

Effect of Methanolic Leaf Extract of *Adansonia digitata* and *Cochorous olitorous* on selected Biochemical parameters in gamma-irradiated Wistar rats

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Abstract

Animal Exposure to radiation increases the physiological free radical concentrations from 25 to 120 ug/dl, thus increasing the spate of oxidative damages. Thus, the study investigated the effect of methanol leaf extract (MLext) of *Adansonia digitata* and *Cochorous olitorous* on heamatological, antioxidant enzymes, lipid profile and liver integrity of gamma-irradiated Wistar rats. A total of 48 rats were grouped into 8 of 6 animals each; Group A received water and rat chow (Positive Control), group B is irradiated but not-treated (Negative Control), group C, D, E, F were irradiated and received MLext of *A. digitata* and *C. olitorous* at 500 and 1000 mg/kg body weight, while group G and H received combination of MLext respectively for 7 days. The rats were sacrificed 24 hours after 7days administration. Blood and tissues were collected for biochemical analysis. Phytochemical screening of MLext of *A. digitata* and *C. olitorous* leaf revealed the presence of saponins, alkaloids, tannins, polyphenols and flavonoids. Heamatological parameters, antioxidant enzymes, lipid profile level and selected liver enzymes were significantly ($P<0.05$) decreased in negative groups when compared with the normal control. However, after 7 days oral administration of the MLext of *A. digitata* and *C. olitorous* leaf, there were significant ($P<0.05$) increase in

the hematological parameter, antioxidant enzymes status and high density lipoproteins (HDL) level of treated group, whereas Alanine amino transferase (ALT), Alanine phosphatase (ALP), Aspartic amino acid transferase (ALT), Low density lipoprotein (LDL), Triglyceride (TRG) were significantly ($P < 0.05$) decreased. We can therefore conclude that MLeet of *A. digitata* and *C. olitorius* may be explored as oral remedy in treatment of free radical induced diseases.

Keywords: Irradiated, Antioxidant, Free Radical, Hemoglobin, *Cochorus olitorius*, *Adasonia digitata* and Phytochemical

1.0 Introduction

Virtually all human are exposed to radiation in one way or the other, either through continuous romance to the several sophisticated technological materials such as phones, laptops, telecommunication gadget and head/ear phones and the very radiotherapy machines, hence the need for protection against exposure and aftermath effect of radiation [1]. Most of the ubiquitous technological materials emit approximately 4-5 grey of gamma radiation, the emitted dose of radiation possesses the potentials to generating and thus increasing the physiological level of free radicals in human system from 24 ug /mol to 120 ug /mol [2], hence an hallmark of oxidative stress.

Radiation can be ionizing or non-ionizing. Human exposure to grey of radiation, result in electron leakages, in the form of a high electron density centered unpaired electron species, called free radicals [3]. Free radical have been implicated in the genesis of various disease and disorders, capable of elongating oxidative stress condition.

Oxidative stress have been implicated in various human diseases, they have been linked to free radical and antioxidant imbalance [4]. They have been studied and have been linked to several diseases and ailment such as cancer, stroke, diabetes, cardiovascular diseases, malaria and ischemia reperfusion diseases [5]. Free radicals attack macromolecules (protein, lipids, nucleic acids) to mediate chain of radical reactions such as in lipid peroxidation, protein oxidation/oligomerization and nucleic acid oxidation, leading to cell damage and such damages is the genesis of pathological aging [6].

Several diseases has been claimed to have been successfully treated with synthetically produced drugs, but most of which have come with several shortcomings, such as accessibility of the drugs, cost implication, adverse drug reaction etc. Hence the need for a more natural products, that is cheaper, accessible, with little or no side effect. Natural product includes fruits, vegetables, water and water. Several Plants materials have been usurped by herbal medicine practitioners in the treatment of several diseases and disorders. The extract of *Pterocarpuss antalinoides* and *Aloe vera* was reported to increase the muscle toning potential .This indicates that such plants may be useful in the management of hypertension [7]. The reversal of dyslipidemia by *Terminalia catappa* could have been reported in the inhibition lipids peroxidation [8].Hence, the aims and objectives of this study is to evaluate the effect of methanol leaf extract of *Adansonia digitata* and *Cochorous olitorous* on selected biochemical parameter associated with free radicals in male Wistar rats. Biochemical parameters such as heamatological, antioxidant enzyme status, lipid profile, liver enzymes and histology. As well as relate the acclaimed pharmacological activities of *Adansonia digitata* and *Cochorous olitorous* to its effects on irradiated Wistar rats.

