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IN-VITRO ANTIOXIDANT, GCMS ANALYSIS, AND PRELIMINARY TOXICOLOGICAL EVALUATION OF AQUEOUS SEED EXTRACT OF *Adansonia digitata* L.

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ABSTRACT

Adansonia digitata L. (*Malvaceae*) is an African medicinal plant known for its nutritional value. The seed of *A. digitata* is used as a thickening agent in soups, a flavoring agent, or roasted as snacks and as anti-diabetics. Toxicity evaluation of medicinal plants and plant products is important in determining the required dosage and observing clinical signs related to the compounds. This study investigated the *in-vitro* antioxidant and toxicological effects of aqueous seed extract of *A. digitata*. The aqueous seed extract of *A. digitata* expressed *in-vitro* antioxidant activities at 0.2-1.0 mg/mL with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (IC_{50} = 0.14±0.00 mg/mL), total antioxidant capacity (IC_{50} = 0.23±0.01 mg/mL), nitric oxide scavenging activity (IC_{50} = 0.85±0.15 mg/mL), and hydrogen peroxide decomposing activity (IC_{50} = 0.17±0.03 mg/mL). No mouse death was recorded in the acute toxicity, and the LD_{50} was found to be above 5000 mg/kg body weight. The administration of aqueous seed extract of *A. digitata* at 500 and 1000 mg/kg body weight did not cause any alteration in lipid profile, plasma total protein, liver total cholesterol, liver triacylglycerol, and liver total protein. However, plasma triacylglycerol and total protein increased at 500 mg/kg body weight compared with the control group after 14 days of administration. The extract did not significantly alter coronary, cardiac, and atherogenic indices, but at 500 mg/kg body weight, cardiac indices significantly decreased. Furthermore, no significant ($P>0.05$) alterations were observed in aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities in plasma and liver after 14 days of administration except at 1000 mg/kg body weight treatment in aspartate aminotransferase. The study concluded that the aqueous seed extract of *A. digitata* possesses *in-vitro* antioxidant properties and is safe at the doses and duration tested.

Keywords: *In vitro,* Antioxidants, *Adansonia digitata*, Seed, LD_{50}, Marker enzyme.

INTRODUCTION

Adansonia digitata (Baobab, *Malvaceae*) is indigenous to Africa. The tree is culturally important and has several traditional uses, including nutritional, medicinal, and cosmetic benefits (Buchmann *et al.,* 2010; De Caluwe *et al.,* 2010). The leaves, stem bark, roots, seeds, fruit pulp, and fruit are important parts of *A. digitata*. They play vital roles as sources of staple food, fibre, medicine, and energy (firewood) in many African countries (Yazzie *et al.,* 1994; Sidibe and Williams, 2002; Chadare *et al.,* 2009; De Caluwe *et al.,* 2010). *A. digitata* seeds are often eaten fresh or dried and ground into flour and applied in stew and soup as thickeners (Nnam and Obiakor, 2003; De Caluwe *et al.,* 2010). In some parts of Northern Nigeria, the seeds are fried with sugar and taken as snacks. Also, *A. digitata* fruit is used to treat dysentery and promote perspiration (Sidibe and Williams, 2002). The fruit pulp is an immune stimulant (Al-Qarawi *et al.,* 2003), while the leaf has analgesic, anti-inflammatory, antipyretic, and febrifuge properties (Ramadan *et al.,* 1994).

A. digitata and other plant materials are increasingly used for the local management and treatment of diseases. However, the inadequate measure of the quality controls, safety values, and efficacy of plant materials used for medicinal purposes has raised concerns (Oyewo *et al.*, 2012). Bioactive compounds are responsible for the therapeutic effects of medicinal plants (Chikezie *et al.*, 2015). They are responsible for medicinal plant activities such as antioxidant, antimalarial, antidiabetic, anticarcinogenic, antimicrobial, and anti-inflammatory (Negi *et al.*, 2011). However, some are accountable for the intoxication record during treatments with medicinal plants (Patel *et al.*, 2013; Prasanth Kumar *et al.,* 2014) and cause damage to vital organs and blood cells. Toxicity evaluation of medicinal plants and plant products

is important in identifying the required dosage and observing clinical signs related to the compounds (Prasanth Kumar *et al.,* 2014). *A. digitata* seeds are eaten as snacks, in condiments, and as traditional medicine for the management of diabetes mellitus (Zahra'u *et al.*, 2014). Thus, there is a need to assess the toxicity or safety profile of the seed. The present study was designed to identify the phytochemical constituents in the seed of *A. digitata* using GCMS analysis and investigate the *in-vitro* antioxidant, antihyperlipidemic, and toxicological effects of aqueous seed extract in normal albino mice.

MATERIALS AND METHODS

Chemicals

Hydrogen peroxide (H_2O_2), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), copper sulphate ($CuSO_4$), phosphomolybdenum, vitamin C, butylated hydroxytoluene (BHT), sodium chloride, sodium hydroxide, glacial acetic acid, and bovine serum albumin were purchased from Sigma Chemical Company, St. Louis, MO, USA. Also, other chemicals were of analytical grade.

Experimental animals

Albino mice of both sexes were purchased from the Pharmacology Animal Unit, Department of Pharmacology, Bauchi State University, Nigeria. The animals were allowed free access to standard mice feed and water *ad libitum*. The Institutional Ethical Review Committee approved the procedures for animal care and handling (Registration number UJ/FPS/F17-00379).

