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In vitro radical scavenging and *in vivo* antiarthritic effects of *Spondias mombin* (L.) stem bark

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Abstract

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that affects about 1% of the population globally. Numerous conventional medications are available for the management of RA. However, their usage is limited due to the associated side effects. *Spondias mombin* is a member of the Anacardiaceae family known for its anti-arthritic effects. This study aimed to evaluate the anti-arthritic activity of *S. mombin* (L.) stem bark in RA. Radical scavenging activity was determined using DPPH assay. The anti-inflammatory effect was determined using protease inhibition and formaldehyde-induced arthritis models. *S. mombin* aqueous fraction (SMAF) exhibited a significant ($p<0.0001$) radical scavenging effect (IC_{50} of 57.87 µg/mL) over the *S. mombin* methanol extract (SMME) and *S. mombin* chloroform fraction (SMCF) when compared to the sham control. SMAF at 500 µg/mL significantly ($p<0.05$) inhibited (56.89 ± 2.02%) the effect of protease when compared to the sham control. SMME significantly ($p<0.001$) reduced inflammation in rat paws when compared to the control with peak effects seen at the dose of 50 mg/kg body weight. The result of the study has revealed that the stem bark has radical scavenging and anti-inflammatory effects.

Keywords: *Spondias mombin*; Radical scavenging; Protease inhibition; Anti-inflammatory; Antiarthritic

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that affects about 1% of the population globally with 70% of the people affected being women between the ages of 50 and 60 years [1]. The sequelae of events associated with RA provoke an inflammatory response that destroys synovium, underlying cartilage and subchondral bone. The hallmark of these attacks is articular disability due to

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joint destruction and deformity, which could also have systemic impacts on the endocrine, neurological, pulmonary and cardiovascular organs.[2,3] Some of the risk factors associated with RA are genetic factors, smoking tobacco, exposure to silica, coffee-tea consumption and infections. However, genetic factors constitute about 50% of the risk associated with RA development [1]. Conventional therapies available for the management of RA such as synthetic disease-modifying antirheumatic drugs (DMARDs), nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids (GCs) are saddled with the challenges of untoward side effects for instance oral and gastrointestinal ulcers, pneumonitis, endocrine pathologies and ophthalmological diseases [4–6]. Patient compliance with conventional therapies for RA is usually low due to the associated side effects of long-term usage, which makes alternative medicine a subject of choice when it comes to the management of RA ailments.

S. mombin Linn (Anacardiaceae) is a rainforest tree that grows to about 22 meters tall, it emits a brown resinous substance following deep incisions on the bark [7]. Locally called Iyeye (Yorubas) and Uvuru (Igbo) by the native people of Nigeria, it is used ethnomedicinally for the treatments of urethritis, diarrhoea, and gonorrhoea. There are reports in the literature that *S. mombin* bark contains astringent tannin constituent useful for the treatment of diarrhoea and dysentery. The stem bark has been used for the treatment of haemorrhoids and to arrest bleeding [8]. Anti-inflammatory activity against tonsillitis and laryngitis has also been reported [9]. Saponins, flavonoids, tannins and alkaloids are present in the stem bark of the plant [10]. Lupeol and gamma-sitosterol have been identified from the leaf of the plant [11]. Several compounds with antimicrobial, antioxidant, and anti-inflammatory effects have been discovered in the stem bark of *Spondias mombin*. These compounds include Aspidofractinine-3-methanol, Phthalic acid 2-ethylhexyl tetradecyl ester, Phthalic acid di(2-propyl pentyl) ester, 9-(2',2'-Dimethylpropanoyl hydrazono)-3,6-dichloro-2,7-bis-[2-diethyl amino)ethoxy]fluorine, Terephthalic acid, and dodecyl 2-ethylhexyl ester [12].

Some members of the Anacardiaceae family have been known to show activity against RA such as *Anacardium occidentale* [13], *Toxicodendrum pubescens* [14] *Semecarpus anacardium* [15] and *Spondias mangifera* [16]. However, information is lacking on the anti-arthritic effect of *S. mombin*. Therefore, this study aimed to investigate the effect of the plant on arthritis in rats.

