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Solvents Fractions of Methanol Leaves Extracts of *Adansonia digitata* and *Corchorus olitorius* in Gamma-irradiation-induced Nf-kB, Nrf-2 and antioxidant alterations in Rats

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ABSTRACT

Rats' exposure to radiation causes increased free radical level from sub-toxic (24 µg/mol) to a toxic concentration (120 µg/mol) in the system of rats. Free radical level above 120 µg/mol, leads to dysregulated nuclear factor kappa-B (Nf-kB) and nuclear related factor-2 (Nrf-2), thus exacerbating oxidative stress and cellular alterations in rats.

This study investigated the effects of solvents (n-hexane, ethylacetate and n-butanol) fractions of *Adansonia digitata* and *Corchorus olitorius* leaves in radiation-induced Nf-kB, Nrf-2 and antioxidant system alterations in Cellular System of Rats.

A total of 48 rats (198 ± 5.00g) were distributed into 8 groups of 6 each. Group I were fed with rat chow and distilled water only, all other groups were exposed to a single dose of 6 grey gamma irradiation, such that groups II, III, IV, V, VI, VII and VIII were administered distilled water, n-hexane fractions of *A. digitata* and *C. olitorius*, ethylacetate fractions of *A. digitata* and *C. olitorius*, n-butanol fractions of *A. digitata* and *C. olitorius* at 1000 mg/kg body weight respectively for 7 days, While group IX received 2 mg/kg bwt of Vitamin-C.

Secondary metabolites constituents of *A. digitata* and *C. olitorius* revealed the presence of flavonoids, polyphenol, alkaloids, tannins and saponins. Irradiation significantly *($p<0.05$)* increased the level of Nf-kB, activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and significantly ($p<0.05$) reduced the level of Nrf-2 and activities of antioxidant enzymes capacity. Administration of solvents fractions at 1000 mg/kg bwt significantly *($p<0.05$)* reduced Nf-kB level, activities of ALP, ALT and significantly *($p<0.05$)* increased Nrf-2 level and antioxidant capacity of rats in all the treated groups, such that, n-butanol fraction is the most effective when compared with the negative control. We can therefore concludes that n-butanol fractions of *A. digitata* and *C. olitorius*, might contain the most active bioactive constituents that is responsible for the attenuations of all the alterations.

Keywords: Free Radical, *Adansonia digitata*, *Corchorus olitorius*, γ-irradiation, Nf-kB, Nrf-2 and cellular alterations, micro nuclei DNA, antioxidant

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INTRODUCTION

Human exposure to radiation increased free radical level, to a level that is capable of generating reactive oxygen species (ROS) and reactive nitrogen species (RNS) in human (IRES, 2010). ROS/RNS attack cells, tissues, organs and macromolecules (DNA, proteins and lipids) resulting in peroxidation, these are hallmark of oxidative stress and cellular alterations (Jhur-shur, 2009). Oxidative stress catalyzed protein unfolding and misfolding, unregulated activation of 26s proteasomes pathway and dysregulated Nrf-2 and Nf-kB (Said and Aiman, 2014). Free radical at above sub-toxic concentration, exacerbate Nf-kB and Nrf-2 level in rat system exposed to thymol alcohol (Fusco *et al.,* 2007). All these ultimately provoke alterations the systemic antioxidant capacity. Nf-kB also known as Nuclear Factor Kappa-B, it's a basic leucin containing factors, with a molecular weight of 234kdalton. It's mostly conserved in the cytosol as an inactive protein trimer/complex in the form of p50-p52-IkB (Scheuring *et al*.,1999). IkB (one of the trimer of Nf-kB) prevents the translocation of an inactive Nf-kB to the nucleus, thus preventing it binding to the DNA binding region (DBR) of genes in the nucleus, thus blocking gene expression (Talalay, 2003).

Radiation causes increased Free radical level (120ug/mol), an increased level of radicals mediates phosphorylation of the inactive Nf-kB in the cytosol (Talalay, 2003). Phosphorylated Nf-kB is matured for degradation in the 26s proteasome Pathway (Ogbe *et al*., 2010). Degradation allows for IkB to be cleaved-off from Nf-kB to become an active dimer (p50-p52), it is then translocated into the nucleus, where its bind to DNA Binding Region (DBR) of gene expressing for majorly pro-oxidant proteins such as prostaglandins, cyclooxygenase-2, inducible nitric oxide synthase, hexokinase-2, anti-apoptotic protein (BCL-2 and BAX), interleukens2 (IL-2) and interleukins family (Scheuring *et al*., 1999).

Over expression of these pro-oxidant proteins (interleukins, prostaglandins, Bcl-2 and BAX proteins) have been implicated in induction of inflammations through ROS production. Overexpression of prostaglandins have been implicated in the growth of cancer (Scheuring *et al*., 2001). Over expression of hexokinase-2 have also been implicated in the growth of neoplastic cells (Patel *et al.,* 2001; Hall and Giaccia, 2006). Over expression of pro and inflammatory proteins have been implicated in the overwhelming of the endogenous antioxidant capacity, this significantly exacerbate oxidative stress and cellular alterations of the cellular system of rat exposed to irradiation (Patel *et al.,* 2001; Hall and Giaccia, 2006).

The nuclear factor related factor-2 (Nrf-2) is cytosolically repressed by Keap-1. It exists as an inactive trimmer too in the cytosols just like Nf-kB (Moi *et al.,* 1994a). Keap-1 prevent Nrf-2 translocation into the nucleus, thus preventing its binding to the antioxidant responsive element (ARE) of DNA of genes, where it expresses for antioxidant enzymes (Moi *et al.,* 1994b).

At physiological condition (less oxidative stress), this condition mediate Nrf-2 activation by phosphorylation, activated Nrf-2 dissociates from Keap-1 and translocate into the nucleus, where it binds the DNA through the antioxidant responsive region (Patel *et al.,* 2001; Hall and Giaccia, 2006). It is also important to mention that secondary metabolites in plant also activate Nrf-2 (Itoh *et al.,* 2004), such that Nrf-2 have been implicated in the induction of antioxidant enzymes such as glutathione-S-transferase, superoxide dismutase, glutathione peroxidases and haem oxygenase in human system (Itoh *et al.,* 2004). While radiation have been implicated in the production of an increased free radicals, it is important to know that increased free represses Nrf-2 activation, thus repressing antioxidant enzymes

induction, hence exacerbating cellular alterations (Farombi, 2001)

Hence, this study investigated the effects of solvent fractions (n-hexane, ethylacetate and n-butanol fractions) of methanol leaves extract of *A. digitata* and *C. olitorius* on the Nf-kB, Nrf-2 and antioxidant capacity of gamma irradiated rat's cellular system.

