/mL}$  to  $94.34 \pm 0.07\%$  at  $200 \mu\text{g/mL}$ , indicating its superior DPPH radical scavenging activity. By contrast, the antioxidant effect of the n-hexane leaf extract was moderate, as evidenced by the increase in inhibition percentage from  $12.49 \pm 0.23\%$  at  $10 \mu\text{g/mL}$  to  $83.27 \pm 0.17\%$  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. All samples displayed a clear concentration-dependent increase in DPPH radical scavenging activity, consistent with typical antioxidant behavior. Increase in the concentration of the extracts resulted in suppression of DPPH radicals, indicating high radical scavenging activity.

**Table 6:** DPPH radical scavenging activity (% inhibition) of *S. mombin* leaf extracts

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

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.

### Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity assessment provides critical insights into the antioxidant potential of natural compounds. From the result in Table 7 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<sub>2</sub>O<sub>2</sub> scavenging activity.

Both leaf extracts of *S. mombin* showed a concentration-dependent increase in % inhibition, but no statistically significant difference in their H<sub>2</sub>O<sub>2</sub> scavenging activities. Comparatively, the methanol extract exhibited slightly higher H<sub>2</sub>O<sub>2</sub> scavenging activity than the n-hexane extract at most concentrations, particularly at 50 µg/mL and above.

**Table 7:**Hydrogen peroxide scavenging activity (% inhibition) of *S. mombin* leaf extracts

| Concentration ( $\mu\text{g/mL}$ ) | Ascorbic                      | n-Hexane                      | Methanol                      |
|------------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 10                                 | 59.24 $\pm$ 0.28 <sup>b</sup> | 17.28 $\pm$ 0.74 <sup>a</sup> | 18.53 $\pm$ 0.41 <sup>a</sup> |
| 25                                 | 84.57 $\pm$ 0.25 <sup>b</sup> | 22.77 $\pm$ 0.57 <sup>a</sup> | 24.67 $\pm$ 0.33 <sup>a</sup> |
| 50                                 | 88.10 $\pm$ 0.33 <sup>b</sup> | 39.84 $\pm$ 0.49 <sup>a</sup> | 45.82 $\pm$ 0.34 <sup>a</sup> |
| 100                                | 91.90 $\pm$ 0.34 <sup>b</sup> | 61.36 $\pm$ 0.43 <sup>a</sup> | 68.53 $\pm$ 0.33 <sup>a</sup> |
| 200                                | 92.77 $\pm$ 0.57 <sup>b</sup> | 79.67 $\pm$ 0.41 <sup>a</sup> | 85.60 $\pm$ 0.47 <sup>a</sup> |

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

### ABTS Radical Scavenging Assay

Table 8 shows the findings of the ABTS scavenging activities of *S. mombin* leaf extracts and ascorbic acid at different concentrations. Ascorbic acid consistently exhibited the highest % inhibition across all concentrations, from 65.73 $\pm$ 0.07% at 10  $\mu\text{g/mL}$  to 96.35 $\pm$ 0.13% at 200  $\mu\text{g/mL}$ , demonstrating its superior antioxidant capacity. Both leaf extracts showed a concentration-dependent increase in ABTS scavenging activity. Again no statistically significant difference is observed in their ABTS scavenging activities.

**Table 8:** ABTS scavenging activity (% inhibition) of *S. mombin* leaf extracts

| Concentration ( $\mu\text{g/mL}$ ) | Ascorbic                      | n-Hexane                      | Methanol                      |
|------------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 10                                 | 65.73 $\pm$ 0.07 <sup>b</sup> | 13.91 $\pm$ 0.12 <sup>a</sup> | 7.72 $\pm$ 0.07 <sup>a</sup>  |
| 25                                 | 92.70 $\pm$ 0.07 <sup>b</sup> | 22.67 $\pm$ 0.12 <sup>a</sup> | 12.14 $\pm$ 0.07 <sup>a</sup> |
| 50                                 | 93.97 $\pm$ 0.07 <sup>b</sup> | 59.09 $\pm$ 0.12 <sup>a</sup> | 56.51 $\pm$ 0.07 <sup>a</sup> |
| 100                                | 94.89 $\pm$ 0.07 <sup>b</sup> | 79.87 $\pm$ 0.07 <sup>a</sup> | 80.87 $\pm$ 0.07 <sup>a</sup> |
| 200                                | 96.35 $\pm$ 0.13 <sup>b</sup> | 87.90 $\pm$ 0.12 <sup>a</sup> | 87.02 $\pm$ 0.07 <sup>a</sup> |

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

### Anti-Inflammatory Activities

#### 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  $0.195 \pm 0.007$ , establishing a baseline for maximum vascular permeability and inflammation. This indicates a successful induction of inflammatory response using acetic acid, a standard method for evaluating anti-inflammatory agents [37]. This also serves as a baseline for comparison with the treatment groups.