MATERIALS AND METHODS

Chemicals and reagents. Analytical grade reagents and solvents were utilized for the study such as solvents from the British Drug House (BDH) and reagents from Sigma Aldrich, England.

Plant material. The stem bark of *S. mombin* was collected from the Iwo community in Nigeria, in April 2022. The plant was identified and authenticated at the herbarium unit of the Department of Pharmacognosy and Natural Products, Afe Babalola University, Ekiti State. A voucher specimen was deposited with specimen number ABUAD/PHM/345.

Extraction and partitioning. The stem bark of the plant was air dried under shade, pulverized and stored in an appropriate container until required. 1 kg of the powdered plant's part was extracted with methanol (Analytical grade) using a Soxhlet extractor for twelve days. The extract was concentrated to dryness *in vacuo*. The concentrated *S. mombin* methanol extract (SMME) was weighed, kept in a labelled bottle, and stored in the refrigerator at 4ºC until used. 50 grams of SMME was dissolved in distilled water and successively partitioned against chloroform until exhausted in the separating funnel. The

fractions (Aqueous and chloroform phases) obtained were concentrated to dryness using a rotary evaporator and water bath. The weight of the chloroform fraction (SMCF) was 5.5 g while the weight of the aqueous fraction (SMAF) was 40 g.

Experimental animals. Albino rats of either sex weighing from 200-300 g were used. The rats were housed under standard conditions with 12 hours of light and 12 hours of the dark cycle. The National Institute of Health (NIH) protocols for the use and care of laboratory Animals were strictly followed. They were allowed free access to standard pellets and water *ad libitum*. Ethical approval (LS171189) was secured from the Animal and Experimental Ethics Committee, Faculty of Life Science, University of Benin, Edo state, Nigeria.

Determination of DPPH radical scavenging activity of *S. mombin* methanol extract and solvent fractions. 50 µL of vitamin C (20, 40, 60, 80 and 100 µg/mL), SMME, SMAF and SMCF all at the same concentration range were mixed with 50 µL of 200 µM DPPH solution in ethanol and 150 µL ethanol (absolute, AR grade) in a 96-well microplate. The mixture was agitated for 15 s at 300 rpm and was allowed to stay in the dark for 30 minutes at room temperature. The absorbance of the mixture was measured at 520 nm using a multiplate reader. The experiment was conducted in triplicate. The DPPH radical scavenging activity was calculated using the formula:

DPPH scavenging effect (%) = 100 − [(Asample-Ablank)/Acontrol × 100] [17].

Determination of protease inhibition activity of *S. mombin* methanol extract and solvent fractions. The reaction mixture (2 mL) made up of trypsin (0.06 mL), Tris HCl buffer (1 mL; 20 mM; pH 7.4) and 1 mL of Aspirin (100. 300 and 500 µg/mL), then 1 mL of SMME, SMAF and SMCF all at the same concentration were incubated for 10 min at 37ºC. Thereafter, casein (1 mL of 0.65% w/v) was added, and the mixture incubated again for 20 minutes. Then, perchloric acid (2 mL; 2 M) was added to the mixture and centrifuged at 7830 rpm for 15 min. The supernatant liquid was measured at 280 nm wavelength. Tris-HCl buffer solution served as a control. Percentage inhibition was calculated using the formula:

Inhibition (%) = (1 − Ac/At) x 100
Ac= absorbance of control sample whereas
At= absorbance of the test sample [18].