Adansonia digitata

Adansonia digitata also known as baobab, belongs to the *Malvaceae* family, is widespread throughout the hot, drier regions of tropical Africa [9]. It's commonly called tree of life in English, “Kukah” in Northern Nigeria, “Luru” in the South-western Nigerian [10]. Baobab leaves have been investigated to contain secondary metabolite responsible for its pharmacology use as well as properties beneficial to overall health. Baobab leaves are used medicinally as a diaphoretic, astringent, expectorant and prophylactic against fever [8, 9, 11, and 12]. Baobab leaves, bark and fruits are used as food as well as medicinal in many parts of Africa. The leaves have been established to be traditionally used in the treatment of aneamia, inflammation, dysentery, diseases of the urinary tract, ophthalmic and otitis in some parts of Africa [13]. Studies have reported that baobab leaves is an important source of iron, having a high iron content compared to numerous other wild – gathered foods and are a rich source of calcium [11, 13]. Baobab leaves possess antioxidant properties, several reviewed information on the phytochemistry and pharmacology of baobabs have been reported by [14].

Cochorous olitorous

Cochorous olitorous belongs to the family *Tiliaceae* and is known as a fiber crop. It is commonly called Jute mallow in English and “Ewedu” in the South-western Nigeria [15]. It is an annual herb with a slender stem and an important green leafy vegetable in many tropical area including Egypt, Sudan, India, Bangladesh [16]. The leaves either fresh or dried are cooked into a thick viscous soup or added to stew or soup and are rich sources of vitamins and minerals [17]. It has been reported to be demulcent, deobstruent, diuretic, lactagogue, purgative and tonic [17,18]. *Cochorous olitorous* is used in folklore medicine for aches, pains, dysentery, enteritis, fever, pectoral pains, and tumors [19, 20 21]. It has also been reported to possess antimicrobial activity [20]. A study reported its antifungal activity in different solvent of the leaves [21]. They are important because microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases [22]. The increase in resistance of microorganisms is in part responsible for the search for natural antimicrobial agent from various medicinal plants [22].

2.0. Materials and Methods

Plant materials

Adansonia digitata and *Corchorus olitorous* leaves were obtained from the University of Ilorin and Oja-oba Market, Ilorin, Kwara State. They were authenticated at the Department of Plant Biology, University of Ilorin and the Voucher Numbers for *Adansonia digitata* and *Corchorus olitorous* are UICH/001/951 and UITH/002/154 respectively. The leaves of the two plants were removed from their stalks, washed thoroughly with clean water and dried at room temperature for one week. The samples were then pulverized to powdery form and stored in an air dried polythene bag.

Plant extraction

The pulverized plant was macerated in 2,000 ml (2 L) of methanol (solvent) contained in a glass cylinder. After 72 hours, the solution was filtered with Whatman no. 4 filter paper, the resulting filtrate was then concentrated using rotary evaporator. The resulting percentage yield of *A. digitata* was 14.8% and *C. olitorous* was 4.6% were then reconstituted into

aqueous solution and administered to irradiated rats at 500 and 1000 mg/kg body weight respectively.

Animals

Forty-eight (48) adult male albino rats weighing of 200-210g were purchased from the animal Unit of Department of Biochemistry, University of Ilorin, Nigeria. They were housed in clean and well ventilated cages and maintained on normal rat feed and clean water for two weeks. The use of experimental animal for this research was consider and approved by the University of Ilorin ethical committee.

Radiation exposure

The animals were exposed to a single dose of 6 Grey whole body gamma radiation. The radiation exposure was carried out in the Department of Radiotherapy, College of Medicine, UITH, Ilorin. The animals were kept in an improvised cage to restrict their movement and to ensure uniform and effective exposure. The animal distribution into groups were as follows.

Animal distribution into groups

Control	Non-irradiated, non-treated
IR	Irradiated, Non-treated
IR+ E ₁ 500mg/kg weight (RE _{1 500})	Irradiated animals treated with 500mg/kg body weight
IR + E ₁ 1000mg/kg weight (RE _{1 1000})	Irradiated animals treated with 1000mg/kg body weight
IR +E ₂ 500 mg/kg weight (RE _{2 500})	Irradiated animals treated with 500mg/kg body weight
IR +E ₂ 1000mg/kg weight (RE _{2 1000})	Irradiated animals treated with 1000ml/kg body weight
IR+E ₁ +E ₂ 500mg/kg weight (R E _{1+ E₂ 500})	Irradiated animals treated with 500ml/kg body weight
IR+ E ₁ +E ₂ 1000mg/kg weight (R E _{1+ E₂ 1000})	Irradiated animals treated with 1000ml/kg body weight

