

## Research Article

## Bioactivity-Guided Fractionation of Antimalarial Active Extract of *Spondias mombin* Linn Stem bark

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## ABSTRACT

Herbs have proven to be viable therapeutic alternatives for treatment of diseases like malaria. This study explores the phytochemical, nutritional, antioxidant and antimalarial potentials of *Spondias mombin* Linn. stem bark. Hexane, methanol, ethanol and aqueous extracts were separately obtained from dried pulverized stem bark, while the most active extracts were fractionated by column chromatography. Extracts/fractions were screened for phytochemical, nutritional and antioxidant properties using established protocols, while antimalarial activity was against *Plasmodium berghei* NK65 in mice using the chemo-suppressive, prophylactic, curative and mean survival time (MST) tests. Phenolics, flavonoids and tannins were detected in all the extracts. Percentage nutritional composition of the plant material showed it contained moisture ( $5.14 \pm 0.01\%$ ), crude fibre ( $1.00 \pm 0.01\%$ ), nitrogen free extract ( $66.18 \pm 0.42\%$ ), crude protein ( $7.42 \pm 0.10\%$ ), crude fat ( $18.24 \pm 1.01\%$ ) and crude ash ( $2.03 \pm 0.01\%$ ). The extracts scavenged ferrous and DPPH radicals with methanol extract having the highest antioxidant activity. Extracts reduced parasitaemia and prolonged MST relative to infected untreated group. Aqueous and ethanol extracts were the most active in the chemo-suppressive (81.63%: MST  $20.00 \pm 2.21$  days) and prophylactic (87.48%: MST  $18.20 \pm 2.48$  days) tests respectively. The hydro-ethanol extract was partitioned into hexane, hexane:ethylacetate-HE, ethylacetate-EA, ethylacetate:methanol-EM and methanol residue-MR fractions. The fractions had varying antimalarial activity with some almost doubling MST relative to infected untreated group. The highest activity was in EA administered group with chemo-suppression 78.32%, MST:  $17.80 \pm 1.12$  days; prophylaxis 66.51%, MST:  $15.00 \pm 0.31$  days; curative 76.70%, MST:  $16.80 \pm 0.48$  days. Therefore, *Spondias mombin* stem bark has rich phyto-nutritional constituents possibly linked to its antimalarial activity.

**Keywords:** *Spondias mombin*, Fractionation, Antimalarial, Antioxidants, *Plasmodium berghei*

## INTRODUCTION

Traditional medicine has gained attraction in recent years with a good number of the population of about a hundred and seventy countries around the world depending on it for the treatment and management of various ailments (WHO, 2019). Chinese traditional medicine, Ayurveda and African traditional medicine have proven to complement orthodox medicines, thus providing relief as well as alternatives in

view of their easy accessibility and applicability, cost and fewer side effects (WHO, 2019). Under these systems, the use of herbs is common practice. These herbs or medicinal plants possess phytochemicals that have bioactivities against several disease-causing organisms such as bacteria, viruses, fungi, nematodes and parasites, (Coates *et al.*, 1994, Yuan *et al.*, 2016; Orumwensodia *et al.*, 2021), or the consequences of the actions of these organisms, hence could be deployed in the treatment and/or management of diseases associated

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with these organisms. Phytochemicals could function alone, in synergy or antagonism with other molecules in order to produce the desired pharmacological effect (Yuan *et al.*, 2016). Worthy of note is that some drugs in clinical practice today were derived from plant sources and their discoveries were based on usage in traditional practice (Fabricant and Farnsworth, 2001; Li-Weber, 2009; Padmavathi, 2013). An example of such herbs is *Spondias mombin* Linn., which has been exploited within traditional set-ups for the treatment of diseases (Aigbokhan, 2014; Orumwensodia *et al.* 2021). Well adapted to the humid tropical climates of Asia, South America and Africa (Ayoka *et al.*, 2008; Orwa *et al.*, 2009), *S. mombin* adorns the semi-deciduous, evergreen lowland, waterlogged or dense canopy forest areas of these continents with a fat trunk and majestic height of around 30 m at maturity. It belongs to the family of Anacardiaceae and sub-family, Spondiadoideae, and has common names like mombin, yellow mombin, yellow Spanish plum and hog plum (Aigbokhan, 2014). Some of the ethno-pharmacological uses of this plant include anti-microbial (Abo *et al.*, 1999), antiviral (Corthout *et al.*, 1992), molluscicidal (Corthout *et al.*, 1994), anti-diarrhoea, anti-helminthic (Ademola *et al.*, 2005), antibacterial (Corthout *et al.*, 1994), antimalarial (Carabalo *et al.*, 2004), etc. Malaria remains a burden within the public health space with increasing clinical cases and mortality. The World Health Organization reports that Africa is the epicenter of this disease with about 95% of cases and 96% of mortality world-wide, while Nigeria accounts for over 31% of these figures (WHO, 2023). The disease is caused by *Plasmodium* spp., and mostly affects children under five years of age, pregnant women and immune-compromised individuals. In the absence of a viable vaccine, relatively less expensive and accessible drugs, and the capacity to deal with the menacing phenomenon of resistance from the mutating parasite, the quest for more effective alternatives is expedient (WHO, 2019).

