https://dx.doi.org/10.4314/ijs.v26i3.4

EVALUATION OF *Cryptolepis sanguinolenta* STEM ETHANOL EXTRACT IN ANIMAL MODELS

Nwikwe, D. C.[1,2*], Raji, Z. I.[1], Balogun, E. A.[1]

[1]University of Ilorin, Faculty of Life Sciences, Department of Biochemistry, Ilorin, Nigeria.

[2]Kings University, Faculty of Science, Department of Chemical Sciences (Biochemistry Unit), Odeomu, Osun State, Nigeria.

[*]Corresponding Author's Email: davidnwikwe@gmail.com (dc.nwikwe@kingsuniversity.edu.ng)

(Received: 19th April, 2024; Accepted: 17th September, 2024)

ABSTRACT

Although herbal medicines are less potent compared to synthetic drugs in some cases, but are still considered less toxic less side effects. *Cryptolepis sanguinolenta* stem ethanol extract (CSSE) is a reportedly potent antimalarial plant with dearth of data on the safety and efficacy on the brain and heart of animals. This study evaluated the safety and efficacy of CSSE in animal models. Thirty albino rats were randomly distributed into five groups (n=6). Group A=distilled water (control), Groups B-E=250, 500, 1000, and 2000 mg/kg body weight extract, respectively, for 21 days. Phytochemicals and biochemical analyses were performed using standard method. Total protein (TP), direct bilirubin (DB), total bilirubin (TB), alanine transaminase (ALT), and aspartate transaminase (AST) were evaluated in liver. Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase, and AST were evaluated in heart. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and nitric oxide (NO) were evaluated in brain. Lipid profiles, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) were evaluated in serum as well. The results revealed that CSSE contained alkaloids, glycosides, steroids, terpenoids and proteins at 35.11, 9.80, 52.35, 22.61 and 30.32 mg/100g, respectively. CSSE significantly increased ($p<0.05$) liver TB and AST, heart AST, and MDA and GST, while total cholesterol and AChE was reduced. However, no significant difference ($p>0.05$) was observed in triglycerides, high density lipoprotein cholesterol, TP, DB, liver ALT, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase, BChE, NO, SOD and CAT in the subjects. CSSE also kept histo-architecture of the subjects intact. Hence, CSSE induced mild alterations in biochemical parameters and tissues of the subjects without an observable damage, hence relatively safe for consumption.

Keywords: Antimalarial; *Cryptolepis sanguinolenta,* Ethanol, Herbal medicine, Safety, Synthetic drugs.

INTRODUCTION

Medicinal plants have impacted about 80% of individuals in developing nations who depend on them for their healthcare necessities (Oguntibeju *et al.,* 2018). The growing public awareness in phytomedicine, alongside speedy expansion of pharmaceutical industries in modern days, has stimulated the increased global appreciation of medicinal plants (Savithramma *et al.,* 2011; Ahad *et al*., 2021; Opoku-Agyemang *et al*., 2022). This claim is supported by the statement that about 80% of traditional medicine uses plant extracts, suggesting that these plants are still significant in contemporary medicine, thereby playing indispensable roles in the introduction of new therapeutic agents (Guan and He, 2015). Since several medicinally-useful composites presently used as contemporary drugs are products of medicinal plants, the use of extracts derived from plants (wholly or semi-purified) for the treatment/management of diseases as applied in the traditional medicine practices can be viewed as a realistic and practical solution to overcome the issue. A crucial medicinal field where usefulness of herbal medicine as an alternative medicine has attracted notable consideration is associated with the treatment of liver abnormalities, heart and brain (Liu *et al.,* 2011; Nofal *et al*., 2023), as well as other vital tissues.

However, numerous epidemiological studies have shown that several phytochemicals deposited in medicinal plants possess potent anti-malarial, anti-inflammatory, anti-atherosclerotic, anti-mutagenic, anti-carcinogenic, anti-bacterial, anti-tumor, and anti-viral activity (Owen *et al.,* 2000; Sala *et al.,* 2002; Balogun *et al.,* 2010). Furthermore, they are linked with lower risks of cancer, diabetes, cardiovascular diseases (CVDs), and even decreased death rates of numerous human ailments (Anderson *et al.,* 2001; Sun *et al.,* 2002).

Cryptolepis sanguinolenta, commonly called 'yellow-die root', is a known thin-stemmed twining and climbing shrub with orange-coloured sap which belongs to the family *Apocynaceae* (subfamily: *Periplocoideae*), and mainly seen in tropical rainforests, copses, and mountainous ecosystems of West Africa (Paulo and Houghton, 2003; Ajayi *et al.,* 2012; Osafo *et al.,* 2017; Opoku-Agyemang *et al.*, 2022). It is a medicinal plant used by numerous traditional herbalists in the treatment of fever as well as respiratory tract infections (Komlaga *et al.,* 2015; Osafo *et al.,* 2017). This plant has been documented for its potent anti-malarial (Bugyei *et al.,* 2010; Nofal *et al.*, 2023), and anti-diabetic activity (Osafo *et al.,* 2017), including its ability to slowdown glucose absorption and transport from the guts and increase insulin production by the pancreas from probably the hypertrophied β-cells in rat models (Ajayi *et al.,* 2012). Sub-acute and acute toxicity evaluation of *C. sanguinolenta* aqueous root extract by Ansah *et al.* (2009) suggested that the plant at doses <500 mg/kg body weight extract is generally safe on the blood, kidney and liver, having showed possible central nervous system (CNS) toxicity and evident marginal enlargement of hepatocytes and nephrocytes of rats at 2000 mg/kg body weight. However, Ansah *et al.* (2009) based its observed CNS effects on behavioural changes of the treated rats without any neurobiochemical parameters evaluated in tandem with the study by Ansah *et al.* (2008) that also reported significant anxiogenic effects in rats reportedly similar to reference caffeine.

Therefore, the widely reported anti-malarial potency of *C. sanguinolenta* has characterized it as a plant of interest in the quest for cheap cost-effective anti-malarial drug, with no any side effect on systemic organs, which can be derived from natural sources. The problem of appropriate choice of dosage, however, has widely characterized the exploration of various plants in the quest for cure of various ailments such as fever, malaria, diabetes, etc. This has not excluded *C. sanguinolenta* which may have yet to be confirmed toxicological implications on the crucial organs of the body. However, there is dearth of data on the possible effect of *C. sanguinolenta* extracts in the heart and brain of both rats and humans, with no known published study so far evaluating the potential acute toxicity of *C. sanguinolenta* ethanol stem extract on the brain and heart in either rats or humans. It is therefore pertinent to establish the safety of *C. sanguinolenta* ethanol stem extract through toxicological assessments in selected liver, heart and brain indices of albino rats.

MATERIALS AND METHODS

Chemicals and reagents

Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Ca^{2+}-Mg^{2+}-ATPase, Na^{+}-K^{+}-ATPase, Acetylcholinesterase (AChE), Butyrylchlinesterase (BChE), Malondialdehyde (MDA), Catalase (CAT), Glutathione S-Transferase (GST), Superoxide Dismutase (SOD), Total Protein (TP), Nitric Oxide (NO), Albumin, Bilirubin, Urea, Creatinine, and lipid profiles kits were obtained from Randox Laboratories Limited, Co-Atrim, UK. Ethanol was a product of BDH England Chemicals Ltd., Poole, England. All other chemicals used in this study were of analytical grades.

