International Journal of Science Academic Research
Vol. 01, Issue 06, pp.326-332, September, 2020
Available online at http://www.scienceijsar.com

ISSN: 2582-6425

Research Article

THE *IN VITRO* AND *IN VIVO* ANTIOXIDANT PROPERTIES OF *CITRULLUS LANATUS* (WATER MELON) FRUIT-PARTS EXTRACTS

[1]Asogwa, Mercy Ebere, [2]Enemali, Michael Okey, [1]Nweze, Chibuzo Carole, [1,*]Haruna, Gambo Sunday and [2]Ogana Joy

[1]Department of Biochemistry and Molecular Biology, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, Nigeria
[2]Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

Received 24th July 2020; **Accepted** 20th August 2020; **Published online** 30th September 2020

Abstract

This research evaluated the antioxidant capacity (*in vitro* and in *vivo*) of the aqueous extracts of mesocarp, exocarp and the ethanol extract of the seeds of *C. lanatus*. DPPH scavenging ability, FRAP, serum MDA concentration, CAT, GPx and SOD activities were determined following standard methods. Results showed that the highest percentage DPPH radical scavenging activity in the endocarp was 96.4500±.58%, for the mesocarp, the highest percentage of DPPH scavenging activity was 96.76±.61%, for the seed extract, the highest percentage DPPH radical scavenging activity was 69.98±1.09%.The endocarp showed the highest DPPH radical scavenging ability followed by the mesocarp. FRAP activity of extracts of the plant parts (endocarp/exocarp, mesocarp and the seeds) were all lower than that of Gallic acid used as standard at all concentrations. For the endocarp/exocarp, the highest ferric reducing antioxidant power scavenging activities was 0.04±.00 μMfe^{2+}/g extract. For mesocarp, the FRAP was 0.01±0.00 μMfe^{2+}/g extract. The highest ferric reducing antioxidant power for the seeds extracts was 0.05±0.00 μMfe^{2+}/g extract. The endocarp showed the highest FRAP radical scavenging ability. Groups 4,5,6,7,8 and 9 (3.03±0.28, 3.07±0.14, 2.75±0.15, 2.46±0.38, 2.62±0.54 and 2.81±0.04 respectively) decreased MDA concentration significantly ($p < 0.05$) when compared to group 2 (5.95±0.64). The test groups 4 and 5 (20.55±0.99 and 26.03±1.08) increased catalase (CAT) activities significantly ($p < 0.05$) when compared to group 2 (13.77±1.49) but the catalase activities in the test groups 6, 8 and 9 (11.87±0.64, 11.21±0.67 and 11.64±1.54 respectively) decreased significantly ($p < 0.05$) when compared to group 2 (13.77±1.49). The GPx activity was significantly higher in groups 6, 7, 8 and 9 (25.21±0.43, 24.23±2.19, 24.845±0.25 and 9 respectively) compared to group 2 (21.29±1.90). The aqueous extract of *C. lanatus* endocarp had the highest DPPH scavenging activities followed by the mesocarp, the aqueous extract of the endocarp also showed the highest activity of FRAP. The mesocarp, endocarp and seed extracts showed significant degrees of endogenous antioxidant activities by reducing MDA concentrations and stabilizing endogenous antioxidants.

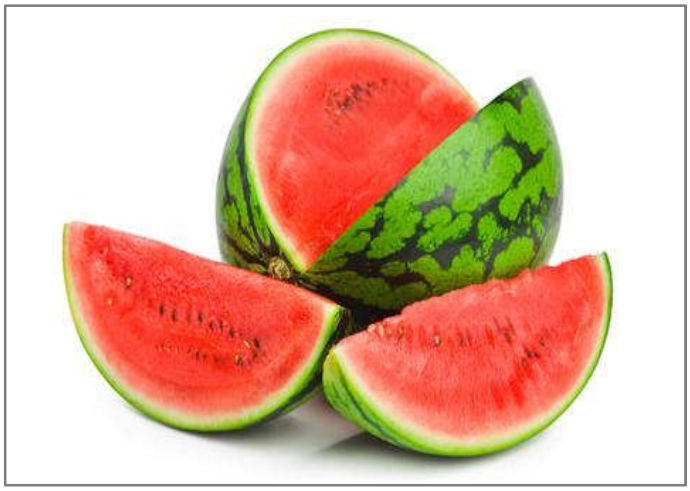
Keywords: *Citrullus lanatus*, Antioxidant properties, Mesocarp, Endocarp, Seeds, Radical scavenging.