Bioactivity-guided fractionation remains a guide to the isolation of lead molecules with biological activity from medicinal plants (Agidew *et al.*, 2013). Therefore, this study explores the plant, *S. mombin* Linn. stem bark, in relation to its phytochemical, nutritional, antioxidant and antimalarial potentials with a view to providing scientific bases for its usage in traditional medicine and setting the stage for the isolation of potentially active antimalarial candidate(s).

## MATERIALS AND METHODS

### Chemicals and Drugs

The chemicals and reagents used for the experiments were Giemsa stock (Trust Chemical Lab, India), immersion oil (Scisco Research Lab, India), hexane, ethylacetate, methanol

and ethanol (Sigma Aldrich, Germany), normal saline, phosphate buffered saline, ferric chloride, ferrozine, DPPH, vitamin C, glacial acetic acid, EDTA, HCl, H<sub>2</sub>SO<sub>4</sub>, mayers reagent, ammonia, olive oil and Chloroquine (Silver Health Diagnostics, Nigeria). All reagents were procured from certified suppliers and were of analytical grade.

### Collection and Preparation of Plant Material

Fresh stem bark of *S. mombin* Linn. was obtained from the forest area of Ijebu-Itele, Ogun State, Southwest Nigeria. The plant was identified and authenticated (voucher No. - UBHa210) in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria. Samples were cleaned and air-dried at room temperature under shade, and pulverized using mortar and pestle. The coarse pulverized sample was stored at room temperature in air-tight plastic container until ready for use.

### Extraction of Plant Material

Four weighed portions (400 g each) of the pulverized plant material were separately macerated in 1.5 L of hexane, methanol, ethanol and distilled water for 3 days under repeated stirring. The extracts were filtered with Whatman filter paper No. 1 (Whatman, England), while the recovered meshes were repeatedly macerated two more times (Cannell, 2006). All filtrates from the respective maceration cycles were separately combined and dried *in vacuo* at 45 °C using a rotary evaporator (Buchi R-200, Germany). The respective extracts were stored under refrigerated condition in air-tight glass containers, until further use.

### Chromatographic Fractionation of Active Extracts

The active hydro-ethanol (30/70 v/v) extract was derived from repeated maceration of 5.2 kg of dried stem bark sample of *Spondias mombin* as described earlier. Slurry of the concentrated hydro-ethanol extract (220 g) was fractionated over a well packed silica gel column (150 x 9.5 cm). The column was eluted successively using solvent combinations of hexane (100%), hexane-ethylacetate (50:50), ethylacetate (100%), ethylacetate-methanol (50:50) and methanol (100%). Eluates of 250 ml were successively collected and monitored with well-developed thin layer chromatography (TLC) plates under ultraviolet (UV) lamp at 254 and 366 nm. Fractions with similar TLC profile were batched for further analyses.

### Phytochemical Screening

Portions of each extract (5 g) were separately mixed in a solution of 75 mL distilled water and boiled for 30 min. Cooling was allowed after a process of hot filtration, while filtrates obtained were used to qualitatively determine the presence of the following phytochemicals; alkaloids, flavonoids, phenolics, tannins, cardiac glycosides and

saponins according to the methods of Stahl, 1973; Sofwora, 1982; Harborne, 1998 and Evans, 2002.

#### Determination of Nutritional Content

The methods of AOAC (2000) were used to ascertain the nutritional composition of the dry plant material. Water loss on drying (moisture), crude fibre, nitrogen free extract (NFE), crude protein, crude fat and crude ash were determined.

#### Determination of Antioxidant Capacity

The antioxidant activity of the plant extracts was measured against ferrous ions and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical using the methods of Dinis *et al.* (1994) and Brand-Williams *et al.* (1995), respectively as modified by Orumwensodia and Uadia (2022).

#### Evaluation of the *in Vivo* Antimalarial Activity of the Extracts/Fractions

##### Animal handling and maintenance

Male Swiss albino mice (6 – 8 weeks old) and weighing  $20 \pm 2$  g were used for this study. They were acquired from the Animal House of Igbinedion University, Okada, Edo State and housed in plastic cages with wood shavings as beddings under a 12 h light-dark cycle. The animals were acclimatized for a week and fed rat chow, and water *ad libitum* for the period of experiment. Animals were handled according to guidelines of the Institute for Laboratory Animal Research (ILAR, 2011).