Several synthetic drugs have been used, either to scavenge or stabilize free radicals in experimental rats, for example synthetic vitamin E protects the integrity of acetyl choline receptors in normal neurons and prevents toxicity and apoptosis induced-ROS in male rabbits (Kennedy *et al*., 2001; Behl and Moosmann, 2002). Synthetic vitamin C, stabilize reactive intermediate as well as neutralize free radical in albino rat (Adaramoye *et al.,* 2001). Zalcitabin is used against radiation-induced diseases, by preventing free radical production through blocking of Cytochrome-C.

Most of these synthetic drugs are either with one or more side adverse effect, as well as high cost, as well as accessibility. Natural product (plants) such as vegetables and fruits, have been established to contain secondary metabolites constituents (flavonoids, polyphenols, tannins, saponins, glycosides, vitamins) and are cheap, accessible and mostly safe. They have been implicate in scavenging free radicals and stabilises reactive species (Adaramoye *et al.,* 2001).

A. digitata is a member of the baobabs family and commonly known as *Kukah* in Hausa, *luru* in Yoruba and tree of life in English (Dharmendra *et al*., 2006). The leaf of the is a commonly used traditional plant consumed in food or used in the treatment of diseases such as anaemia, diabetes, lipid peroxidation disorders, ischemia reperfusion diseases and inflammatory bowel syndrome in South-west Nigeria (Lewanda *et al.,* 2007). The leaves are used as blood booster, anti-asthmatic, anti-histamine and anti-tension, treatment of insect bites, guinea worm, internal pains, dysentery and diseases of the urinary tract, opthalmia and otitis (Scheuring *et al*., 1999). Baobab leaves are used medicinally as a diaphoretic, astringent, expectorant and as a prophylactic against fever (Scheuring *et al*., 1999). The carotenoid content of baobab leaves has also been established to contribute to the prevention of oxidation in the human cell and lots of other diseases.

*Corchorus olitorius (*Linn) is a leafy vegetable that belongs to the family *Tiliaceae*, it is known as Jute mallow in English and *Ewedu* in Yoruba. It is an annual herb with a slender stem and an important green leafy vegetable in many tropical areas including Nigeria, Egypt, Sudan, India, Bangladesh, in tropical Asia such as Philippine and Malaysia. 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 (Branda *et al*., 2004) with various therapeutic uses in herbal medicine. The leaves extracts have been reported to possess hypotensive (Thyagarajan *et al.,* 1988), anti-diabetic (Odetola & Akojenu, 2000), anti-malarial (Sripanidkulchai *et al*., 2002), hepatoprotective, anti-microbial (Adeneye *et al.,* 2006), anti-inflammatory (Baskurt *et al.,* 2009) and analgesics (Thyagarajan *et al.,* 1988) in properties .

MATERIALS AND METHODOLOGY

Materials

Collection of Plant Materials and Authentication

Fresh leaves of *A. digitata* and *C. olitorius* were purchased from 'Ipata' Market, Ilorin, Kwara State, Nigeria. The plants were identified and authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Kwara State. Voucher Specimen Number (UICH/001/951 and UITH/002/154) were deposited in the Herbarium respectively.

Preparation of Plant Extracts

Fresh leaves of *Adansonia digitata* and *Corchorus olitorius* were washed with clean water, cut into pieces and dried at room

temperature (27ºC) for two weeks. The dried plants were grinded using blender (Steelman K207, H.M and co Ltd, China). A known quantity (50 g) of powdered *Adansonia digitata* and *Corchorus olitorius* leaves were extracted in 200 ml Methanol (98.8 %) for 72 hours. The extracts were filtered using Whatman No. 4 filter paper. The filtrates were concentrated using Rotary evaporator (RE, k25, Longfin, Australia).

Preparation of n-hexane, ethylacetate and n-butanol Fractions of *A. digitata* and *C. olitorius*

n-hexane, ethylacetate and n-butanol partitioned fractions of *A. digitata* and *C. olitorius* were prepared by adopting the method described by Mbaoji *et al.* (2014).

Table 1: Percentage yield of the three solvent partitioned extracts of *A. digitata and C. olitorius*

Solvent Fraction	n-Hexane (%)	Ethylacetate (%)	n-Butanol (%)
Adansonia digitata	14.41	12.89	16.21
Corchorus olitorius	16.73	11.45	14.12

Ethical Approval

Ethical Approval was obtained from the University of Ilorin Ethical Committee, University of Ilorin. With the ethical approval number obtained as follow UERC/ASN/2018/1409 on the 13th September, 2018. The study was conducted following the guidelines on the care and use of laboratory animals.

Experimental Animals

A total of fifty-four (54) healthy male Wistar rats (198 ± 5.00g) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in clean cages and adequately-ventilated house condition. They were allowed free access to rat pellets and clean tap water. The cages were cleaned daily and the study was conducted following the guidelines on the care and use of laboratory animals. Ethical approval was obtained from the University of Ilorin Ethical Committee, University of Ilorin.

Exposure of Animal to Radiation

Male wistar rats were exposed to single dose 6 grey whole-body gamma radiations by the method described by (Kamani *et al.* (2008) and modified by Nwozo and Bello (2014). Radiotherapy Machine with Cobalt 60 was the source of radiation. The exposure was carried out in the Department of Radiotherapy, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Drugs, Chemicals and Assay Kits

The drugs and other chemicals used in the study were commercially obtained from Monobind International, Lake Forest, USA. ELISA kits were products of Cayman Laboratory LTD., USA. All other chemicals will be of analytical grades and prepared in all-glass distilled water.

Secondary Metabolite Constituents of *Adansonia digitata* and *Corchorus olitorius*

Qualitative and quantitative secondary metabolites screening of *A. digitata* and *C. olitorius* leaves were determined by adopting the methods described by Harborne (1993); Sofowora (1993) and Ajayi *et al.* (2010). All determinations were done in triplicates.