Plant sample and extraction

Fresh stems of *Cryptolepis sanguinolenta* plant were bought at "Ipata" commercial market (longitude 4° 25' E and 4° 65' E, latitude 8° 20' N and 8° 50' N), Ilorin, Nigeria, in 2021. It was identified and authenticated in Department of Plant Biology Herbarium, University of Ilorin, with deposited voucher specimen number UILH/001/1427/2021. The stems were collected, rinsed in clean water, dried at room temperature, and then pulverized into powder employing an electric blender (Mazeda Mill, MT 4100, Japan). The powder (1750 g) was extracted in 7 litres ethanol by maceration for 48 hrs with continuous stirring. A filter paper (Whatman No. 1; Springfield, Maidstone, Kent, UK) was used to filter the mixture, and evaporated to dryness in rotary evaporator (RE-300B model, Henan Touch Science product, China), lyophilized and preserved in an airtight container and then refrigerated at 4 ^{0}C for use.

Grouping of experimental animals

Thirty adult albino rats with average weight 150 g were obtained from the Department of Biochemistry Animal Breeding Unit in University

of Ilorin. They were preserved in normal laboratory state of humidity, temperature (25 ± 2 ^{0}C) and light (12 hr: 12 hr night) for 7 days, and allowed free access to food and water *ad libitum*. They were randomized into five groups (n=6 rats/group). Group A-control (NC), while groups B–E orally received 250, 500, 1000 and 2000 mg/kg body weight *C. sanguinolenta* ethanol extract, respectively, using distilled water as vehicle for 21 days.

Blood collection and serum preparation

Twenty-four hours after the last administration, the animals were sacrificed under diethyl ether anaesthesia. Jugular venous blood was collected into plain and EDTA bottles for biochemical and haematological analyses, respectively. The blood in plain bottles were subjected to centrifugation at 5,000 rpm for 10 minutes, the serum were carefully separated into well-labeled clean tubes. They were preserved frozen until desired for oxidative status markers. The liver, heart and brain of each rat were carefully removed, cleansed of superficial connective tissues/blood, weighed and homogenized in cold 0.25 M sucrose solution (1:5 w/v). They were thawed thereafter and centrifuged at 10,000 rpm for 10 minutes in a refrigerated centrifuge and the supernatants pipetted into new sample tubes. The samples were stored at -20 ^{0}C for biochemical analyses.

Phytochemical screening

The secondary metabolites present in *C. sanguinolenta* stem ethanol extract were quantitatively determined using the methods of Odebiyi and Sofowora (1978), Trease and Evans (1989), Obadoni and Ochuko (2001), and Jagadish *et al.* (2009).

Biochemical analysis

Standard methods were adopted for the determination of various biochemical parameters; ALT and AST activities (Reitman and Frankel, 1957), ALP activity (Wright *et al.,* 1972), TP (Gornall *et al.,* 1949), serum albumin (Doumas *et al.,* 1971), serum bilirubin (Evelyn and Malloy, 1938), serum urea (Veniamin and Vakirtzi-Lemonias, 1970), serum creatinine (Bartels *et al.,* 1972), serum MDA level (Buege and Aust, 1978), serum SOD activity (Misra and Fridovich, 1975); serum CAT activity (Beers and Sizer, 1952), serum GST activity (Habig *et al.,* 1974), AChE and BChE activities (Ellman *et al.*, 1961), Ca^{2+}-Mg^{2+}-ATPase activity (Ronner *et al.,* 1977), Na^{+}-K^{+}-ATPase activity (Bewaji *et al.,* 1985), NO level (Haussmann and Werringloer, 1985), serum total cholesterol (TC) level (Fredrickson *et al.*, 1967), serum triglyceride (TRIG) level (Tietz, 1995), serum high density lipoprotein cholesterol (HDL-c) level (Lopes-Virella *et al.*, 1977), and serum low density lipoprotein cholesterol (LDL-c) level (Friedwald *et al.*, 1972).

Histology of the liver, heart, and brain

The brain, liver, and heart of the rats were excised, cleansed with blotting paper, weighed, and fixed in 10% (v/v) formalin. The excised organs were dehydrated using ascending grades of ethanol (70, 90 and 95% v/v); this was further dressed in xylene and fixed in paraffin wax (56 ^{o}C melting point). The procedure described by Drury *et al.* (1967) was adopted for the tissue sections, afterwards stained with haematoxylin/eosin. The slides of the fixed samples were read using a light microscope (OLYMPUS, Model CX21FSI, Philippines).

Statistical analysis

The generated data were evaluated for statistical significance by one-way analysis of variance (ANOVA) and Duncan's *Post-hoc* multiple comparisons using IBM SPSS Statistical package for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Differences at $p<0.05$ were considered significant. The plotted graphs were constructed using GraphPad Prism 6 Software for Windows (GraphPad Software, California, USA).

RESULTS

Secondary metabolites of *C. sanguinolenta* stem extract

Table 1 shows the phytochemical screening of *C. sanguinolenta* stem ethanol extract (CSSE). CSSE contained some amounts of steroids (52.35 ± 0.44 mg/100g), alkaloids (35.11 ± 0.00 mg/100g), terpenoids (22.61 ± 0.07), glycosides (9.80 ± 0.41 mg/100g), and proteins (30.32 ± 2.73 mg/100g).

Table 1. Phytochemical screening of the secondary metabolites present in *C. sanguinolenta* stem ethanol extract (CSSE) in rats.

Phytochemical	Mean composition (mg/100g)
Alkaloids	35.11 ± 0.00
Glycosides	9.80 ± 0.41
Proteins	30.32 ± 2.73
Steroids	52.35 ± 0.44
Terpenoids	22.61 ± 0.07

Values represent mean of 3 replicates ± standard errors of mean (SEM).

The result of the effects of CSSE on MDA and antioxidant enzyme levels of rats treated with CSSE is presented in Table 2. The MDA levels, being a measure for lipid peroxidation, increased significantly ($p<0.05$) compared to the control. The SOD and CAT activities in the treated subjects compared favorably ($p>0.05$) with the control, while the GST activity increased significantly.

Table 2. Effects of *C. sanguinolenta* stem ethanol extract on serum lipid peroxidation and antioxidants in rats.

Parameters	SOD (mmol/min/mg protein)	CAT (mmol/min/mg protein)	GST (mmol/min/mg protein)	MDA (µmol/mg protein)
Control	2.70 ± 0.10^{a}	43.89 ± 1.20^{a}	0.77 ± 0.05^{a}	0.53 ± 0.04^{a}
250 mg/kg	2.84 ± 0.21^{a}	46.03 ± 1.40^{a}	1.38 ± 0.10^{b}	0.63 ± 0.08^{b}
500 mg/kg	3.15 ± 0.17^{a}	45.05 ± 1.36^{a}	1.18 ± 0.08^{c}	0.71 ± 0.02^{b}
1000 mg/kg	3.19 ± 0.15^{a}	47.82 ± 0.68^{a}	1.18 ± 0.10^{c}	0.69 ± 0.05^{b}
2000 mg/kg	3.03 ± 0.10^{a}	50.01 ± 1.23^{a}	1.27 ± 0.10^{bc}	0.67 ± 0.05^{b}

Values are means of six replicates ± S.E.M. Values in the same column with different superscripts are different ($p<0.05$) significantly. **SOD**: Superoxide dismutase; **CAT**: Catalase; **GST: G**lutathione S-transferase; **MDA:** Malondialdehyde

Table 3 presents results of the effects of CSSE on lipid profile indices in rats. The total cholesterol levels in the treated rats significantly decreased ($p<0.05$) compared to the control. However, the triacylglycerol and high density lipoprotein cholesterol levels in the treated subjects compared favorably ($p>0.05$) to the control.