##### Grouping and dosing of animals

The study was in two phases: the first phase involved the extracts- methanol, ethanol and aqueous (with the exclusion of hexane, owing to low yield), while the second phase involved hydro-ethanol extract and its partially purified fractions. However, both phases had similar groupings. Male Swiss albino mice were randomized into three main groups comprising five mice per group for the respective experimental models. Groups A and B served as controls, while group C with multiple sub-groups served as test groups for the various extracts and fractions. Mice in Group A which served as positive controls, were infected and treated with the vehicle (PBS). Group B mice which served as reference drug controls were infected and treated with chloroquine (10 mg/kg bw of mice in the first phase and 25 mg/kg bw of mice in the second phase). Group C (test groups) mice were infected and treated with

extracts/fractions (800 mg/kg bw of mice). Extracts, fractions and the reference drug were administered orally, once daily, while infection with *P. berghei* ( $10^7$ ) was done once intraperitoneally (ip).

Infection and treatments were according to the different experimental model employed (i.e., whether suppressive, curative or prophylactic) as described below.

#### Experimental Models

##### Four-day chemo-suppressive test

The suppressive activity of extracts/fractions was determined according to the modified method of Fidock *et al.* (2004). Briefly, inoculation and then treatment of mice were conducted on the same day (D0), with a two-hour interval between both procedures. Treatments were continued for the next three days (i.e., D0 to D3), while on D4, parasitaemia was determined under microscope (Olympus XSZ-107, Japan) from Giemsa-stained thin smears with blood obtained from the tail of each mouse.

##### Prophylactic Test

Prophylaxis potential of the extracts/fractions was determined in accordance with the modified method of Fidock *et al.* (2004). Mice were first treated for four consecutive days (D0 to D3) and subsequently infected with the standard inoculum on D4. After 72 h of infection (D7), parasitaemia was monitored under a microscope from thin blood smears obtained from the tail of each mouse.

##### Estimation of Curative Activity (Rane's test)

The schizontocidal activity of extracts/fraction on established infection was evaluated using the method described by Ryley and Peters (1970). The mice were infected with the inoculum, on the first day (D0). Then, seventy-two hours post-infection (D3), treatments commenced and lasted for the next four days. Parasitaemia level of each mouse was monitored from tail-blood smears under a microscope.

##### Estimation of Parasitaemia

Percent parasitaemia and percent suppression were determined in line with the methods of Fidock *et al.* (2004) and Kalra *et al.* (2006) by counting the number of pRBCs in random fields of microscope view under  $\times 100$  objective lens.

##### Estimation of Mean Survival Time (MST)

The MST was determined by recording daily mortality and number of days of survival from moment of infection to death of mice. Values were noted for each mouse in the control and treatment groups for the duration of experiment.

$$\% \text{ Parasitaemia} = \frac{\text{Number of pRBCs}}{\text{Total number of RBCs}} \times 100$$

$$\% \text{ Suppression} = \frac{(\% \text{ Parasitaemia of Negative Control} - \% \text{ Parasitaemia of Treated group})}{\% \text{ Parasitaemia of Negative Control}}$$

$$\text{MST (days)} = \frac{\text{Sum of days of survival of mouse in a group}}{\text{Total number of mouse in the group}}$$

### Data Analysis

Data were analyzed using GraphPad Prism 5 and presented as mean  $\pm$  Standard Error of Mean (SEM). The differences among means were compared using one-way ANOVA, followed by Turkey's HSD multiple comparison tests with significance set at  $p < 0.05$ .

## RESULTS

### Phytochemical Content

The phytochemical content of extracts of *S. mombin* stem bark is presented in Table 1. Phenolics, flavonoids and tannins were detected in all extracts. However, cardiac glycosides, alkaloids and saponins were not detected in the hexane extract, while alkaloids were not detected in the aqueous extract.

### Nutritional Content

Figure 1 represents the percentage nutritional content of *S. mombin* stem bark. The sample was found to contain the following nutrients moisture ( $5.14 \pm 0.01\%$ ), crude fibre ( $1.00 \pm 0.01\%$ ), nitrogen free extract ( $66.18 \pm 0.42\%$ ), crude protein ( $7.42 \pm 0.10\%$ ), crude fat ( $18.24 \pm 1.01\%$ ) and crude ash ( $2.03 \pm 0.01\%$ ).

### Antioxidant Activity

Table 2 shows the antioxidant activity of the *S. mombin* stem bark extracts (hexane, methanol, ethanol and aqueous) against ferrous ion and DPPH radicals. From the results,

hexane extract had significant ( $p < 0.05$ ) ferrous ion chelating and DPPH scavenging activity, while the ethanol extract had significant ( $p < 0.05$ ) DPPH scavenging activity. However, other extracts had non-significant ( $p > 0.05$ ) activity compared to the reference antioxidants (ascorbic acid and EDTA).