INTRODUCTION

In this study, we examined and compared the *in vitro* and *in vivo* antioxidant activities of the aqueous extracts of the mesocarp and endocarp as well as the ethanol extract of the seeds of *C. lanatus* (watermelon). The plant *C. lanatus* is an annual plant that grows up to 3m long and new growth has yellow or brown hairs. Leaves are 60 to 200 mm long and 40 to 150 mm wide. These usually have three lobes which are themselves lobed or doubly lobed. The plant have both male and female flowers on 40-mm-long hairy stalks. It's long, weak, trailing or climbing stems which are five-angled (five-sided) and up to 3 m (10 ft) long. Young growth is densely woolly with yellowish-brown hairs which disappear as the plant ages. The leaves are large, coarse, hairy pinnately-lobed and alternate; they get stiff and rough when old. The plant has branching tendrils. The white to yellow flowers grow singly in the leaf axils and the corolla is white or yellow inside and greenish-yellow on the outside. The flowers are unisexual, with male and female flowers occurring on the same plant (monoecious). The male flowers predominate at the beginning of the season; the female flowers, which develop later, have inferior ovaries, the styles are united into a single column. The *C. lanatus*fruits are usually large with a kind of modified berry called a pepo with a thick rind (exocarp) and fleshy center (mesocarp and endocarp). The wild type have fruits up to 20 cm (8 in) in diameter, while cultivated varieties may exceed 60 cm (24 in).

Corresponding Author:** ***Haruna, Gambo Sunday
Department of Biochemistry and Molecular Biology, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, Nigeria.

The rind of the fruit is mid- to dark green and usually mottled or striped, and the flesh, containing numerous pips spread throughout the inside, can be red or pink (most commonly), orange, yellow, green or white (Maynard *et al.*, 2012). The plant (*C. lanatus*) is one of such medicinal plant that has attracted scientific interest due to its bioactivities (Erhirhie *et al.*, 2013). *C. lanatus sp*. is a natural source of antioxidants such as beta carotene (Charoensiri *et al.*, 2009), vitamin C (Altaş *et al*, 2011), citrulline (Collins *et al.*, 2007). The red fleshy part makes *C. lanatus* an excellent source of lycopene (Perkins-Veazie *et al.*, 2001). The tissue protective effects of its juice have been reported (Akashi *et al.*, 2004).
C. lanatus fruit



Taxonomy of *C. lanatus*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Cucurbitales
Family:	Cucurbitaceae
Scientific name:	Citrullus lanatus
Synonyms:	Citrullus vulgaris
Genus:	*Citrullus*
Species:	*Lanatus*

(NPDC, 2000).

The phrase "Reactive Oxygen Species (ROS)" is used to describe a number of reactive Biomolecules and free radicals derived from molecular oxygen. Oxygen-based radicals are usually produced during aerobic respiration. These molecules, produced as byproducts during the mitochondrial electron transport of aerobic respiration or by oxidoreductases and metal ion catalyzed oxidation, have the potential to cause a number of deleterious events. It was originally thought that only phagocytic cells were responsible for ROS production as their part in host cell defense mechanisms. Recent work has demonstrated that ROS have a role in cell signaling, including; apoptosis; gene expression; and the activation of cell signaling cascades (Hancock *et al.,* 2001). A number of environmental factors (e.g., UV radiation, ionizing radiation, xenobiotics, tobacco smoke) as well as an activation of superficial receptors of the cell may contribute to the increased production of ROS in those organisms (Bartsch and Nair, 2000). The presence of antioxidants help ells to quench the effect of ROS and free radicals. Plants usually produce secondary metabolites known as phytochemicals that help to protect them against oxidative attacks resulting from metabolic activities, when animals eat such plant parts such as fruits, leaves etc. they tend to also acquire those plant secondary products which may also serve the same purpose as antioxidants. Different mechanisms may be followed in the antioxidant pathways but the generally known fact is that antioxidant quenches the effect of free radicals by donating lone pair electrons to them thereby making them stable.

MATERIALS

Plant Material

The plant material used in this study include the exocarp, fleshy mesocarp and seeds of the ripe fruit of *C. lanatus* (watermelon). The ripe watermelon fruit was purchased from Keffi market and identified at the Department of Plant Science and Biotechnology of Nasarawa state University, Keffi, Nigeria.

Experimental animal models

The *in vivo* study was carried out using 45 male albino rats, weighing between 79-220g which were purchased from National Veterinary Research Institute (NVRI) Vom, Jos, Nigeria. The animals were housed in well ventilated cages in the animal house, Department of Biochemistry and Molecular Biology, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi. They were fed with water and starter mash (vital feed) for two weeks (14 days) to acclimatize them to laboratory condition before commencement of the study.