Table 3. Effects of *C. sanguinolenta* stem ethanol extract on serum lipid profiles in rats.

Parameters	Total cholesterol (Meq/L)	Triglycerides (Meq/L)	HDL cholesterol (Meq/L)
Control	24.63 ± 0.77^{a}	8.00 ± 0.25^{a}	7.80 ± 0.12^{a}
250 mg/kg	18.32 ± 1.17^{b}	7.65 ± 0.40^{a}	8.00 ± 0.20^{a}
500 mg/kg	16.02 ± 0.54^{b}	6.76 ± 0.39^{a}	8.52 ± 0.30^{a}
1000 mg/kg	18.63 ± 0.70^{b}	7.12 ± 0.20^{a}	7.79 ± 0.18^{a}
2000 mg/kg	17.75 ± 0.80^{b}	7.56 ± 0.70^{a}	8.36 ± 0.30^{a}

Values are means of six replicates ± S.E.M. Values in the same column with the similar superscripts are comparably ($p>0.05$) significantly.

The results of the effects of CSSE on serum protein and bilirubin concentrations in rats are presented on Figures 1 and 2. The serum protein concentrations in the treated subjects compared significantly ($p>0.05$) to the controls at 500, 1000, and 2000 mg/kg body weight, but increased at 250 mg/kg body weight. The serum total bilirubin concentrations in the treated subjects significantly decreased ($p<0.05$) compared to the control.

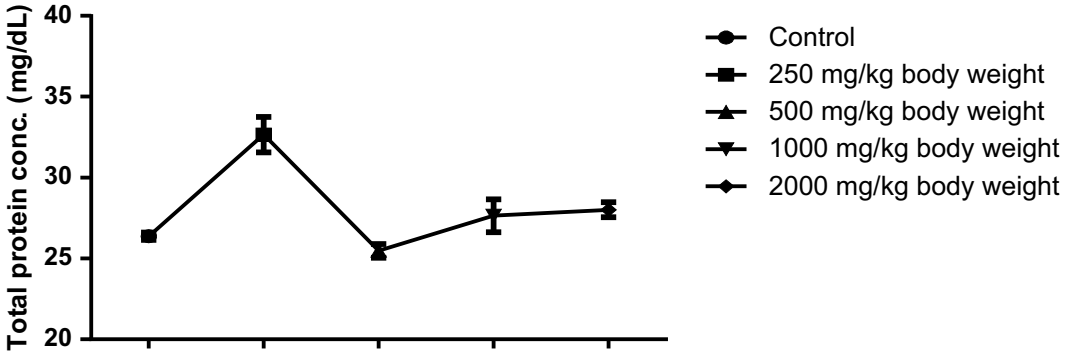

Figure 1. Effects of *C. sanguinolenta* stem ethanol extract on serum total protein concentration in rats.

Figure 2. Effects of *C. sanguinolenta* stem ethanol extract on serum bilirubin concentration in rats.

Figures 3 and 4 depict the effects of CSSE on AST and ALT in rats. The serum, liver and heart AST activities in the treated subjects increased significantly ($p<0.05$) compared to the controls. However, the serum ALT activity significantly decreased ($p<0.05$) compared to the control, while the hepatic ALT activity was not altered.

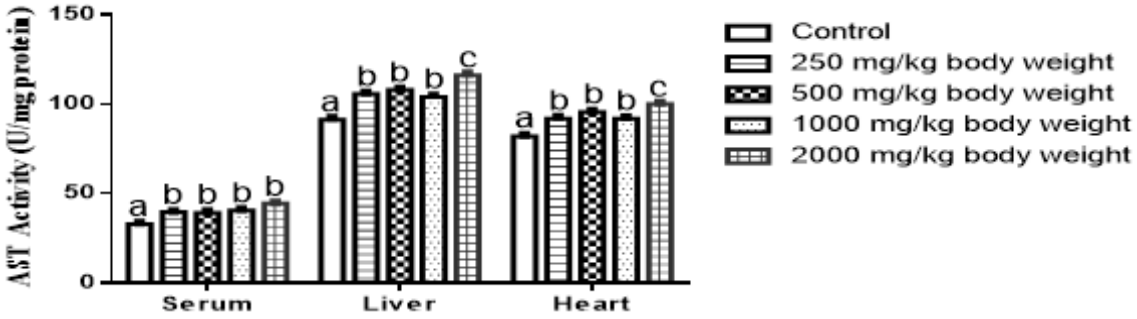

Figure 3. Effects of *C. sanguinolenta* stem ethanol extract on aspartate transaminase (AST) activity in rats.

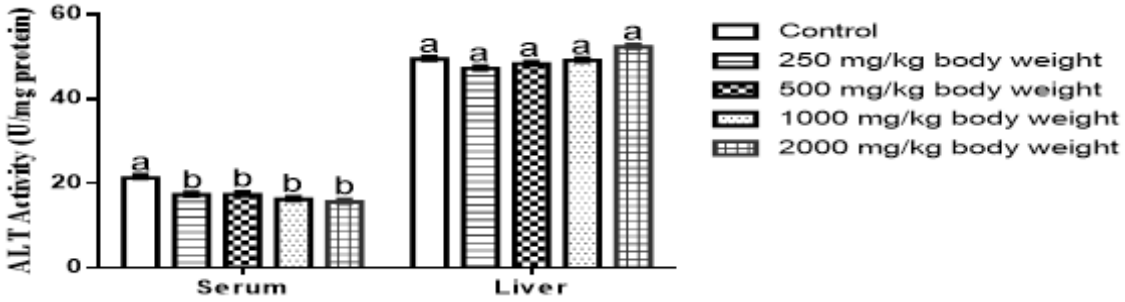

Figure 4. Effects of *C. sanguinolenta* stem ethanol extract on alanine transaminase (ALT) activity in rats.

Figure 5 depicts the effects of CSSE on Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in the heart of treated subjects. The activity of these enzymes compared favourably ($p>0.05$) in the heart of the treated subjects compared to control.

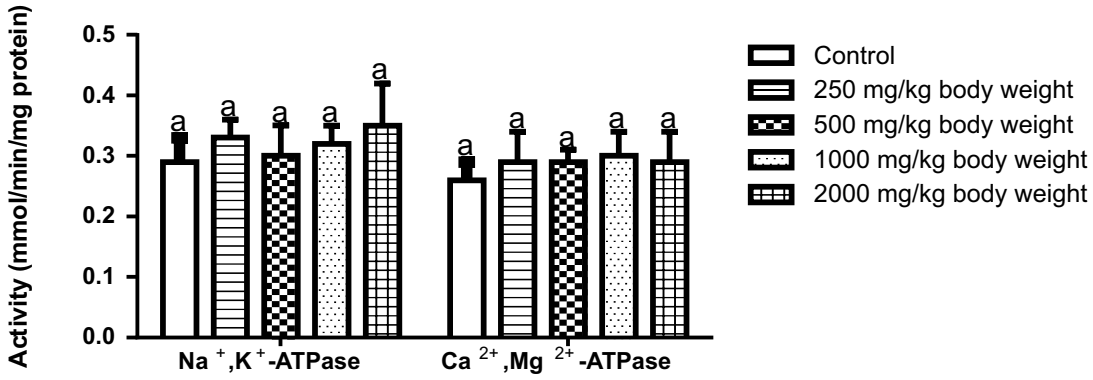

Figure 5. Effects of *C. sanguinolenta* stem ethanol extract on Na^{+},K^{+}-ATPase and Ca^{2+},Mg^{2+}-ATPase activities in rats.