### Chemo-suppressive Antimalarial Activity of Extracts

Chemo-suppressive antimalarial activity of crude extracts of *Spondias mombin* stem bark is presented in Table 3. The growth of *P. berghei* was significantly ( $p < 0.05$ ) suppressed by the extracts, which also significantly ( $p < 0.05$ ) increased MST in treated groups compared to the untreated control group (PBS- pH 7.4). Suppressive activity reduced in the following order aqueous  $>$  ethanol  $>$  methanol, though chloroquine had better activity compared to the extracts.

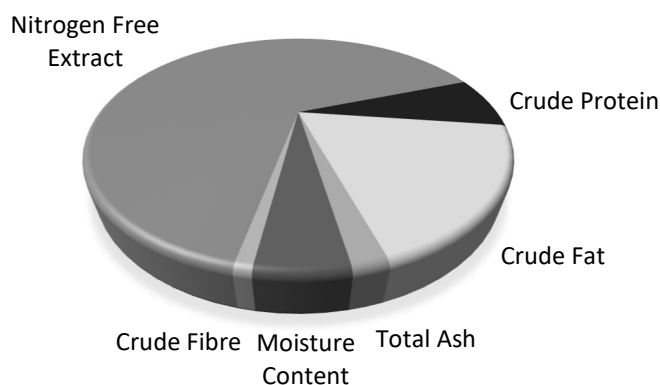
### Prophylactic Antimalarial Activity of Extracts

The prophylactic antimalarial activity of crude stem bark extracts of *Spondias mombin* against *P. berghei* infection in mice is presented in Table 4. The extracts demonstrated significant ( $p < 0.05$ ) prophylaxis against *P. berghei* infection in treated group compared to the untreated control group (PBS- pH 7.4). Prophylaxis increased accordingly; methanol  $<$  aqueous  $<$  ethanol. MST with ethanol extract almost doubling survival time compared to the infected untreated control group.

**Table 1.** Phytochemical Content of Extracts of *Spondias mombin* Stem Bark

Extracts	Phenolics	Flavonoid	Cardiac glycoside	Tannins	Alkaloids	Saponins
Hexane	+	+	–	+	–	–
Methanol	+	+	+	+	+	+
Ethanol	+	+	+	+	+	+
Aqueous	+	+	+	+	–	+

+ detected; – not detected



**Figure 1.** Nutritional Content of *Spondias mombin* Stem Bark.

Data represent percentage mean  $\pm$  SEM of triplicate determinations.

**Table 2.** Antioxidant Activity of Extracts of *Spondias mombin* Stem Bark

Extracts/Reference	Ferrous Ion Chelating IC <sub>50</sub> ( $\mu\text{g/ml}$ )	DPPH IC <sub>50</sub> ( $\mu\text{g/ml}$ )
Hexane Extract	1.38 $\pm$ 0.15 <sup>a</sup>	1.865 $\pm$ 0.41 <sup>a</sup>
Methanol Extract	144.75 $\pm$ 0.50 <sup>b</sup>	1.10 $\times 10^5 \pm$ 0.50 <sup>b</sup>
Ethanol Extract	12.14 $\pm$ 0.42 <sup>b</sup>	1.40 $\times 10^{-3} \pm$ 0.12 <sup>a</sup>
Aqueous Extract	2.66 $\times 10^{19} \pm$ 0.21 <sup>b</sup>	2.67 $\times 10^2 \pm$ 0.34 <sup>b</sup>
EDTA	4.03 $\pm$ 0.25	–
Ascorbic acid	–	12.81 $\pm$ 0.11

IC<sub>50</sub> values of triplicate determinations (n = 3/group). a = significant (p < 0.05), b = non-significant (p > 0.05) relative to the standard controls, EDTA and ascorbic acid.

### Percentage Yield and Physical Characteristics of Fractions

The percentage yield and physical appearance of solvent fractions of crude hydro-ethanol extract of *Spondias mombin*

stem bark are shown in Figure 2. Ethylacetate: Methanol-soluble fraction (50:50 v/v) had the highest yield while hexane-soluble fraction (100%) had the lowest yield. They had varying physical appearance in colour and texture.

**Table 3.** Four-day Chemo-suppressive Activity of *Spondias mombin* Stem Bark Extracts against *P. berghei* Infection in Mice