Animal groupings: Administration of the solvent fractions to irradiated rats

Groups

I	Non-irradiated, fed with rat chow and distilled water only
II	Irradiated animal and not treated at all
III	Pre-administered with 1000 mg/kg bwt. ethyl acetate fraction of *A. digitata* before exposure to irradiation
IV	Pre-administered with 1000 mg/kg bwt. ethyl acetate fraction of *C. olitorius* before exposure to irradiation
V	Pre-administered with 1000 mg/kg bwt. n-butanol fraction of *A. digitata* before exposure to irradiation
VI	Pre-administered with 1000 mg/kg bwt. n-butanol fraction of *C. olitorius* before exposure to irradiation
VII	Pre-administered with 1000 mg/kg bwt. n-hexane fraction of *A. digitata* before exposure to irradiation
VIII	Pre-administered with 1000 mg/kg bwt. n-hexane fraction of *C. olitorius* before exposure to irradiation
IX	Pre-administered with 2mg/kg bwt. Vitamin-C before exposure to irradiation

Bwt: body weight. The rats were pre-administered with solvents fractions for seven (7) days before they were all exposed to radiation. After the single dose whole body gamma irradiation, they were left for 7days before sacrificed

Blood was collected by the method described by Akanji and Ngaha (1989)

Serum was collected by the method described by Yakubu *et al.* (2003)

Nf-kB and Nrf-2 assay was carried out by the method adopted by Cayman *et al.* (2003) using the principles of enzyme linked immunosorbent assay (ELISA)

Preparation of Tissue Homogenate

The rats were sacrificed by cervical dislocation, the rats were dissected to isolate the liver. The isolated liver was gently cleansed with 1.14 % Kcl washing solution to remove blood stains and dirty. The liver was weighed and immediately stored in ice cold 0.25M sucrose solution. 1g of the liver was cut with a clean scalpel, homogenized in ice-cold 0.25M sucrose solution ($1:5^{w}/_{v}$). The homogenates were cold centrifuge at 12000g for 15 minutes, the supernatant was pipetted into a clean sample tube and stored in the freezer ($-5^{o}C$) until required for further analysis.

Alkaline phosphatase activities assay was carried out by the method adopted by Reitman and Frankel (1960b); aspartate amino transaminase activities assay was carried out by the method adopted by Reitman and Frankel (1960b); and alanine transaminase assay were carried by the method adopted by Reitman and Frankel (1957).

Superoxide dismutase activities was carried out by the method adopted by Haliwel *et al*. (2001); Catalase by the method adopted by Yhoung-shur (1980); Glutathione-s-transferase by the method adopted by Yhoung-shur (1980); Glutathione peroxidase by the method adopted by Abolaji (2015) and Glutathione by the method adopted by Lazekhs *et al.* (1982b).

Statistical Analysis

Data were expressed as mean ± SEM of six determinations. Duncan Multiple Range Test was used and complemented with Student's T-test. Statistical Package for Social Sciences, version 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Statistical significance was set at 95% confidence interval (Mahajan, 1997).

RESULTS

The secondary metabolites of *Adansonia digitata* and *Corchorus olitorius* leaves revealed the presence of saponins, alkaloids, polyphenols, tannins, steroids and terpenoids respectively as shown in Table 1. Anthraquinone, cardiac glycosides and phlobatannins were absent. Quantitative screening of the methanol leaves extracts of *Adansonia digitata* and *Corchorus olitorius* revealed that tannins (198.98 mg/L) were the most abundant in *Adansonia digitata* whereas

polyphenols (203.14 mg/L) occurred the most in *Corchorus olitorius* as shown in Table 1 below

Table 1: Secondary Metabolites Constituents of Methanolic Extract of *A. digitata* and *C. olitorius* leaves

Metabolites	*Adansonia digitata* g/dL	*Corchorus olitorius*
Alkaloids	**78.76 ± 0.43**	**72.36 ± 2.05**
Anthraquinone	**ND**	**ND**
Cardiac glycosides	**ND**	**ND**
Flavonoids	**13.42 ± 1.03**	**104.81 ± 0.18**
Polyphenols	**147.90 ± 0.68**	**203.14 ± 0.32**
Phlobatannins	**ND**	**ND**
Saponins	**16.59 ± 1.85**	**22.17 ± 0.24**
Tannins	**198.98 ± 0.14**	**127.40 ± 0.16**
Terpenoids	**42.45 ± 0.45**	**21.25 ± 1.08**
Steroids	**30.45 ± 0.51**	**12.59 ± 1.85**

ND-Non detected

Nf-kB

Exposure of the rats to 6 grey whole body gamma radiation significantly ($p<0.05$) increased the level of Nf-kB of irradiated rats by 88 % when compared with the non-irradiated control as shown in figure 1a, b and c, below. In contrast, administration of 1000 mg/kg body weight (bwt) n-hexane, ethylacetate and n-butanol fractions of *A. digitata*, *C. olitorius* and combined *A. digitata* and *C. olitorius* leaves before exposure, have significantly ($p<0.05$) reduced the effects of radiation on the pre-administered groups respectively as shown in Figure 1a, b and c respectively. Such that the most effective of the solvent fraction is n-butanol fraction.

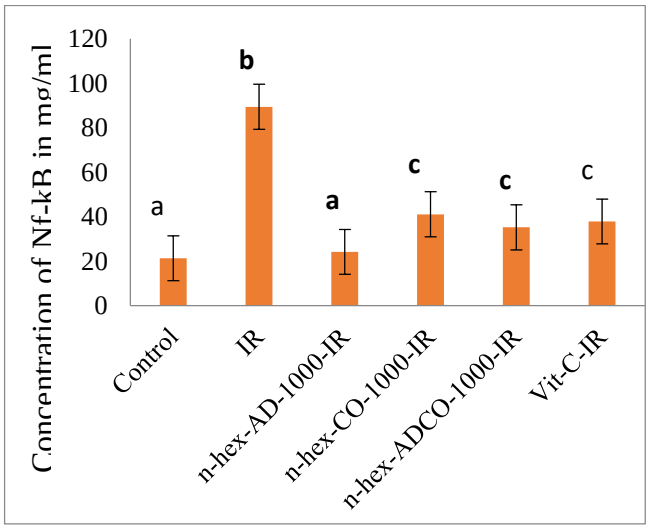


Figure 1a: Effects of n-hexane fraction of Methanol Leaf Extract of *Adansonia digitata* and *Corchorus olitorius* on Nf-kB status of irradiated Rats.

Means ± SEM; n=6, ($P < 0.05$);

IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO: *Corchorus olitorius*, ADCO: *A. digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight.