The results for the effects of CSSE on AChE and BChE activities in the brain of treated subjects are shown in Figure 6. The brain AChE activity decreased significantly ($p<0.05$) compared to control. Conversely, the brain BChE activity significantly compared favourably ($p>0.05$) to control.

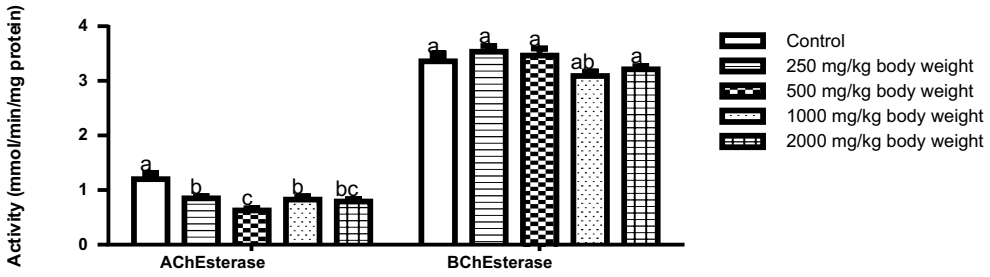

Figure 6. Effects of *C. sanguinolenta* stem ethanol extract on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity in rats.

Figure 7 reveals the effects of CSSE on NO concentrations in the brain of subjects treated with CSSE. The result showed that the brain NO levels significantly compared favourably ($p>0.05$) to control.

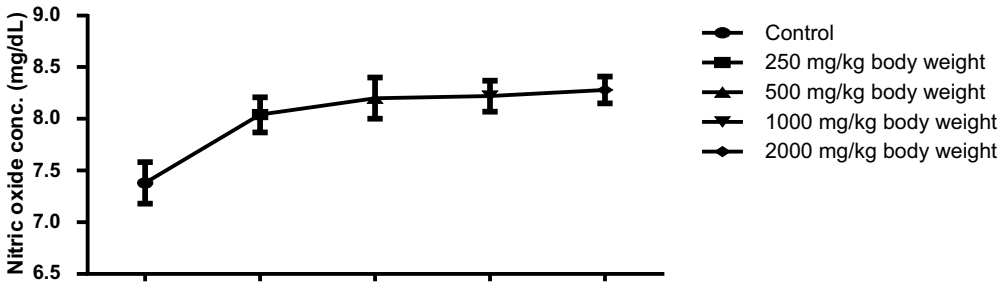

Figure 7. Effects of *C. sanguinolenta* stem ethanol extract on nitric oxide (NO) concentration in rats.

Plates 1-15 show the photomicrographs of cross-sections of liver, heart and brain tissues of *C. sanguinolenta* stem ethanol extract (CSSE)-treated rats and the control. The results show no evidence of degenerative changes in the brain tissue of the extract-treated rats after 21 days oral administration of varying concentrations of CSSE. Similarly, there was no evidence of injury or damage in liver and heart tissues of extract-treated subjects although mild alterations were observed in each tissue.

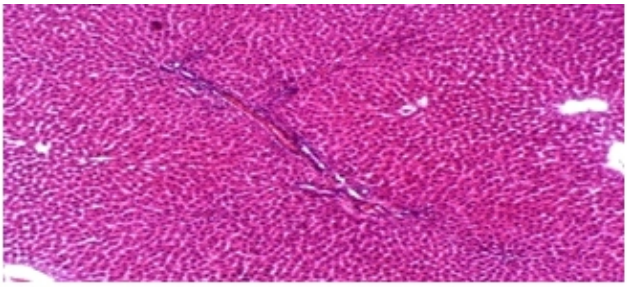

Plate 1. Photomicrograph of the liver of control after 21 days with normal hepatocytes showing no features of inflammation or acute/chronic damage (mag. ×400); routine hematoxylin eosin (H&E) staining

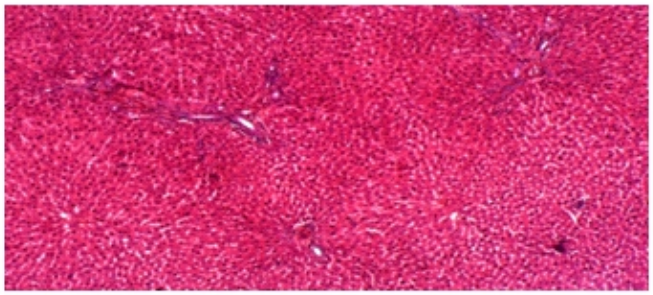

Plate 2. Photomicrograph of the liver of rat administered 250 mg/kg bwt extract after 21 days with normal hepatocytes, portal tracts and central vein with damage. (mag. ×400); routine hematoxylin eosin (H&E) staining

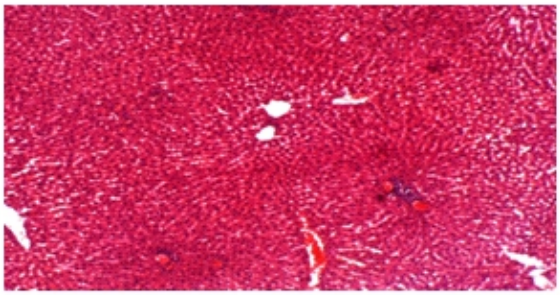

Plate 3. Photomicrograph of liver of rat treated 500 mg/kg bwt extract after 21 days with mild hepatocyte, central vein; with no signs of inflammation or acute/chronic disease (mag. ×400); routine hematoxylin and eosin staining

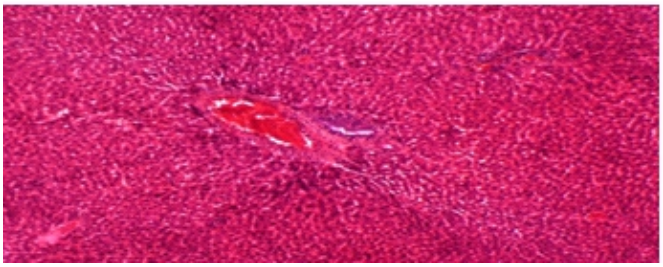

Plate 4. Photomicrograph of the liver of rat treated 1000 mg/kg bwt extract after 21 days with mild hepatocyte, central vein with no signs of inflammation or acute/chronic disease (mag. ×400); routine hematoxylin and eosin staining

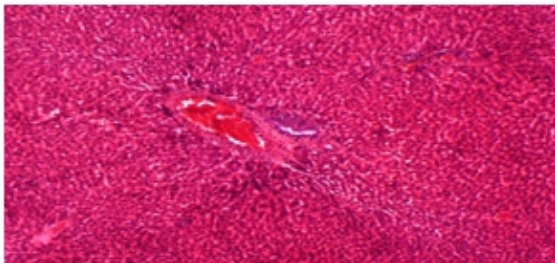

Plate 5. Photomicrograph of liver of rat treated 2000 mg/kg bwt extract after 21 days with mild hepatocyte, central vein with no signs of inflammation or acute/chronic disease (mag. ×400); routine hematoxylin and eosin staining