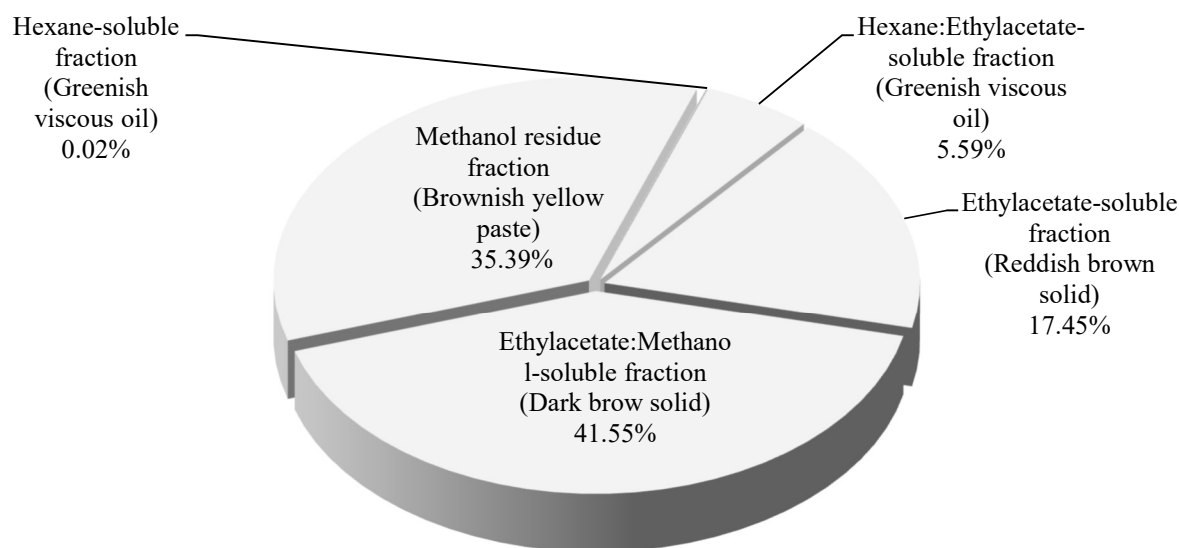
Treatment	Dose (mg/kg)	% Parasitaemia	% Chemo-suppression	Mean Survival Time (days)
Methanol extract	800	2.66 $\pm$ 1.23	65.09 <sup>ab</sup>	14.50 $\pm$ 1.26 <sup>ab</sup>
Ethanol extract	800	2.13 $\pm$ 0.19	72.05 <sup>ab</sup>	17.80 $\pm$ 2.48 <sup>ab</sup>
Aqueous extract	800	1.40 $\pm$ 0.57	81.63 <sup>ab</sup>	20.00 $\pm$ 2.21 <sup>a</sup>
PBS (pH 7.4)	-	7.62 $\pm$ 0.13	-	10.80 $\pm$ 0.73 <sup>b</sup>
Chloroquine	10	0.33 $\pm$ 0.35	95.67	21.00 $\pm$ 0.98

Values are presented as mean  $\pm$  SEM; n = 5. a = significant (p < 0.05) compared to infected untreated control (Phosphate Buffered Saline- PBS- pH 7.4), b = significant (p < 0.05) compared to reference drug, chloroquine. p < 0.05.

**Table 4.** Prophylactic Activity of *Spondias mombin* Stem Bark Extracts against *P. berghei* Infection in Mice

Treatment	Dose (mg/kg)	% Parasitaemia	% Chemo-suppression	Mean Survival Time (days)
Methanol extract	800	1.77 $\pm$ 0.61	71.95 <sup>ab</sup>	15.80 $\pm$ 1.26 <sup>ab</sup>
Ethanol extract	800	0.79 $\pm$ 0.15	87.48 <sup>a</sup>	18.20 $\pm$ 2.48 <sup>a</sup>
Aqueous extract	800	0.96 $\pm$ 0.18	84.79 <sup>ab</sup>	16.80 $\pm$ 2.21 <sup>ab</sup>
PBS (pH 7.4)	-	6.31 $\pm$ 0.24	-	9.80 $\pm$ 0.21 <sup>b</sup>
Chloroquine	10	0.54 $\pm$ 0.81	91.44	19.20 $\pm$ 0.67

Values are represented as mean  $\pm$  SEM; n = 5. a = significant (p < 0.05) compared to infected untreated control (Phosphate Buffered Saline- PBS- pH 7.4), b = significant (p < 0.05) compared to reference drug, chloroquine. p < 0.05.



**Figure 2.** Percentage Yield and Physical Characteristics of Fractions of *Spondias mombin* Stem Bark

#### Chemo-suppressive Antimalarial Activity of Hydro-Ethanol Extracts and Fractions

Chemo-suppressive antimalarial activity of crude hydro-ethanol (30/70 v/v) extract and fractions of *Spondias mombin* stem bark are shown in Table 5. Suppression of *P. berghei* growth was significant ( $p < 0.05$ ) in all treated groups when compared with the untreated control group (PBS- pH 7.4). They also significantly ( $p < 0.05$ ) increased MST relative to the untreated control group. The highest and lowest activities were recorded in the groups administered ethylacetate (EA) and methanol residue (MR) fractions, respectively. However, chloroquine was more effective than the test candidates.

#### Prophylactic Antimalarial Activity of Hydro-Ethanol Extracts and Fractions

The prophylactic antimalarial activity of crude hydro-ethanol extract and fractions of *Spondias mombin* stem bark against *P. berghei* infection in mice is shown in Table 6. They all demonstrated significant ( $p < 0.05$ ) prophylaxis in treated groups compared to the infected untreated control group (PBS- pH 7.4). They also increased MST though only the EA fraction and crude hydro-ethanol extract were significant ( $p < 0.05$ ) when compared with the infected untreated control group. However, they were not as active as the reference drug, chloroquine.