Preparation of aqueous seed extract of *A. digitata*

Fresh mature seeds of *Adansonia digitata* were collected in June 2019 from the Azare market of Bauchi State, Nigeria, and identified at the Department of Biological Sciences, Bauchi State University, Gadau, Nigeria. A voucher specimen was deposited at the Federal College of Forestry, Jos, with the voucher number FHJ287. The fruit pulp was washed from the seed using clean water and dried under shade for three days. Thereafter, 100 g of the dried seed was pounded into powder and extracted with 250 mL of distilled water. The aqueous extract was concentrated using a rotary evaporator at 45 °C. The extract was kept in a tight stopper bottle until required for use.

GCMS analysis

The identification of phytochemical constituents from aqueous seed extract of *A. digitata* was conducted using Gas Chromatography Mass-Spectrometry (GCMS), Agilent-7890A (GC) instrument coupled with a Mass Spectrometer detector.

In-vitro antioxidant assays

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH), total antioxidant capacity (TAC), nitric oxide (NO), and hydrogen peroxide (H_2O_2) scavenging activities were determined according to the methods of Ruch *et al.* (1989), Prieto *et al.* (1999), McCune and Johns, (2002), and Fiorentino *et al.* (2008), respectively.

DPPH radical scavenging assay

The scavenging activity against DPPH radical was determined according to the method of McCune and Johns (2002). The method determines the abilities of the extract to reduce methanolic DPPH in the presence of hydrogen-donating antioxidants. Briefly, 500 µL of 0.11 mM methanolic DPPH was added to 500 µL of different concentrations (0.2-1.0 mg/mL) of samples and incubated at room temperature in the dark for 15 min. The absorbance was measured at 517 nm for the determination of DPPH activity using the formula = ((Abs Blank-Abs Sample)/Abs Blank)*100.

Total antioxidant capacity

The total antioxidant capacity (TAC) was evaluated using the phosphomolybdenum assay (Prieto *et al.*, 1999). The method evaluates the reduction of molybdenum VI (Mo(VI) to Mo(V) by the samples and the subsequent formation of green phosphate/Mo(V) complex in an acidic medium. Briefly, 0.1 mL of samples (0.2-1.0 mg/mL) was added to 1 mL working reagent containing 0.6 M tetraoxosulphate (VI) acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was incubated at 95 °C for 90 min. Absorbance was measured at 695 nm after cooling to room temperature, after which the TAC was determined using the formula ((Abs Blank-Abs Sample)/Abs Blank)*100.

Nitric oxide scavenging capacity

Nitric oxide (NO) scavenging capacity was

measured according to the method of Fiorentino *et al.* (2008). The method involves the generation of NO from sodium nitroprusside (SNP) and then measured as nitrite by Griess reaction. Briefly, 2 mL of 5 mM SNP (in 0.1M phosphate buffer, pH 7.4) was added to 0.5 mL of the sample (0.2-1.0 mg/mL). The assay mixture was incubated at 37 °C for 2 h. Thereafter, 0.1 mL of the reaction mixture was withdrawn and added to 0.1 mL of Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 5% phosphoric acid). The mixture was kept in the dark for 10 min at room temperature and followed by measurement of their absorbance at 530 nm. NO scavenging capacity was determined using the formula ((Abs Blank-Abs Sample)/Abs Blank)*100.

Hydrogen peroxide scavenging activities

Hydrogen peroxide (H_2O_2) scavenging activity was determined according to the procedure described by Ruch *et al.* (1989). Briefly, 3.4 mL of sample (0.2-1.0 mg/mL) in phosphate buffer saline (pH 7.4) was mixed with 0.6 mL of 40 mM H_2O_2. The absorbance was read at 230 nm after 10 min incubation at room temperature. Percentage activities were calculated using the expression ((Abs Blank-Abs Sample)/Abs Blank)*100.

Acute toxicity assay in swiss albino mice

Eighteen albino mice were randomly distributed into six groups with three mice per group. The animal was administered with 0.2 mL of distilled water, 500, 1000, 2000, 3000, and 5000 mg/kg body weights of aqueous seed extracts of *A. digitata*,, respectively after overnight fasting for 12 h. After the first phase, nine mice in three groups were treated with 10, 100, and 1000 mg/kg body weight of aqueous seed extracts of *A. digitata*, respectively. The animals were regularly and individually observed for behavioural changes and general toxicity signs after dosing for the first 30 min at the first 24 h and followed by interval observation for seven days. Mortality, body weight, and physical and behavioural changes were recorded (Lorke, 1982).

Sub-acute toxicity assay in Swiss albino mice

Fifteen (15) albino mice were grouped into three (3) with five (5) mice per group. Group one was treated with 0.2 mL distilled water, and groups two and three received 500 and 1000 mg/kg body weight of aqueous seed extract of *A. digitata*, respectively. The animals were treated for 14 days consecutively. All animals were sacrificed on the 15th day (24 h after the last extract administration) under diethyl ether anaesthesia. The blood sample was collected in an EDTA bottle for plasma preparation, and the liver was excised, cleansed of blood, and homogenized in 0.25 M sucrose solution (1/5, w/v). The organ homogenized was centrifuged at 1,500 g for 10 min, and the supernatant was aspirated into a new sample bottle and stored refrigerated until required for use.

Lipid profile assays

Total cholesterol (Tchol), triacylglycerol (TGs), and High-Density Lipoprotein cholesterol (HDLChol.) were determined according to the method described by Tietz (1995) and Friedwald *et al.* (1972). Very Low-Density Lipoprotein Cholesterol (VLDL-chol.) and Low-Density Lipoprotein Cholesterol (LDL-chol.) were determined using the formula described by Friedwald *et al.* (1972). Atherogenic index (AI) was calculated using the formula described by Lamarche *et al.* (1996), while cardiac index and coronary artery index were determined as previously described by Kang *et al.* (2004).