Where E_1 is *Adasonia digitata* and E_2 is *Cochorous olitorous*

Preparation of tissue homogenates, serum and histology

The sera and liver supernatants were prepared as previously described (23). The rats were anesthetized in a glass jar containing cotton wool soaked in diethyl ether. The unconscious rat was quickly removed and the neck area cleared of fur. The jugular veins were cut and the blood collected into clean and dry test tubes were allowed to clot at room temperature for 10 min and centrifuged at $4500 \times g$ for 15min. The rats were then dissected, the liver removed and cleaned, weighed and homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were then centrifuged at $10,000 \times g$ for 10min to obtain the supernatants, which were later transferred into specimen bottles and kept Frozen (24). The sera and supernatants were used within 24h for their respective assays. Some part of the tissue (liver) was cut and weighed into a universal test tube containing formalin solution to preserve the tissue for histological analysis.

Chemicals and reagents

All chemical and assay kits were products of Randox Laboratories, County Antrim, UK. Vitamin C were products of Sigma-Aldrich, St. Louis, USA. All other reagents were of analytical grade and prepared with distilled water.

Determination of biochemical parameters

The biochemical parameters were determined using standard procedures as described for protein, albumin [22, 23] and bilirubin [25a], ALP [25b] AST and ALT [25c].

Determination of Alkaline Phosphatase Activity

The procedure described by [26b] was employed for the determination of alkaline phosphatase activity. A known volume (0.2 ml) of the appropriately diluted homogenate was pipetted into the test tube, and then, 2.2 ml of Carbonate buffer (0.1M) and 0.1 ml of $MgSO_4 \cdot 7H_2O$ (0.1M) was added. The samples was incubated for 30 minutes at $37^{\circ}C$. Then, 0.5 ml of the substrate (p-nitrophenyl phosphate 10 mM) was added. The mixtures was thoroughly mixed and incubated for 30 minutes at $37^{\circ}C$. The reaction was stopped by adding 2.0 ml of 1N NaOH. Blank was obtained by

following the same procedure, only that 0.2 ml of distilled water was used in lieu of 0.2 ml of diluted homogenates. The absorbance was read spectrophotometrically at 400 nm. Enzyme activity was calculated using the following expression;

$$\text{Enzyme activity (nM/min/ml)} = \frac{\text{AB/min} \times 1000 \times \text{TV} \times \text{F}}{9.9 \times \text{SV} \times \text{L}}$$

Determination of Aspartate aminotransferase activity

The procedure described by [27] was used to assay the activity of aspartate amino transferase. 0.1 ml of diluted sample was pipetted into a test tube and 0.5 ml of reagent A was added. Also, 0.5 ml of reagent A was added to 0.1 ml of distilled water in another test tube to constitute the blank. The mixture was incubated at 37⁰C for 30 minutes. Then, a known volume (0.5 ml) of reagent B was added into all the test tubes. The mixtures was mixed and left undisturbed for 20 minutes at 25⁰C. The reaction was stopped by adding 0.5 ml of 0.4N NaOH. The solution was mixed and absorbance was read against blank after 5 minutes at 468 nm. The enzyme activity will be obtained from the calibration curve.

Determination of Alanine Aminotransferase Activity

The procedure described by Reitman and Frankel [27] was used for the determination of the activity of alanine aminotransferase. 0.1 ml of the diluted sample was pipetted into a test tube and 0.5 ml of reagent A was added. Also, 0.5 ml of reagent A was added to 0.1 ml of distilled water in another test tube to constitute the blank. The mixture was incubated at 37⁰C for 30 minutes. Then, 0.5 ml of reagent B was added into all the test tubes. The mixtures was mixed and left undisturbed for 20 minutes at 25⁰C. The reaction was stopped by adding 0.5 ml of 0.4N NaOH. The solution was mixed and absorbance was read against blank after 5 minutes at 546 nm. The enzyme activity was obtained from the calibration curve.

Protein

Protein concentration in tissues was determined using the Biuret method [28]; A known volume (1 ml) of the appropriately diluted homogenate was pipetted into test tube and 4 ml of Biuret reagent was added. This was thoroughly mixed and left undisturbed for 30 minutes at room temperature. 4 ml of Biuret reagent was added to 1 ml of distilled water in

another test which constituted the blank. The absorbance was read against the blank at 540 nm. Protein concentrations of the samples was calculated from a calibration curve obtained using Bovine Serum Albumin (BSA).

