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Biosafety assessment and *In vitro* antioxidant activity of *Musanga cecropioides* root sap

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Abstract

The present study evaluated the biosafety and in vitro antioxidant activity of *Musanga cecropioides* root sap, a traditional remedy known for its health benefits. The sap was found to be rich in bioactive compounds, including alkaloids, tannins, saponins, flavonoids, and phenols, confirming existing literature. Proximate analysis revealed high moisture, fat, and protein content, alongside significant levels of essential vitamins and amino acids, indicating potential nutritional and therapeutic benefits. Acute toxicity assessments in mice demonstrated safety at doses up to 100 ml/kg, while the sub-acute toxicity assessment using rats showed no significant changes in haematological parameters or liver and kidney function markers. Histological examinations showed minor, non-significant alterations in liver and lung tissues. Antioxidant assays (DPPH and ABTS) revealed considerable radical scavenging activities, attributed to the presence of known antioxidant compounds. These findings support the traditional use of *Musanga cecropioides* root sap, highlighting its potential as a natural therapeutic agent. Further research is recommended to explore its mechanisms and clinical efficacy, paving the way for its development into a standardized natural remedy.

Keywords: *Musanga cecropioides*; Bioactive compounds; Antioxidant activity; Toxicity assessment; Traditional medicine; phytochemicals

1. Introduction

Musanga cecropioides, commonly known as the African corkwood tree, belongs to the family Urticaceae and is widely distributed across tropical Africa. Traditionally, various parts of this plant have been utilized for their medicinal properties, particularly in rural communities. The root sap of *Musanga cecropioides*, in particular, is reputed for its health and nutritional benefits. This sap is commonly used by traditional healers in the parts of Nigeria and other African countries for treating a variety of ailments in both humans and livestock. The sap is used to manage and cure diarrhoea, stimulate breast milk production and to ease menstrual cramps, treat gonorrhoea, vaginal candidiasis, pulmonary complaints, treatment of upper respiratory tract infection (cough), trypanosomiasis, treatment of dirty wounds and skin diseases, otitis, rheumatism, epilepsy, oedema, and in childbirth. It is also used to cure conjunctivitis, headache and as a laxative [1;2;3;4;5].

Despite the widespread use of *Musanga cecropioides* root sap in traditional medicine, scientific validation of its bioactive components, safety profile, and pharmacological activities is limited [6]. Understanding the phytochemical composition and elemental constituents of the root sap is crucial for corroborating its traditional uses and ensuring its safety for consumption. Phytochemicals, such as tannins, saponins, alkaloids, flavonoids, and phenols, are known to contribute to the medicinal properties of many plants due to their antioxidant, antimicrobial, and anti-inflammatory activities [7].

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Furthermore, evaluating the mineral content of the root sap provides insight into its nutritional value. Essential minerals such as potassium, calcium, magnesium, and phosphorus play vital roles in various physiological processes [8; 9]. Conversely, the presence of heavy metals, even in trace amounts, necessitates a thorough investigation to prevent potential toxicity [10].

Antioxidant activity is another significant attribute of medicinal plants, contributing to their therapeutic effects by neutralizing free radicals and preventing oxidative stress-related diseases [11]. Therefore, assessing the antioxidant capacity of *Musanga cecropioides* root sap through in vitro assays can provide valuable information on its potential health benefits.

In this study, we aimed to quantitatively analyze the bioactive and elemental composition of *Musanga cecropioides* root sap and evaluate its antioxidant activities. Additionally, we assessed the acute and sub-acute toxicity of the sap in animal models to establish its safety profile. Through these comprehensive investigations, we seek to validate the traditional use of the sample and ascertain the safety of the sample for use.

2. Material and methods

2.1. Plant Sample Collection

A young branch of *Musanga cecropioides* bearing leaves, flowers and fruit was collected from a private farmland in Ulorin village, Ovia North Local Government Area of Edo State, Nigeria in for identification and authenticated by Prof. MacDonald Idu of the Department of Plant Biology and Biotechnology, UNIBEN, Benin City. The voucher specimen was deposited in the same Department and voucher specimen number obtained (UBH-M500). The root sap for this study was however collected overnight by making an incision on the root and placing a collection bowl underneath for the fluid to flow. The sap was stored in fitly covered containers and refrigerated at $4^{o}C$ prior to use.

2.2. Qualitative Phytochemical Screening

Qualitative screening of phytochemicals was done following standard protocol as described and adopted by Okwu and Okwu [12] for alkaloids, Odebiyi and Sofowora [13] for saponins, Ayeni and Yahaya [14] for tannin, flavonoid, phenol and cyanogenic glycoside, Ugochukwu *et al*. [15] for terpenoid, sterol/Steroids and oxalate and Trease and Evans [16] for anthraquinone.

2.3. Quantitative Phytochemical Screening

Determination of total alkaloid content and total saponin content was done following the methods adopted by Ifemeje *et al.* [17]. The method described by Folin-Denis and modified by Polshettiwar *et al.* [18] was adopted in the determination of total tannin content. Total flavonoid content was estimated using Aluminum chloride method described by Chang *et al.* [19]. Total phenolic content was estimated using the Folin and Ciocalteau's method [20]. Total terpenoid content and total glycoside content were estimated following methods described by Harborne [21] and Sofowora [22].

2.4. Proximate Analysis

The proximate composition (moisture, ash, fat, fibre, carbohydrate and protein content) of *Musanga cecropioides* root sap was analyzed following the methods described by AOAC [23].

2.4.1. Vitamins Analysis

Vitamin composition in *Musanga cecropioides* root sap was analysed with High Performance Liquid Chromatography following methods described by AOAC [23] and Idu *et al.* [24].

2.5. Amino Acids Analysis

Analysis of amino acids in *Musanga cecropioides* root sap was done following methods described by AOAC [23], using Gas Chromatography (HP 6890) equipped with capillary column EZ and pulsed flame photometric detector.

2.5.1. Mineral Analysis

Musanga cecropioides root sap were analysed for mineral elements following methods described by AOAC [23] using UNICAM 929 London Atomic Absorption Spectrophotometer powered by the SOLAAR software.

2.6. Acute Toxicity Assessment

The acute oral toxicity assessment of *Musanga cecropioides* root sap was evaluated in albino mice using the method described by Lorke [25], while adopting minimal modifications described by Usman *et al.* [26].

2.7. Experimental Animals

Acute toxicity assessment was carried out in male and female albino mice with weight range of 20 – 35 g, which were purchased from an animal farm in Ibadan, Oyo State, Nigeria. They were then kept to acclimatize for two weeks in the Edo University Animal house, Iyamho. They were housed in standard plastic cages and allowed free access to animal pellets (Vital Feeds, Nigeria) and free access to water. The animals were kept and handled as outlined in the standard protocols for the use of laboratory animals [27]. The study was approved and overseen by the Faculty of Science ethical committee, Edo State University Uzairue. The sap was administered by using orogastric gavage.

2.8. Experimental Design

This experiment was done in two phases as follows:

- Phase one: animals were shared in three groups of three mice for each treatment. The doses administered at this stage were 10, 20 and 40 ml/kg body weight. All animals were watched closely hourly, 12 hours long and once daily for the next 2 weeks for signs of toxicity namely salivation, stretching of the body, weakness, brushing of nose on the floor, sleep, coma and death.
- Phase two: animals were also shared in three groups of two mice for each treatment dose. The doses administered at this stage were 60, 80 and 100 ml/kg body weight was administered for *Musanga cecropioides* root sap. The animals were observed every hour for 12 hours and up to two weeks for any signs of toxicity or death.

The LD_{50} was determined with the following formula:

$$LD_{50} = \sqrt{(D_o \times D_{100})}$$

Where D_o = highest dose with no death

And D_{100} = lowest dose with death

2.9. Sub-Acute Toxicity Assessment

The sub-acute toxicity study of *Musanga cecropioides* root sap was done following the methods described by Oshomoh *et al.* (2013) and Olaniyan *et al.* (2016). The animals were administered *Musanga cecropioides* root sap (5, 10, 25 ml/kg body weight for 28 days. At the end of the drug administration, the rats were fasted overnight and anaesthetized with chloroform in a chamber. The animals were then sacrificed and 5 ml of blood was obtained through the cardiac vein for further analysis. Organ weight was measured and vital organs harvested for histology.