Biomarker enzyme assays

The method described by Reitman and Frankel (1957) was used to determine the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma and liver homogenates. Also, the method described by Wright *et al.* (1972) was used to determine alkaline phosphatase (ALP) activity in plasma and liver homogenates.

Statistical analysis

Data were subjected to statistical analysis using a one-way analysis of variance followed by the Duncan multiple range test (SPSS version 20, SPSS Inc., Chicago. IL, USA). Data (where applicable) was presented as mean ± SEM. Significance levels were considered at $P<0.05$ while the graphs were generated using GraphPad Prism 6 software (GraphPad Software, California, USA). IC_{50} values were calculated using Origin

software, version 7, USA.

RESULTS

GCMS analysis of aqueous seed extract of *A. digitata*

GCMS analysis indicated the presence of several phytochemical constituents in the aqueous seed extract of *A. digitata*. The most abundant phytochemicals detected were 8-Octadecenoic acid (5.95%), 3,4-Dimethyldihydrofuran-2,5-dione (5.88%) and cyclopentadecanone (3.42%) (Figure 1, Table 1).

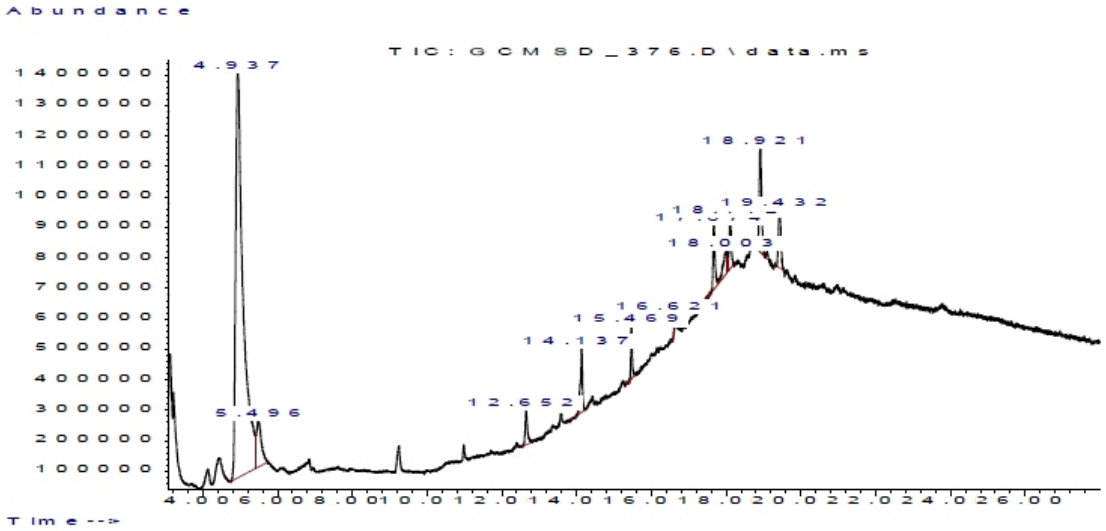

Figure 1: GCMS chromatogram of aqueous seed extract of *Adansonia digitata*.

Table 1: Detected phytochemical constituents of aqueous seed extract of *Adansonia digitata*.

Components	Peak Number	Retention Time	Area	Peak Height	Concentration (%)
8-Octadecenoic acid	10	18.921	5.95	338112	5.952%
3,4-Dimethyldihydrofuran-2,5-dione	2	5.496	5.88	147361	5.880%
Cyclopentadecanone	11	19.432	3.42	184627	3.423%
Cycloheptasiloxane	4	14.137	3.04	206195	3.040%
Hexadecanoic acid	7	17.674	3.03	209248	3.030%
Hexadecanoic acid	9	18.112	2.54	163803	2.537%
1-Methyl-1-(2-hydroxyethyl)-1-silacyclobutane	8	18.003	1.87	72945	1.867%
Cyclohexasiloxane	3	12.652	1.86	109501	1.859%
2,5-Dihydroxybenzoic acid	5	15.469	1.66	169326	1.662%
1-Butene, 1-(methylthio)-, (Z)-	6	16.621	0.28	748136	0.278%
Dopamine,					
1-Butanol	1	4.937	70.47	1366205	70.472%

In-vitro antioxidant effects of aqueous seed extract of *A. digitata*

The DPPH scavenging radical properties of aqueous seed extract of *A. digitata* were significantly higher ($P<0.05$) compared to BHT with IC_{50} values of 0.14±0.00 mg/mL and 0.17±0.00 mg/mL, respectively (Figure 2, Table 2). However, the total antioxidant capacity of BHT was significantly higher ($P<0.05$) compared with the aqueous seed extract of *A. digitata* with IC_{50} values of 0.15±0.00 mg/mL and 0.23±0.01 mg/mL, respectively (Figure 3, Table 2). Also, the aqueous seed extract of *A. digitata* possesses significantly lower ($P<0.05$) nitric oxide reducing power (Figure 4) and significantly higher ($P<0.05$) hydrogen peroxide decomposing activities compared with vitamin C and BHT (Figure 5, Table 2).