Albumin

The procedure described by [21] was used for the determination of albumin in the serum of the animals. A known volume (0.01 ml) of the appropriately diluted serum was pipetted into a test tube and 3 ml of reagent B was added. Also, 3 ml of reagent B was added to 0.01 ml of distilled water in another test tube to constitute the blank. For the standard, 3 ml of reagent B was added to 0.01 ml of reagent A in another test tube. The mixture was incubated at 37°C for 10 minutes. Absorbance was read spectrophotometrically at 630 nm. Concentration of standard = 50.3 g/L.

Calculation:

$$\text{Albumin concentration (g/L)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Concentration of standard}$$

Serum Bilirubin

Bilirubin was determined by adopting the procedure described by [21]. For total bilirubin, 0.2 ml of reagent A, a drop of reagent B and 1 ml of reagent C was pipetted into a test tube. A known volume (0.2 ml) of the appropriately diluted serum was pipetted into the test tube. 0.2 ml of reagent A, a drop of reagent B and 0.1 ml of reagent C was also be pipetted into another test tube, and 0.2 ml of distilled water was added to serve as the blank. The mixture was incubated at 37°C for 30 minutes. A known volume (1 ml) of reagent D was added into all the test tubes. The mixtures was incubated further at 25°C for 30 minutes. Absorbance was read at 578 nm.

Antioxidant assay

Malondealdehyde (MDA) [27], Glutathione-s-transferase (GST) and Glutathione peroxidase (GPx) [28], Catalase (CAT), Super oxide dismutase (SOD) [25].

Glutathion-S-Transferase assay

The principle is based on the fact that all known glutathione-S-transferase demonstrate a relatively high activity with 1-chloro-2, 4 - dinitrobenzene as the second substrate, consequently, the conventional assay for glutathione-S-transfease activity utilizes 1-chloro-2, 4, - dinitrobenzene as substrate. When this substance is conjugated with reduced glutathione, its absorption maximum shifts to a longer wavelength. The absorption increases at the new wavelength of 340 nm and provides a direct measurement of the enzymatic reaction [27].

Glutathione peroxidase (GPx)

In order to determine the glutathione peroxidase 500 ul phosphate buffer was added to 100 ul NaN₃, after which 200 ul GSH was added to the grand mixture, then 100 ul H₂O₂ solution was further added. Added to the entire mixture was 500 ul of the sample, and 600 ul distilled water. The whole mixture will be incubated at 37 oC for 3minutes after which 0.5 ml of TCA was added and thereafter centrifuge at 3000 rpm for 5minutes. To 1 ml of each of the supernatant, 2 ml of K₂HPO₄ and 1 ml DNTB was added and the absorbance was read at 412 nm against the blank.

Super oxide dismutase (SOD)

The level of SOD was determined by the method [27]. One ml of sample was diluted in 9 ml of distilled water to make 1: 10 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The absorbance at 480 nm was monitored every 30 seconds for 150 seconds. One unit of SOD activity was provided as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline % SOD inhabitation= increase in absorbance for substrate/increase in absorbance of blank × 100

Heamatological Studies

Automated Heamatological Analyzer (Sysmex Heamatology Systems, Sysmex Ameria Inc., model no. KX-21N, Kobe, Japan) was used to

determine the levels of heamoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), lymphocytes and platelets.

Lipid profile assay

Malondialdehyde (MDA)

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) that maybe produced during lipid peroxidation as described by [26]. This method is based on the reaction between 2- thiobarbituric acid (TBA) and malondialdehyde: an end product of lipid peroxidation. On heating in acidic medium, the product is pink-coloured compound known as chromophore which absorbs maximally at 532 nm and which is extractable into organic solvents such as butanol. The results are expressed as the amount of free MDA produced.

Triglycerides

Triglycerides were measured by colorimetric method as described by [27]. Triglycerides, normally are hydrolysed in the presence of lipase into glycerol and fatty acids, and the product, glycerol, is acted upon by glycerol kinase to produce glycerol-3-phosphate and adenosine diphosphate. The activated glycerol (glycerol-3-phosphate) is further oxidized by glycerol phosphate oxidase to form dihydroxy acetone phosphate and hydrogen peroxide. The hydrogen peroxide formed reacts with 4-aminophenazone and 4-chlorophenol in the presence of peroxidase to give quinineimine, an indicator of triglyceride that can be measured spectrophotomerically at 500 nm. Trygliceride concentration = $\frac{\text{absorbance (sample)}}{\text{absorbance (standard)}} \times 200 \text{ mg/dl}$

HDL-cholesterol (High density lipoprotein-cholesterol)

HDL-cholesterol was determined in serum by the method of [20]. The Randox HDL-cholesterol precipitant Kit was used. Low density lipoproteins (LDL and VLDL) and chylomicron fractions precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions after centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, was determined.