2.10. Experimental Animals

Sub-acute toxicity assessment was performed using male and female albino rats, weighing 100 – 200 g. The animals were obtained from the animal house in AAU, Ekpoma, and allowed to acclimatize for 2 weeks, with unrestricted access to pelletized Vital growers feed and clean water before the experiment. The animals were kept and handled as outlined in the standard protocols for the use of laboratory animals [27]. This study was also overseen by the Edo State University Faculty of Science ethical committee. The sap was administered by using orogastric tubes.

2.11. Experimental Design

The treatment groups are as follows:

Control administered 1 ml/kg DW;

5 ml/kg MCCRS;

10 ml/kg MCCRS;

25 ml/kg MCCRS.

2.12. Haematology Analysis

2 ml of blood was collected in 5 ml EDTA (ethylenediaminetetra acetic acid) bottles and estimated for concentration of various haematological parameters. The parameters considered were white blood cell count, lymphocytes, monocytes, granulocytes, red blood cell count, haemoglobin concentration, packed cell volume and platelet count. The haematological parameters were analysed using automatic haematology analyser (Model BC-2800) within 24 hours of blood collection.

2.13. Biochemical Analysis

3 – 5 ml of blood was collected in 5 ml Lithium heparin containers and was separated using a centrifuge at 2500 rpm for 10 minutes. The plasma was then put in sterile bottles for biochemical analysis.

2.14. Liver Function

Alanine aminotransferase and Aspartate aminotransferase activities were estimated following the method of Reitman and Frankel [30] while Alkaline phosphatase was estimated following the method described by Plummer [31] using commercially available test kit (BioVision Inc, USA). Gamma glutamyl transferase activity was estimated using the kinetic colorimetric method as described by Persijn and Van der Slik [32]. Total bilirubin, conjugated bilirubin and unconjugated bilirubin were determined following the colorimetric method described by Jendrassik and Grof [33]. Total protein was assayed using the Biuret method Doumas *et al.* [34].

2.15. Renal Function

Creatinine and urea were assayed using commercial kits and in accordance with principles previously reported by Larsen [35] and Taylor and Vadgama [36]. Sodium, chloride bicarbonate and potassium were measured using standard Flame Atomic Absorption Photometry procedure as described by Howe [37].

2.16. Lipid Profile

Total cholesterol was determined enzymatically by hydrolysis and oxidation as described by Trinder [38]. Triacylglycerol was estimated after enzymatic hydrolysis by lipases following the Trinder [38] and Tietz [39] methods. HDL was estimated using Randox kit. Low density lipoprotein and very low density lipoprotein were calculated using the Friedewald formula [40].

2.17. Plasma Antioxidant

Malondialdehyde concentration was determined following the method described by Varshney and Kale [41]. The assay is based on the reaction of malondialdehyde with thiobarbituric acid, forming a red colour complex that absorbs light at 535 nm. SOD was determined following the method described by Misra and Fridovich [42]. CAT concentration was determined according to the method described by Cohen *et al*. [43]. Gluthathione peroxidase activity was determined following the method described by Flohe and Gunzler [44]. Glutathione reductase activity was determined following the method described by Teitze [45].

2.18. Histology of Vital Organs

The vital organs where isolated from the sacrificed rats and weighed in a digital scale and then fixed in 10% formal saline for 24 hours, dehydrated in an alcohol-xylene series and embedded in paraffin. Sections were stained with haematoxylin and eosin for histological examination and photomicrographs were taken. The organs considered include heart, liver, lungs, and kidney.

2.19. DPPH Radical Scavenging Activity

DPPH radical scavenging activity of *Musanga cecropioides* crude root sap was determined according to the method described by Adebiyi *et al.* [46]. DPPH was dissolved in methanol to a 0.025 g/l. Ascorbic acid (standard) and the crude root sap was used as its stock solution. The various concentrations (10, 20, 40, 60, 80 and 100 µg/ml) of the stock solution was added to each test tube containing 1 ml of DPPH working solution. After a 20 minutes reaction at room temperature, the absorbance of the solution was measured at 515 nm. The free radical scavenging activity of each sample was determined by comparing its absorbance with the DPPH solution. The ability to scavenge the DPPH radical was expressed as percentage inhibition and calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A0 - A1}{A0} \times 100$$

Where A0 is the absorbance of the blank solution and A1 is the absorbance of the sample.

2.20. ABTS Radical Scavenging Activity

ABTS radical scavenging activity of *Musanga cecropioides* crude root sap was assessed following the procedure outlined by Re *et al.* [47]. ABTS radical cation ($ABTS^{+}$) was formed by reacting 5 ml ABTS stock with 5 ml of 2.45 mM (ammonium) potassium persulfate solutions, kept away from light at room temperature for 16 hours. Prior to use, the solution was topped with DW till an absorbance of 0.70 ± 0.02 at 734 nm at $30^{o}C$ was obtained. *Musanga cecropioides* sap was prepared with DW to obtain the required concentrations. 5 lL of each plant concentration was mixed with 195 lL ABTS_ +, which was incubated at room temperature for 6 minutes and its absorbance was read at 734 nm. The percentage inhibition of ABTS by *Musanga cecropioides* was calculated with the formula below:

$$\% \text{ Inhibition} = \frac{B0 - B1}{B0} \times 100$$

Where

B0 = absorbance of control

B1 = absorbance of solution with plant extract.

2.21. Data Analysis

Data were subjected to one way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons using Graphpad prism software (version 6.01). Results are expressed as mean ± SEM and values of $P < 0.05$ were considered as statistically significant

3. Results

3.1. Qualitative Phytochemical Screening

Table 1 shows the results on the qualitative phytochemical screening of *Musanga cecropioides* crude root sap. Alkaloid, tannin, saponin, flavonoid and phenol was found present.

Table 1 Qualitative Phytochemical Screening of *Musanga cecropioides* aqueous and methanol leaf extracts, root extracts and crude root sap

Phytochemicals	**MCCRS**
Alkaloid	+
Tannin	+
Saponin	+
Flavonoid	+
Phenol	+
Terpenoid	-
Cyanogenic Glycoside	-
Anthraquinone	-
Sterol/Steroid	-
Oxalate	-

_ Negative; + positive

3.2. Quantitative Phytochemical Screening

Table 2 shows the results on the quantitative phytochemical screening of *Musanga cecropioides* root sap.

Table 2 Quantitative phytochemical screening of *Musanga cecropioides* root sap

Phytochemicals	Root sap
Alkaloid (%)	1.75 ± 1.25
Tannin (µg/ml)	5.14 ± 0.00
Saponin (%)	0.30 ± 0.10
Flavonoid (µg/ml)	11.29 ± 0.00
Phenol (µg/ml)	5.97 ± 0.00

3.3. Proximate Analysis

Table 3 shows the results on the proximate analysis of *Musanga cecropioides* root sap.

Table 3 Proximate analysis of *Musanga cecropioides* root sap

Parameters	Root sap
Moisture %	84.59
Ash %	-
Fat %	15.00
Fibre %	-
Soluble Carbohydrate %	-
Protein %	0.41

3.4. Vitamin Content

Table 4 shows the results on the vitamin content of *Musanga cecropioides* root sap. Vitamins A, B1, B2, B3, B5, B6, B7, B9, B12, C, D, E and K1 were present in *Musanga cecropioides* root sap.

Table 4 Vitamin Composition of Musanga cecropioides Root Sap

Vitamins	Recommended Intake (mg)	Root Sap (mg/100g)
A	0.600	0.426
B1 (thiamin)	4.000	0.075
B2 (riboflavin)	6.000	0.065
B3 (niacin)	18.000	0.649
B5 (pantothenic acid)	6.000	0.281
B6 (pyridoxine)	2.000	0.194
B7 (biotin)	0.030	0.001
B9 (folate)	0.400	0.001
B12 (cobalamine)	0.006	0.028
C (ascorbic acid)	75.000	17.200

D (ergocalciferol)	0.015	0.002
E (tocopherol)	10.000	0.367
K1 (phylloquinone)	0.080	0.005

3.5. Amino Acids

Table 5 shows the results on the amino acid contents of *Musanga cecropioides* root sap. *Musanga cecropioides* root sap showed the presence of essential and non-essential amino acids with the highest concentrations of aspartate, glutamate and histidine at 9.13, 13.09. 4.37 g/100 g protein respectively.

