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In vitro antioxidant and toxicity studies of *Sorindeia warneckei* Engl. (Anacardiaceae) stem methanol extract in laboratory animals

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Abstract
Sorindeia warneckei (SW) Engl. (Anacardiaceae) is a plant of medicinal and economic significance. Its edible fruits are used in medicinal baths and stems as traditional toothbrushes. This study evaluates the antioxidant activity and safety profile of SW stem extract. Antioxidant activity was assessed using the diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Acute and sub-acute toxicity tests were performed following the protocol described by the Organization for Economic Cooperation and Development (OECD). The methanol extract of SW was rich in phytochemicals, with total phenolic and flavonoid contents measured at 149.64 ± 0.50 mg/g rutin equivalent and 172.097 ± 0.28 mg/g gallic acid equivalent respectively. SW demonstrated dose-dependent antioxidant activity (IC_{50} = 0.0246 mg/mL), comparable to ascorbic acid (IC_{50} = 0.0195 mg/mL). Acute toxicity studies indicated a lethal dose (LD_{50}) > 2000 mg/mL. Haematological parameters were not adversely affected. However, certain biochemical markers exhibited significant alterations ($p<0.05$) following 28 days of treatment. Histological analysis revealed structural changes in renal and hepatic tissues, including hepatocyte hyperpigmentation and disorganization of the renal parenchyma. The findings highlight SW stem methanol extract's antioxidant potential but suggest caution regarding prolonged use due to signs of toxicity.

Keywords: Antioxidant; DPPH; Phenolic content; *Sorindeia warneckei*; Toxicity

INTRODUCTION

Oxidative stress remains a significant health concern, contributing to the development or progression of various neurological, immunological, metabolic, and cardiovascular conditions [1]. It is characterized by an imbalance between the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by cells and tissues and their removal. Prolonged imbalance can lead to the accumulation of these reactive species, resulting in cellular damage through interactions with macromolecules such as lipids, proteins, and nucleic acids [2]. ROS are commonly generated as by-products of several biochemical processes in biological systems and at normal levels, they play a significant role in certain body processes such as signalling pathways, phosphorylation,

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immunity, transcriptions, apoptosis, and differentiation [3]. ROS comprises highly reactive and unstable free radicals such as Hydroxyl (OH^-) groups, superoxide radicals (SO_2^-), singlet oxygen (O_2), ozone (O_3), and non-free radicals like hydrogen peroxides (H_2O_2), hypochlorous acid (HOCl), and hypobromous acid (HBrO). RNS mainly refers to nitrogen derivatives like nitrous oxides (NO) and peroxynitriles ($ONOO^-$) [4]. The generation of ROS can be triggered by a variety of intrinsic and extrinsic factors. Intrinsic factors include aging, physical and mental stress, underlying medical conditions, and biochemical processes such as respiratory activity, prostaglandin synthesis, phagocytosis, and the cytochrome P450 system [1]. Extrinsic factors such as exposure to heavy metals, hazardous chemicals, exhaust fumes, domestic fumes, as well as radiation, also stimulate ROS production [1]. Maintaining equilibrium between production, accumulation, and elimination of ROS is crucial for human health [4]. Antioxidants are chemical compounds that interact with oxidative species, stabilize, and facilitate their removal from the body. They also inhibit the production of ROS and enhance the expression of natural antioxidants [5]. Based on their sources and mode of action, they can be categorized as exogenous or endogenous compounds, and enzymatic or non-enzymatic antioxidants respectively. Enzymatic endogenous antioxidants include superoxide dismutase, glutathione peroxidase, and catalase, while non-enzymatic antioxidants include carotenoids, vitamins E and C, and polyphenols [6]. When the biological system fails to produce sufficient antioxidants to maintain equilibrium, reactive species accumulate within cells, potentially leading to cellular damage and death.

Plants synthesize a variety of phytochemicals including alkaloids, flavonoids, polyphenols, saponins, terpenoids, sterols, glycosides, and tannins, which play crucial roles in plant development, defence mechanisms, and responses to adverse environmental conditions [7,8]. These compounds, found in different plant parts such as leaves, stem bark, roots, seeds, and fruits, possess diverse structures with functional groups that serve protective functions in the body, by acting as antioxidants or anti-inflammatory agents [8,9]. They can directly mitigate oxidative damage by interacting with oxidative species or modulating genes and enzymes involved in the production of endogenous antioxidants. Phytochemicals have been documented to alleviate the onset and progression of various chronic diseases including cancer, diabetes, Alzheimer, and cardiovascular diseases, underscoring their significance in general physiological functions [8-9].

Sorindeia warneckei (SW) is a climbing shrub to a small tree belonging to the Anacardiaceae family and it holds economic significance, particularly in South-West Nigeria, where its stem is commonly utilized and marketed as a botanical toothbrush [13]. Its edible fruits change colour from yellow to black as they ripen, and these fruits are used in medicinal baths and as a tattoo dye in cosmetology [13]. It is predominantly found in West African countries and locally referred to as Bùjé were and Umu-Ahia amongst the Yoruba and Igbo ethnic groups respectively, in Nigeria [13.14]. Previous studies have highlighted the *in-vitro* antioxidant and alpha-amylase inhibitory properties of SW with secondary metabolites such as flavonoids, saponins, and triterpenes reportedly present in its leaves and stem bark extracts [14-16]. Moreover, antimicrobial activities against important pathological, dental bacteria, and fungi have also been reported in the literature [15,17]. Using the reversed HPLC, gallic acid, rutin, and quercetin have been isolated from the leaves of this plant [14]. Though various *in vitro* studies suggest the medicinal potentials of SW, there's limited information on its

pharmacological effects following oral administration compared to other species within the same genus. This study aims to investigate the *in vitro* antioxidant potential and *in vivo* toxicity of SW stem methanol extract.