**Table 5.** Comparison of Four-day Chemo-suppressive Activity of Crude Hydro-ethanol Extract and Partially Purified Fractions of *Spondias mombin* Stem Bark against *P. berghei* Infection in Mice

Treatment	Dose (mg/kg)	% Parasitaemia	% Chemo-suppression	MST (Days)
HEF	800	2.68 ± 0.17	62.52 <sup>ab</sup>	14.40 ± 0.45 <sup>ab</sup>
EAF	800	1.55 ± 0.70	78.32 <sup>ab</sup>	17.80 ± 1.12 <sup>ab</sup>
EMF	800	3.09 ± 0.40	56.78 <sup>ab</sup>	13.00 ± 0.39 <sup>cb</sup>
MRF	800	4.18 ± 0.27	41.54 <sup>ab</sup>	12.50 ± 1.47 <sup>cb</sup>
Crude plant extract	800	1.95 ± 0.54	72.73 <sup>ab</sup>	16.80 ± 0.96 <sup>ab</sup>
Negative control	-	7.15 ± 0.35	0.00	9.00 ± 0.1 <sup>b</sup>
Chloroquine	25	0.19 ± 0.14	97.34	> 28

Values represent mean ± SEM; n = 5. HEF = hexane:ethylacetate fraction, EAF = ethylacetate fraction, EMF = ethylacetate:methanol fraction, MRF = methanol residue fraction, MST = mean survival time. a = significant ( $p < 0.05$ ) when compared with negative (infected untreated) control; b = significant ( $p < 0.05$ ) when compared with chloroquine control. c = non-significant ( $p < 0.05$ ) compared to negative (infected untreated) control.

**Table 6.** Comparison of Prophylactic Activity of Crude Hydro-ethanol Extract and Partially Purified Fractions of *Spondias mombin* Stem Bark against *P. berghei* Infection in Mice

Treatment	Dose (mg/kg)	% Parasitaemia	% Chemo-suppression	MST (Days)
HEF	800	3.41 ± 0.81	47.38 <sup>ab</sup>	12.40 ± 1.09 <sup>bc</sup>
EAF	800	2.17 ± 0.34	66.51 <sup>ab</sup>	15.00 ± 0.31 <sup>ab</sup>
EMF	800	4.34 ± 1.12	33.02 <sup>ab</sup>	11.50 ± 0.44 <sup>bc</sup>
MR	800	5.12 ± 0.47	20.99 <sup>ab</sup>	10.20 ± 2.01 <sup>bc</sup>
Crude plant extract	800	3.02 ± 0.21	53.40 <sup>ab</sup>	15.80 ± 1.54 <sup>ab</sup>
Negative control	-	6.48 ± 0.62	0.00	8.80 ± 0.19 <sup>b</sup>
Chloroquine	25	0.14 ± 0.55	97.83	> 28

Values represent mean ± SEM; n = 5. HEF = hexane:ethylacetate fraction, EAF = ethylacetate fraction, EMF = ethylacetate:methanol fraction, MRF = methanol residue fraction, MST = mean survival time. a = significant (p < 0.05) when compared to negative (infected untreated) control; b = significant (p < 0.05) when compared with chloroquine control. c = non-significant (p > 0.05) compared to negative (infected untreated) control.

### Curative Antimalarial Activity of Hydro-Ethanol Extracts and Fractions

The curative activity of crude hydro-ethanol extract and fractions of *Spondias mombin* stem bark against *P. berghei* infection in mice on the 9<sup>th</sup> day of peak parasitaemia is shown in Table 7. All test candidates significantly (p < 0.05) suppressed the progression of malaria with EAF and MRF having the highest and lowest activities, respectively.

The extract and fractions also increased MST of treated mice with significance recorded for HEF, crude extract and EAF. Again, chloroquine was most active with mice in the reference group surviving beyond the 28-day period of study.

**Table 7.** Comparison of Antimalarial Curative Activity of Crude Extract and Partially Purified Fractions of *Spondias mombin* Stem bark against *P. berghei* Infection in Mice (9<sup>th</sup> Day)

Treatment	Dose (mg/kg)	% Parasitaemia	% Chemo-suppression	MST (Days)
HEF	800	8.12 ± 0.42	56.90 <sup>ab</sup>	15.00 ± 0.21 <sup>ab</sup>
EAF	800	4.39 ± 0.54	76.70 <sup>ab</sup>	16.80 ± 0.48 <sup>ab</sup>
EMF	800	9.89 ± 0.83	47.51 <sup>ab</sup>	12.50 ± 1.33 <sup>bc</sup>
MRF	800	15.44 ± 0.31	18.04 <sup>ab</sup>	9.50 ± 0.50 <sup>bc</sup>
Crude plant extract	800	6.71 ± 0.22	64.38 <sup>ab</sup>	16.20 ± 0.88 <sup>ab</sup>
Negative control	-	18.84 ± 0.56	0.00	9.20 ± 1.14 <sup>b</sup>
Chloroquine	25	0.11 ± 0.21	99.42	> 28