Determination of the anti-arthritis activity of *S. mombin* methanol extract and solvent fractions using formaldehyde-induced arthritis in rats. Albino rats of either sex were randomly assigned to 11 groups of 5 rats each. Group I served as sham control and received distilled water (3 mL/kg; p. o.). Group II received the standard drug (aspirin at 100 mg/kg p. o). Similarly, Groups III, IV, and V received SMME at 50, 100, and 200 mg/kg p. o. respectively. Group VI, VII, and VIII were given SMCF at 50, 100, and 200 mg/kg p.o. respectively. Group IX, X, and XI were treated with SMAF at 50, 100 and 200 mg/kg p.o. respectively. All administrations were done for a period of 10 days. On the first day, 30 min post-drug administration, arthritis was induced by a sub-planter injection of 0.1 mL of 2% formaldehyde solution and was repeated on the third day. The mean paw diameter of the test animals was measured for a period of 10 days through the use of a digital Vernier calipers.
The percentage inhibition of oedema was estimated using the formula:

Percentage inhibition of edema = (1-Vt)/Vc x 100
Where Vt and Vc are the joint diameters of treated and control rats [18].

Statistical analysis. Values are expressed as mean ± SEM. Differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test at a 95% confidence interval using GraphPad Prism version 6.01.

RESULTS AND DISCUSSION

Figure 1 shows the relative scavenging activities of the crude and solvent fractions of *S. mombin* against the oxidative stress generated by the free radicals of DPPH. SMAF exhibited a significant ($p<0.0001$) radical scavenging effect (IC_{50} of 57.87 µg/mL) over SMME and SMCF when compared to the control. This enhanced effect could be due to the pooling of molecules with free valence shell electrons in the aqueous fraction as well as the bioavailability of molecules in the ionic form in polar solvents such as ethanol. SMME is a conglomeration of secondary metabolites (those with and without free radical scavenging activities) which could result in the water-down effect of the concentration evaluated, although it significantly attenuated the effect of oxidative stress elicited by DPPH. SMCF is not water-soluble thus impeding the complete ionization of the compounds in polar solvents as evidenced in the IC_{50} value of 510.13 µg/mL (Table 1). Their respective radical scavenging capacity is shown in the IC_{50} of the extract and solvent fractions (Table 1). The crude extract and the aqueous fraction neutralized the oxidative stress generated by DPPH. The generation of oxidative stress during RA development results in the damage of synovial tissue in arthritic joints [19]. The high reactive oxygen species (ROS) generated by hypoxia during RA pathophysiology is reported to activate factors responsible for angiogenic processes and cartilage erosion in the synovium [20]. Therefore, the ability of plant extract to quench the ROS generated during RA events can ameliorate the associated symptoms.

Table 2 reveals the protease inhibition activity of the extract and solvent fractions of *S. mombin*. The stem bark and solvent fractions significantly ($p<0.05$) inhibited the effect of protease in a concentration-dependent manner relative to the control. A peak effect was observed in 500 µg/mL of SMAF with a percentage inhibition of 56.89 ± 2.02. Several proteolytic enzymes have been implicated in the development and progression of RA such as metalloproteinases which are known to be involved in synovium degradation [21]. Inhibition of protease exhibited by the stem bark of *S. mombin* indicates that the extract exerted anti-inflammatory activity that can attenuate the sequelae of events that lead to the initiation and sustenance of RA. Reports in the literature have indicated the potential of plant-based therapy as an anti-rheumatoid agent [22,23]. Figures 2-4 show the anti-inflammatory strength of the stem bark of *S. mombin*. The extract and solvent fractions significantly suppressed the inflammatory effects of formaldehyde in rat's paw. SMME significantly ($p<0.001$) reduced inflammation in rat paws when compared to the sham control with peak effects seen at the dose of 50 mg/kg body weight (Figure 2). There was no significant difference in the anti-inflammatory effect of SMAF on day one but shows a delayed onset of activity from the second day with a significant ($p<0.0001$) reduction in the level of inflammation when compared to the sham control (Figure 3). The peak effect is equally observed at the dose of 50 mg/kg body weight of the test animals. SMCF did not exert a significant reduction in inflammation when compared to the sham control (Figure 4). Inflammatory factors and pathways such as nuclear factor-kB (NF-κB), activator protein-1 (AP-1), tissue necrotic factor-α (TNFα) and interleukin-1β (IL-1β) are excited during RA pathogenesis and thus play a vital role in the initiation and progression of the disease [24,25]. The activation of these factors destroys joint tissues and loss of joint functions.