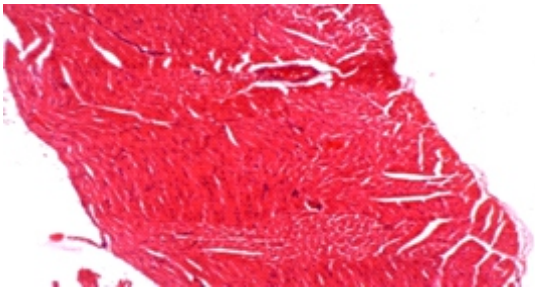

Plate 6. Photomicrograph of the heart of control rat after 21 days administration showing muscular wall composed of branched myocardial muscle fibre bundles with interspersed vascular channels. (mag. ×400); routine hematoxylin and eosin staining

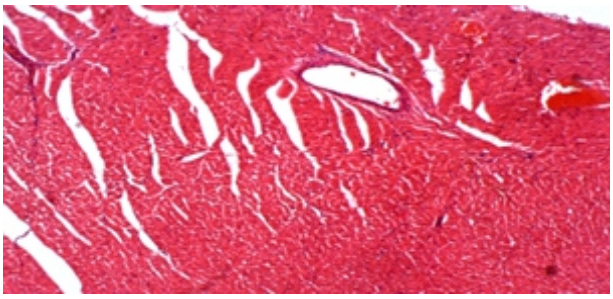

Plate 7: Photomicrograph of heart of rat treated 250 mg/kg showing muscular wall composed of branched myocardial muscle fibre bundles with interspersed vascular channels. (mag. ×400); routine H&E staining. No atherosclerotic lesions or muscular degeneration or inflammation.

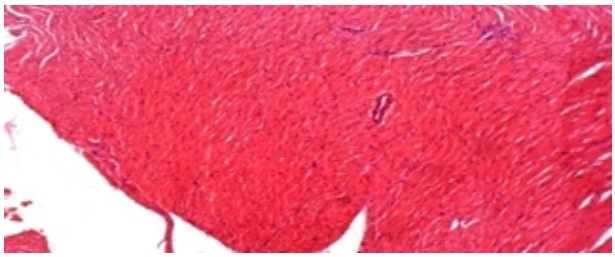

Plate 8: Photomicrograph of the heart of rat treated 500 mg/kg showing muscular wall composed of branched myocardial muscle fibre bundles with interspersed vascular channels. (mag. ×400); routine H&E staining. No atherosclerotic lesions.

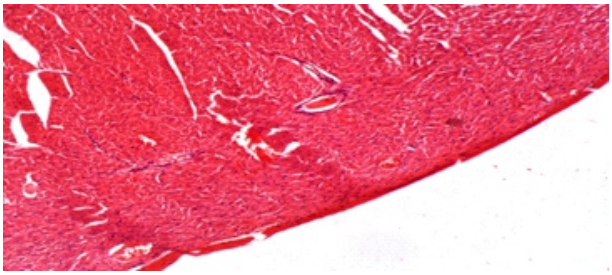

Plate 9: Photomicrograph of heart of rat treated 1000 mg/kg showing muscular wall composed of branched myocardial muscle fibre bundles with interspersed vascular channels. (mag. ×400); routine H&E staining. No atherosclerotic lesions or muscular degeneration/inflammation feature

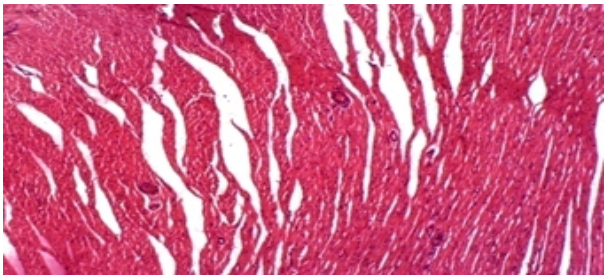

Plate 10: Photomicrograph of heart of rat treated 2000 mg/kg showing muscular wall composed of branched myocardial muscle fibre bundles with interspersed vascular channels. mag. ×400); routine H&E staining. No atherosclerotic lesions or features of muscular degeneration or inflammation.

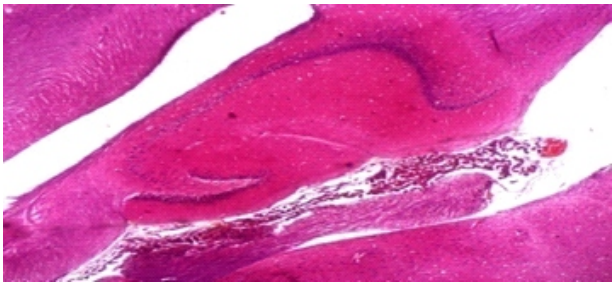

Plate 11: Photomicrograph of the brain of control rat with normal cerebral, hippocampal and cerebellar regions characterized by preserved neurons and white mater without degenerative changes. (mag. ×400); routine H&E) staining

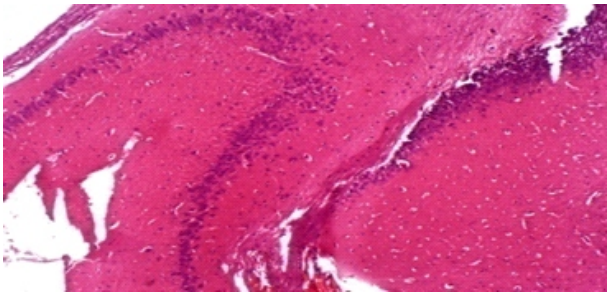

Plate 12: Photomicrograph of brain of rat treated 250 mg/kg with normal cerebral, hippocampal and cerebellar regions characterized by preserved neurons and white mater; without degenerative changes. (mag. ×400); routine H&E staining

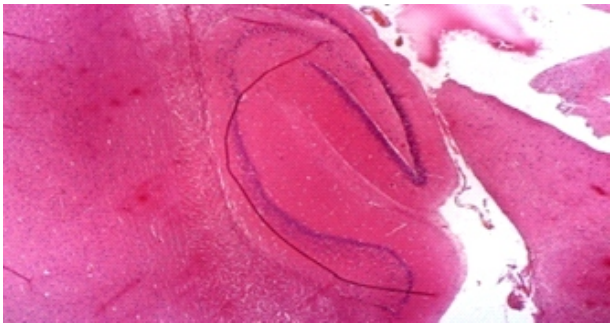

Plate 13: Photomicrograph of brain of rat treated 500 mg/kg with normal cerebral, hippocampal and cerebellar regions characterized by preserved neurons and white mater; without degenerative changes. mag. ×400); routine H&E staining

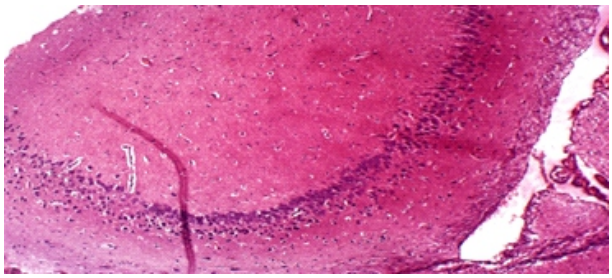

Plate 14: Photomicrograph of the brain of rats administered 1000mg/kg with normal cerebral, hippocampal and cerebellar regions characterized by preserved neurons and white mater; without degenerative changes. (mag. ×400); routine H&E) staining

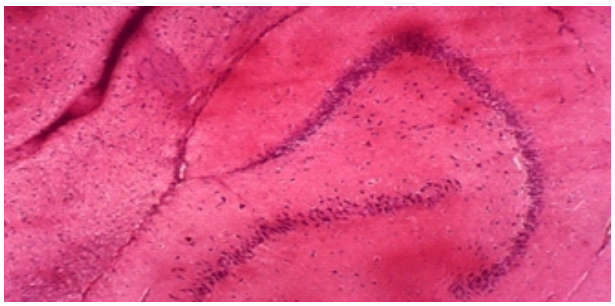

Plate 15: Photomicrograph of the brain of rats administered 2000mg/kg with normal cerebral, hippocampal and cerebellar regions characterized by preserved neurons and white mater; without degenerative changes. (mag. ×400); routine H&E) staining