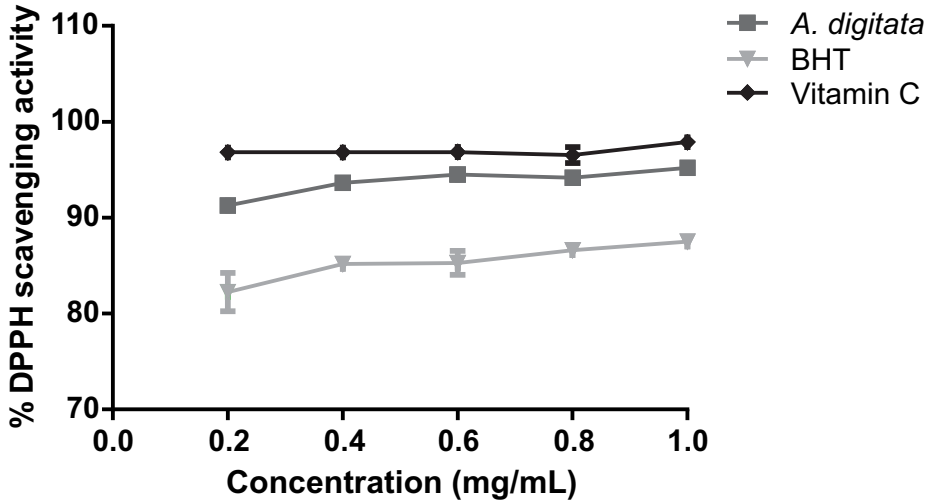

Figure 2: DPPH scavenging activities of aqueous seed extract of *Adansonia digitata*. Values are means ± SEM of triplicate determinations, BHT = Butylated hydroxytoluene.

Table 2: IC_{50} values for various *in-vitro* antioxidant activity of aqueous seed extract of *Adansonia digitata*.

	IC_{50} (mg/mL)		
Parameter	A. *digitata*	BHT	Vitamin C
DPPH	0.14±0.00[a]	0.17±0.00[b]	0.13±0.00[c]
TAC	0.23±0.01[a]	0.15±0.00[b]	0.94±0.00[c]
NO	0.85±0.15[a]	0.30±0.05[b]	0.34±0.02[b]
H_2O_2	0.17±0.03[a]	0.15±0.00[a]	0.15±0.01[a]

Values are means ± SEM of triplicate determinations

BHT = Butylated hydroxytoluene, DPPH = 2,2-diphenyl-1-picrylhydrazyl scavenging activities, TAC = Total antioxidant capacity, NO = Nitric oxide reducing power, H_2O_2 = Hydrogen peroxide decomposing activity, IC_{50} = Half maximal inhibitory concentration. Values with different superscripts across the row are significantly different at $P<0.05$.

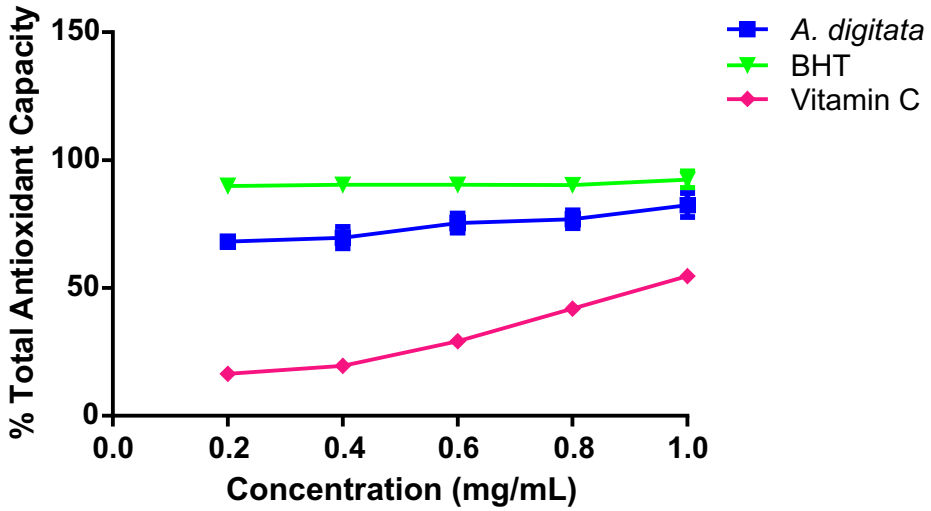

Figure 3: Total antioxidant capacity of aqueous seed extract of *Adansonia digitata*. Values are means ± SEM of triplicate determinations, BHT = Butylated hydroxytoluene.

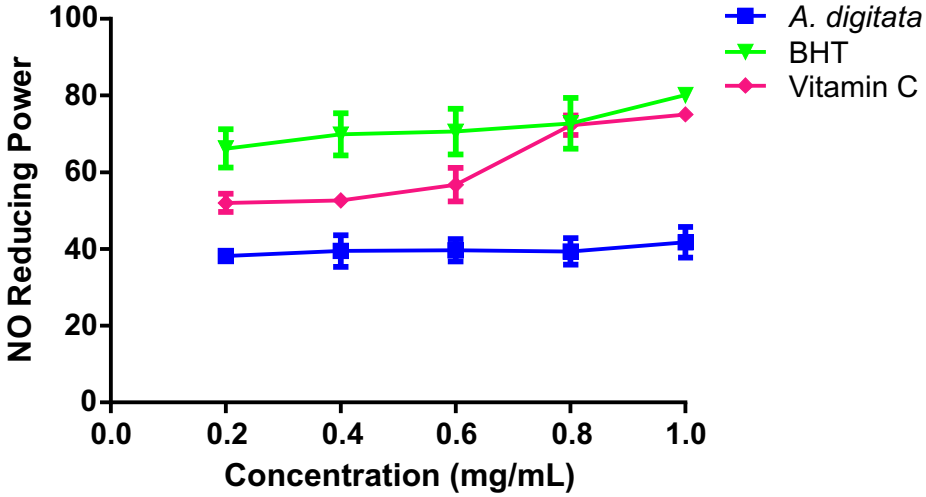

Figure 4: Nitric oxide reducing power of aqueous seed extract of *Adansonia digitata*. Values are means ± SEM of triplicate determinations, BHT = Butylated hydroxytoluene.