Acetaminophen/Silymarin

The drug, acetaminophen was supplied as a research support from Emzor Pharmaceutical Ltd., Lagos while the Silymarin, a branded drug (Sylibon 140) from Micro Laboratory, India, was purchased from a pharmaceutical store in Keffi town. Solutions was ere made in appropriate volume of distilled water in sample bottles and refrigerated in readiness for the study.

METHODS

Preparation of the *C. lanatus* sample

The water melon fruit was washed under a running tap and cut into medium pieces using knife. The fleshy mesocarp, exocarp/endocarp of the water melon were blended seperately with blending machine set at high speed for some minutes and separately soaked in distilled water for 24 hours for extraction of the bioactive constituents, after which it was sieved using muslin cloth. The juice was filtered using filter paper, the filtrate was concentrated using water bath to get the extract. The seeds was air dried for two weeks and pounded then soaked in ethanol and tightly covered. It was allowed to extract for 48 hours, after which it was sieved and filtered, the filtrates was concentrated using water bath to get the seed extract. The crude extracts were stored in a refrigerator until commencement of the experiment.

Determination of DPPH radical-scavenging activity:

The Free radical scavenging activity of different extracts of *C. lanatus* (mesocarp, endocarp/exocarp and the seeds) were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH). "Briefly, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1ml) was added to 3 ml of different extracts in ethanol at different concentrations (5, 10, 15, 20, 25, 30 µl/ml). Here, only those extracts are used which are solubilized in ethanol and their various concentrations were prepared by dilution method (Vaidyaratnam *et al*., 2002)". The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes, the absorbance was measured at 517 nm by using spectrophotometer (Ahmed *et al*., 2013). Reference standard compound being used was ascorbic acid and the experiment was done in triplicate according to the method of (Patel and Petel, 2011). The IC_{50} value of the sample, which is the concentration of the sample required to inhibit 50% of the DPPH free radical, was calculated using log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity (Koleva *et al*., 2002). The percent DPPH scavenging effect was calculated by using the equation

DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of tests or standard samples.

Determination of Ferric Reducing/Antioxidant Power (FRAP)

FRAP assay was carried out according to the method of Benzie and Strain (1996) with minor modifications for assay on a 96-well microplate. "The FRAP reagent was prepared by mixing 25 ml of 300.0 mmol/L acetate buffer, 2.5 ml of 10 mmol/L TPTZ solution, and 2.5 ml of 20 mmol/L FeCl3 solution in a 10:1:1 ratio. 10 µL of sample was mixed with 200 µL of FRAP reagent; the contents were mixed vigorously and was warmed

to 37°C in oven prior use. Ferric tripyridyltriazine (FeIII-TPTZ) complex is reduced to ferrous tripyridyltriazine (FeII-TPTZ) form in the presence of antioxidants and develops anintense blue color, with maximum absorption at 593 nm. A total of 50 µl samples extract were added to 1.5 ml of the FRAP reagent and mixed well. The absorbance was measured in three replicates. Standard curve of iron (II) sulphate ($Fe_2(SO_4)$) solution (200, 400, 600, 800, 1000 ppm) was prepared using the similar procedure. The results were expressed as µmol Fe (II)/100g extract sample".

Experimental Design for *in vivo* studies

The test Albino rats were weighed and divided into nine (9) groups of five (5) animals per group and treated thus;

Group 1 (normal control): The rats in this group were given only water and starter mash.

Group 2 (negative control): They were given water, feed and Acetaminophen (2g/kg).

Group 3 (standard control); were given water, feed and silymarin (200mg/kg) pre-treatment for two (2) weeks, then fasted for up to seven hours, followed by intoxication with Acetaminophen (2g/kg).

Group 4; were given water, feed and aqueous extract of water melon mesocarp (200mg/kg) pre-treatment for two (2) weeks, then fasted for seven hours, followed by intoxication with Acetaminophen (2g/kg).

Group 5; were given water, feed and aqueous extract of water melon mesocarp (400mg/kg) pre-treatment for two (2) weeks, then fasted for seven hours, followed by intoxication with Acetaminophen (2g/kg)

Group 6: The rats were given water, feed and aqueous extract of water melon exocarp (200mg/kg) pre-treatment for two (2) weeks, then fasted for up to seven hours, followed by intoxication with Acetaminophen (2g/kg).

Group 7: The rats were given water, feed and aqueous extract of water melon exocarp (400mg/kg) pre-treatment for two (2) weeks, then fasted for up to seven hours, followed by intoxication with Acetaminophen (2g/kg).

Group 8: The rats were given water, feed and ethanol extract of water melon seeds (200mg/kg) pre-treatment for two (2) weeks, then fasted for up to seven hours, followed by intoxication with acetaminophen (2g/kg)

Group 9: The animals were given water, feed and ethanol extract of the water melon sees (400mg/kg) pre-treatment for two (2) weeks, then fasted for up to seven hours, followed by intoxication with acetaminophen (2g/kg).