Table 5 Amino Acid Composition of *Musanga cecropioides* Root Sap

	Amino Acids	**Root Sap**
		g/100 g protein
Non-essential amino acids	Glycine	2.53
	Alanine	2.39
	Serine	2.49
	Aspartate	9.13
	Glutamate	13.09
	Tyrosine	1.74
	Cysteine	0.79
	Proline	3.50
Essential amino acids	Valine	2.92
	Methionine	0.80
	Phenylalanine	2.80
	Histidine	4.37
	Arginine	4.33
	Threonine	3.65
	Isoleucine	2.67
	Leucine	4.80
	Tryptophan	0.29
	Lysine	4.02

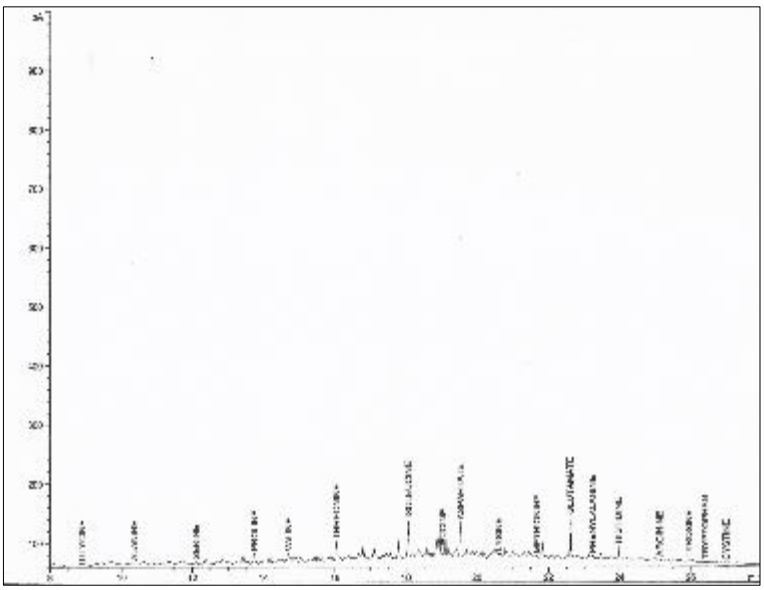

Figure 1 Chromatogram of amino acid content of *Musanga cecropioides* root sap

3.6. Elemental Analysis

Table 6 shows the results on the elemental analysis of *Musanga cecropioides* root sap.

Table 6 Concentration of Elements in *Musanga cecropioides* Root Sap

Elements	Recommended Daily Intake/ Permissible Limits (mg)	Root Sap (mg/L)
Zinc	15	0.05
Copper	2	0.09
Iron	3.9	0.30
Manganese	5	0.10
Potassium	3500	6.90
Calcium	300	5.65
Magnesium	350	2.25
Sodium	2400	4.12
Lead	< 1	0.00
Nickel	< 1	0.00

3.7. Acute Toxicity Assessment

Table 7 shows the results on the acute toxicity assessment of *Musanga cecropioides* crude root sap. No mortality was observed at all doses administered (10, 20, 40, 60, 80, 100 ml/kg), thus the LD_{50} was indeterminable.

Table 7 Result of Acute Toxicity Assessment on *Musanga cecropioides* Crude Root Sap in Mice

STAGE 1			
Dose (ml/kg)	**Number of death**	**Mortality (%)**	**Symptoms**
10	0/3	0	None
20	0/3	0	None
40	0/3	0	None
STAGE 2			
Dose (ml/kg)	**Number of death**	**Mortality (%)**	**Symptoms**
60	0/3	0	None
80	0/3	0	None
100	0/3	0	None

(0/3)= 0= Number of death, 3= Number of rats used for the test

3.8. Sub-Acute Toxicity Assessment

3.8.1. Changes in whole body weight

Table 8 shows results on the changes in whole body weight of animals treated with *Musanga cecropioides* crude root sap in the 28 days sub-acute toxicity assessment. The groups treated with *Musanga cecropioides* crude root sap at 10 and 25 ml/kg body weight showed significantly lower values at $P < 0.05$ when compared to the control group, however an increase in the weight of the animals was observed at the end of the experiment.

Table 8 Changes in whole body weight of rats treated daily with *Musanga cecropioides* crude root sap for 28 days

Treatment groups/Dose	**Mean weight of animals (g) during experiment**				
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control 1 ml/kg DW	133.2 ± 33.67	141.8 ± 28.88	163.2 ± 23.52	167.3 ± 26.40	182.9 ± 29.69
MCCRS 5 ml/kg	101.3 ± 8.76	108.8 ± 7.48	116.3 ± 5.14	118.7 ± 2.54	122.1 ± 1.72
MCCRS 10 ml/kg	101.3 ± 4.87	100.2 ± 5.10	102.3 ± 7.40*	105.0 ± 5.71*	107.9 ± 5.20*
MCCRS 25 ml/kg	106.7 ± 8.20	106.3 ± 8.75	101.0 ± 9.39*	103.1 ± 10.43*	106.5 ± 12.09*

Values are expressed as Mean ± SEM, $P < 0.05$; n=4 MCCRS: *Musanga cecropioides* crude root sap; DW: distilled water; * Significant values at $P < 0.05$, when compared with the control group

3.8.2. Organ-to-body weight ratio

Table 9 Organ-to-body weight ratios of rats treated daily with *Musanga cecropioides* crude root sap for 28 days

Treatment groups/Dose	**Ratios ($\times 10^{-3}$)**			
	LV:BW	**H:BW**	**K:BW**	**L:BW**
Control 1 ml/kg DW	35.88 ± 2.30	3.48 ± 0.13	6.12 ± 0.44	8.03 ± 0.97
MCCRS 5 ml/kg	30.28 ± 1.71	3.18 ± 0.11	5.33 ± 0.33	6.96 ± 0.62
MCCRS 10 ml/kg	30.23 ± 0.90	3.46 ± 0.52	5.91 ± 0.30	9.62 ± 1.42
MCCRS 25 ml/kg	30.53 ± 0.73	3.47 ± 0.26	5.66 ± 0.12	7.56 ± 0.98

Values are expressed as Mean ± SEM, $P < 0.05$; n=4; MCCRS: *Musanga cecropioides* crude root sap; DW: distilled water; LV: Liver; H: Heart; K: kidney; L: Lungs; No Significant difference observed at $P < 0.05$, when compared with the control group

Table 9 shows results on the organ-to-body weight ratio of animals treated with *Musanga cecropioides* crude root sap in the 28 days sub-acute toxicity assessment respectively. On the 28th day of administration of crude root sap (at 5, 10 and 25 ml/kg), no significant difference ($P < 0.05$) was observed in the liver, heart, kidney and lungs-to-body weight ratio when compared to the control group.

3.9. Haematology

Table 10 shows results on the haematology analysis of animals treated with *Musanga cecropioides* crude root sap in the 28 days sub-acute toxicity assessment respectively. Administration of *Musanga cecropioides* crude root sap (at 5, 10 and 25 ml/kg), did not significantly alter the white blood cell count, lymphocytes, monocytes, granulocytes, red blood cell count, haemoglobin concentration, haematocrit and platelet count when compared to the control group expect for the granulocytes which was significantly higher at $P < 0.05$ (2.63 ± 0.73) when compared to the control group (1.03 ± 0.60).