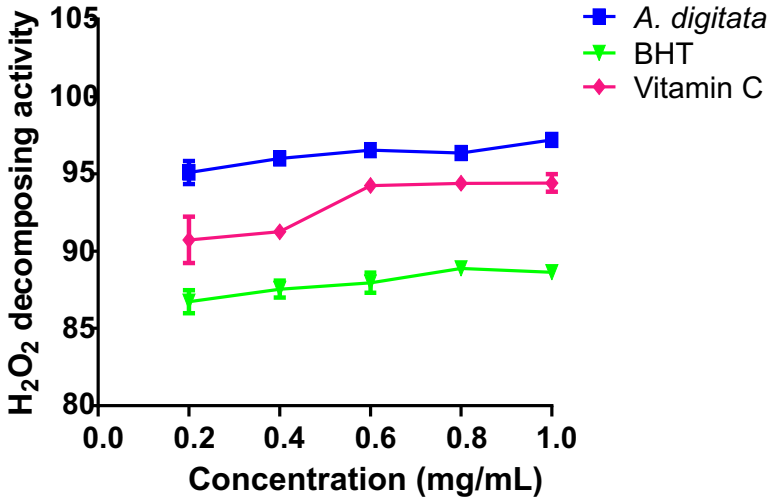

Figure 5: Hydrogen peroxide decomposing activity of aqueous seed extract of *Adansonia digitata*. Values are means ± SEM of triplicate determinations, BHT = Butylated hydroxytoluene.

Acute and sub-acute toxicity studies of aqueous seed extract of *A. digitata*

In the acute toxicity test, no death was recorded in the first and second phases of the study. Mild increases in breathing and hypoactivity were recorded at doses above 2000 mg/kg body weight aqueous seed extract of *A. digitata* (Table 3). Other toxicity signs such as gasping, writhing, loss of appetite, and change in the colour of the eye or fur texture were not observed. The LD_{50} was estimated at above 5000 mg/kg body weight.

Table 3: Acute toxicity of aqueous seed extract of *Adansonia digitata* in Swiss albino mice.

Groups	Dosage mg/kg bwt	Number of mice/group	Number of dead mice/group	% death of mice	Toxicity Signs
Group 1	0	n=3	0/3	0	None
Group 2	500	n=3	0/3	0	None
Group 3	1000	n=3	0/3	0	None
Group 4	2000	n=3	0/3	0	Increase breathing rate
Group 5	3000	n=3	0/3	0	Increase breathing rate
Group 6	5000	n=3	0/3	0	Hypoactive

Significant liver-body weight increase ($P<0.05$) was observed in 1000 mg/kg body weight treated mice after 14 days of treatment compared with the control (Figure 6). However, no significant ($P>0.05$) alterations were observed in plasma total protein concentration at 500 and 1000 mg/kg body weight compared with control mice (Figure 7).

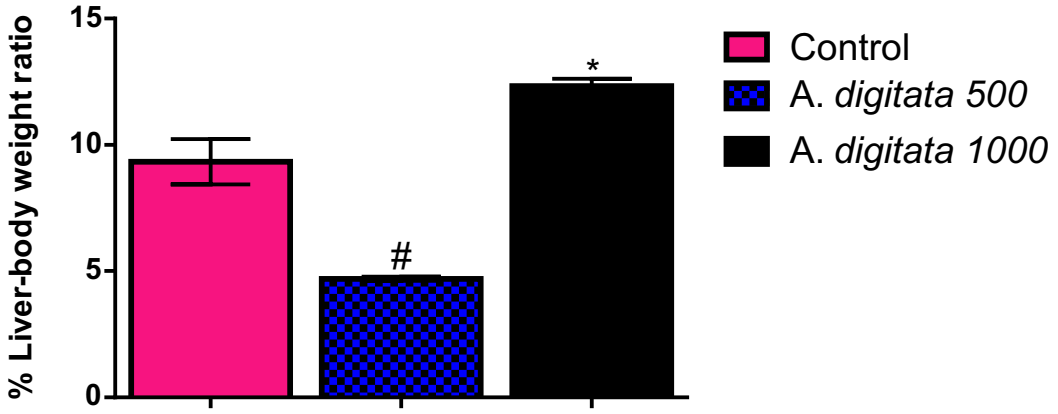

Figure 6: Liver-body weight ratio of mice after 14 days of administration of *Adansonia digitata* seed extract. Values are mean ± SEM, n=5, #*Superscript are significantly different at $P<0.05$ from the control.

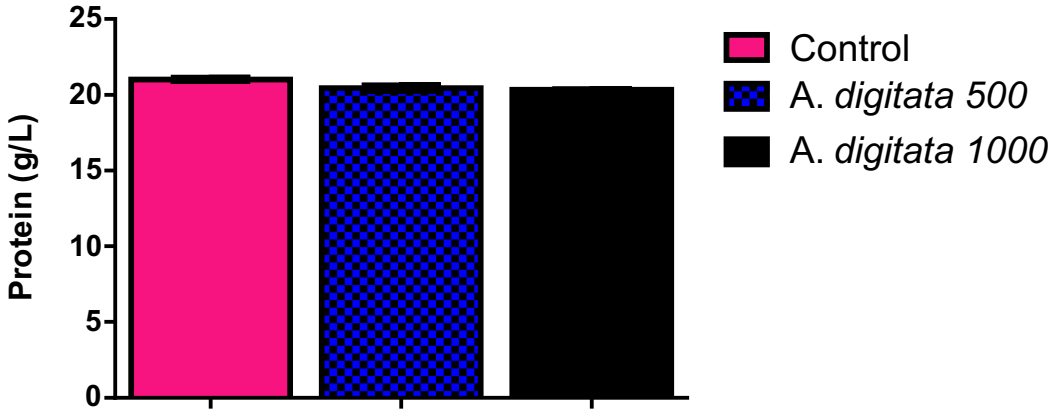

Figure 7: Effects of aqueous seed extract of *Adansonia digitata* on plasma total protein concentration of mice after 14 days of administration. Values are mean ± SEM, n=5.