Values represent mean ± SEM; n = 5. HEF = hexane:ethylacetate fraction, EAF = ethylacetate fraction, EMF = ethylacetate:methanol fraction, MRF = methanol residue fraction, MST = mean survival time. a = significant (p < 0.05) when compared to negative (infected untreated) control; b = significant (p < 0.05) when compared with chloroquine control. c = non-significant (p > 0.05) compared to negative (infected untreated) control.

## DISCUSSION

The challenges occasioned by emerging strains of disease-causing organisms, resistance from existing ones and the burden from those diseases without known cure has continued to widen the scope for the discovery of newer drugs or drug candidates (WHO, 2019). Prospects from alternative medicine have rekindled hopes of breakthrough in this ever-challenging field. For instance, the menace adduced to malaria has been tackled though not sufficiently by the use of medicinal plants otherwise known as herbs in local parlance. The dependence on these herbs by many homes in sub-Saharan Africa has largely complemented orthodox medications, which remain expensive and inaccessible. One of such plants is *Spondias mombin*, herein evaluated for its phytochemical, nutritional, antioxidant and antimalarial potentials.

Results from Table 1, revealed the presence of phenolics, flavonoids, cardiac glycosides, tannins, alkaloids and saponins in the various extracts with few exceptions. Cardiac glycosides, alkaloids and saponins were not detected in the hexane extract, while the aqueous extract was devoid of alkaloids. These phytochemicals are believed to participate in secondary metabolism in plants including an enhanced phyto-defense system against invading organisms (Alvarez, 2014). Therefore, *S. mombin* as a repository of rich phytochemicals required for defence against disease causing organisms is further confirmed (Raes *et al.*, 2015; Orumwensodia *et al.*, 2021; Yu *et al.*, 2021).

Also, the nutritional content of *S. mombin* (Figure 1) showed it was rich in desirable nutrients. For example,

carbohydrates, fats and proteins were detected in relatively good amount, suggesting the plant to be a potential source of these nutrients, thus, making it nutritionally suitable for consumption, aid recovery during ailments and by extension enhance the sustenance of life (Ochs, 2014; Nelson and Cox, 2017; Orumwensodia and Uadia, 2022). Also, moisture was below or within permissible range (6 - 8%) for a crude drug (African Pharmacopoeia, 1986). This suggests that the crude plant material could be free from susceptible degradation or hydrolytic break down of its chemical constituents by microbes.

Antioxidant capacity is one of the indices of assessment of herbal efficacy in medicinal chemistry. Antioxidants are known to neutralize the actions of reactive oxygen species (associated with disease causing organisms) thus protecting the cells from their debilitating effects, through several mechanisms including radicals scavenging and chelation of metals among others. Free transition metals such as iron and copper, play key roles in disease pathology where they initiate lipid peroxidation and produce radical species that attack vital components of the cells (Atamna and Ginsburg, 1993; Guha *et al.*, 2006; Percário *et al.*, 2012). For instance, ferrous ion released by the *Plasmodium* parasite during malaria infection can catalyze the Haber–Weiss reaction *in vivo* and induce superoxide ions which in turn generate hydroxyl radicals that could pose danger to cellular processes (Percário *et al.*, 2012). However, antioxidants are able to chelate these metals and prevent them from causing harmful cellular damage. On the other hand, the DPPH scavenging assay which is also a rapid radical test, evaluates the ability of these candidates to scavenge DPPH radicals by donating electrons to them which results in decolourization of the DPPH solution. Decrease in absorbance of the reaction mixture connotes significant free radical scavenging activity of the test candidate (Gyamfi *et al.*, 1999). Results from Table 2 demonstrate the plant's antioxidant potentials in comparison with reference antioxidants; EDTA and ascorbic acid. *S. mombin* extracts (hexane, methanol, ethanol and aqueous) were able to chelate ferrous ions and mop up DPPH radicals. However, the hexane and ethanol extracts had significantly ( $p < 0.05$ ) higher ferrous ions chelating and DPPH scavenging antioxidant activity compared to their respective reference antioxidant molecules. These results confirm the presence of phytochemicals in *S. mombin* with antioxidant capacity.