EXPERIMENTAL METHODS

Collection and preparation of plant material. The stem of SW was collected in Ibadan, Oyo state, South-West, Nigeria, and identified by Dr S.A. Odewo and Mr. B.A. Ajani of the forestry herbarium unit of the Forestry Reserve Institute of Nigeria (FRIN), Ibadan, Oyo state, Nigeria. The herbarium specimen of the plant with voucher number FHI 113586 was prepared and deposited at the herbarium unit of FRIN. After collection, the stem was chopped into smaller pieces and air-dried at room temperature for two weeks, subsequently followed by oven drying at 45^{o}C before milling. Powdered stem sample (700 g) obtained after milling was extracted by cold maceration in absolute methanol (3 L) for 72 hours with occasional shaking. The extract was filtered, concentrated using a rotary evaporator, and evaporated to dryness over a water bath (40^{o}C). The dried extract was stored in an air-tight glass container for further analysis. The percentage yield was calculated using the formula described below.

$$\text{Percentage yield} = \frac{Weight\ of\ dried\ extract}{Weight\ of\ starting\ material} \times 100$$

Determination of phytochemical constituents. The phytochemical composition of the crude methanol extract of SW stem was qualitatively analysed using standard procedures [18,19].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The antioxidant potential of the SW stem methanol extract was evaluated by assessing its free radical scavenging activity through the DPPH assay in a 96-well plate [20]. Distilled water (100 µL) was added to each well, and a stock solution of SW extract (2.5 mg/mL) was prepared. Starting from the first well, 100 µL of the extract was serially diluted across the plate to achieve a concentration range of 1.25 mg/mL to 0.0097 mg/mL. The DPPH was dissolved in methanol to create a concentration of 0.08 mg/mL, and 100 µL of this solution was added to each well and allowed to react with the extract. The mixtures were thoroughly mixed and then incubated at 25^{o}C in a dark cabinet for 30 minutes. Free radical scavenging activity was quantified spectrophotometrically by measuring absorbance at 540 nm using a microtiter plate reader FLUOstar Omega (BMG LABTECH, Germany) and analysed using the Omega data analysis. The same procedure was repeated for ascorbic acid (standard drug) at equivalent concentrations serving as the positive control. IC_{50} value was determined using GraphPad Prism and percentage DPPH inhibition was calculated from absorbance values using the following equation:

$$\text{Percentage DPPH Inhibition} = \frac{(Abs.of\ control\ -Abs.of\ extract)}{(Absorbance\ of\ control)} \times 100$$

Determination of total phenol content (TPC). The total phenol content of SW stem methanol crude extract was determined spectroscopically according to the Folin–Ciocalteu method [14]. Gallic acid was used to set up the standard curve (3.9 µg/mL – 500µg/mL). About 0.25 mL of SW extract (1 mg/mL) was mixed with 0.5mL Folin-Ciocalteu phenol reagent and allowed to stand for 5 min. To the mixture was added 5 mL of 7.5% sodium carbonate solution and 65 mL distilled water. The mixture was thoroughly mixed and incubated for 90 minutes at 25°C. Absorbance readings were taken thereafter at 765 nm. The total phenol content of the stem methanol crude extract was evaluated from a gallic acid standard curve and expressed as mg/g of GAE (Gallic Acid Equivalent) of extract. Distilled water was used as blank.

Determination of total flavonoid content (TFC). Total flavonoid content was determined using the aluminium chloride method [21]. One mL of extract (1 mg/mL) was mixed with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate, and made up to 10 mL with distilled water. This mixture was thoroughly mixed and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm using a UV-visible spectrophotometer. The total flavonoid content was determined from the calibration curve of rutin as standard (0-100 µg/mL in methanol). The concentration of total flavonoids was expressed as mg/g of rutin equivalent (RE) of plant extract.

Experimental animals. Animals used for the study were purchased from the central animal house, Olabisi Onabanjo University, Sagamu campus, Ogun state, Nigeria, and transferred to the laboratory animal house of the same institute where the study took place. They were kept in clean and well-ventilated cages maintained under standard environmental conditions. Animals were acclimatized for two weeks before the commencement of the study and fed with pelletized feed (Vital Feeds Ltd) and allowed free access to water *ad libitum*.

Acute toxicity test. The acute toxicity of SW stem methanol extract was evaluated according to procedures described by the Organization for Economic Cooperation Development (OECD) guide 423 [22] and [23]. The test as described in the guide allows the use of a minimum number of animals (3 animals of single sex per step) with stepwise administration of a fixed dose of test substance believed to provide sufficient scientific information, classification, and ranking according to Global Harmonized System (GHS). Female animals are usually preferred because they are considered generally slightly more sensitive than the male animals [22]. The dried extract was reconstituted in distilled water and administered orally at doses of 300mg/kg, and 2000 mg/kg in a volume of 1 mL. A total of nine (9) Wistar albino mice (weight 17–22 g) of 16-20 weeks of age were used for the study and grouped into three (n=3). The animals were fasted on food but not water for 4 hours before the experimental period and 1 hour after administration of the test substance to avoid food and drug interaction. Group 1 received 300 mg/kg SW stem methanol extract and animals were observed for clinical toxicity signs and mortality every 30 min for the first 4 hours, 12 hourly for the next 48 hours, and daily for 14 days. According to OECD guide 423 where there is an absence of mortality or presence of not more than one mortality at a fixed dose of 300mg/kg, the next fixed dose should be initiated [22]. Regarding this, the same experiment was conducted for group 2 of mice which received 2000 mg/kg of SW stem methanol extract. Animals in group 3 served as the control and received distilled water. All animals were observed for toxicity signs, onset of toxicity, and length of recovery. They were also closely monitored for changes in behavioural patterns, skin, and fur colour, feeding habits, and eyes and mucous membranes. Furthermore, observations were made for signs such as convulsion, tremor, diarrhoea, lethargy, sleep, coma, and mortality. Animals' body weight was taken before administration of extract and vehicle on the first day of the experiment and at the end of the experiment. Percentage body weight change was calculated using the formula:

$$\text{Percentage change in body weight} = \frac{Body\ wt\ after\ treament\ -\ Body\ wt\ before\ treatment}{Body\ weight\ before\ treatment} \times 100$$

Sub-acute toxicity test. Sub-acute toxicity is carried out to assess the toxicity of a substance over repeated exposure for a long time. The assessment was carried out using the 28 – days repeated dosing method [24,25]. A limit test dose of 1000 mg/kg of SW stem methanol extract was administered daily for 28 days. A total of ten (10) Wistar albino rats (wt. 186 g

to 228 g) aged 16 - 20 weeks were used for the study and grouped into two. Group one (n=5) received 1000 mg/kg SW stem extract dissolved in distilled water while group two (n=5) received distilled water and served as the negative control group. All administrations were done orally once daily for 28 days. Physical observation of animals was done at intervals of 30 min for the first 4 hours, and daily for 28 days. Animals were observed for behavioural changes, clinical signs of toxicity, and mortality. On the last day of the experiment, animals were euthanized and blood and tissue samples were collected for haematological, biochemical, and histological studies.

Collection of blood and organ samples. Animals were euthanized on day 28 (sub-acute toxicity test) with diethyl ether. Blood samples were collected by puncturing the prominent jugular vein with a syringe and needle and collected into properly heparinized ethylene diamine tetra acetate (EDTA) bottles (for haematological study) and non-heparinized (without anticoagulant) bottles (for biochemical analysis). Liver and kidney tissues were isolated, washed in normal saline, and stored in properly bottles containing 10% formaldehyde for histological study.

Haematological study. Haematological indices such as red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), haematocrit (Hct), neutrophils, leucocytes, eosinophil, basophils, monocytes, platelets, packed cell volume (PCV) were quantified in the sample using automated haematology analyser BC-3600 Mundray Guagzhon Medsinglong medical equipment Co. Ltd., China. Differential Leucocyte counts were done using optical microscopy (400x) [25].

Biochemical parameters. A biochemical study was carried out on blood samples collected into non-heparinized bottles. The blood was allowed to clot at room temperature and centrifuged at 3000 RPM for 10 min to obtain serum. Subsequently, the serum was used to quantify levels of biochemical markers. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB), and conjugated bilirubin (CB) as well as urea and creatinine levels in the blood were used to determine liver and kidney functions respectively. Cholesterol indices were also characterized. Biochemical assessment was carried out using Sigma-Aldrich bio-assay kits and an automated biochemical analyser [25].

Histological analysis. Samples of kidney and liver tissues were harvested for histological study. The organs were subjected to dehydration with increasing concentration of alcohol (70–100%) followed by diaphanization in xylol, impregnation, and embedded in paraffin. Tissue samples were sectioned using a microtome and stained with haematoxylin-eosin followed by microscopical examination [23].

Ethical approval. Guidelines for animal handling were strictly adhered to [26]. The experiment was also approved by the Health Research Ethics Committee (HREC), Olabisi Onabanjo University Teaching Hospital with approval number OOUTH/HREC/662/2023AP.

Statistical analysis. The data were analysed statistically by comparing the treated and untreated groups using an unpaired t-test. A significance level of $\alpha < 0.05$ was set to determine statistical significance. All statistical analyses and graphical representations were performed using GraphPad Prism (Version 8.0.1).

RESULTS

Percentage yield and phytochemical screening. The percentage yield of extract obtained from SW stem using methanol was 20.7%. A preliminary phytochemical study of SW methanol crude stem extract reveals the presence of flavonoids, saponins, terpenes, and tannins while anthraquinone, alkaloids, and

cardiac glycosides were absent (Table 1). TPC and TFC as extrapolated from the standard curves of rutin and gallic acid respectively were found to be 149.64 ± 0.50 mg/g rutin equivalent (RE) and 172.097 ± 0.28 mg/g gallic acid equivalent (GAE) (Table 2).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. The antioxidant capacity of the SW stem methanol extract was evaluated using a DPPH radical scavenging assay. The methanol extract exhibited concentration-dependent antioxidant activity (Figure 1) with an IC_{50} value of 0.0246 mg/mL, comparable to the positive control, ascorbic acid (IC_{50} = 0.0195 mg/mL).

Acute toxicity. The acute toxicity study of the SW stem methanol extract on treated female mice following a single-dose treatment of 300 mg/kg and 2000 mg/kg revealed the absence of toxic central nervous system (CNS) symptoms, convulsions, nerve damage, or coma throughout the entire 14-day observation period. There was also no observable change in the physical appearance, respiratory and behavioural patterns, skin colour, and feeding habits of treated animals as compared to animals in the untreated group. No mortality was recorded throughout the study period. According to OECD guide 423 [22], where there is no mortality recorded at a dose of 2000 mg/kg body weight, SW stem methanol extract can be considered to have a LD_{50} >2000 mg/kg. In addition, both the treated and untreated groups exhibited weight gain, as presented in Table 3.