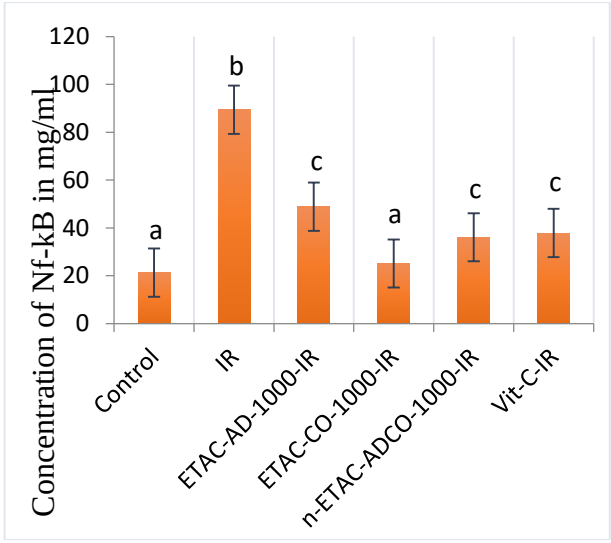


Figure 1b: Effects of ethylacetate partitioned-fraction of Methanol Leaf Extract of *Adansonia digitata* and *Corchorus olitorius* on Nf-kB status of irradiated Rats.

Means ± SEM; n=6, (P < 0.05); IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO: *Corchorus olitorius*, ADCO: *A. digitata* + *C. olitorius*,1000; 1000 mg/ kg body weight.

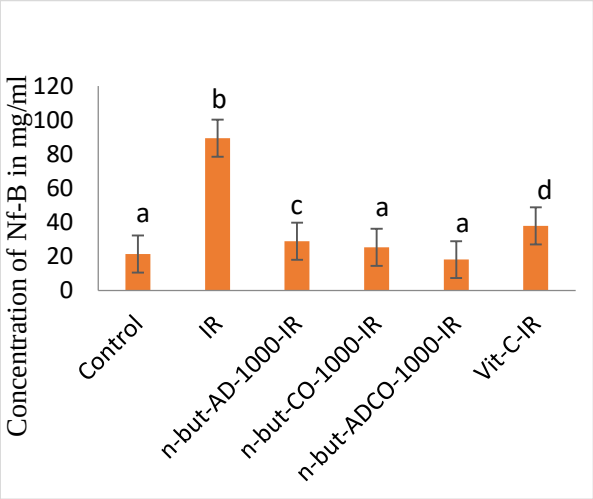


Figure 1c: Effects of n-butanol fraction of Methanol Leaf Extract of *Adansonia digitata* and *Corchorus olitorius* on Nf-kB status of irradiated Rats.

Means ± SEM; n=6, (P < 0.05); IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO: *Corchorus olitorius*; 1000; 1000 mg/ kg body weight.

Nrf-2

Exposure of the rats to 6 grey whole body gamma radiation significantly ($p<0.05$) reduced the level of **Nrf-2** of irradiated rats when compared with the non-irradiated control as shown in figure 2a, b and c, below. In contrast, administration of 1000 mg/kg body weight (bwt) n-hexane, ethylacetate and n-butanol fractions of *A. digitata*, *C. olitorius* and combined *A. digitata* and *C. olitorius* leaves before exposure, have significantly ($p<0.05$) reduced the effects of radiation on the pre-administered groups respectively. Such that the most effective of the solvent fraction is n-butanol fraction.

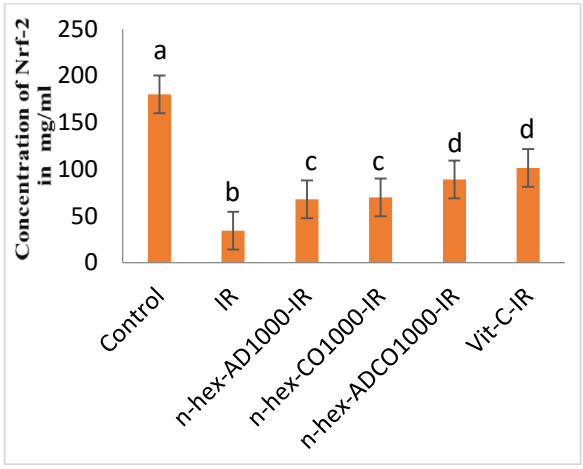


Figure 2a: Effects of n-Hexane, partitioned-fraction of Methanol Leaves Extract of *Adansonia digitata* and *Corchorus olitorius* on Nrf-2 status of irradiated Rats.

Means ± SEM; n=6, (P < 0.05);

IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO: *Corchorus olitorius*, ADCO: *A.digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight.

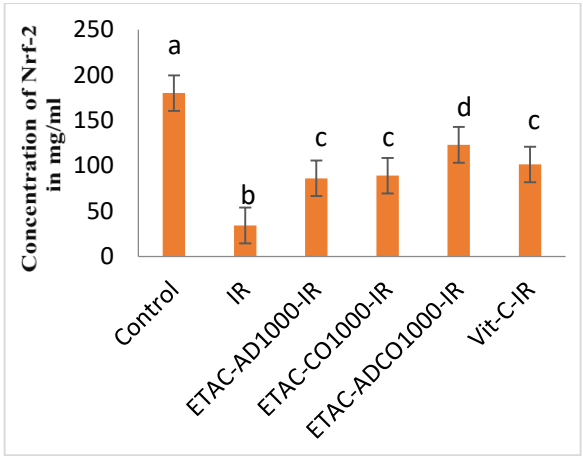


Fig 2b: Effects of Ethylacetate fraction of Methanol Leaves Extract of *Adansonia digitata* and *Corchorus olitorius* on Nrf-2 level of irradiated Rats.

Means ± SEM; n=6, (P < 0.05);

IR; irradiation, ETAC: ethylacetate, AD: *A. digitata* CO: *C. olitorius*, ADCO: *A.digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight.