Lipid profile effects of aqueous seed extract of *A. digitata*

The administration of aqueous seed extract of *A. digitata* for 14 days at 500 and 1000 mg/kg body weight did not cause any significant alteration (P>0.05) in plasma total cholesterol, high density, and low-density lipoprotein cholesterol compared with the control (Figure 8). However, a significant increase (P<0.05) was observed in plasma triacylglycerol at 500 mg/kg body. In the liver, significant increases (P<0.05) were observed in liver cholesterol and liver protein at 500 mg/kg body weight compared with the control (Figure 9). The administration of the aqueous seed extract of *A. digitata* did not significantly (P>0.05) alter coronary artery, cardiac and atherogenic indices, except at 500 mg/kg body weight which was significantly decreased (Figure 10).

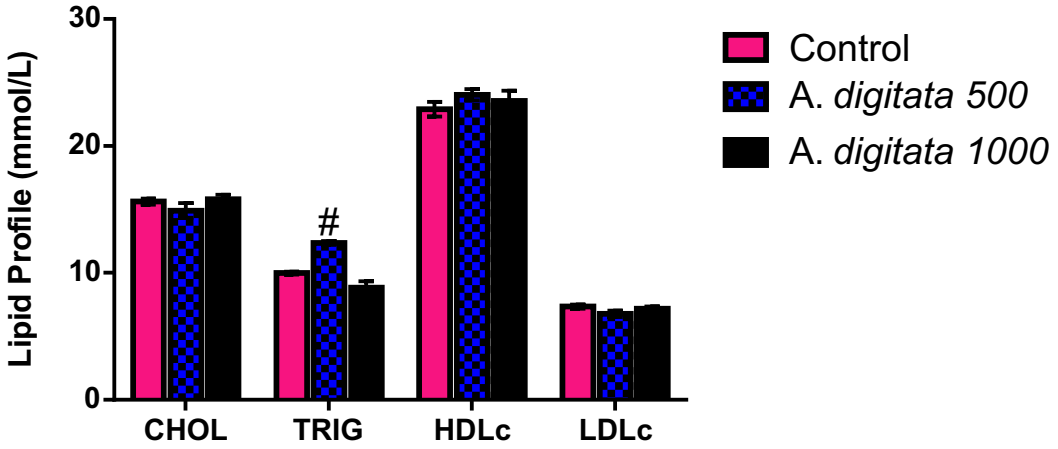

Figure 8: Effects of aqueous seed extract of *Adansonia digitata* on plasma lipid profile of mice after 14 days of administration. Values are Mean ± SEM, n=5, #Superscript are significantly different at P<0.05 from the control. CHOL = Total cholesterol, TRIG = Triacylglycerol, HDLc = High Density Lipoprotein cholesterol, LDLc= Low Density Lipoprotein Cholesterol.

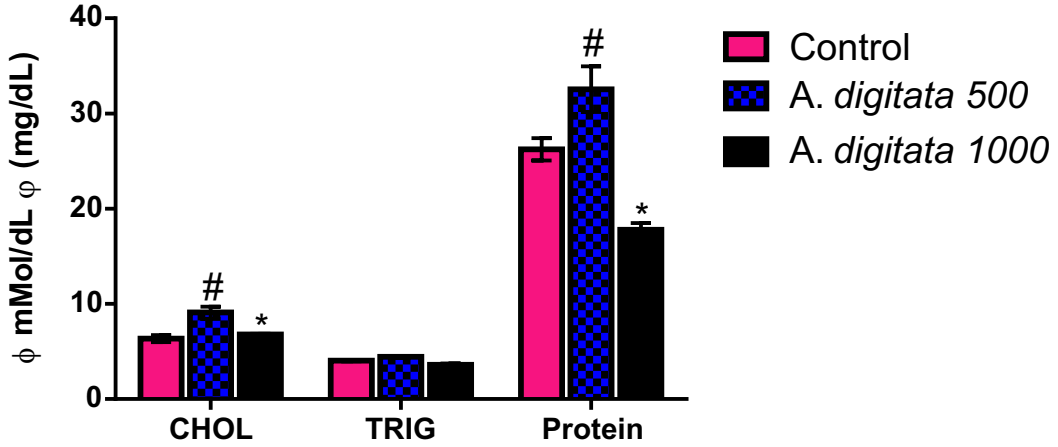

Figure 9: Effects of aqueous seed extract of *Adansonia digitata* on liver total cholesterol, triacylglycerol, and total protein concentration of mice after 14 days of administration. Values are mean ± SEM, n=5, #*Superscript are significantly different at P<0.05 from the control. ϕ = units for cholesterol and triacylglycerol, φ = units for protein. CHOL = Total cholesterol, TRIG = Triacylglycerol.