Table 10 Effect of *Musanga cecropioides* crude root sap on the haematological parameters of rats in 28 days sub-acute toxicity study

Parameters	WBC 10^{-3}/uL	LYM 10^{-3}/uL	MON 10^{-3}/uL	GRAN 10^{-3}/uL	RBC 10^{-6}UL	HGB g/dL	HCT %	PLT 10^{-3}/uL
Control 1 ml/kg DW	10.83 ± 3.02	9.00 ± 2.65	0.80 ± 0.30	1.03 ± 0.60	5.55 ± 0.44	12.50 ± 0.87	48.55 ± 0.59	693.8 ± 95.39
MCCRS 5 ml/kg	8.43 ± 2.74	6.80 ± 2.17	0.53 ± 0.23	1.10 ± 0.35	5.76 ± 0.20	13.80 ± 0.68	48.30 ± 4.53	568.5 ± 133.5
MCCRS 10 ml/kg	11.00 ± 2.90	7.83 ± 2.30	0.80 ± 0.16	1.70 ± 0.38	5.64 ± 0.55	13.35 ± 1.11	46.53 ± 3.82	476.8 ± 26.05
MCCRS 25 ml/kg	10.15 ± 2.56	6.58 ± 1.77	0.95 ± 0.26	2.63 ± 0.73*	5.635 ± 0.44	13.48 ± 1.18	44.33 ± 3.68	375.3 ± 37.02

Values are expressed as Mean ± SEM, $P < 0.05$; n=4; MCCRS: *Musanga cecropioides* Crude Root Sap; DW: distilled water

WBC: White blood cell count; LYM: Lymphocytes; MON: Monocytes; GRAN: Granulocytes; RBC: Red blood cell count; HGB: Haemoglobin concentration; HCT: Haematocrit; PLT: Platelet count; * Significant values at $P < 0.05$, when compared to control group

3.10. Liver Function

Table 11 shows results on the liver function tests of animals treated with *Musanga cecropioides* crude root sap in the 28 days sub-acute toxicity assessment. *Musanga cecropioides* crude root sap did not significantly alter any of the liver function parameters when compared to the control group.

Table 11 Effect of *Musanga cecropioides* crude root sap on the liver function parameters of rats in 28 days sub-acute toxicity study

Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	TB × 10^{-2} (mg/dl)	CB × 10^{-2} (mg/dl)	UB × 10^{-2} (mg/dl)	TP (g/dl)
Control 1 ml/kg DW	29.59 ± 0.75	48.21 ± 0.48	15.41 ± 0.43	0.61 ± 0.01	5.50 ± 0.65	3.25 ± 0.48	2.25 ± 0.25	15.28 ± 0.78
MCCRS 5 ml/kg	29.61 ± 0.63	48.56 ± 0.62	17.33 ± 1.07	0.68 ± 0.04	5.25 ± 0.95	2.75 ± 0.48	2.50 ± 0.50	12.67 ± 1.31
MCCRS 10 ml/kg	30.32 ± 0.41	47.42 ± 0.20	17.40 ± 0.41	0.66 ± 0.03	5.25 ± 0.48	2.75 ± 0.48	2.50 ± 0.29	13.32 ± 0.84
MCCRS 25 ml/kg	30.58 ± 0.43	47.99 ± 0.34	16.76 ± 0.80	0.65 ± 0.04	6.00 ± 0.82	3.75 ± 0.63	2.25 ± 0.25	13.25 ± 0.56

Values are expressed as Mean ± SEM, $P < 0.05$; n=4; MCCRS: *Musanga cecropioides* crude root sap; DW: distilled water; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphate; GGT: Gamma glutamyl tranferase; TB: Total bilirubin; CB: Conjugated bilirubin; UB: Unconjugated bilirubin; TP: Total protein; no significant difference when groups were compared to control group

3.11. Renal Function

Table 12 shows results on the renal function tests of animals treated with *Musanga cecropioides* crude root sap in the 28 days sub-acute toxicity assessment respectively. *Musanga cecropioides* crude root sap at all administered doses (5, 10, 25 ml/kg body weight) did not significantly alter the renal function parameters at $P < 0.05$ when compared with the control group.

Table 12 Effect of *Musanga cecropioides* crude root sap on the renal function parameters of rats in 28 days sub-acute toxicity study

Parameters	Urea (mg/dl)	Creatinine (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Control 1 ml/kg DW	25.24 ± 0.75	0.36 ± 0.01	99.49 ± 0.48	25.80 ± 0.24	65.95 ± 0.47	13.05 ± 0.67
MCCRS 5 ml/kg	25.71 ± 0.41	0.37 ± 0.01	100.5 ± 0.51	25.50 ± 0.43	68.04 ± 0.89	13.30 ± 0.61
MCCRS 10 ml/kg	25.41 ± 0.53	0.37 ± 0.01	101.0 ± 0.87	26.27 ± 0.96	69.16 ± 0.65	12.78 ± 0.73
MCCRS 25 ml/kg	25.83 ± 0.64	0.38 ± 0.01	101.5 ± 0.40	27.11 ± 0.53	69.41 ± 0.99	13.04 ± 0.68

Values are expressed as Mean ± SEM, $P < 0.05$; n=4; MCCRS: *Musanga cecropioides* crude root sap; DW: distilled water; No Significant difference in various groups when compared to control group.

3.12. Lipid Profile

Table 13 shows results on the lipid profile of animals treated with *Musanga cecropioides* crude root sap in the 28 days sub-acute toxicity assessment. *Musanga cecropioides* crude root sap did not significantly alter lipid profile parameters, only the high density lipoprotein was significantly reduced at $P < 0.05$ in group treated with 5 ml/kg body weight (20.24 ± 0.62 mg/dl) when compared to the control group (23.25 ± 0.48 mg/dl).

Table 13 Effect of *Musanga cecropioides* crude root sap on the lipid profile parameters of rats in 28 days sub-acute toxicity study

Parameters	Cholesterol (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control 1 ml/kg DW	59.15 ± 0.60	217.6 ± 0.47	23.25 ± 0.48	38.88 ± 0.99	43.52 ± 0.09
MCCRS 5 ml/kg	61.92 ± 1.63	217.4 ± 1.43	20.24 ± 0.62*	38.67 ± 1.67	43.49 ± 0.29
MCCRS 10 ml/kg	59.47 ± 1.04	214.6 ± 1.12	21.23 ± 0.46	37.77 ± 1.10	42.92 ± 0.22
MCCRS 25 ml/kg	59.83 ± 0.50	214.5 ± 1.20	20.94 ± 0.77	37.86 ± 0.58	42.90 ± 0.24

Values are expressed as Mean ± SEM, $P < 0.05$; n=4 MCCRS: *Musanga cecropioides* crude root sap; DW: distilled water; TAG: Triacylglycerol; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; * Significant values at $P < 0.05$, when compared to control group

3.12.1. Plasma Antioxidant

Table 14 shows results on the plasma antioxidant concentration of animals treated with *Musanga cecropioides* crude root sap in the 28 days sub-acute toxicity assessment. *Musanga cecropioides* crude root sap significantly reduced the Superoxide dismutase concentration at $P < 0.01$ in the group treated with 10 ml/kg body weight (4.48 ± 0.46 U/ml) when compared with the control group (7.66 ± 0.21 U/ml). Glutathione peroxidase concentration was also significantly reduced at $P < 0.05$ in the group treated with 25 ml/kg body weight (131.8 ± 1.39 U/ml) when compared with the control group (150.3 ± 0.86 U/ml).

Table 14 Effect of *Musanga cecropioides* crude root sap on the plasma antioxidant parameters of rats in 28 days sub-acute toxicity study

Parameters	MDA (mmole/ml)	SOD (U/ml)	CAT (U/ml)	GPX (U/ml)	GRX (U/ml)
Control 1 ml/kg DW	36.12 ± 0.83	7.66 ± 0.21	255.3 ± 1.04	150.3 ± 0.86	2.92 ± 0.04
MCCRS 5 ml/kg	35.78 ± 0.70	5.67 ± 0.94	232.3 ± 7.81	140.8 ± 8.23	2.78 ± 0.35
MCCRS 10 ml/kg	34.46 ± 0.36	4.48 ± 0.46**	250.9 ± 1.85	146.2 ± 5.10	2.38 ± 0.12
MCCRS 25 ml/kg	34.16 ± 1.57	6.12 ± 0.55	236.1 ± 9.68	131.8 ± 1.39*	2.91 ± 0.21

Values are expressed as Mean ± SEM, $P < 0.05$; n=4; MCCRS: *Musanga cecropioides* crude root sap; DW: distilled water; MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GPX: Glutathione peroxidase; GRX: Glutathione reductase; * Significant values at $P < 0.05$; ** significant values at $P < 0.01$, when compared to control group

3.13. Histology of Vital Organs

3.13.1. Histology of the Heart

Figure 2 shows photomicrographs of sections of the heart of animals treated with *Musanga cecropioides* crude root sap after the 28 days sub-acute toxicity assessment respectively. There were no pathological alterations observed in all treatment groups, all myocytes appeared normal.