LDL-cholesterol

LDL-cholesterol was determined by the method of Friedewald *et al.* (1972).

LDL-Cholesterol = total cholesterol – trygliceride – HDL – cholesterol/2 (mg/dl)

Total cholesterol

The level of cholesterol was determined using Randox kits, a method of enzymatic hydrolysis described by [24]. The cholesterol was determined after enzymatic hydrolysis and oxidation. Cholesterol ester was hydrolysed to free cholesterol and fatty acids by cholesterol esterase. Free cholesterol was then oxidized into cholesterol-3-one and hydrogen peroxide in a reaction catalyzed by cholesterol oxidase. The peroxidase catalyzes the reaction between H₂O₂ and 4-amino phenazone and hvdroxyl benzoate to produce quinoneimine whose colour intensity is proportional to the total holesterol present in the sample. The indicator quinoneime was formed hydrogen-peroxide and 4- aminoantipyrine in the presence of phenol and peroxidase. Than the amount was mixed and incubated for 10 minutes at 20-25 °C or 5 minutes at 37 °C. Measurements using a sample and A standard against reagent blank within 60 minutes at 500 nm was performed. Total cholesterol concentration = absorbance (sample)/ absorbance (standard) × 200 mg/dl

Statistical analysis

Data obtained were expressed as Mean ± Standard error and analyzed using the Analysis of Variance ‘ANOVA, F-ratio and student’s t’ test where applicable. Values at $P=0.05$ and $P<0.05$ were regarded as significant in comparison with appropriate controls.

Result

Table 1: Secondary Metabolites Constituent of *Adasonia digitata* and *Cochorous olitorous* (mg/l)

Metabolites	<i>Adasonia digitata</i>	<i>Corchorus olitorous</i>
Alkaloids	81.56 ± 0.56	68.65 ± 2.05
Saponin	16.59 ± 1.85	22.17 ± 0.24
Tanins	311.98 ± 0.01	287.07 ± 0.16
Flavonoids	25.38 ± 2.88	157.38 ± 0.38
Polyphenols	170.90 ± 0.68	330.07 ± 0.32

Mean ± SEM; $p < 0.05$. Values are Expressed as Means ± SEM; $n=8$, IR: Irradiated, E_2 : *C. olitorous*; E_1 : *A. digitata* Significant difference of $p < 0.05$. Table 1 above shows the result of the quantitative secondary metabolite constituents of methanol leaf extract of *Adansonia digitata* and *Cochorous olitorous*. The screening revealed the presence of significant ($p < 0.05$) amount of alkaloids, saponins, tannins, flavonoids, polyphenols.

Table 2: The effect of methanolic leaf extracts of *A. digitata* and *C. olitorous* on Serum AST, ALT and ALP of irradiated wistar rat (Umol/ml)

Group	AST	ALT	ALP
Control	95.39 ± 0.89 ^a	58.23 ± 0.51 ^a	24.68 ± 0.16 ^a
IR	143.33 ± 0.89 ^b	98.00 ± 0.57 ^b	76.49 ± 0.77 ^b
IR(E_1 500)	136.32 ± 0.36 ^c	84.71 ± 0.16 ^c	60.24 ± 0.24 ^c
IR(E_1 1000)	122.58 ± 0.27 ^d	70.15 ± 0.45 ^d	52.15 ± 0.57 ^d
IR(E_2 500)	135.37 ± 0.71 ^c	83.89 ± 0.23 ^c	60.73 ± 0.37 ^c
IR (E_2 1000)	124.34 ± 1.03 ^d	71.73 ± 0.37 ^d	53.22 ± 0.30 ^d
IR(E_1 + E_2 500)	110.26 ± 0.50 ^e	66.45 ± 0.29 ^e	38.07 ± 0.14 ^e
IR(E_1 + E_2 1000)	94.89 ± 0.11 ^a	57.67 ± 0.67 ^a	23.70 ± 0.39 ^a

Values are Expressed as Means ± SEM; $n=8$, IR: Irradiated, E_2 : *C. olitorous*; E_1 : *A. digitata*. ALT; Alanine aminotransferase. AST; Aspartate aminotransferase and ALP; Alkaline phosphate Significant difference of $p < 0.05$. The result from table 2 above shows significant ($p < 0.05$) increase in ALT, AST and ALP in irradiated group, but was attenuated and

ameliorated after treatment with methanol extract of *A. digitata* and *C. olitorous* at varying dose as shown above.