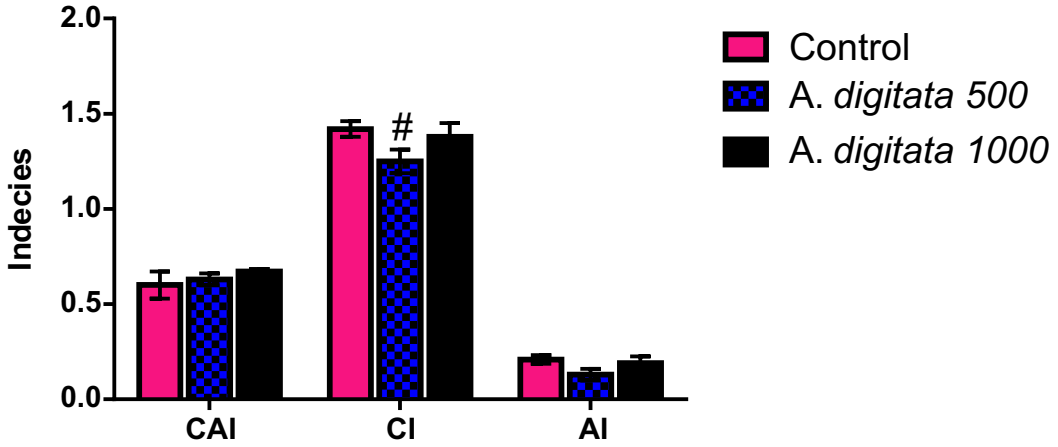

Figure 10: Coronary artery, cardiac and artherogenic indices following treatments of *Adansonia digitata* in mice for 14 days. Values are mean ± SEM, n=5, #Superscript are significantly different at P<0.05 from the control. CAI = Coronary artery indices, AI = Atherogenic indices, CI = Cardiac indices.

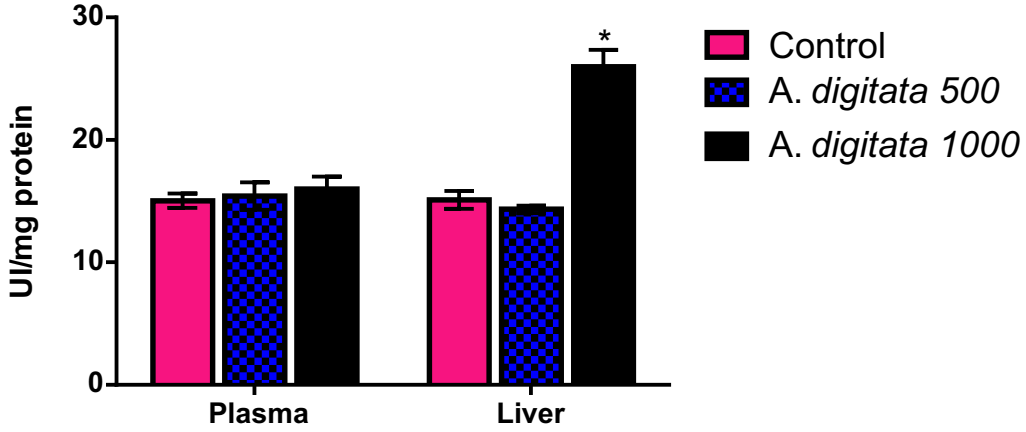

Figure 11: Aspartate aminotransferase activities in plasma and liver of mice after 14 days of administration of aqueous seed extract of *Adansonia digitata*. Values are mean ± SEM, n=5, *Superscript are significantly different at P<0.05 from the control.

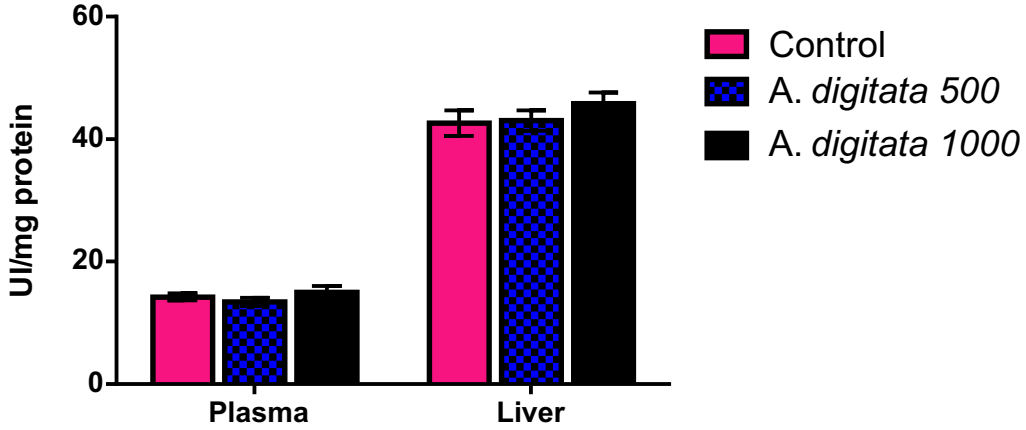

Figure 12: Alanine aminotransferase activities in plasma and liver of mice after 14 days of administration of aqueous seed extract of *Adansonia digitata*. Values are mean ± SEM, n=5.

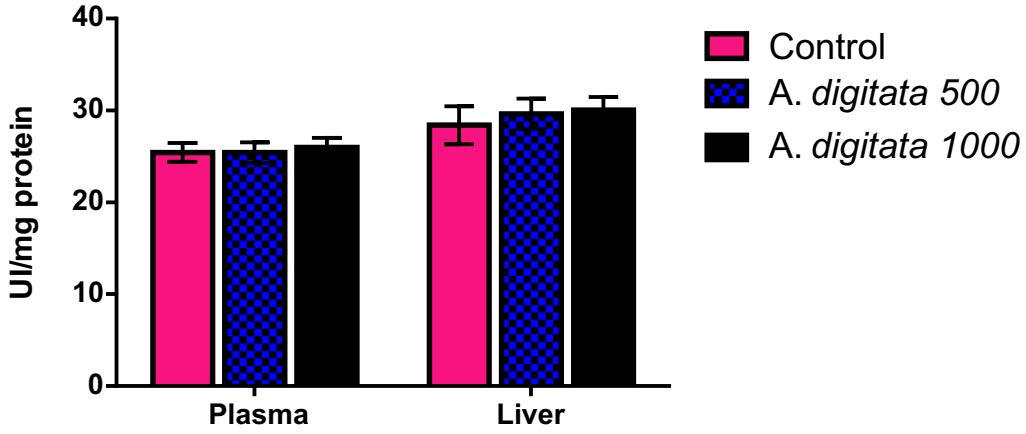

Figure 13: Alkaline phosphatase activities in plasma and liver of mice after 14 days of administration of aqueous seed extract of *Adansonia digitata*. Values are mean ± SEM, n=5.