Sub-acute toxicity study. The SW stem methanol extract was administered orally at a dose of 1000 mg/kg daily to experimental animals for 28 days and its effects on the organs and tissues of animals were assessed. All parameters were compared between treated and untreated groups. The haematology parameters of the treated group were not significantly different when compared with the control group $p<0.05$ (Table 4).

The biochemical analysis of biomarkers in the treated group revealed no significant changes in the levels of AST, ALT, TB, and CB compared to the untreated group. However, levels of plasma creatinine and urea were significantly altered in the treated group (*p*-value = 0.0350 and 0.0284 respectively $\alpha<0.05$, Table 5).

The full Lipid profile test of treated animals was comparable to the control with levels of cholesterol, HDL, and LDL not significantly affected. However, the level of triglyceride was significantly higher in the treated group when compared with the control (*p*-value = 0.0108 $\alpha<0.05$, Table 6).

The liver and kidney tissues of both treated and untreated groups were analysed histologically after the prolonged administration of the crude extract for 28 days. Histological investigation of liver tissue in the treated group showed a normal hepatic central vein. However, hyperchromatic pigmentation, sinusoid dilatation, and activation of Kupffer cells were also observed (Figure 2). Effect of extract on weight of mice was also studied (Figure 3). In the renal tissue, the glomerulus was well-defined, although the parenchyma cells appeared disorganized (Figure 4).

DISCUSSION

The methanol extract of SW stem yielded a percentage of 20.7%. The yield obtained in this study is slightly higher than the 8.05% reported in previous studies [15], which may be attributed to the large volume of solvent used for extraction in this study. Preliminary phytochemical screening of the methanol crude extract showed the presence of flavonoids, saponins, terpenes, and tannins. Similar phytochemical classes have been reported to be present in the leaves and stem of SW as well as in the stem bark of *Sorindeia madagascariensis*, a closely related species [14-16]. In this study, quantitative determination of the total flavonoid and phenolic content of SW stem methanol extract

was found to be 149.64 ± 0.50 mg/g RE and 172.097 ± 0.28 mg/g GAE respectively (Table 2). The presence of these polyphenols suggests that SW may be a promising candidate for pharmacological applications. This result is comparable to the total flavonoid and total phenolic contents in the leaves of SW, reported by Adesegun et al. to be 25.54 ± 0.47 QE (quercetin equivalent) and 41.24 ± 1.18 GAE (gallic acid equivalent)[14]. This indicates that different parts of the plant contain varying levels of phytochemicals with pharmacological significance. The result of the DPPH radical scavenging activity of SW methanol extract as presented in Figure 1 revealed that the extract exhibited a dose-dependent antioxidant capacity by its ability to scavenge free DPPH radicals.

Table 1: Summary of phytochemical screening of *Sorindeia warneckei* stem methanol extract

	Flavonoids	Saponins	Alkaloids	Anthraquinones	Tannins	Cardiac glycosides	Terpenes
SW	+	+	-	-	+	-	+

(+) = Present (-) = Absent

Table 2: Total flavonoid and phenolic contents of *Sorindeia warneckei* stem methanol extract

Parameters	Concentration	Regression equation
Total flavonoid content	149.640 ± 0.50 mg/g RE	y = 0.0014x+0.532 r^2 = 0.9434
Total phenolic content	172.097 ± 0.28 mg/g GAE	y = 0.0031x+0.0333 r^2 = 0.9649

Data set presented as mean ± S.E.M. n=3. RE = rutin equivalent, GAE = gallic acid equivalent, y = absorbance, x = concentration, r^2 = coefficient of regression

Table 3: Effect of *Sorindeia warneckei* stem methanol extract on the weight of mice before and after treatment

	Average weight of mice (grams)		
	Day 1	Day 14	Percentage body weight change
Group A (300 mg/kg)	19.5 ± 0.40	21.6 ± 0.24	10.77%
Group B (2000 mg/kg)	17.0 ± 0.37	18.6 ± 0.79	9.41%
Group C (Distilled water)	21.4 ± 0.55	23.3 ± 0.40	8.88%

Data values are presented as mean ± S.E.M. (n=3).

Table 4: Effect of *Sorindeia warneckei* stem methanol extract on haematological indices of treated and untreated rats after 28 days of oral administration

Blood parameters	Control group (Distilled water)	Experimental group (1000mg/Kg)
PCV (g/dL))	61.00 ± 1.00	60.00 ± 2.00
Hb (%)	20.40 ± 0.20	19.95 ± 0.65
WBC ($x10^3$µL)	1600.00 ± 200.00	1800.00 ± 400.00
N (%)	56.00 ± 6.00	44.00 ± 11.00
L (%)	42.00 ± 4.00	54.00 ± 11.00
E (%)	0.50 ± 0.01	1.00 ± 0.00
M (%)	1.00 ± 0.00	0.60 ± 0.05
B (%)	0.5 ± 0.01	0.50 ± 0.00
PLT ($x10^3$µL)	177.50 ± 2.50	230.00 ± 20.00
RBC ($x10^6$µL)	5.90 ± 0.10	5.75 ± 0.15

Data presented as mean ± S.E.M. (n=5). Significance level ($p < 0.05$) was determined using an unpaired t-test comparing the treated group to the untreated group. PCV (packed cell volume), Hb (haemoglobin), WBC (white blood cell), N (neutrophils), L (leucocytes), E (eosinophils), M (monocytes), B (basophils), PLT (platelets), RBC (red blood cell)