Table 3: Effects of methanolic leaf extracts of *A. digitata* and *C. olitorous* on the antioxidant enzyme system and Malondealdehyde status of irradiated Wistar rats

Treatment Group	GST	GPX	CAT	SOD	MDA
Control	2.95 ± 0.302 ^a	2.67 ± 0.06 ^a	57.88 ± 0.01 ^a	4.59 ± 0.02 ^a	0.230 ± 0.03 ^a
IR	1.20 ± 0.01 ^b	1.08 ± 0.02 ^b	21.79 ± 0.29 ^b	1.89 ± 0.03 ^b	0.845 ± 0.01 ^b
IR(E ₁ 500)	1.89 ± 0.01 ^c	1.63 ± 0.04 ^c	29.82 ± 0.24 ^c	2.56 ± 0.02 ^c	0.789 ± 0.04 ^c
IR(E ₁ 1000)	2.45 ± 0.03 ^d	1.78 ± 0.01 ^d	36.08 ± 0.01 ^d	2.90 ± 0.01 ^d	0.572 ± 0.01 ^d
IR(E ₂ 500)	1.93 ± 0.01 ^c	1.65 ± 0.02 ^c	31.00 ± 0.20 ^c	2.60 ± 0.01 ^c	0.746 ± 0.03 ^c
IR (E ₂ 1000)	2.49 ± 0.01 ^d	1.80 ± 0.01 ^d	38.14 ± 0.07 ^d	2.98 ± 0.01 ^d	0.540 ± 0.05 ^d
IR(E ₁ +E ₂ 500)	2.73 ± 0.01 ^e	2.26 ± 0.01 ^e	44.23 ± 0.04 ^e	3.92 ± 0.01 ^e	0.324 ± 0.06 ^a
IR(E ₁ +E ₂ 1000)	3.04 ± 0.02 ^a	2.73 ± 0.00 ^a	58.77 ± 0.97 ^a	4.71 ± 0.01 ^a	0.221 ± 0.05 ^a
IR(VIT C)	1.97 ± 0.01 ^c	1.60 ± 0.01 ^c	31.56 ± 0.22 ^c	2.37 ± 0.01 ^c	0.548 ± 0.02 ^c

Values are Expressed as Means ± SEM; n=8, IR: Irradiated, E₂: *C. olitorous*; E₁: *A. digitata* Significant difference of $p < 0.05$. GST; glutathione-s-transferase. GPX; glutathione peroxidase. CAT; catalase. SOD; superoxide dismutase. MDA; Malondealdehyde. The result from table 3 above show significant ($p < 0.05$) reduction in the level of antioxidant enzymes in irradiated rats, but was significantly ($p < 0.05$) exacerbated after treatment with methanol leaf extract of *A. digitata* and *C. olitorous* at varying doses

Table 4: The Effects of methanolic leaf extracts of *A. digitata* and *C. olitorous* on the heamatological parameters of rats exposed to 6 grey of Radiation.

Treatment Group	PCV (%)	HB (%)	RBC (%)	WBC($10^9/L$)	LYMPS	PLT($(10^5/\mu L)$)
NORMAL	36.29±0.36 ^a	12.11±0.21 ^a	8.84±0.06 ^a	8.55±0.04 ^a	47.23±0.39 ^a	834.00±33.06 ^a
IR	20.97±0.89 ^b	6.40±0.12 ^b	4.76±0.01 ^b	13.51±0.33 ^b	81.52±0.29 ^b	671.67±20.81 ^b
IR(E ₁ 500)	24.70±0.16 ^c	7.82±0.10 ^c	5.37±0.04 ^c	11.96±0.03 ^c	65.48±0.29 ^c	718.33±14.43 ^c
IR(E ₁ 1000)	28.81±0.42 ^d	8.89±0.26 ^d	7.44±0.26 ^d	11.16±0.04 ^d	60.33±0.33 ^d	879.33±39.48 ^d
R(E ₂ 500)	25.14±0.32 ^c	7.54±0.03 ^c	5.31±0.05 ^c	12.07±0.07 ^c	64.48±0.29 ^b	715.00±63.02 ^c
IR (E ₂ 1000)	30.99±0.20 ^d	9.07±0.12 ^d	7.47±0.04 ^d	11.05±0.08 ^d	61.20±0.31 ^d	884.33±25.36 ^d
IR(E ₁ +E ₂ 500)	33.51±0.38 ^e	11.20±0.11 ^e	9.99±0.01 ^e	9.99±0.02 ^e	51.74±0.13 ^e	908.33±60.87 ^e
IR(E ₁ +E ₂ 1000)	39.21±0.34 ^f	15.33±0.17 ^f	12.73±0.09 ^f	8.85±0.09 ^f	44.99±0.07 ^f	929.67±29.35 ^f