Table 1: IC_{50} values of DPPH radical scavenging effect of extract and solvent fractions of *S. mombin*

Extract and fractions	IC_{50} µg/mL
Ascorbic acid	27.78
SMAF	57.67
SMME	86.00
SMCF	510.13

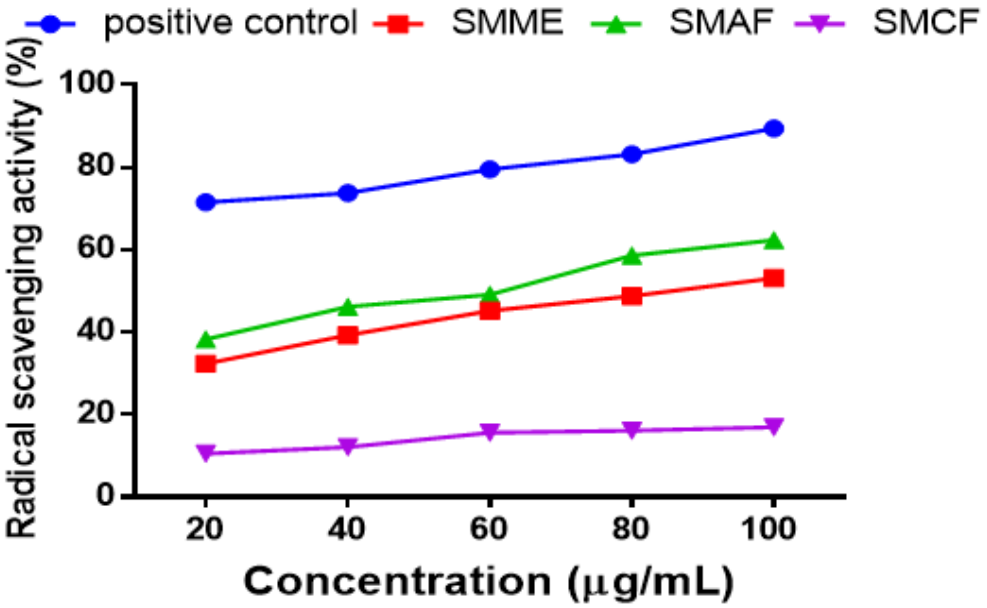

Figure 1. Radical scavenging effects of crude and solvent fractions of *S. mombin* on DPPH. Values are expressed as mean ± SEM. Data were significant at $p<0.0001$ when compared to the positive control group using two-way ANOVA and Dunnett's post hoc test. n=5

Table 2: Protease inhibition effect of extract and solvent fractions of *S. mombin*

Treatment	Concentration (µg/mL)	Inhibition (%)
Positive control	100	41.00±2.96
Positive control	300	68.54±2.90
Positive control	500	88.34±2.90
SMME	100	39.35±2.16
SMME	300	$45.23±2.23^{a}$
SMME	500	$51.78±2.23^{b}$
SMCF	100	$11.44±1.29^{c}$
SMCF	300	$11.12±1.19^{d}$
SMCF	500	$12.91±1.19^{e}$
SMAF	100	39.48±2.19
SMAF	300	$44.21±2.02^{f}$
SMAF	500	$56.89±2.02^{g}$

Values are expressed as mean ± SEM. Data with superscript a, b, c, d, f, g are significant at $p<0.05$ when compared to the positive control group using one-way ANOVA and Dunnett's post hoc test. n=5