DISCUSSION

Natural plant sources have remained an essential part of human endeavors since time immemorial. In countries like Nigeria, the first choice for 60% children suffering with high fever and presumptuously malaria is herbal medicines; and *Cryptolepis Sanguinolenta* has been successfully used in the management of these ailments and other health-related diseases such as diabetes. In this present study, the *Cryptolepis sanguinolenta* ethanol stem extract (CSSE) contained relatively moderate amounts of steroids (52.35 mg/100g) and alkaloids (35.11 mg/100g). The terpenoids (22.61 mg/100g) and glycosides (9.80 mg/100g) levels were recorded in the extract. Secondary metabolites such as saponins, alkaloids, cardiac glycosides, terpenoids, steroids, and proteins have been reported to demonstrate pharmacological and biological activities against innumerable chronic diseases like cancer, gastrointestinal disorders, and cardiovascular diseases (Badam *et al.,* 2002; Gupta and Tandon, 2004; Kamalakkannan *et al.,* 2005; Chew *et al.,* 2009), as well as against malaria. The high amounts of these compounds may have conferred on its antimalarial activity, since these phytochemicals are reported to possess antiplasmodial activities (Belay *et al.,* 2018; Misganaw *et al.,* 2019). Flavonoids and alkaloids may also confer antioxidant activity (Ayoola *et al.,* 2008), which is beneficial in malaria treatment because flavonoid-rich (plant-based) diets may perform significant roles in highly-endemic malaria areas (Ferreira *et al.,* 2010). Alkaloids, terpenoids, glycosides and steroids present in this plant are reported to have antioxidant and antimalarial activities (Zofou *et al.,* 2011; Balogun *et al.,* 2014; Ntie-Kang *et al.,* 2014; Osafo *et al.,* 2017).

Increased lipid peroxidation resulting from oxidative damage of cells by reactive oxygen species (ROS) is usually linked with malaria thereby affecting the membrane of both infected and uninfected erythrocytes (Das and Nanda, 1999; Omodeo-Sale *et al.*, 2003). Increased MDA levels in the treated subjects in this study may suggest the reduction in damaging effect of infection on host cell plasma membrane caused by ROS. However, antioxidant enzymes are known to scavenging radicals, donate electrons or hydrogen, decompose peroxides, and/or chelate metal ions (Lobo *et al.*, 2010). Enhanced activity of cellular

antioxidants ensure that free radicals are effectively neutralized (Pal and Nimse, 2006), therefore preventing their damaging effects to cells. Decreased antioxidants or antioxidant enzymes' inhibition can cause oxidative stress, which may damage or kill the cells (Valko *et al.,* 2007). Elevated levels of these enzymes have been reported to protect cells against ROS from malaria infections (Wheeler *et al.,* 2001; Becker et al., 2004; Elchuri *et al.*, 2005; Osman *et al.,* 2016). In this study, the SOD and CAT activities compared favorably with the control suggesting that their scavenging effects were not altered in the cells of the treated rats. However, GST activity increased significantly compared, thereby suggesting its function in metabolizing reactive species and offering protection against oxidative stress (Osman *et al.,* 2016).

Serum lipid profile variations have been observed in malaria infection but not much is known about their main biological mechanism of action (Visser *et al.,* 2013). Alterations in the levels of TC, LDL-c, LDL-c, and triglycerides are primary risk factors of atherosclerosis (As *et al.*, 2013; Nemati *et al.,* 2013). Decrease in TC levels of the treated compared to control without alterations in triglycerides and HDL-c levels may imply that CSSE has low cholesterol concentrations in the cardiovascular system of the subjects, which could be an indication of cardioprotective effects of CSSE as evident in the histology. This corroborates the report of Ajayi *et al.* (2012) who reported a significant TC reduction in ethanol extract of *C. sanguinolenta* stem.

Bilirubin and total protein concentrations are biochemical parameters that can be used to diagnose liver damage and dysfunction (Rosenberg *et al.,* 2018). Aminotransferases (AST and ALT) were also considered as major hepatic toxicity markers (Thapa and Walia, 2007; Hussein *et al.,* 2013). The decrease in total bilirubin without a change in direct bilirubin and serum protein levels may infer that the synthetic proficiency of the hepatocytes was preserved by the extract without causing excessive haem degradation. Similarly, elevated serum protein concentrations observed in the treated subjects administered 250 mg/kg body weight extract may also be an indication that the synthetic and metabolic functions of the hepatocytes were not impaired significantly by the extract. Elevation in AST activity of the treated compared to control in the tissues may suggest that the extract may not have caused biliary obstruction in the tissues. However, the low levels of ALT in treated rats without effects in the hepatocytes may also suggest that the enzyme caused no effects on the hepatocytes of the rats treated with the extract.

The impairment of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase is often linked to heart failure, ischemia, and hypertension (Bundgaard and Kjelden 1996; Suhail, 2010). The levels of these enzymes in the treated subjects were not altered significantly. This may be an indication that the extract could possess cardioprotective effects on the subjects without having adverse effects on the plasma membrane of the cardiac cells at the duration investigated.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities, and nitric oxide (NO) concentrations are used as peripheral biomarkers of neurotoxicity. Alterations in these biochemical parameters might reflect similar changes in the nervous system (Costa, 1987; Lees, 1993; Gonçalves *et al.,* 2012). The brain BChE and NO levels were not altered in the treated subjects, while the AChE activity decreased significantly. This may suggest that the extract may not adversely affect cholinergic neurotransmission in the BChE and NO levels of the subjects, but may be neurotoxic in the AChE activity at the duration investigated. This may lead to the disruption of neurotransmission process which may eventually lead to neurodegenerative diseases as evident on the histological tissues.

CONCLUSION

Cryptolepis sanguinolenta stem ethanol extract (CSSE) induced mild alterations in biochemical parameters and histo-architecture of rats. However, it kept tissues of the rats intact without feasible damage, hence is relatively safe for consumption.

CONFLICT OF INTEREST

Authors of this work declare that the publication of this manuscript has no conflict of interest.

REFERENCES

Ahad, B., Shahri, W., Rasool, H., Reshi, Z. A., Rasool, S. and Hussain, T. 2021. Medicinal plants and herbal drugs: An overview. *Medicinal and Aromatic Plants: Healthcare and Industrial Applications*, 2021:1-40.

Ajayi, A. F., Akhigbe, R. E., Iyiola, T. O., Adewumi, O. M. and Olaleye, S. B. 2012. "Gastric secretagogue action of *Cryptolepis sanguinolenta* in the perfused stomach of anesthetized rats," *International Journal of Medicine and Biomedical Research,* 1(1):62-67.

Anderson, K. J., Teuber, S. S., Gobeille, A., Cremin, P., Waterhouse, A. L. and Steinberg, F. M. 2001. Walnut polyphenolics inhibit *in vitro* human plasma and LDL oxidation. *The Journal of Nutrition*, 131(11):2837-2842.

Ansah, C., Mfoafo, E. A., Woode, E., Opoku-Okrah, C., Owiredu, W. K. B. A., Duwiejua, M. 2008. "Toxicological evaluation of the anti-malarial herb *Cryptolepis sanguinolenta* in rodents," *Journal of Pharmacology and Toxicology*, 3(5):335-343.