Furthermore, using activity guided techniques, preliminary screening of extracts (excluding hexane extract, owing to low yield) of *S. mombin* Linn. stem bark for their antimalarial efficacy against *Plasmodium berghei* NK65 infection in mice was conducted. The four-day chemo-

suppressive, prophylactic and mean survival time- MST assays were used for this aspect of the study, and results indicate the plant had antimalarial potential which was comparable to the reference drug, chloroquine. From the results (Tables 3 and 4) activity was more in the aqueous and ethanol extracts treated groups for the suppressive and prophylactic studies, respectively, though all three extracts had significant ( $p < 0.05$ ) ameliorative effect compared to the infected untreated control group. Also, MST was almost doubled in treated groups compared to their infected untreated counterpart. It was noticed that activity increased with increasing polarity of solvent of extraction, leaving the possible inference that the active principles were possibly polar in nature. Therefore, the aqueous and ethanol extracts were adopted for fractionation following their activity index and ability to prolong the MST of infected mice. The duo was combined (hydro-ethanol: 30/70 v/v) and fractionated; resulting in five fractions (Figure 2), which (again, excluding the hexane extract, owing to yield) were further screened for their antimalarial efficacy. The efficacies of crude extracts are sometimes overshadowed or obliterated by an array of diverse phytochemical presence, wherein they either antagonize or interfere with activity. Even still, some of these constituents could pose danger in form of toxicity or undesired side-effect when consumed in their crude form as whole plant extract. Through bioactive guided fractionation, partial purification is achieved and activity narrowed, while undesired constituents are foreclosed, thereby, achieving the purpose of optimization of therapeutic effectiveness and the possible discovery of therapeutic leads (Agidew *et al.*, 2013).

Results obtained from the screening of the fractions (Tables 5 and 6) showed activity resided most in the ethylacetate fraction- EAF and least in the methanol residue fraction- MRF with chemo-suppression of 4.61 and 2.05 folds, respectively, over the infected untreated control. This antimalarial activity can possibly be tied to the presence of phytochemicals as detected in this study. Phytochemicals are known to have structural diversity, molecular properties and biochemical function (Newman and Cragg, 2020; Yu *et al.*, 2021) that adapt them to play vital roles in combating diseases such as malaria. For instance, alkaloids have been found to inhibit the transformation of heme in food vacuole of *Plasmodium* parasites (Inbaneson *et al.*, 2012). Also, flavonoids (a member of the phenolic family) exert their antimalarial function via interference with protein biosynthesis, chelation of nucleic acid base pairs of *Plasmodium* as well as generation of free radicals (which kill the parasites) from hemozoin formation during heme degradation (Okokon *et al.*, 2017; Abdussalam *et al.*, 2018). These classes and more were detected in the extracts of *S.*



*mombin*. Meanwhile, further purification of whole plant extract could lead to either increased activity or loss of it in the ensuing fractions (Etame *et al.* 2018; Orumwensodia and Uadia, 2023). However, in this case there was improved activity with purification. Summarily, antimalarial activity of the extract and fractions of *S. mombin* stem bark declined accordingly; EAF > CPE > HEF > EMF > MRF, thus suggesting the active principle(s) were more soluble in ethylacetate (a mid-polar solvent) and further purification could lead to their isolation.

Also, the curative potential of the crude hydro-ethanol extract and fractions on the day of peak parasitaemia (9<sup>th</sup> day) was tested (Table 7). The trend in activity was similar to that obtained in the suppressive and prophylactic studies (Tables 5 and 6), as EAF retained its activity ahead of the crude extract and other fractions when compared with the infected untreated control. There was significant ( $p < 0.05$ ) increase in survival time for groups treated EAF, HEF and the crude hydro-ethanol plant extract. Elongation of survival time has been linked to parasite clearance and activity of the drug candidate (Mulisa *et al.*, 2018; Abdullahi *et al.*, 2022). This relationship is linear. That is the more active a drug candidate is, the greater the chances of survival of a treated infected animal. This derives from the fact that when the menacing activities of the parasite (such as increased inflammation and oxidative stress, depletion of vital biomolecules like glucose, lipids, proteins, including erythrocytes, etc.) are tackled, events leading to sustenance of life are restored. In all studies, chloroquine had better antimalarial activity and significantly ( $p < 0.05$ ) prolonged survival time of infected mice with values greater than those expressed by the various extracts and fractions. This is perhaps due in part to the rapid degradation of these extracts (Muluye, *et al.*, 2015) or the interplay of other contending phytoconstituents. Nonetheless, the endowment of *S. mombin* with rich nutrients and the presence of phytochemicals with antioxidant property could be the reason behind its activity and use in traditional settings.

## CONCLUSION

Aside the rich phyto-nutritional constituents and antimalarial activity revealed by the stem bark of *Spondias mombin* Linn. in this study, fractionation of its crude hydro-ethanol extract yielded an ethylacetate fraction with enhanced antimalarial activity. Therefore, isolation from this active fraction with a view of zeroing on the antimalarial active candidate(s) is recommended.

## AUTHORS' CONTRIBUTIONS

KOO conceptualized the study, optimized the protocol used, carried out bench work, performed statistical analysis and wrote, reviewed, and edited the manuscript. POU conceptualized, supervised the study and edited the manuscript.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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