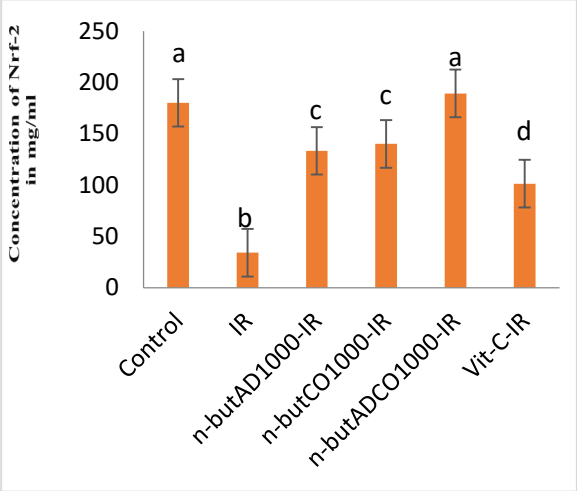


Figure 2c: Effects of n-Butanol fraction of Methanol Leaves Extract of *Adansonia digitata* and *Corchorus olitorius* on Nrf-2 level of irradiated Rats.
Means ± SEM; n=6, (P < 0.05),
IR; irradiation, n-but: n-butanol, AD: *A. digitata* CO*: C. olitorius*; ADCO: *A.digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight.

Antioxidant System

Exposure of the rats to 6 grey whole-body gamma irradiations significantly ($p<0.05$) reduced the level of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), whereas it significantly ($p<0.05$) increased Malondialdehyde (MDA)s level of irradiated Rats, when compared with the non-irradiated control as shown in Figure 3 below. In contrast, administration of 1000 mg/kg body weight (bwt) n-hexane, ethylacetate and n-butanol fractions of *A. digitata*, *C. olitorius* and combined *A. digitata* and *C. olitorius* leaves before exposure, have significantly ($p<0.05$) increased the level of increased SOD, CAT, GST and GPx ($p<0.05$) and reduced MDA.

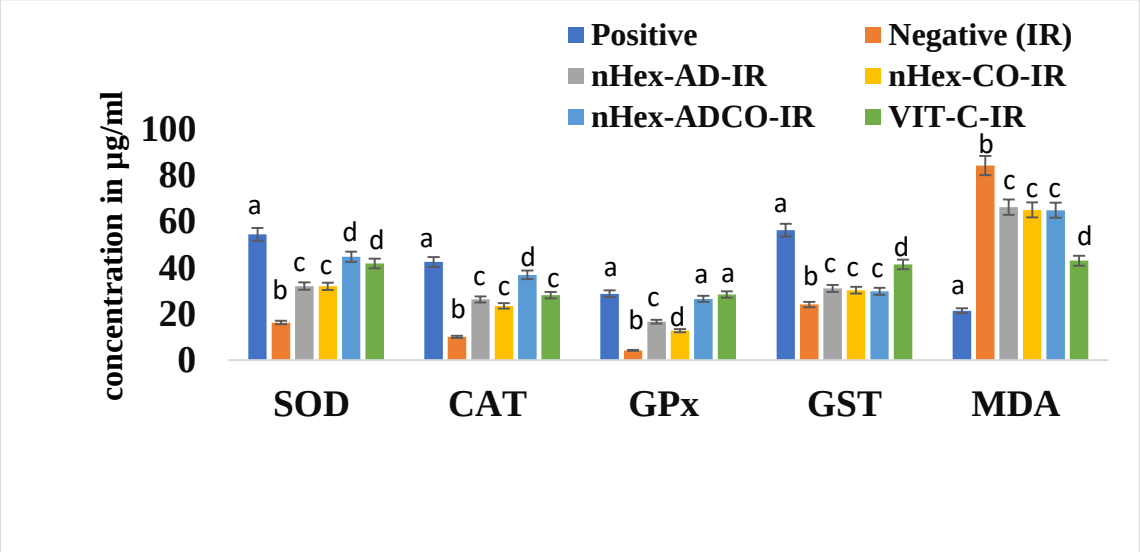


Fig 3a: Effects of n-Hexane Fraction of Methanol Leaves Extract of *Adansonia digitata* and *Corchorus olitorius* on Antioxidant Capacity of Irradiated Rats
Means ± SEM; n=6, (P < 0.05);
IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO: *Corchorus olitorius*, ADCO: *A.digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight. SOD; Superoxide dismutase, CAT; Catalase, GPx; Glutathione peroxidase, GST; Glutathione-s-transferase, MDA: Malondialdehyde

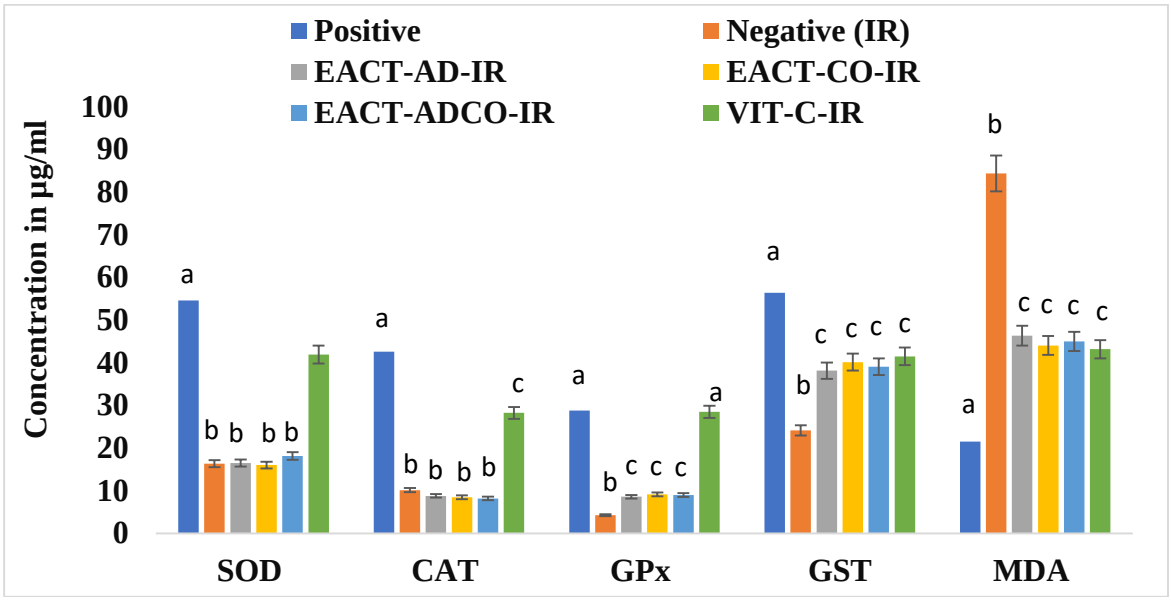


Fig 3b: Effects of Ethylacetate Fraction of Methanol Leaves Extract of *Adansonia digitata* and *Corchorus olitorius* on Antioxidant Capacity of Irradiated Rats.
Means ± SEM; n=6, (P < 0.05);
IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO: *Corchorus olitorius*, ADCO: *A.digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight. SOD; Superoxide dismutase, CAT; Catalase, GPx; Glutathione peroxidase, GST; Glutathione-s-transferase, MDA: Malondialdehyde