Values are Expressed as Means $\pm$ SEM; n=8, IR: Irradiated, E₂: *C. olitorous*; E₁: *A. digitata* Significant difference of $p<0.05$. PCV; parked cell volume. HB; heamoglobin. RBC; red blood cell. WBC; white blood cell. LYMPS; lymphocyte. PLT; platelet. The result from table 4 above show significant ($p<0.05$) effect of radiation on the heamatological parameters of Irradiated-wistar rat, but after treatment with methanol extract of *A. digitata* and *C. olitorous* , the effect was significantly ($p<0.05$) attenuated as shown above

Table 5: Effects of methanolic leaf extracts of *A. digitata* and *C. olitorous* on the lipid profile of irradiated male wistar rats.

Treatment Group	HDL-CHOL	LDL-CHOL	TOTAL-CHOL	TRIGLYCERIDES
NORMAL	288.74 $\pm$ 0.26a	158.39 $\pm$ 0.50a	164.89 $\pm$ 0.59a	102.08 $\pm$ 0.39a
IR	208.75 $\pm$ 0.57b	191.09 $\pm$ 0.53b	105.63 $\pm$ 0.41b	177.81 $\pm$ 0.42b
IR(E ₁ 500)	237.00 $\pm$ 1.59c	181.36 $\pm$ 3.07c	125.96 $\pm$ 2.97c	158.57 $\pm$ 1.42c
IR(E ₁ 1000)	254.18 $\pm$ 0.34d	165.98 $\pm$ 0.62d	138.54 $\pm$ 0.27d	127.50 $\pm$ 0.29d
IR(E ₂ 500)	239.67 $\pm$ 1.46c	179.77 $\pm$ 1.60c	129.22 $\pm$ 0.41c	156.64 $\pm$ 0.45c
IR (E ₂ 1000)	258.40 $\pm$ 2.07d	164.47 $\pm$ 1.80d	140.59 $\pm$ 0.71d	123.31 $\pm$ 0.68
IR(E ₁ +E ₂ 500)	278.33 $\pm$ 0.88e	152.17 $\pm$ 2.60a	158.00 $\pm$ 1.16e	113.67 $\pm$ 0.33
IR(E ₁ +E ₂ 1000)	297.20 $\pm$ 1.41a	165.26 $\pm$ 3.90a	172.44 $\pm$ 3.24a	101.00 $\pm$ 0.58

Values are Expressed as Means $\pm$ SEM; n=8, R: Irradiated, E₂: *C. olitorous*; E₁: *A. digitata* Significant difference of $p<0.05$.HDL-CHOL; high density lipoprotein cholesterol. LDL-CHOL; low density lipoprotein cholesterol.TOT-CHOL; total cholesterol. The result from table 5 above show significant ($p<0.05$) effect of radiation on the lipid profile parameters of Irradiated-wistar rat, but after treatment with methanol extract of *A. digitata* and *C. olitorous* , the effect was significantly ($p<0.05$) attenuated as shown above.

Discussion

The phytochemical screening of the methanolic extract of *Adansonia digitata* and *Cochorous olitorous* leaf revealed the presence of alkaloids, terpenoids, tannins, phenolics, saponins and flavonoids as shown in Table1. Secondary metabolites in plant have been validated by several report to be responsible for the pharmacological properties of plant and as well as influencing their biological effects of such plat. Thus the resulting pharmacological effect of the MLExt of *A. digitata* and *C. olitorous* against radiation induced damaged in Wistar rat maybe due to the presence of reasonable quantity of the secondary metabolites therein.

Human exposure to Radiation have been established to increase the physiological concentration of free radicals from 20 to 120 ug/dl, thus increasing the spate of oxidative stress and oxidative stress induced diseases (atherosclerosis, dyslipidemia, dementia, ulcer, liver damage and cancer) [18]. Radiation-induced free radical have the potential of causing serious cellular alteration such as those caused in the experimental rat exposed to radiation in the present research. While administration of methanol leaf extract of *A. digitata* and *C. olitorous* to irradiated rat, it was bale to attenuate, prevent and treat the cellular alteration caused by radiation-induced free radical.