Ansah, C., Otsyina, H. R., Duwiejua, M., Woode, E., Aboagye, F. A. and Aning, K. G. 2009. Toxicological assessment of *Cryptolepis sanguinolenta* for possible use in veterinary medicine. *Journal of Veterinary Medicine and Animal Health*, 1(1):011-016.

As, S., Sahukar, S., Murthy, J. and Kumar, K. 2013. A study of serum apolipoprotein A_1, apolipoprotein B and lipid profile in stroke. *Journal of Clinical and Diagnostic Research*, 7(7):1303-6.

Ayoola, G., Coker, H., Adesegun, S., Adepoju, B., Obaweye, K., Ezennia, E. and Atangbayila, T. 2008. Phtyochemical screening and antioxidant activity of some selected medicinal plants used for malaria therapy in South Western Nigeria. *Tropical Journal of Pharmaceutical Research*, 7(3):1019-24.

Badam, L., Bedekar, S. S., Sonawane, K. B. and Joshi, S. P. 2002. *In vitro* antiviral activity of bael (*Aegle marmelos* Corr.) upon human coxsackie viruses B1-B6. *Journal of Communicable Diseases*, 34(2):88-99.

Balogun, E. A., Adebayo, J. O., Zailani, A., Kolawole, O. and Ademowo, O. 2010. Activity of ethanolic extract of *C. violaceum* leaves against *P. berghei* in mice. *Agriculture and Biology Journal of North America*, 1(3):307-12.

Balogun, E. A., Zailani, A. and Adebayo, J. O. (2014). Augmentation of antioxidant system: Contribution to antimalarial activity of *Clerodendrum violaceum* leaf extract. *TANG*, 4(4):e26.

Bartels, H., Böhmer, M. and Heierli, C. 1972. Serum creatinine determination without protein precipitation. *Clinica chimica* Acta; *International Journal of Clinical Chemistry*, 37:193.

Becker, K., Tilley, L., Vennerstrom, J., Roberts, D., Rogerson, S. and Ginsburg, H. 2004. Oxidative stress in malaria parasite-infected RBCs: host-parasite interactions. *International Journal of Parasitology*, 34(2):163-89.

Beers, R. F. and Sizer, I. W. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, 195(1):133-140.

Belay, W., Gurmu, A. and Wubneh, Z. 2018. Antimalarial activity of stem bark of *P. linearifolia* during early and established *Plasmodium* infection in mice. *Evidence-Based Complementary and Alternative Medicine*, 1-7.

Bewaji, C. O., Olorunsogo, O. O. and Bababunmi, E. A. 1985. Comparison of the membrane-bound (Ca^{2+},Mg^{2+})-ATPase in erythrocyte ghosts from some mammalian species. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 82(1):117-22.

Buege, J. A. and Aust, S. D. 1978. Microsomal lipid peroxidation. In: Flesicher, S. and Packer, L. (eds.), Methods in Enzymology, vol. 52, Academic Press, New-York, pp. 302-10.

Bugyei, K. A., Boye, G. L. and Addy, M. E. 2010. Clinical efficacy of a tea-bag formulation of *Cryptolepis sanguinolenta* root in the treatment of acute uncomplicated *falciparum* malaria. *Ghana Medical Journal*, 44(1).

Bundgaard, H. and Kjeldsen, K. 1996. Human myocardial Na,K-ATPase concentration in heart failure. *Molecular and Cellular Biochemistry*, 163-164:277-83.

Chew, Y. L., Goh, J. K. and Lim, Y. Y. 2009. Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in peninsular Malaysia. *Food Chemistry*, 116(1):13-18.

Costa, L. G. 1987. Peripheral models for the study of neurotransmitter receptors: their potential application to occupational health. In: V. Foa, E.A. Emmet, M. Maroni and A. Colombi (eds.), Occupational and environmental chemical hazards, Ellis Horwood Ltd., Chichester, UK, pp. 524-528.

Das, B. S. and Nanda, N. K. 1999. Evidence for erythrocyte lipid peroxidation in acute *falciparum* malaria. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 93:58-62.

Doumas, B. T., Watson, W. A. and Biggs, H. G. 1971. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta*, 31(1):87-92.

Drury, R. A. B., Wallington, E. A. and Cameron, S. R. 1967. Carleton's histological technique, London. 4th Edn., Oxford University Press, NY. USA, pp: 279-280.

Elchuri, S., Oberley, T., Qi, W., Eisenstein, R., Roberts, L., Van Remmen, H., Epstein, C. and Huang, T. 2005. Cu-Zn-SOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene*, 24(3):367-80.

Ellman, G. L., Courtney, K. D., Andres Jr, V. and Featherstone, R. M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7:88-90.

Evelyn, K. A. and Malloy, H. T. 1938. Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. *Journal of Biological Chemistry*, 126(2):655-662.

Ferreira, J., Luthia, D., Sasaki, T. and Heyerick, A. 2010. Flavonoids from *A. annua* as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecule*, 15(5):3135-70.

Fredrickson, D. S., Levy, R. I. and Lees, R. S. 1967. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. *New England Journal of Medicine*, 276(3):148-56.

Friedwald, W. T., Levey, R. I. and Friedrickson, D. S. 1972. Estimation of the concentration of LDL in plasma without the use of preparative concentrating. *Clinical Chemistry*, 18:499-502.

Gonçalves, J. F., Nicoloso, F. T., da Costa, P., Farias, J. G., Carvalho, F. B., da Rosa, M. M., Gutierres, J. M., Abdalla, F. H., Pereira, J. S., Dias, G. R., Barbosa, N. B., Dressler, V. L., Rubin, M. A., Morsch, V. M. and Schetinger, M. R. 2012. Behavior and brain enzymatic changes after long-termintoxication with cadmium salt or contaminated potatoes. *Food and Chemical Toxicology*, 50:3709-18.

Gornall, A. G., Bardawill, C. J. and David, M. M. 1949. Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, 177(2):751-766.

Guan, Y. S. and He, Q. 2015. Plants consumption and liver health. *Evidence-Based Complementary and Alternative Medicine*, 1:2015.

Gupta, A. K. and Tandon, N. 2004. *Reviews on Indian Medicinal Plants. Vol. 1. New Delhi, India: Indian Council of Medicinal Research*, 2004:543.

Habig, W. H., Pabst, M. J. and Jacoby, W. B. 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, 249:7130-9.

Haussmann, H. J. and Werringloer, J. 1985. Nitric oxide and nitrite formation 4during degradation of N-nitrosamines. *Naunyn-Schmiedeberg's Archives of Pharmacology Scimago*, 329:R21.

Hussein, R. R., Soliman, R., Ali, A., Tawfeik, M. and Abdelrahim, M. 2013. Effects of antiepileptic drugs on liver enzymes. *Beni-Suef University Journal of Basic and Applied Sciences,* 2(1):14-9.

Jagadish, L. K., Krishnan, V. V., Shenbhagaraman, R. and Kaviyarasan, V. 2009. Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricusbisporus imbach* before and after boiling. *African Journal of Biotechnology*, 8:654-661.

Kamalakkannan, N., Stanely, M. and Prince, P. 2005. Antihyperlipidaemiceffect of*Aegle marmelos* fruit extract in streptozotocin-induced diabetes in rats. *Journal of the Science of Food and Agriculture*, 85:569-573.