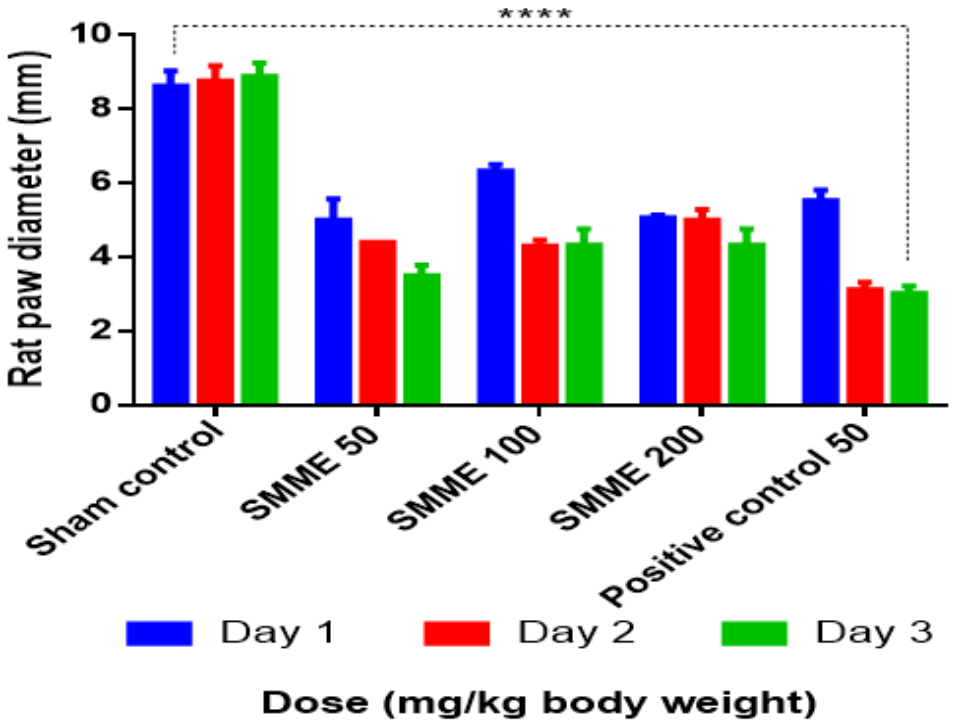

Figure 2: Effect of SMME on rat paw diameter
The values are expressed as mean ± SEM. Data with superscript **** were significant at p<0.0001 when compared to the sham control group using two-way ANOVA and Dunnett's post hoc test. n=5