Flavonoids and tannins contained in *A. digitata* and *C. olitorous* as showed in the table 1 above, have been reported to poses antioxidant inducing potential, thus offering protection against oxidative deterioration caused by free radical generated from exposure to radiation [15, 26]. Also [27] documented the induction of antioxidant enzymes such as catalase, superoxide dismutase, glutathione transferase and peroxidases by Polyphenols and flavonoids hence the presence of reasonable amount of flavonoids, polyphenols and tannins as shown in Table1, maybe responsible for the significant ($p < 0.05$) increase in the antioxidant enzymes status in irradiated-rat –extract treated group, thereby offering protection against radiation induced free radical cellular damage.

Contained in cucumber is polyphenols, which induces Heam oxygenase-1, phase-2 enzymes (Uridyl-glucuronosyl transferase, Sulfotransferase, methionine transferase) and several antioxidant enzymes (Glutathione-S-transferase, Glutathione peroxidase) [27]. In the present study, that methanolic extract of *A. digitata* and *C. olitorous* contain

significant ($P < 0.05$) amount of polyphenol, this has been attributed to an increase in induction of SOD, CAT, GST and GSH in irradiated-rat-extract treated group when compared to irradiated not treated group. It is therefore logical to conclude that the pharmacological activities of *A. digitata* and *C. olitorous* against radiation induced cellular alteration may be attributed to significant amount of polyphenols and flavonoids.

The hepatoprotective potentials of flavonoids have also been reported, as well as prevention of coronary heart diseases [26]. In the present study the hepatic architecture was found damaged, with lesion and Periportal Damages at the Centrilobular region of the liver of irradiated rat, but were repaired after administration of methanol leaf extract of *A. digitata* and *C. olitorous*, this further lends credence to the hepatoprotective potentials of the methanol leaf extract of *A. digitata* and *C. olitorous* in the treated group as shown in table 2.

Jubili formula, an herbal preparation made from three medicinal herbs (*Parquetina nigrescens*, *Sorghum bicolor* and *Harungana madagascariensis*) has been successfully used in the treatment of blood diseases, this herbal extract was screened and revealed the presence of significant ($P < 0.05$) amount of saponins [2]. Saponins stabilize hemoglobin structure and prevent hemoglobin aggregation and oligomerization, thus preventing Heinz body formation [29]. In the present study, significant ($P < 0.05$) amount of saponins in *A. digitata* and *C. olitorous*, may be suggestive of the increase level of hemoglobin in the irradiated-extract treated group as shown in Table 4.

Hematological assessment is a very important tool to assess blood invasion by foreign components and to also assess blood-cell related damage [30]. The significant ($P < 0.05$) reduction in the RBC, hemoglobin and other related blood parameters in the rats exposed to 6 grey radiation may suggest damage to red blood and red blood cell and other blood parameters. The administration of methanol extract of *A. digitata* and *C. olitorous* leaves at the varying doses to irradiated rat significantly ($P < 0.05$) reverse the damage, an indication that both the individual plants and the combination might have induced the production of more erythropoietin and invariably RBC, thus preventing blood disorder [30]. Hemoglobin is an iron containing protein, its mediates oxygen carrying capacity in the blood, thus, the observed increase in hemoglobin concentration in the extract treated group, may be attributed to increase

RBC production, thus increasing the oxygen carrying capacity of hemoglobin, thus abating any risk of anemia, which maybe pronounced in irradiated group as shown in table 4.

Malondialdehyde (MDA) is a product of lipid peroxidation, high level of MDA narrows the blood vessels and thus increasing blood flow pressure, which is hallmark of hypertension, atherosclerosis, cardiovascular and several other central metabolic diseases [31]. The significant ($p < 0.05$) reversal in the MDA, Triglyceride and LDL-cholesterol level of irradiated-extract treated group when compared with the negative group as shown in table 4 above, is an indication that the plant extract poses the potential to prevent free radical-induced lipid peroxidation, hence prevent atherosclerosis. HDL-cholesterol have been documented to transport chylomicrons or lipid plaque out of the heart to the adipose tissues, where it is stored as fat, while LDL-cholesterol reversed the transportation. The reversal of significant ($p < 0.05$) amount HDL-cholesterol in the irradiated but extract treated group may prevent narrowing of the blood vessels by lipid plaque thus preventing hypertension and atherosclerosis.

Conclusion

The combined administration of the methanolic extract of *A. digitata* and *C. olitorius* leaves wistar rats at the concentrations used in the present research cause no selective or overall damage to metabolic processes in the organism. It is interesting to know that it induce more of the endogenous antioxidant enzyme system and may reduce the risk of oxidative stress and several disorders that may be mediated by exposure to radiation. In view of this, it is therefore logical to conclude that, the plant maybe used as oral remedy in the treatment of free radical induced diseases.

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