DISCUSSION

Gas Chromatography Mass-Spectrometry (GCMS) revealed important phytoconstituents in the aqueous seed extract of *A. digitata*. It possesses several important constituents including magnesium, potassium, iron, phosphorous, and calcium in the seeds (Nkafamiya *et al.,* 2007). Thus, it was suggested that the seed could contribute significantly to the overall daily intake of these elements (Gadanya *et al.,* 2014). Furthermore, the seed is an important source of energy, proteins, oils, and relatively low-fat value (Igboeli *et al.,* 1997; Nnam and Obiakor, 2003).

The aqueous seed extract of *A. digitata* expressed promising *in-vitro* antioxidant activities, which in some cases were higher than the reference compound vitamin C and BHT. The phytochemical contents present in plant extract have been attributed to their biological activities. For instance, the antiallergenic, anticarcinogenic, anti-inflammatory, antimicrobial, antiviral, and antioxidant properties have been linked to the presence of some compounds in plant extracts (Ralte *et al*., 2022). This implies that the aqueous seed extract of *A. digitata* could provide beneficial antioxidant activities, such as protecting the cells from the deleterious effects of reactive oxygen species (ROS) by scavenging them. Furthermore, they could prevent the spread of oxidative stress and intercept free radical chain reactions of lipid peroxidation.

The acute toxicity of aqueous seed extract of *A. digitata* in experimental mice did not cause mortality of the animals or sign of gasping, writhing, loss of appetite, or change in eye colour or fur texture. The calculated LD_{50} was greater than 5000 mg/kg body weight. This suggests that the extract is relatively safe. The organ-body weight ratio was significantly lower ($P<0.05$) at a low dose and increased at a high dose in the sub-acute toxicity test. This observation suggests that the extract could stimulate the feeding patterns of the animals with no signs of shrinking of the liver or its enlargement. Also, no significant alterations were observed in a majority of the biochemical parameters assayed.

Atherogenic indices are indicators of the risk of developing cardiovascular diseases (CVD). The higher the value, the higher the risk of developing CVD (Kazemi *et al*., 2018). The aqueous seed extract of *A. digitata* did not significantly alter the lipid profile of animals during the 14 days of administration and did not significantly alter cardiac indices for the duration of administration. These observations suggest that the extract will not predispose animals to cardiovascular diseases. Also, the extract did not affect protein concentrations in plasma and liver homogenate. This observation indicates that the protein synthesis is not adversely affected by the extract and will not affect protein functions such as the transportation of biomolecules. The results obtained from acute and sub-acute toxicity indicated that aqueous seed extract of *A. digitata* demonstrated a high safety margin. The animals tolerated up to 5000 mg/kg body weight of the extract orally with no death of mice recorded and no significant alterations were observed in the

assayed biochemical parameters. Other safety issues of *A. digitata* parts have been reported. For instance, the methanol extract of the leaf is protective against sodium arsenite-induced toxicities in rats, protecting against circumstances of co-exposure and cases of arsenicosis (Adegoke *et al.*, 2017).

Investigations were carried out to determine the effect of aqueous seed extract of *A. digitata* on the plasma and liver of mice after 14 days of treatment. Key enzymes evaluated included aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities in plasma and the liver. The ALT and AST are markers of liver damage and are used to assess liver cytolysis, while ALT is a more sensitive biomarker for liver toxicity than AST (Pramyothin *et al.*, 2006). AST catalyses the conversion of aspartate and alpha keto-glutarate to oxaloacetate and glutamate (Kimmich *et al.*, 2002). The treatment with aqueous seed extract of *A. digitata* showed a non-significant increase in AST activities in the plasma and liver except at a dose of 1000 mg/kg body weight compared with the control. This increase could suggest increasing synthesis of the enzymes in the liver rather than damage to the hepatocytes or the leakage of the enzyme as no corresponding increase in plasma AST activity was observed (Malomo, 2000; Ogunlana *et al.*, 2013).

ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum, and it is found in blood cells, the pancreas, the brain, the kidney, skeletal muscle, the heart, the bone, and the liver (Timbrell, 1996). ALP is used to assess the integrity of the plasma membrane and as a standard marker of biliary tract obstruction (Akanji *et al.,* 1993; Manjunatha *et al.*, 2005). No significant increase was observed in ALP activities in the plasma and liver after 14 days of treatment, indicating no adverse effects on the activities of the enzyme. *A. digitata* leaves and stem-bark extracts have demonstrated their protective abilities by decreasing elevated liver marker enzymes in alloxan-induced diabetic rats (Yakubu *et al.*, 2020). This was attributed to the presence of free radical scavenging bioactive compounds in the extracts (Li *et al.*, 2015)

CONCLUSION

The study suggests that aqueous seed extracts of *A. digitata* are practically safe at the lower doses of 500 and 1000 mg/kg body weight. The seed contains important bioactive compounds and possesses significant *in vitro* antioxidant activities.

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