Komlaga, G., Agyare, C., Dickson, R. A., Mensah, M. L. K., Annan, K., Loiseau, P. M. and Champy, P. 2015. Medicinal plants and finished marketed herbal prod-ucts used in the treatment of malaria in the Ashanti region, Ghana. *Journal of Ethnopharmacology*, 172:333-46.

Lees, G. J. 1993. Contributory mechanisms in the causation of neurodegenerative disorders. *Neuroscience*, 54:287-322.

Liu, M. L., Chien, L. Y., Tai, C. J., Lin, K. C. and Tai, C. J. 2011. Effectiveness of traditional Chinese medicine for liver protection and chemotherapy completion among cancer patients. *Evidence-Based Complementary And Alternative Medicine*, 2011:291843.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4(8):118-26.

Lopes-Virella, M. F., Stone, P., Ellis, S. and Colwell, J. A. 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical Chemistry*, 23(5):882-4.

Misganaw, D., Engidawork, E. and Nedi, T. 2019. Evaluation of the antimalarial activity of crude extract and solvent fractions of the leaves of *Olea europaea* (Oleaceae) in mice. *BMC Complementary and Alternative Medicine*, 19:171.

Misra, H. P. and Fridovich, I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247(10):3170-5.

Nemati, H., Khodarahmi, R., Rahmani, A., Ebrahimi, A., Amani, M. and Eftekhari, K. 2013. Serum lipid profile in psoriatic patients: correlation between vascular adhesion protein 1 and lipoprotein (a). *Cell Biochemistry and Function*, 31:36-40.

Nofal, A. E., Elmongy, E. I., Hassan, E. A., Tousson, E., Ahmed, A. A. S., El Sayed, I. E. T., Binsuwaidan, R. and Sakr, M. 2023. Impact of Synthesized Indoloquinoline Analog to Isolates from *Cryptolepis sanguinolenta* on Tumor Growth Inhibition and Hepatotoxicity in Ehrlich Solid Tumor-Bearing Female Mice. *Cells*, 12(7):1024.

Ntie-Kang, F., Onguene, P., Lifongo, L., Ndom, J., Sippl, W. and Mbaze, L. 2014. The potential of antimalarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. *Malaria Journal*, 13:81.

Obadoni, B. O. and Ochuko, P. O. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, 8:203-208.

Odebiyi, O. O. and Sofowora, E. A. 1978. Phytochemical Screening of Nigerian. *Medicinal Plants Part II: IIoydia*, 41:1-25.

Oguntibeju, O. O. 2018. Medicinal plants with anti-inflammatory activity from selected countries and regions of Africa. *Journal of Inflammation Research*, (11):307-17.

Omodeo-Sale, M. F., Motti, A., Basilico, N., Parapini, S. and Olliaro, P. 2003. Accelerated senescence of human erythrocytes cultured with *P. falciparum*. *Blood*, 102:705-711.

Opoku-Agyemang, F., Dodoo, J. N., Hlomador, T. E., Gilday, K. and Amissah, J. N. 2022. Conservation and Sustainable Use of Crytolepis sanguinolenta. InHerbs and Spices-New Advances 2022 Oct 30. IntechOpen. doi: 10.5772/intechopen.108249

Osafo, N., Mensah, K. B. and Yeboah, O. K. 2017. "Phytochemical and pharmacological review of *Cryptolepis sanguinolenta* (Lindl.) Schlechter," *Advances in Pharmacological Sciences*, 2017(Article I D 3026370):1-13.

Osman, A. G., Chittiboyina, A. G. and Khan, I. A. 2016. Cytoprotective Role of Dietary Phytochemicals against Cancer Development via Induction of Phase II and Antioxidant Enzymes. In: Fishbein, J.C. and Heilman, J.M. (eds.), Advances in Molecular Toxicology, Academic Press, San Diego, pp. 99-137.

Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., Spiegelhalder, B. and Bartsch, H. 2000. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *European Journal of Cancer*, 36(10):1235-1247.

Pal, D. and Nimse, S. 2006. Screening of antioxidant activity of *H. verticillata*. *Advanced Journal of Chemistry*, 18:3004-8.

Paulo, A. and Houghton, P. J. 2003. Chemotaxonomic analysis of the genus Cryptolepis. *Biochemical Systematics and Ecology*, 31(2):155-166.

Reitman, S. and Frankel, S. A. 1957. Colorimetric method for the determination of serum glutamate-oxaloacetate and pyruvate transaminases. *American Journal of Clinical Pathology*, 28:56-63.

Ronner, P., Gazzotti, P. and Carafoli, E. 1977. A lipid requirement for the (Ca^{2+}-Mg^{2+})-activated ATPase of erythrocyte membranes. *Archives of Biochemistry and Biophysics*, 179(2):578-83.

Rosenberg, W., Tony-Badrick, T. and Tanwar, S. 2018. Liver disease. In: N. Rafai, A.R. Horvath, and C.T. Wittwer (Eds.), Tietz textbook of clinical chemistry and molecular diagnostics, 6th ed., Elsevier Inc, pp. 1348-97.

Sala, A., Recio, M. D. C., Giner, R. M., Máñez, S., Tournier, H., Schinella, G. and Ríos, J. L. 2002. Anti-inflammatory and antioxidant properties of *Helichrysum italicum*. *Journal of Pharmacy and Pharmacology*, 54(3):365-371.

Savithramma, N., Rao, M. L. and Suhrulatha, D. 2011. Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research*, 8(3):579-584.

Suhail, M. 2010. Na^+, K^+-ATPase: Ubiquitous multifunctional transmembrane protein and its relevance to various pathophysiological conditions. *Journal of Clinical Medicine Research*, 2(1):1.

Sun, J., Chu, Y. F., Wu, X. and Liu, R. H. 2002. Antioxidant and antiproliferative activity of common fruits. *Journal of Agricultural and Food Chemistry*, 50(25):7449-7454.

Thapa, B. R. and Walia, A. 2007. Liver function tests and their interpretation. *India Journal of Pediatrics*, 74:663.

Tietz, N. W. 1995. Clinical Guide to Laboratory Tests. 3rd Edition, W.B Saunders Company, Philadephia. Pp. 1096-1096.

Trease, G. E. and Evans, W. C. 1989. A Textbook of Pharmacognosy. 13th ed. Bailliere-Tindall Ltd., London, 245-250.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. and Mazur, M. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, 39(1):44-84.

Veniamin, M. P. and Vakirtzi-Lemonias, C. 1970. Chemical basis of the carbamidodiacetyl micromethod for estimation of urea, citrulline and carbamyl derivatives. *Clinical Chemistry*, 16(1):3-6.

Visser, B. J., Wieten, R., Nagel, I. and Grobusch, M. 2013. Serum lipids and lipoproteins in malaria - a systematic review and meta-analysis. *Malaria Journal*, 12:442.

Wheeler, M., Nakagami, M., Bradford, B., Uesugi, T., Mason, R., Connor, H., Dikalova, A., Kadiiska, M. and Thurman, R. 2001. Overexpression of manganese superoxide dismutase prevents alcohol-induced liver injury in the rat. *Journal of Biological Chemistry*, 276(39):36664-72.

Wright, P., Leathwood, A. and Plummer, D. 1972. Enzymes in rat urine: Alkaline phosphatase. *Enzymology*, 42(4):317-27.

Zofou, D., Kowa, T. K., Wabo, H., Ngemenya, M., Tane, P. and Titanji, V. 2011. *H. lanceolatum* as a potential source of new antimalarials: bioassay-guided fractionation of stern bark. *Malaria Journal*, 10(1):1-7.