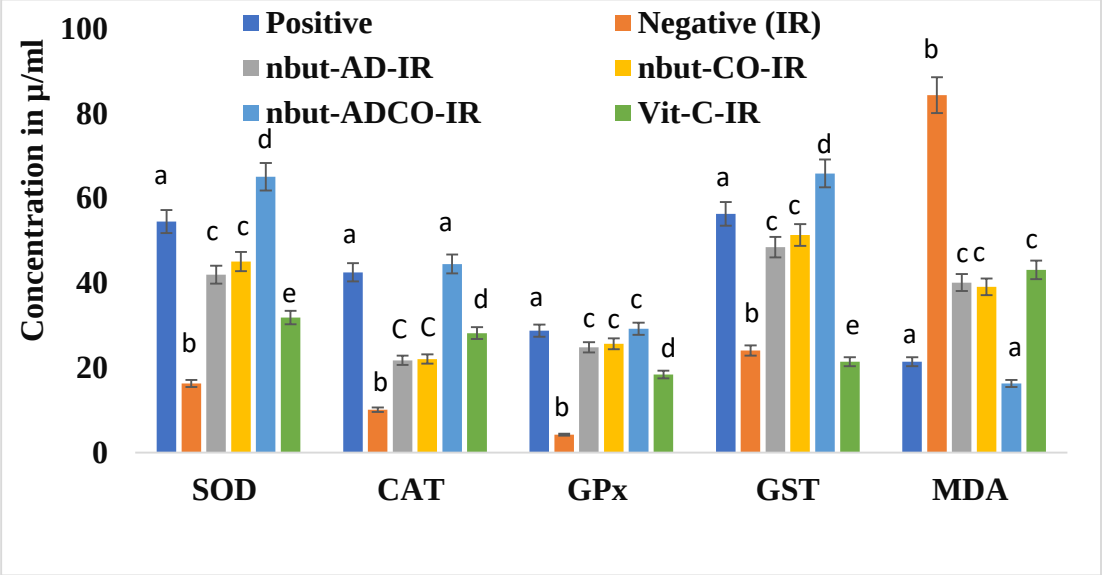


Fig 3c: Effects of n-Butanol Fraction of Methanol Leaves Extract of *Adansonia digitata* and *Corchorus olitorius* on Antioxidant Capacity of Irradiated Rats

Means ± SEM; n=6, (P < 0.05); IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO: *Corchorus olitorius*, ADCO: *A.digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight. SOD; Superoxide dismutase, CAT; Catalase, GPx; Glutathione peroxidase, GST; Glutathione-s-transferase, MDA: Malondialdehyde.

Toxicity Profile

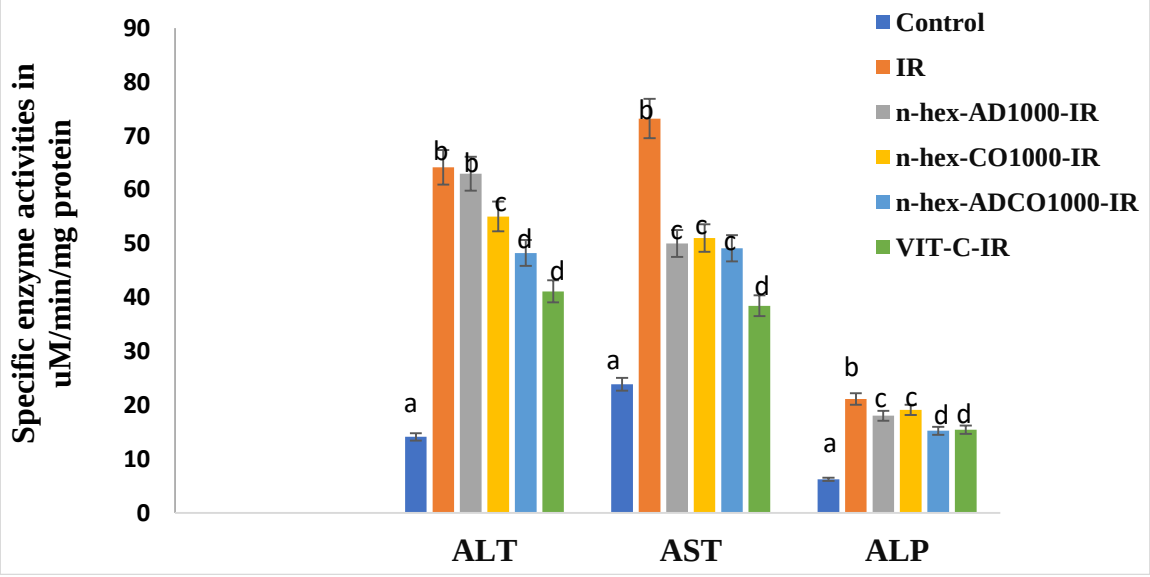


Fig 4a: Effects of n-hexane Fraction of Methanol Leaves Extract of *Adansonia digitata* and *Corchorus olitorius* on ALT, AST and ALP of Irradiated Rats

Means ± SEM; n=6, (P < 0.05); IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO: *Corchorus olitorius*, ADCO: *A.digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight.