Table 5: Effect of *Sorindeia warneckei* stem methanol extract on biochemical parameters of treated and untreated rats after 28 days of oral administration

Blood parameters	Control group (Distilled water)	Experimental group (1000mg/kg)
Urea (mmol/L)	48.50 ± 4.50	73.00 ± 8.00*
Creatinine (mg/dL)	1.65 ± 0.15	2.50 ± 0.30*
AST (U/L)	8.00 ± 1.00	8.00 ± 3.00
ALT (U/L)	4.00 ± 0.01	3.50 ± 0.50
TB (mg/dL)	0.03 ± 0.00	0.04 ± 0.01
CB (mg/dL)	0.02 ± 0.00	0.03 ± 0.00

Data presented as mean ± S.E.M (n=5). *Significance level ($p < 0.05$) was determined using an unpaired t-test comparing the treated group to the untreated group. AST (aspartate aminotransferase), ALT (alanine aminotransferase), TB (total bilirubin), CB (conjugated bilirubin)

Table 6: Effect of *Sorindea warneckei* stem methanol extract on lipid profile in treated and untreated rats 28 days of post administration

Blood parameters	Control group (Distilled water)	Experimental group (1000 mg/Kg)
Chol (mg/dL)	40.50 ± 3.50	46.00 ± 9.00
Trig (mg/dL)	99.00 ± 2.00	136.50 ± 12.50*
HDL (mg/dL)	52.50 ± 7.50	49.50 ± 4.50
LDL (mg/dL)	32.00 ± 11.00	31.00 ± 11.00

Data presented as mean ± S.E.M. (n=5). *Significance level ($p < 0.05$) was determined using an unpaired t-test comparing the treated group to the untreated group. Chol (cholesterol), Trig (Triglycerides), HDL (High-density lipoprotein), LDL (Low-density lipoprotein)

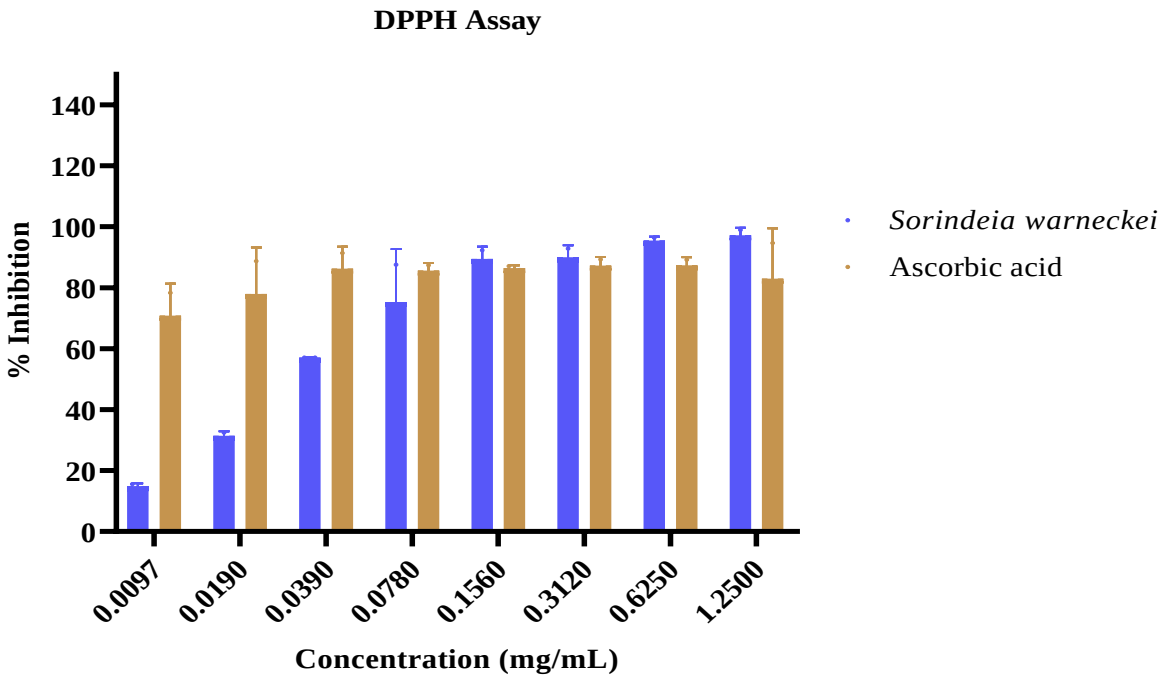

Figure 1: DPPH radical scavenging activity of *Sorindea warneckei* (SW) stem methanol extract (Data set presented as mean ± S.E.M. based on two independent experiments)