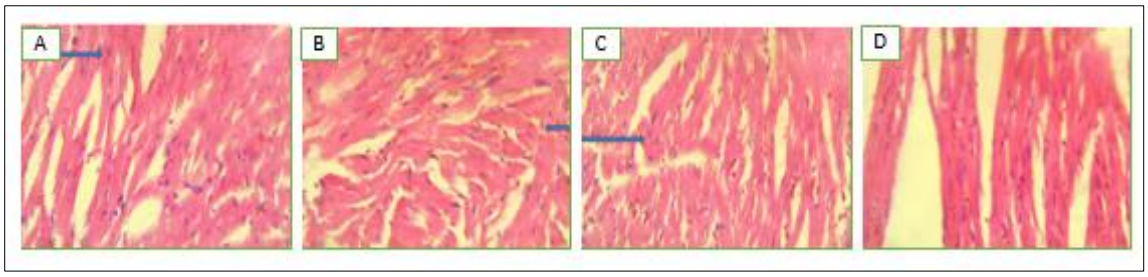

Figure 2 Effect of *Musanga cecropioides* crude root sap on the histology of the heart in of rats in 28 days sub-acute toxicity study

A: Normal control shows normal myocyte; B: MCCRS (5 ml/kg) shows normal myocyte; C: MCCRS (10 ml/kg) shows normal myocyte; D: MCCRS (25 ml/kg) shows normal myocyte: H and E ×400

3.14. Histology of the Kidney

Figure 3 shows photomicrographs of sections of the kidney of animals treated with *Musanga cecropioides* crude root sap after the 28 days sub-acute toxicity assessment respectively. There were no visible signs of pathological alterations observed, all photomicrographs of the various treatments showed normal glomerulus and normal tubules.

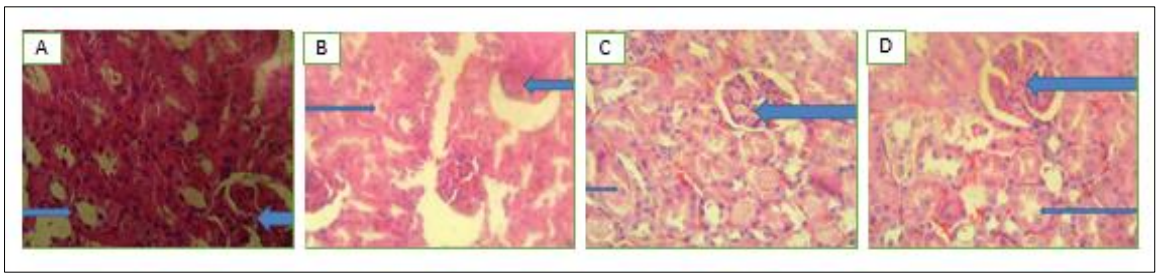

Figure 3 Effect of *Musanga cecropioides* crude root sap on the histology of the kidney in of rats in 28 days sub-acute toxicity study

A: Normal control, thick arrow shows normal glomerulus and thin arrow shows normal tubules; B: MCCRS (5 ml/kg) normal glomerulus and normal tubules seen; C: MCCRS (10 ml/kg) normal glomerulus and normal tubules seen; D: MCCRS (25 ml/kg) normal glomerulus and normal tubules seen: H and E ×400

3.15. Histology of the Liver

Figure 4 shows photomicrographs of sections of the liver of animals treated with *Musanga cecropioides* crude root sap after the 28 days sub-acute toxicity assessment respectively. All treatment groups showed signs of pathological alterations with a few exceptions.

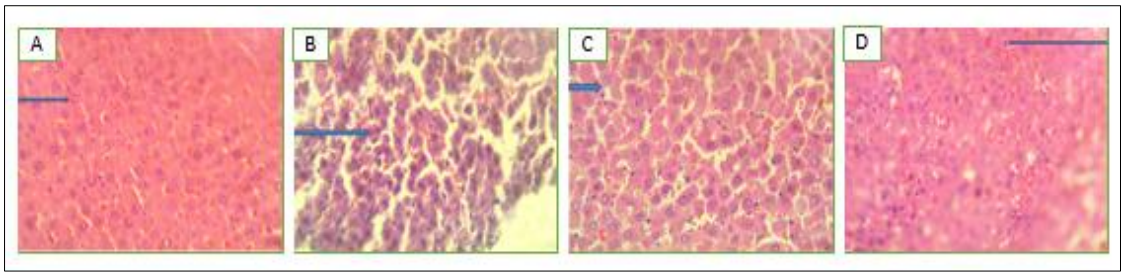

Figure 4 Effect of *Musanga cecropioides* crude root sap on the histology of the liver in of rats in 28 days sub-acute toxicity study

A: Normal control shows normal hepatocyte; B: MCCRS (5 ml/kg) arrow shows hepatocyte with pyknotic nuclei (sign of necrosis); C: MCCRS (10 ml/kg) shows normal hepatocyte; D: MCCRS (25 ml/kg) shows hepatocyte with pyknotic nuclei : H and E ×400

3.16. Histology of the Lungs

Figure 5 shows photomicrographs of sections of the lungs of animals treated with *Musanga cecropioides* crude root sap after the 28 days sub-acute toxicity assessment respectively. Thickening of the alveoli septae was observed in most treatment groups with a few exceptions.

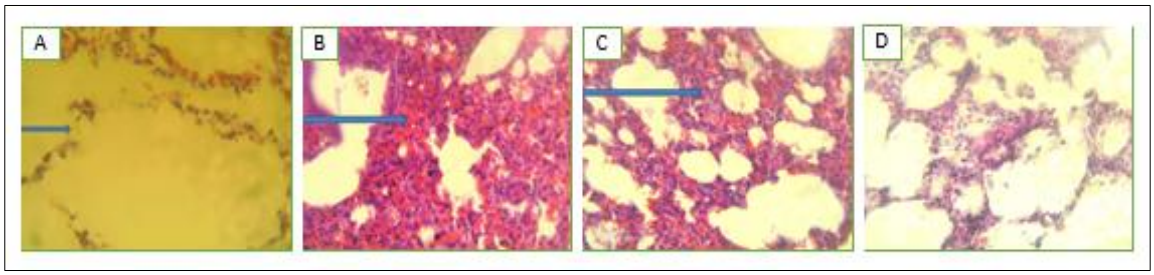

Figure 5 Effect of *Musanga cecropioides* crude root sap on the histology of the lungs in of rats in 28 days sub-acute toxicity study

A: Normal control shows normal alveoli space; B: MCCRS (5 ml/kg) shows thickening of the alveoli septae; C: MCCRS (10 ml/kg) shows thickening of the alveoli septae; D: MCCRS (25 ml/kg) appears normal: H and E ×400

3.17. Anti-Oxidant Assessment

3.17.1. DPPH Radical Scavenging Activity

Figure 6 shows DPPH radical scavenging activity of *Musanga cecropioides* crude root sap. Less than 50% percentage inhibition was also observed however a concentration or dose dependent increase in percentage inhibition was observed.

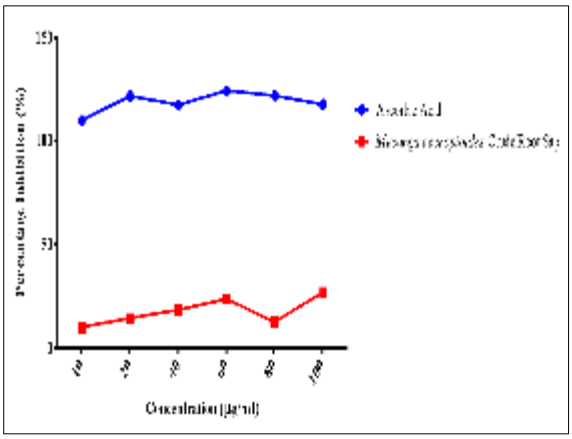

Figure 6 DPPH radical scavenging activity of *Musanga cecropioides* crude root sap

3.17.2. ABTS Radical Scavenging Activity

Figure 7 shows the ABTS radical scavenging activity of *Musanga cecropioides* crude root sap. This sample showed better antioxidant activity in the ABTS model than in the DPPH model.