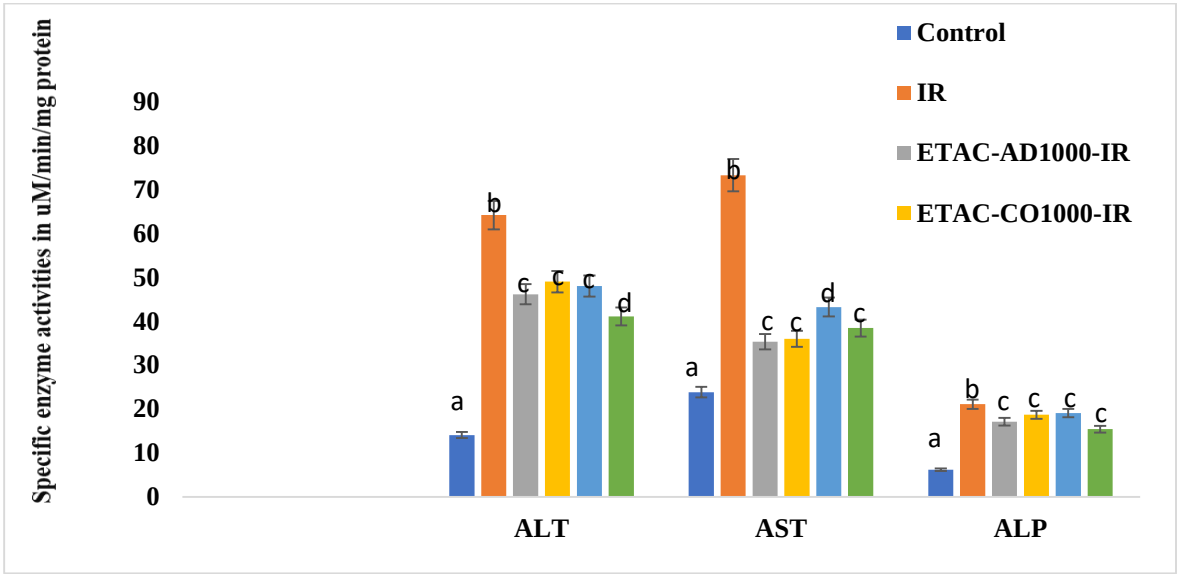


Fig 4b: Effects of Ethylacetate Fraction of Methanol Leaves Extract of *Adansonia digitata* and *Corchorus olitorius* on ALT, AST and ALP of Irradiated Rats

Means ± SEM; n=6, (P < 0.05); IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO*: Corchorus olitorius*, ADCO: *A. digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight

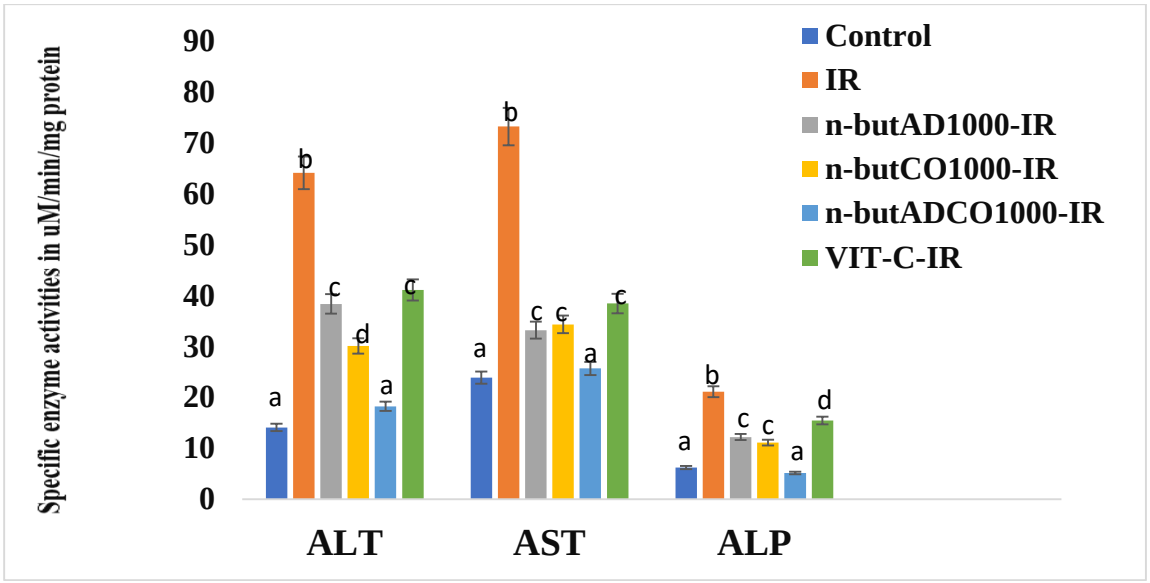


Fig 4c: Effects of n-butanol Fraction of Methanol Leaves Extract of *Adansonia digitata* and *Corchorus olitorius* on ALT, AST and ALP of Irradiated Rats

Means ± SEM; n=6, (P < 0.05); IR: irradiation, n-hex: n-hexane, AD: *Adansonia digitata*, CO*: Corchorus olitorius*, ADCO: *A.digitata* + *C. olitorius*, 1000; 1000 mg/ kg body weight

DISCUSSION

Exposure of rats to 6 grey gamma radiation altered cellular system of rats, by altering cellular parameters such as Nf-kB, Nrf-2, ALT, AST, SOD and CAT associated with physiological cellular system (Haliwell, 2001). Free radical level above physiological concentration (from 20ug/mol) is an underlying mechanism through which radiation altered cellular parameters (Simões, 2009). As revealed by the results obtained from this study, radiation significantly ($p<0.05$) altered Nf-kB, Nrf-2, SOD, CAT, GPx, ALT, ALP and AST level of rats that are not pre-treated with solvent fractions of *A. digitata* and *C. olitorius* as shown in Figure 1, 2 and 3.

The result obtained from the irradiated not-treated group in figure 3a. b and c further alluded to the alteration caused by irradiation, as it significantly *($p>0.05$)* reduced the activities of SOD, CAT, GPx and GST of irradiated rats.

Medicinal quality of plants is associated with the bioactive constituents therein the plants, this qualities are the underlying factors responsible for the pharmacological functions of most plant (Baskurt *et al.,* 2009). The pharmacological influences of the solvent fractions of *Adansonia digitata* and *Corchorus olitorius* leaves on cellular parameters and metabolism of irradiated rats as shown in this study, have strongly been implicated by the presence of flavonoids, polyphenols, tannins, saponins, alkaloids as shown in Table 1 above.

The use of secondary metabolites in plants to regulate cellular metabolism and offer pharmacological protection have long been validated (Olanlokun, 2013). Many plants with medicinal properties have been used in the treatment and protection against cellular alterations. Such as *A. digitata* and *C. olitorius* in this study; Polyphenols have been established to mediate the activation of Nrf-2 and deactivate Nf-kB (Giuliano *et al.,* 1999). Nrf-2 have been implicated in the induction of superoxide dismutase, catalase and Glutathione, whereas Nf-kB mediate their repression (Bossy-Wetzel *et al.,* 2004). The significant *($p<0.05$)* increasased in the level of Nrf-2 and decreased of Nf-kB as shown in figure 1 (a, b and c) and 2 (a, b and c) maybe associated with the presence of appreciable amounts of polyphenols in n-hexane, ethylacetate and n-butanol fraction of methanol leaves extract of *Adansonia digitata* and *Corchorus olitorius*, with n-butanol fraction been the most effective of the three solvent as shown in figure 1 and 2 above.