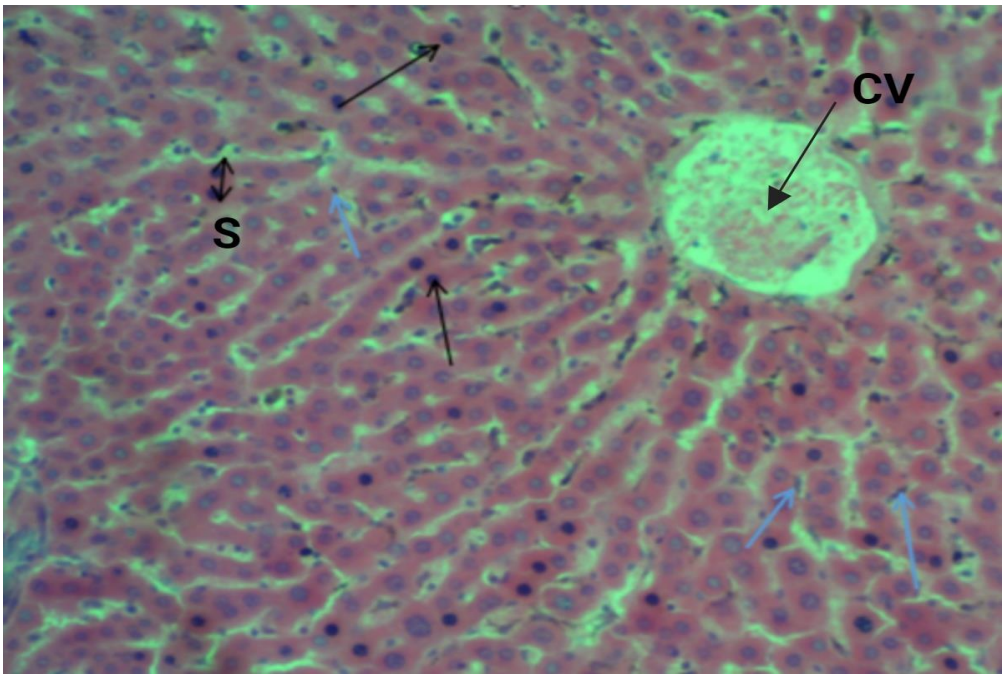

Figure 2: Photomicrograph showing rat liver in experimental group revealing normal central vein (CV). The result also reveals hyperchromatic pigmentation (Black arrow) with sinusoids (s) appearing very prominent. There was also the presence of Kupffer cells (blue arrow) (H&E 40x)

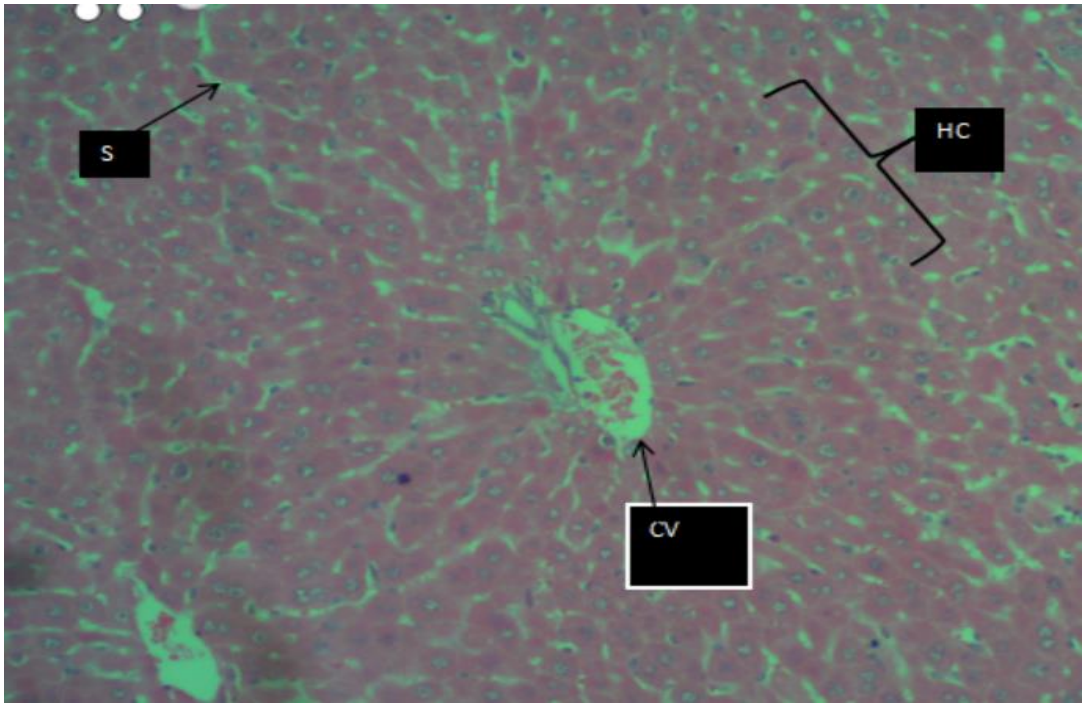

Figure 3: Photomicrograph of rat liver in control group showing sinusoids (S), central vein (CV), and the hepatic cells (HC), all revealing the normal architecture of the liver histology. (H&E 40x)