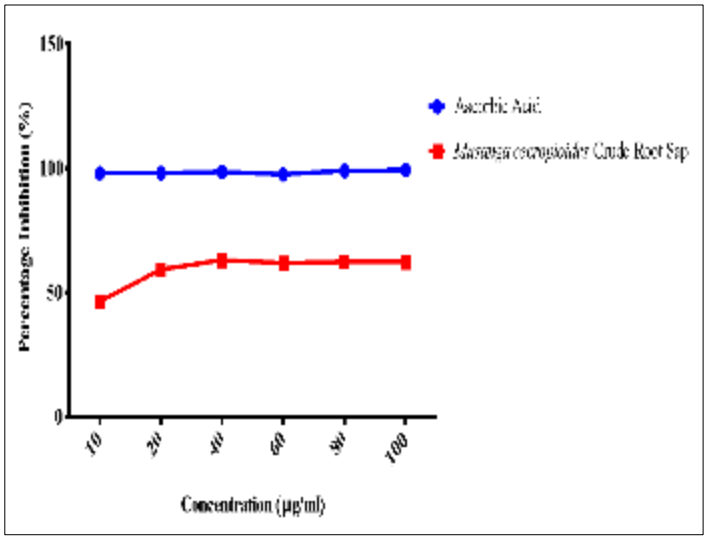

Figure 7 ABTS radical scavenging activity of *Musanga cecropioides* crude root sap

4. Discussion

The present study assessed the biosafety and in vitro antioxidant activity of *Musanga cecropioides* root sap, a traditional remedy used for various health and nutritional purposes. The sap was found to be rich in bioactive compounds, including alkaloids, tannins, saponins, flavonoids, and phenols, which are known for their medicinal properties.

The presence of phytochemicals in *Musanga cecropioides* root sap is in conformity with existing literature which identified alkaloids, catechic tannins, phlobatannins, flavonoids (or bioflavonoids), triterpenes, sterols, coumarins, tannins (gallic), free and bound anthraquinone, anthocyanosides, oxalate, phenols, cyanogenic glycosides, saponin and cardiac glycosides in the leaves, stem bark, stem sap and root sap [48; 49; 50; 51; 52; 53].

Proximate composition of *Musanga cecropioides* root sap revealed high moisture content which is in agreement with the findings of Shemishere *et al.* [54]. The high moisture content of the sample indicates that they may be easily susceptible to spoilage if not well preserved [55]. The sample also showed the presence of considerable fat and protein content, making it a prospective option for beverage and energy value. The protein content indicates that the plant sap would facilitate tissue replacement and repair [56].

Micronutrients are credited to having indispensable roles to play in metabolism and maintenance of tissue function, however, in excess quantities, they may be harmful, especially when administered to people who do not need them [57].

The presence of vitamins further indicates the possible health benefits *Musanga cecropioides* root sap may elicit. The high contents of vitamin A in the root sap may be due to the high carotene content as carotene is synthesized to vitamin A. The sample showed high concentrations of vitamins A, C, B7, and E, this is an indication that the plant materials would possess good antioxidant and immune-boosting properties [54].

Amino acids are precursors for the synthesis of proteins and phytochemicals such as alkaloids [58]. They are involved in osmolyte synthesis, cell metabolism, ammonia detoxification, antioxidant activity and alkaloid synthesis. Dietary supplementation of glycine has been reported effective in treating metabolic disorders in patients with cardiovascular diseases, several inflammatory diseases, obesity, cancers, and diabetes [59]. Aspartic acid function is essential for purine, pyrimidine, asparagine and inositol synthesis. Glutamic acid and glycine participate in the synthesis of glutathione increasing the antioxidant capacity. Valine maintains the balance of branched-chain amino acids, whereas alanine is involved in hepatic autophagy, gluconeogenesis, and transamination. Leucine regulates protein turnover and gene expression [60; 61]. Glycine, lysine, threonine and glutamate help to maintain intestinal integrity and health [62; 63]. Histidine is important in the growth and repair of worn-out tissues, maintenance of the myelin sheaths that protect nerve cells, and is needed for the production of both red and white blood cells [64]. In this study, *Musanga cecropioides* root sap revealed the presence of both non-essential and essential amino acids in various concentrations. The essential amino acids found present are valine, methionine, phenylalanine, histidine, arginine, threonine, isoleucine, leucine, tryptophan and lysine, however, the concentrations of valine, arginine, and leucine were relatively high. While the non-essential amino acids found present are glycine, alanine, serine, aspartate, glutamate, tyrosine, cysteine and proline, while glycine, alanine, aspartate and glutamate concentrations were relatively high. These results are in agreement with previous studies [54]. The presence of both essential and non-essential amino acids in *Musanga cecropioides* root sap also serves as a pointer to the possible roles of the plant material in protein synthesis and general metabolism [61;65].

Potassium, calcium, magnesium, and sodium were also present in relatively high concentrations, indicating the plant samples possess health benefits related to these mineral elements as indicated in literature [66;67;68;69;54]. The low concentrations of lead and nickel also indicate that the sample may be safe for use.

The acute toxicity assessment in mice indicated that *Musanga cecropioides* root sap is safe at doses up to 100 ml/kg, with no observed mortality or adverse symptoms. The sub-acute toxicity study over 28 days showed no significant alterations in organ-to-body weight ratios at doses of 5, 10, and 25 ml/kg. However, a decrease in body weight was observed at higher doses, which warrants further investigation. Generally, reductions in body weight and internal organ weights are considered to be simple and sensitive indices of toxicity after prolonged exposure to toxic substances [70]. Subsequently, an increase in the relative weight of the vital organs would suggest that the extract might have caused hypertrophy of the vital organs [71]. The changes in both the body weight and organ weights of the experimental animals administered all doses in the sub-acute toxicity study are not statistically or toxicologically significant. The fact that the various doses of *Musanga cecropioides* root sap administered in this study did not significantly increase the overall weights of the experimental animals agrees with the submissions of Shemishere *et al.* [54], that *Musanga cecropioides* may be useful in a weight loss diet.

The haematopoietic system is considered a sensitive target of toxic compounds and is an important index of physiological and pathological status in man and animals [48]. Haematology parameters tested were not significantly altered in all groups administered the various doses when compared with the control group after the 28 days sub-acute toxicity study. The results suggest that *Musanga cecropioides* root sap has no direct effect on the haematopoietic system of the experimental animals and is in agreement with the findings of Adeneye *et al.* [48].

Liver and renal function tests are vital in evaluating the toxicity of drugs and plant extracts [29]. The biochemical analysis revealed no significant changes in liver and kidney function markers, suggesting that *Musanga cecropioides* root sap does not cause hepatic or renal toxicity, suggesting that the crude root sap maybe incapable of interference with mechanisms associated with the development of cardiovascular diseases or they may not in any way promote the development of related diseases [72].

Malondialdehyde concentration was not increased in all treatment groups when compared with the control group. This is an indication that lipid peroxidation was not initiated by *Musanga cecropioides* root sap at the high doses administered, which is in agreement with the reports of Oduola *et al.* [73], who reported that ingestion of high doses of *Phyllanthus amarus* leaf extract did not induce lipid peroxidation in a sub-acute toxicity study.

However, the activities of the enzymatic antioxidants superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase were not significantly increased across experimental groups, rather some of the values were significantly lower ($P < 0.05$) in the treatment groups when compared with the control group. While it may be agreed that *Musanga cecropioides* does not initiate lipid peroxidation, the extracts may not also have a direct effect on enzymatic antioxidant activities. This may also be because exogenous toxicants or radicals were not introduced to the experimental animals [74].

In the histological examinations of the vital organs, the treatments showed no visible alterations in the heart and kidney however the photomicrographs of the liver and lungs showed signs of necrosis and thickening of the alveoli septae respectively. The pathological alterations observed in the liver at the various administered treatments were not significantly supported by the results obtained in the liver function tests, this may be a marker that these changes are not pathologically significant or they may be reversible [75].

DPPH and ABTS radical scavenging assays reveal the antioxidant activity of test samples [76]. In this study, *Musanga cecropioides* root sap showed considerable radical scavenging activities. In the ABTS assay, the sample showed better antioxidant potentials than the DPPH assay. This may be due to the stereoselectivity of radicals and the solubility of extracts in diverse solvent systems [77]. However, the ability of the extracts to scavenge both radicals shows they are useful agents in reversing diverse pathological changes caused by different types of radicals. The antioxidant activity of *Musanga cecropioides* root sap may be due to the presence of alkaloids, tannin, saponin, flavonoid, phenols and vitamins A, C and E which are known antioxidant compounds and this is in agreement with other studies [78;46]. The antioxidant activity of phytochemicals is due to their ability to act as reducing agents, hydrogen donors, metal chelators in redox reactions [79].