Flavonoids have been implicated in the prevention of lipid peroxidation and formation of Malondialdehyde in rats cellular system. Polyphenols impedes the formation of chain of lipid peroxidation (Finkel, 2012). Malondialdehyde is a product of lipids peroxidation, and have been studied to be involved in the genesis of cardiovascular diseases, hypertension, ischemia reperfusion diseases, atherosclerosis etc. (Kuete, 2010), thus the significant *($p<0.05$)* reduction in the level of MDA, as shown in figure 3 a, b and c, in the rat preadministered with n-hexane, ethylacetate and n-butanol fraction of *Adansonia digitata* and *Corchorus olitorius* Ieaves, maybe alluded to the presence of significant *($p<0.05$)* amount of polyphenols in the plant as shown in figure 3 above. Even though n-butanol fraction showed the most effective pharmacological effects of the three solvent fractions.

As observed in the irradiated not treated group in figure 1, 2 and 3 above, there was significant *($p<0.05$)* reduction in the level of antioxidant enzymes of the irradiated rats. This have been implicated in the genesis of cellular alterations in irradiated rat. Farombi and Yhong-sur (2001) revealed that epigallocatechin gallate scarvenge free radicals in mice feed with aspatame. The significant *($p<0.05$)* reduction in the antioxidant capacity of rat in this research as shown in figure 1, 2 and 3 above, maybe due to radiation-induced free radical in rats.

flavonoids have been implicated in the upregulation of Nrf-2; Nrf-2 mediated the induction and expression of antioxidant enzymes respnonsible for the scarvenging of free radicals in rats exposed to oral antracene (Olanlokun and Akomolafe, 2013). alkaloids mediates the expression and induction of Gpx. Gpx is responsible for the stabilization of ROS/RNS generated by benzo(a)pyrene in rabbits (Rushmore, Morton and Pickett, 1991). The significant *(p<0.05)* increased in SOD, CAT, GST, Gpx and GSH in irradiated rats administered with n-hexane, ethylacetate and n-butanol fractions of methanolic extract of *A. digitata* and *C. olitorius* leaves maybe due to the presence of significant *(p<0.05)* amount of catechin, quercetin and epigallocatechin in the leaves of *A. digitata* and *C. olitorius.* They might also be responsible for the increase in the induction of the antioxidant enzymes of the treated rats groups in this sudy.

As observed in the irradiated not treated group from figure 1 above, there was significant *(p<0.05)* increase in the level of activtated Nf-kB, suggesting that radiation-induced free radical have exercebated Nf-kB activations as shown in the figure 1. Nf-kB, have been implicated in the expression of pro-inflamatory proteins and supression of antioxidant enzyme synthesis (Rushmore and Pickett, 1990). The activation of Nf-kB, by irradiation might be responsible for the significant *(p<0.05)* reduction of SOD, catalase, GPx and GST in irradiated not treated group as shown in figure 3a, b and c above.

Luteolin and eleagnin are flavonids and polyphenols respectively, they have been validated to prevent the activation of an inactive Nf-kB in the cytosol (Motohashi, 2004). Luteolin and eleagnin also mediated the induction of antioxidant enzymes, that have been implicated to scarvenged free radicals that are capable of exercebating pathological condition and cellular alterations in rat (Olanlokun, 2013). As revealed by the result from figure 1a, b and c, above, irradiation have significantly *(p<0.05)* exacerbated Nf-kB activation in irradiated rats, but administration of n-hexane, ethylacetate and n-butanol fractions of methanolic extract of *A. digitata* and *C. olitorius* leaves significantly *(p<0.0)* reduced the activation of Nf-kB in rat exposed to radiation, such that n-butanol fraction is the most effective of the solvent fraction as shown in Figure 1a, b and c above.

As observed in the irradiated not treated group from figure 4a, b and c above , there were significant *(p>0.05)* increase in the level of serum ALT, AST and ALP of irradiated rats, this suggest, that there is irradiation-induced compromised of the hepatocyte(liver) function (Malomo *et al*., 2001). This action of free radicals on the liver membrane lead to compromise in functions of the membrane channels, thus resulting in efflux of ALT, AST and ALP out the membrane-bound into the serum stream (Adesokan *et al.,* 2001). The significant *(p<0.05)* increase in the activities of serum ALT, AST and ALP of irradiated rats as shown in figure 4a, b and c above, maybe due to the exposure of the rats to irradiation, which inturns increased free radical generation and attack on the membrane.

The administration of n-hexane, ethylacetate and n-butanol fractions of methanol leaves extract of *A. digitata* and *C. olitorius* to irradiated rats, significantly *(p<0.05)* reduced the activities of ALT, AST and ALP of the treated group, such that, n-butanol is the most effective of the three solvent.

The signifciant *(p<0.05)* reduction in the activities of ALP, AST and ALP in the treatment group, as shown in figure 4a, b and c maybe due to the presence of significant *(p<0.05)* amount of Flavonoids in the n-hexane, ethylacetate and n-butanol fractions of the methanol leaves extract of *A. digitata* and *C. olitorius* (Djeussi *et al.,* 2013). Astragalin and eleagnine (flavonids and polyphenols) have been implicated in the

reversal of temporary liver damage in colchicine-induced liver damage in mice (Odunola and Owumi, 2008). Thus, the presence of significant ($P<0.05$) amounts of flavonoids and polyphenols in n-hexane, ethylacetate and n-butanol fractions of methanol leaves extract of *A. digitata* and *C. olitorius* might be responsible for the reversal of the damages observed in the administered groups.

CONCLUSION

Radiation induced cellular alteration in rat and administration of n-butanol fraction of methanol leaves extract of *Adansonia digitata* and *Corchorus olitorius* is the most effective of the solvent fraction, such that it was able to effectively protect and attenuates radiation-induced cellular alteration in rats. We can therefore conclude, that *Adansonia digitata* and *Corchorus olitorius* leaves can be explored as oral remedy against radiation-induced cellular alterations.

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Conflict of Interest

There is/are no conflicts of interest on this work be it at the level of research or manuscript submission.

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