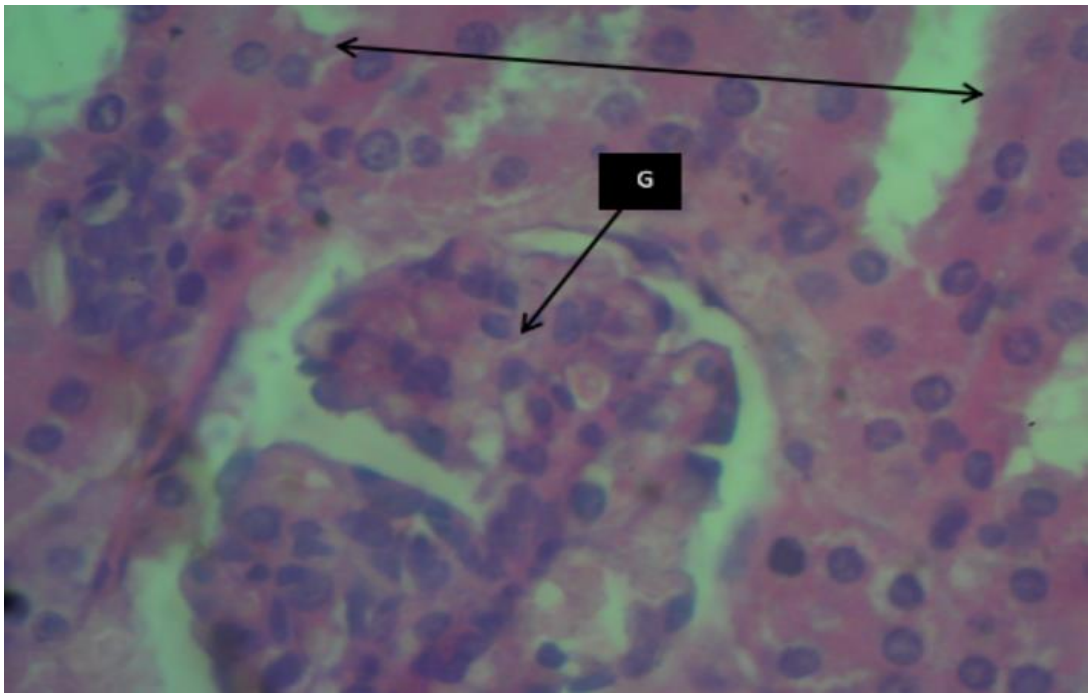

Figure 4: Photomicrograph showing rat kidney in experimental group revealing normal structure of the glomerulus (G). The kidney parenchyma appears a bit disorganized. (H&E 40x)

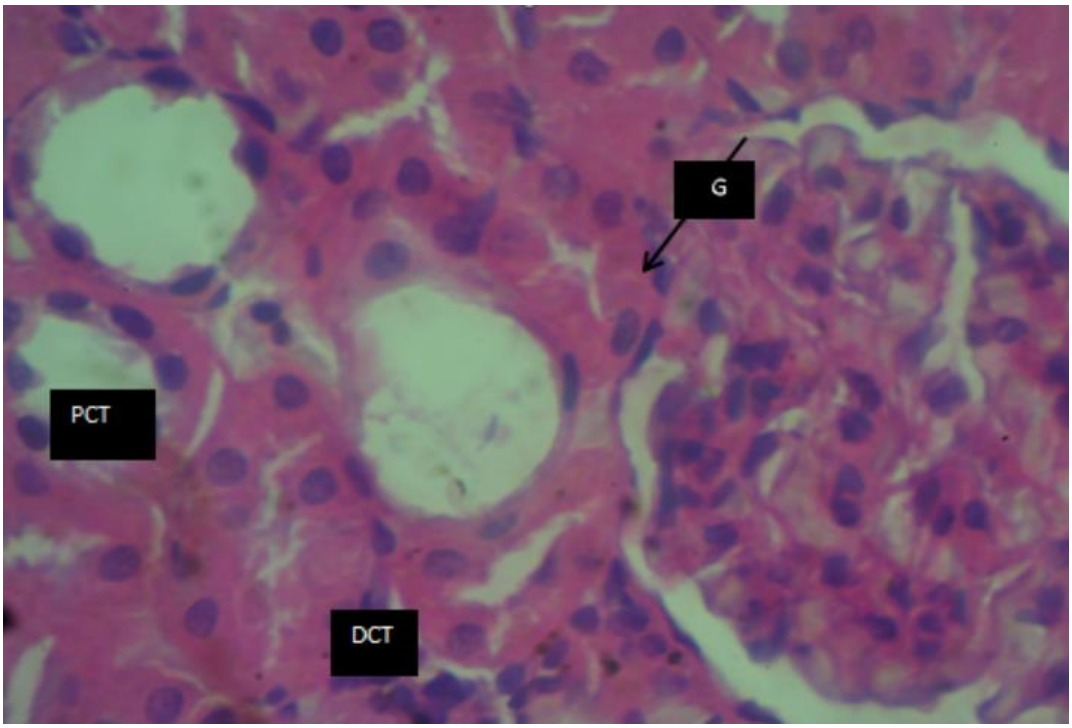

Figure 5: Photomicrograph of rat kidney in control group showing normal histoarchitecture of the kidney with Distal Convoluted Tubule (DCT) and Proximal convoluted tubule (PCT) well outlined as well as the Bowman's capsule and the glomerulus (G). (H&E 40x)

Free radicals are highly reactive unstable entities that readily accept or donate electrons to attain stability [27]. These radicals, when present in large amounts that surmount the level of the body's natural antioxidant capacity pose serious health risks, and attack cells, nucleic acid, and proteins [28]. DPPH assay is a widely accepted assay in determining the antioxidant capacity of organic agents. DPPH is a free radical with an electron deficit nitrogen atom. In the presence of a hydrogen donor, it is reduced from DPPH (violet color) to a stable hydrazine compound called 2,2-diphenylpicrylhydrazine, DPPH-H (yellow color) whose color intensity is indicative of the number of electrons acquired [29,30]. Phenolic compounds are highly polyhydroxylated, a property that gives them the ability to react with and neutralize the oxidative species by donating electrons to the electron-deficient radical, acting as reducing agents, scavenging for free radicals, and forming complexes with metal oxides [31]. The antioxidant effect of SW stem extract (Fig. 1) may be attributed to its polyphenol contents which readily donate their hydrogen ion to the radical DPPH to form a stable entity. In this study, SW stem methanol extract exhibited a comparable antioxidant capacity to ascorbic acid, with IC_{50} values of 0.0246 mg/mL and 0.0195 mg/mL, respectively. Although ascorbic acid demonstrated a slightly lower IC_{50} value, the antioxidant activity of SW was comparable, suggesting that it contains bioactive compounds with notable radical-scavenging potential (Figure 1). These findings are consistent with those reported by [15]. where the IC_{50} values of SW methanol stem extract and ascorbic acid were 0.72 µg/mL and 0.69 µg/mL, respectively. This observed trend further supports the finding that the methanol stem extract of SW exhibits antioxidant activity comparable to that of ascorbic acid, as demonstrated by the minimal difference in IC_{50} values between the extract and the positive control. In addition, the IC_{50} value observed for the SW methanol stem extract in this study (0.0246 mg/mL) was slightly higher than that reported in the literature (0.72 µg/mL) [15]. This difference could be attributed to the concentration of DPPH used in the respective studies.