5. Conclusion

The study provides comprehensive evidence supporting the biosafety and medicinal potential of *Musanga cecropioides* root sap. The sap's rich phytochemical profile, substantial antioxidant activities, and lack of acute and sub-acute toxicity highlight its potential as a natural therapeutic agent. Further studies are recommended to elucidate the mechanisms underlying its medicinal properties and to evaluate its efficacy in clinical settings. The findings support the traditional use of *Musanga cecropioides* root sap and pave the way for its potential development into a standardized natural remedy.

Disclosure of conflict of interest

All author declares that there is no conflict of interests.

References

[1] Idu M, Obaruyi GO, Erhabor JO. Ethnobotanical uses of plants among the Binis in the treatment of ophthalmic and ENT (Ear, Nose and Throat) ailments. Ethnobotanical Leaflets. 2009, 13:480–496.

[2] Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestree Database: A Tree Reference and Selection Guide Version 4.0, 2009. [Online] [Available at: http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp] [Accessed: 10/01/2017]

[3] Todou G, Meikeu-Kamdem MG. *Musanga cecropioides* R.Br. Ex Tedlie. In: Lemmens, R.H.M.J., Louppe, D. and Oteng-Amoako, A. A. (Editors). Prota. 2011, 7(2): Timbers/Bois D'œuvre 2. [CD-Rom]. PROTA, Wageningen, Netherlands.

[4] Isaac UE. Physicochemical and microbial studies of water obtained from *Musanga cecropioides* (African cork wood or umbrella or "Uno" tree). World Applied Sciences Journal. 2012, 19(2): 287 – 294.

[5] Uwah AF, Otitoju O, Ndem JI, Peter AI. Chemical composition and antimicrobial activities of adventitious root sap of *Musanga cecropioides*. Der Pharmacia Lettre. 2013, 5: 13 – 16.

[6] Enoyoze GE, Idu M. A MINI-REVIEW ON *Musanga cecropioides* R. Br. ex Tedlie. EPRA International Journal of Research and Development (IJRD). 2023 Nov, 8(11): 199-204.

[7] Banu KS, Cathrine L. General techniques involved in phytochemical analysis. International Journal of Advanced Research in Chemical Science. 2015, 2(4): 25 – 32.

[8] Kennedy DO. B Vitamins and the Brain: Mechanisms, Dose and Efficacy—A Review. Nutrients. 2016, 8(2): 68. doi: 10.3390/nu8020068

[9] Azad S. Amino acids: Its types and uses. International Journal of Clinical and Diagnostic Pathology. 2018, 1(1): 13 – 16.

[10] Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. Experientia Supplementum. 2012, 101: 133 – 164.

[11] Sindhi V, Gupta V, Sharma K, Bhatnagar S, Kumari R, Dhaka N. Potential applications of antioxidants – A review. Journal of Pharmacy Research. 2013, 7: 828 – 835.

[12] Okwu DE, Okwu ME. Chemical composition of *Spondias mombin* (Linn.) plant parts. Journal of Sustainable Agriculture and Environment. 2004, 6(2): 140 – 147.

[13] Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants II. Lloydia. 1978, 41(3): 234 – 246.

[14] Ayeni KE, Yahaya SA. Phytochemical screening of three medicinal plants Neem leaf (*Azadirachta indica*), Hibiscus leaf (*Hibiscus rosa-sinensis*) and spear grass leaf (*Imperata cylindrica*). Continental Journal of Pharmaceutical Sciences. 2010, 4: 47 – 50.

[15] Ugochukwu SC, Arukwe UI, Onuoha I. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. Asian Journal of Plant Science and Research. 2013, 3(3): 10 – 13.

[16] Trease GE, Evans WC. Textbook of Pharmacognosy. 15th Edition. WB Sanders Publishers, London, UK. 2002, 393p.

[17] Ifemeje JC, Egbuna C, Eziokwudiaso JO, Ezebuo FC. Determination of the Anti-nutrient Composition of *Ocimum gratissimum*, *Corchorus olitorius, Murraya koenigii* Spreng and *Cucurbita maxima*. International Journal of Innovation and Scientific Research. 2014, 3(2): 127 – 133.

[18] Polshettiwar SA, Ganjiwale RO. Spectrophotometric estimation of total tannins in some ayurvedic eye drops. Indian Journal of Pharmaceutical Sciences. 2007, 69(4): 574 – 576.

[19] Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 2002, 10: 178 – 182.

[20] Folin O, Ciocalteau V. On tyrosine and tryptophane determination in proteins. Journal of Biological Chemistry. 1927, 27: 627 – 650.

[21] Harbone JB. Phytochemical methods. London: Chapman and Hall Ltd. 1973, 279p.

[22] Sofowora A. Medicinal Plants and Tradition Medicine in African. 2nd Edition. Spectrum Books Ltd., Ibadan, Nigeria. 1993, 289p.

[23] AOAC. Official Methods of Analysis. 20th Edition, Association of Official Analytical Chemists Inc. Volume 1, George W. and Latimer, Jnr. (Eds) Washington, D. C., USA. 2016, pp. 89 – 101.

[24] Idu M, Ovuakporie-Uvo O, Omoregie ES, Omosigho M. Physicochemical properties, antioxidant activity and phyto-nutritional composition of cold and hot pressed coconut oils. GSC Biological and Pharmaceutical Sciences. 2018, 5(1): 56 – 66.

[25] Lorke D. A New Approach to Tropical Acute Toxicity Testing. Archives of Toxicology. 1983, 53: 275 – 287.

[26] Usman N, Ozolua RI, Uwaya DO, Osagiede BE, Ugiagbe EE. Acute and Sub-Acute Toxicological Evaluation of Aqueous Leaf Extract of *Nauclea latifolia* (Rubiaceae) in Albino Rats. European Journal of Medicinal Plants. 2016, 12(2): 1 – 10.

[27] NIH. Public Health Service Policy on Humane Care and Use of Laboratory Animals. National Institute of Health. Office of the laboratory animal welfare, USA. 2002, pp 1 – 19.

[28] Oshomoh EO, Idu M, Uwaya OD. Acute and sub-acute toxicity of the aqueous extract of the stem of *Masularia acuminata* (G. Don) Bullock ex Hoyle on albino rats and mice. Pharmacologia. 2013, 4: 606 – 616.

[29] Olaniyan JM, Muhammad HL, Makun HA, Busari MB, Abdullah AS. Acute and sub-acute toxicity studies of aqueous and methanol extracts of *Nelsonia campestris* in rats. Journal of Acute Disease. 2016, 5(1): 62 – 70.

[30] Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic – pyruvate transaminases. American Journal of Clinical Pathology. 1957, 28(1): 56 – 63.

[31] Plummer DT. An Introduction to Practical Biochemistry. 3rd Edition. McGraw – Hill Book Company, Maidenhead, Berkshire. 1978, 347p.

[32] Persijn JP, van der Slik W. A new method for the determination of gamma-glutamyl transferase in serum. Journal of Clinical Chemistry and Biochemistry. 1976, 14(9): 421 – 427.

[33] Jendrassik L, Grof P. Colorimetric method of determination of bilirubin. Biochemische Zeitschrift. 1938, 297: 81 – 82.

[34] Doumas BT, Bayse DD, Carter RJ, Peters T, Schaffer R. A candidate reference method for determination of total protein in serum: I Development and validation. Clinical Chemistry. 1981, 27(10): 1642 – 1650.

[35] Larsen K. Creatinine assay by reaction kinetic principle. Clinica Chimica Acta. 1972, 41: 209 – 217.

[36] Taylor AJ, Vadgama P. Analytical reviews in clinical Biochemistry: The estimation of urea. Annals of Clinical Biochemistry. 1992, 29: 245 – 264.

[37] Howe A, Fung LH, Lalor G, Rattray R, Vutchkov M. Elemental composition of Jamaican foods 1: A survey of five food crop categories. Environmental Geochemistry and Health. 2005, 27: 19 – 30.

[38] Trinder P. A simple turbidimetric method for the determination of serum cholesterol. Annals of Clinical Biochemistry: International Journal of Laboratory Medicine. 1969, 6: 165 – 166.