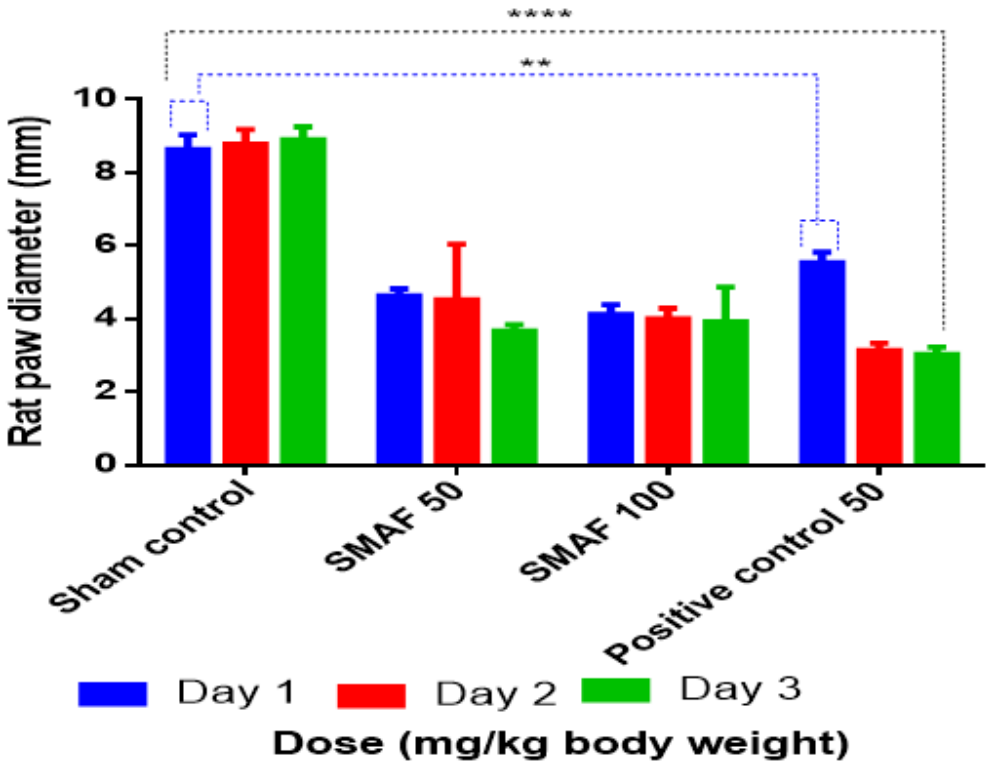

Figure 3: Effect of SMAF on rat paw diameter
The values are expressed as mean ± SEM. Data with superscript **and **** are significant at p<0.01 and p<0.0001 respectively when compared to the sham control group using two-way ANOVA and Dunnett's post hoc test. n=5

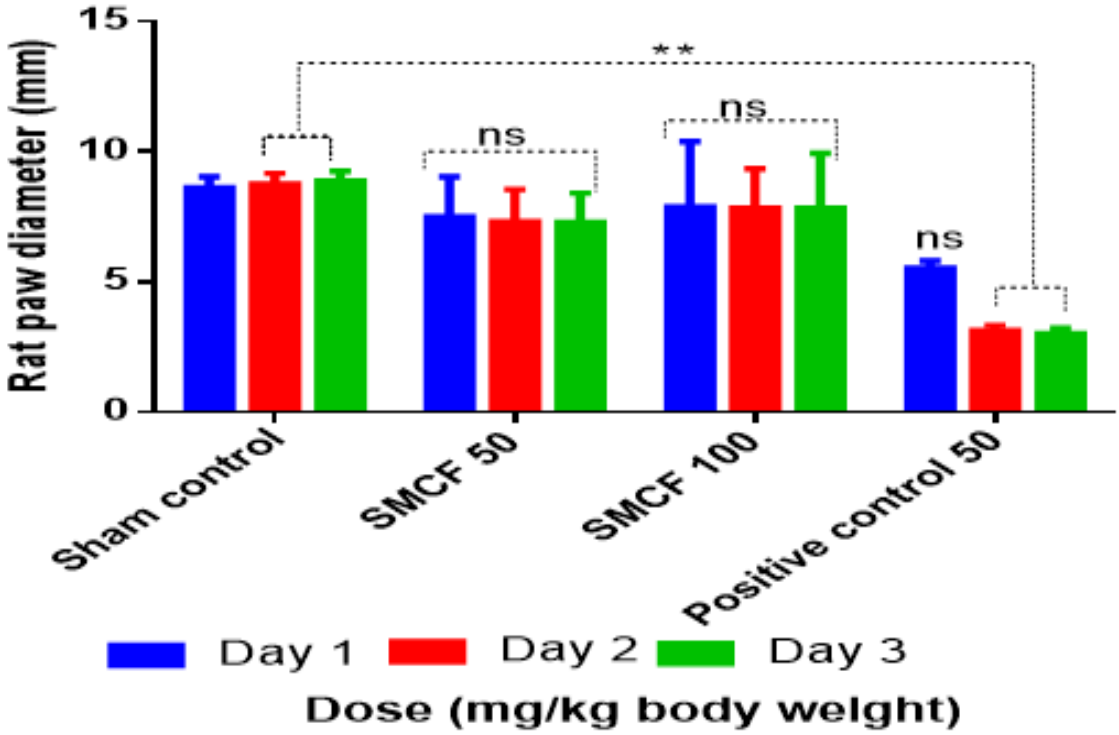

Figure 4: Effect of SMCF on rat paw diameter
Values are expressed as mean ± SEM. Data with superscript ** were significant at p<0.01 and bars with superscript "ns" were not significant when compared to the sham control group using two-way ANOVA and Dunnett's post hoc test. n=5

The reduction in paw diameter indicated inhibition of inflammatory mediators, which suggests the potential of *S. mombin* stem bark as an anti-inflammatory agent for the treatment of RA.

Conclusion. Findings in the study demonstrated that the stem bark extract of the plant exhibited radical scavenging and anti-inflammatory activities. The evidence accumulated from the investigation shows the stem bark of the plant could be used for the management of inflammatory and rheumatoid arthritis cases.

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