The group administered with aspirin (100 mg/kg) demonstrated a significant reduction in OD to  $0.079 \pm 0.011$ , corresponding to a 59.49% inhibition of vascular permeability. Aspirin, a well-known nonsteroidal anti-inflammatory drug (NSAID), exerts its effects by blocking cyclooxygenase enzymes, thereby reducing the synthesis of pro-inflammatory prostaglandins [38]. This substantial inhibition confirms the validity of the experimental model used to assess anti-inflammatory effect.

The *S. mombin* leaf extract at a dosage of 200 mg/kg resulted in an OD of  $0.141 \pm 0.014$ , 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 [10, 11, 30].

**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)                  | $0.079 \pm 0.011^a$ | 59.49%       |
| II           | <i>S. mombin</i> extract (200 mg/kg) | $0.141 \pm 0.014^b$ | 27.69%       |
| III          | <i>S. mombin</i> extract (400 mg/kg) | $0.121 \pm 0.008^c$ | 37.95        |
| IV (Control) | Normal saline (10 mL/kg)             | $0.195 \pm 0.007^d$ | -            |

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

At a higher dose of 400 mg/kg, the extract further reduced the OD to  $0.121 \pm 0.008$ , 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.

### **Carrageenan-induced paw edema**

The results as depicted in Table 10 indicate 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 effectively induced. The methanol leaf extract of *S. mombin* exhibited remarkable anti-inflammatory effect in the experimental rats with carrageenan-induced paw edema, as evidenced by the reduction in paw size (Table 10). There were no significant variances observed among the treatment groups and the control group in the first hour after induction, indicating that the early reaction to inflammation was identical. However, the doses of 200 mg/kg and 400 mg/kg of the extract demonstrated significant 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). The higher dosage of *S. mombin* extract (400 mg/kg) resulted in 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.

The methanol extract of *S. mombin* demonstrated anti-inflammatory effect according to a dose-dependent pattern. The dose of 400 mg/kg displayed greater 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 [39]. These outcomes agree with the research done by Nworuet *al.* [40], which illustrated that administering methanol extract from *S. mombin* leaves at dosages of 100, 200, and 400 mg/kg inhibited carrageenan-induced paw edema in a dose-dependent manner. 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.

**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                |
| <b>I</b>                   | 200 mg/kg | 0.10±0.00               | 0.22±0.07 <sup>a</sup>                            | 0.39±0.01 <sup>c</sup> | 0.43±0.06 <sup>c</sup> | 0.27±0.05 <sup>c</sup> |
| Plant extract              |           |                         | (0.00%)   | (-30.00%)              | (36.76%)               | (30.77%)               |
| <b>II</b>                  | 400 mg/kg | 0.10±0.00               | 0.17±0.05 <sup>a</sup>                            | 0.25±0.02 <sup>a</sup> | 0.29±0.01 <sup>b</sup> | 0.21±0.01 <sup>b</sup> |
| Plant extract              |           |                         | (22.73%)  | (16.67%)               | (57.35%)               | (46.15%)               |
| <b>III</b>                 | 0.2 mL/kg | 0.10±0.00               | 0.22±0.03 <sup>a</sup>                            | 0.30±0.02 <sup>b</sup> | 0.68±0.03 <sup>d</sup> | 0.39±0.08 <sup>d</sup> |
| Distilled H <sub>2</sub> O |           |                         |   |                        |                        |                        |
| <b>IV</b>                  | 200 mg/kg | 0.10±0.00               | 0.17±0.05 <sup>a</sup>                            | 0.23±0.02 <sup>a</sup> | 0.18±0.08 <sup>a</sup> | 0.14±0.02 <sup>a</sup> |

|         |          |          |          |          |
|---------|----------|----------|----------|----------|
| Aspirin | (22.73%) | (23.33%) | (73.53%) | (64.10%) |
|---------|----------|----------|----------|----------|

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

These outcomes indicate that the 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 presence of numerous groups of phytochemicals 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 [10, 11, 30]. These flavonoids have shown to inhibit the production of proinflammatory cytokines like interleukin-6 (IL-6) and enhance the secretion of interleukin-10 (IL-10) under different conditions [11]. Additionally, some flavonoids function as phospholipase inhibitors, thereby blocking the cyclooxygenase and lipoxygenase pathways [41].

### Conclusion

The methanol and n-hexane leaf extracts of *Spondias mombin* were found to contain several bioactive compounds. The existence of these bioactive compounds validates the traditional exploitation of *S. mombin* for the healing of several ailments. The plant also exhibit significant antioxidant and anti-inflammatory properties. 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.

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