[39] Tietz NW. Clinical Guide to Laboratory Tests. 2nd Edition. WB Saunders Company, Philadelphia, PA, USA. 1990, pp 554 – 556.

[40] Friedewald WT, Levi RI, Fredrickson DS. Estimation of the concentration of low density lipoproteins cholesterol in plasma without use of the ultracentrifuge. Clinical Chemistry. 1972, 18(6): 499 – 502.

[41] Varshney R, Kale RK. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *International Journal* of *Radiation Biology.* 1990, 58: 733 – 743.

[42] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 1972, 247(10): 3170 – 3175.

[43] Cohen D, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. Annals of Clinical Biochemistry. 1970, 34: 30 – 38.

[44] Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods in Enzymology. 1984, 105: 114 – 120.

[45] Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Analytical Biochemistry. 1969, 27: 502 – 522.

[46] Adebiyi OE, Olayemi FO, Ning-Hua T, Guang-Zhi Z. In vitro antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*. Beni-Suef University Journal of Basic and Applied Sciences. 2017, 6: 10 – 14.

[47] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999, 26(10): 1231 – 1237.

[48] Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. Journal of Ethnopharmacology. 2006, 105(3): 374 – 379.

[49] Kadiri AB, Ajayi GO. Phyto-anatomical characteristics of the West African {umbrella tree} *Musanga cercropioides* M. Smithii R. Br. (Moraceae). Indian Journal of Science and Technology. 2009, 2(7): 1 – 5.

[50] Mabeku LBK, Roger KJ, Louis OEJ. Screening of some plants used in the Cameroonian folk medicine for the treatment of infectious diseases. International Journal of Biology. 2011, 3(4): 13 – 21.

[51] Tchouya RF, Nantia EA. Phytochemical analysis, antioxidant evaluation and total phenolic content of the leaves and stem bark of *Musanga cecropioides* R.Br. Ex Tedlie (Cecropiaceae), growing in Gabon.. Journal of Pharmacognosy and Phytochemistry. 2015, 3(5): 192 – 195.

[52] Ajagbonna OP, Adeniran LA, Idowu OO. Diuretic effects of aqueous crude extract of *Musanga cecropioides* in normotensive Sprague dawley rat. Journal of Biology, Agriculture and Healthcare. 2015, 5(13): 47 – 53.

[53] Nyunaï N, Yaya AJG, Tabi TGN, Tchamgoue AD, Ngondé MC, Minka CSM. Anti-hyperglycemic and antioxidant potential of water-ethanol extract of *Musanga cecropioides* stem bark. International Journal of Pharmaceutical Sciences and Drug Research. 2016, 8(1): 43 – 49.

[54] Shemishere UB, Taiwo JE, Erhunse N, Omoregie ES. Comparative Study on the Proximate Analysis and Nutritional Composition of *Musanga cecropioides* and *Maesobotyra barteri* leaves. Journal of Applied Sciences and Environmental Management. 2018, 22(2): 287 – 291.

[55] Omoregie ES, Osagie AU. Effect of *Jatropha tanjorensis* leaves supplement on the activities of some antioxidant enzymes, vitamins and lipid peroxidation in rats. Journal of Food Biochemistry. 2011, 35(2): 409 – 424.

[56] Igile GO, Iwara IA, Mgbeje BI, Uboh FE, Ebong PE. Phytochemical, Proximate and Nutrient composition of *Vernonia calvaona* Hook (Asterecea): A Green-leafy vegetable in Nigeria. Journal of Food Research. 2013, 2(6): 111 – 122.

[57] Shenkin A. Micronutrients in health and disease. Postgraduate Medical Journal. 2006, 82: 559 – 567.

[58] Croteau R, Kutchan TM, Lewis NG. Natural products (secondary metabolites). Biochemistry and Molecular Biology of Plants. 2000, 1250 – 1318.

[59] Razak MA, Begum PS, Viswanath B, Rajagopal S. Multifarious Beneficial Effect of Nonessential Amino Acid, Glycine: A Review. Oxidative Medicine and Cellular Longevity, 2017: 1 – 7. Doi: https://doi.org/10.1155/2017/1716701

[60] Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids. 2009, 37: 1 – 17.

[61] Akram M, Asif M, Uzair M, Naveed A, Madni MA, Ali SS, Hasan Z, Khan A. Amino acids: A review article. Journal of Medicinal Plants Research. 2011, 5: 3997 – 4000.

[62] Rhoads MJ, Wu G. Glutamine, arginine, and leucine signaling in the intestine. Amino Acids. 2009, 37: 111 – 122. Wang W, Qiao S, Li D. Amino acids and gut function. Amino Acids. 2009, 37: 105 – 110.

[63] Vázquez-Ortiz FA, Caire G, Higuera-Ciapara I, Hernández G. High performance liquid chromatographic determination of free amino acids in shrimp. Journal of Liquid Chromatography. 1995, 18: 2059 – 2068.

[64] Agbadi RK, Kaukhova IE, Terninko II, Sirichenko TI. Quantitative and Qualitative Analyses of Amino Acids in *Morinda citrifolia* (Rubiaceae). International Journal of Pharmacognosy and Phytochemical Research. 2017, 9(7): 980 – 984.

[65] Okaka JC, Okaka ANO. Food Composition, Spoilage and Shelf-Life Extension. Ocjarco Academic Publishers, Enugu, Nigeria. 2001, pp 54 – 56.

[66] Weaver CM. Potassium and Health 1–3. American Society for Nutrition. Advances in Nutrition. 2013, 4: 368S–377S,

[67] [68] Gröber U, Schmidt J, Kisters K. Magnesium in Prevention and Therapy. Nutrients. 2015, 7: 8199 – 8226.

[68] Faraco G, Brea D, Garcia-Bonilla L, Wang G, Racchumi G, Chang H, Buendia I, Santisteban MM, Segarra SG, Koizumi K, Sugiyama Y. Dietary salt promotes neurovascular and cognitive dysfunction through a gut-initiated TH17 response. Nature Neuroscience. 2018, 21(2): 240 – 249.

[69] Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of Dmethylphenidate and D, L- methylphenidate in Sprague Dawley rats. Toxicology. 2002, 179: 183 – 196.

[70] Yakubu MI, Abbas MY, Chindo BA, Anuka JA. Toxicity Screening of Ethanol Extract of *Cussonia barteri* Leaf seeman (Araliaceae) in Wistar Rats. Nigerian Journal of Pharmaceutical and Biomedical Research. 2018, 3(2): 147 – 153.

[71] Tom ENL, Nyunaï N, Djaouro KG, Medou FM, Nankia, FD, Dimo T. Acute and Subacute Toxicity Evaluation of the Stem Bark Aqueous Extract of *Harungana madagascariensis* in Rodents. Journal of Advanced Pharmaceutical Science and Technology. 2018, 1(4): 1 – 12.

[72] Oduola T, Kakako SL, Tajudeen M, Aiyelabegan F, Olayinka OS, Isah LO. Effect of intake of *Phyllanthus amarus* aqueous leaf extract on lipid peroxidation and some antioxidant factors in wistar rats. Journal of Pharmacognosy and Phytochemistry. 2018, 7(4): 2660 – 2666.

[73] Akande G, Aliu YO, Ambali SF, Ayo JO. Co-treatment of chlorpyrifos and lead induce serum lipid disorders in rats: alleviation by taurine. Toxicology and Industrial Health. 2014, 32: 1328 – 1334.

[74] Boone L, Meyer D, Cusick P, Ennulat D, Bolliger AP, Everds N, Meador V, Elliott G, Honor D, Bounous D, Jordan H. Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies. Veterinary Clinical Pathology. 2005, 34: 182 – 188.

[75] Wu LC, Hsu HW, Chen YC, Chiu CC, Lin YI, Ho JA. Antioxidant and anti-proliferative activities of red pitaya. Food Chemistry. 2006, 95: 319 – 327.

[76] Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M. Free radical scavenging properties of wheat extracts. Journal of Agriculture and Food Chemistry. 2002, 50: 1619 – 1624.

[77] Esua OJ, Makinde OO, Arueya GL, Chin NL. Antioxidant potential, phytochemical and nutrient compositions of Nigerian hog plum (*Spondias mombin*) seed kernel as a new food source. International Food Research Journal. 2016, 23: 179–185.

[78] Pietta PG. Flavonoids as antioxidants. Journal of Natural Products. 2000, 63: 1035 – 1042.