To determine the safety of SW stem methanol extract after oral administration over both short- and medium-term use, acute and sub-acute toxicity studies were conducted. The acute toxicity study of the SW stem extract on treated female mice, following a single-dose treatment up to 2000 mg/kg, revealed the absence of toxicity signs and mortality with an LD_{50} >2000 mg/kg. The weight of treated animals was also not adversely affected as both the treated and untreated groups exhibited weight gain (Table 3) probably due to consistent eating habits. In the sub-acute toxicity test, the animals received an oral administration of 1000 mg/kg of the extract daily for 28 days. Throughout the study, no physical signs of clinical toxicity or behavioral changes were observed, and there were no recorded instances of mortality. Plant extracts contain various Phyto-constituents capable of interacting with diverse physicochemical processes in the body [32]. Investigating and monitoring levels of serum biomarkers, enzymes, non-enzymes, and immune cells are crucial for identifying or detecting substance toxicity. Alterations in the normal levels of these parameters can provide insights into the condition of organs that regulate crucial body processes when exposed to toxic agents, as well as the underlying mechanisms of toxicity [32-34].

Findings from this study revealed that SW stem methanol extract did not significantly alter haematological indices (Table 4). Biochemical markers such as AST, ALT, TB, and CB were comparable to those of the untreated groups (Table 5). These findings are similar to those reported about the leaves of *Sorindeia juglandifolia* (SJ), a specie related to SW where no toxic effect was observed on

AST and ALT post-treatment [35]. However, creatinine and urea levels in the treated group (2.50 mg/dL and 73.00 mmol/L respectively) were significantly higher than the untreated group (1.65 mg/dL and 48.50 mmol/L respectively) $p<0.05$. Urea, a by-product of protein metabolism, is produced in the liver and primarily excreted by the kidneys. Similarly, creatinine, a by-product of creatine and phosphocreatine metabolism, is synthesized in the liver, pancreas, and kidneys [36]. Both urea and creatinine are commonly used as indicators of renal function. Elevated levels of blood urea nitrogen and creatinine may indicate a decline in kidney function, as impaired renal filtration reduces their clearance from the bloodstream [36-37]. Similarly, the lipid profile revealed comparable levels of total cholesterol, HDL, and LDL between the treated and untreated groups, except for triglycerides, where the treated group exhibited elevated levels at 136.50 mg/dL compared to 99.00 mg/dL in the untreated group (Table 6). Elevated triglyceride levels are of clinical importance, as they have been associated with an increased risk of cardiovascular disease and a predisposition to acute pancreatitis [38-39]. Treatment with SW stem methanol extract resulted in an elevation of triglyceride levels in the treated animals, which may contribute to an increased risk of cardiovascular disease.

A histological examination of the liver after 28 days of oral administration of crude SW stem methanol extract at a dose of 1000 mg/Kg revealed a normal morphology of the hepatic central vein with prominent sinusoids. The hepatocytes also appeared hyperpigmented (Figure 2). A study conducted on the aqueous leaf extract of SJ revealed that SJ demonstrated a hepatoprotective and renoprotective effect by alleviating methotrexate-induced hepatic and renal damage in rats while observing a dilation of the sinusoids at a dose of 350 mg/Kg of SJ leaves[40]. The dilation of sinusoids is indicative of changes in hepatic blood circulation [41]. In our study, a similar effect was observed in the liver of treated rats, with the sinusoids appearing prominent after 28 days of repeated exposure. Sinusoidal lining contains Kupffer cells which are the liver macrophages. They are the first point of contact in the liver with substances absorbed from the gastrointestinal tract and serve various physiological roles like liver protection, phagocytosis, and regeneration of liver cells [42,43]. The presence of Kupffer cells in the liver of treated animals suggests their activation to protect the liver against injury. Histological analysis of renal tissue from the treated group revealed disorganization of the parenchymal cells (Figure 4). These structural alterations in the renal parenchyma likely impaired the kidneys' ability to effectively filter and excrete waste products such as creatinine and urea. This disruption probably led to the accumulation of creatinine and urea in the bloodstream, potentially explaining the elevated levels observed. Overall, our findings suggest that SW stem methanol extract possesses significant antioxidant potential. However, signs of toxicity were observed in the liver and kidney tissues at the tested concentration of 1000 mg/mL. To gain a more comprehensive understanding of the extract's safety profile, further studies are recommended to evaluate its effects on other vital organs**.**

Conclusion. In conclusion, the SW stem methanol extract contains secondary metabolites of significant pharmacological importance. The extract is particularly rich in polyphenolic compounds which are likely responsible for its antioxidant potential. However, prolonged exposure to the extract may result in alterations in some biochemical parameters and structural changes in liver and kidney tissues, indicating the need for cautious use.

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