Blood sample collection for endogenous Antioxidant Assay

The rats were humanely sacrificed after 48 hours of intoxication under anaesthesia (Diethyl ether) and blood collected for analysis of antioxidant parameters. The whole blood was collected in covered test tubes and allowed to stand for 60 minutes for clotting to occur. The clotted blood were centrifuged at 3000rpm for 5 minutes to separate the serum which was used for the analysis.

Determination of Malondialdehyde (MDA) Concentration

Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al*. (1993).

Principle: Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) to form a red or pink coloured complex which in acid solution absorbs maximally at 532 nm.

$$MDA + 2TBA \rightarrow MDA: TBA\ adduct + H_2O$$

Procedure: "To a test tube was added 0.1 ml of sample, 0.9 ml of distilled water, 0.5 ml of 25 % TCA and 0.5 ml of 1 % TBA in 0.3 % NaOH. The mixture was shaken thoroughly, incubated at 95 ºC for 40 minutes and cooled in a cold water bath. After the cooling, 0.1 ml of 20% SDS (sodium dodecyl sulphate) was added and the absorbance of the mixture determined at wavelengths of 532 nm and 600 nm against a corresponding blank. The level of lipid peroxidation (MDA concentration) was determined in terms of thiobarbituric acid reactive substances (TBARS) as follows"

$$\%\ TBARS = \frac{A532 - A600}{0.5271 \times 0.1} \times 100 \quad [\text{Slope form standard curve} = y = 0.5208x\ (mg/ml)]$$

Determination of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) was assayed using method described by Xin *et al*. (1991).

Principle: Superoxide dismutase (SOD) reduces superoxide radical to hydrogen peroxide. The theory of this method is based on the competition between SOD activity and iodonitrazolium violet in reacting with superoxide, which is generated by xanthine oxidase (XOD) reaction.

(a) $Xanthine + O_2 \longrightarrow Uric\ acid + O_2$

(b) $O_2^- + O_2 + 2H^+ \xrightarrow{XOD} H_2O_2 + O_2$

(c) $O_2^- + violet \xrightarrow{SOD} oxidized\ detector$

The SOD activity measured is related to 50% inhibition of the detector reduction.

Procedure: "Distilled water (0.9 ml) and serum sample (0.1 ml) was pipetted into a test tube and mixed thoroughly with 0.9 ml of carbonate buffer. 75 µl of xanthine oxidase was then added and the absorbance determined at wavelength of 500 nm for 3 minutes at 20 seconds interval. The changing rate of absorbance was used to determine superoxide dismutase activity"

Determination of Catalase (CAT) Activity

This was determined according to the method described by Aebi (1983).

Principle: The ultraviolet absorption of hydrogen peroxide can be easily measured at 240 nm. On the decomposition of hydrogen peroxide (H_2O_2) with catalase, the absorption decreases with time and from this decrease catalase activity can be measured.

Procedure: "Phosphate buffer (2.5 ml), H_2O_2 (2 ml) and serum sample (0.5 ml) were pipetted into a test tube. To 1 ml of the reaction mixture was added 2 ml of dichromate acetic acid reagent. The absorbance of the mixture was then determined at wavelength of 240 nm at a minute interval into

four places. Catalase activity was calculated using the following equation"

$$\text{Catalytic concentration } (\mu/L) = \frac{0.23 \times log \frac{Absorbance\ 1}{Absorbance\ 2}}{0.00693}$$

Assay for Glutathione peroxidase (GPX)

This was done according to the method of Paglia and Valentine (1967). Glutathione peroxidase (GPX) catalyses the oxidation of glutathion (GSH) by Cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH and $NADP^+$. The decrease in the absorbance at 340 nm is measured.

$$2GSH + ROOH \xrightarrow{GPX} GSSG + ROH + H_2O$$

$$GSSG + NADPH + H^+ \longrightarrow NADP^+ + 2GSH$$

Procedure: "A known volume, 0.5ml of heparinized whole blood was diluted with 2 ml of diluting reagent and this was used for the essay. 50µl of diluted sample was mixed with 1ml of R_1 and R_2 respectively. The initial absorbance of both test and blank were read after 1minute and the timer started simultaneously. Absorbance were read again after 1 and 2 minutes intervals at 340nm.

Glutathione peroxide activity was calculated from the formula below,

µ/l of haemolysate = 8412 x ΔA 340nm/min."

Statistical analysis

Data obtained were analyzed using one-way ANOVA with the help of the software, SPSS. Further test for level of significance were carried out using LSD (least significant difference) and Duncan tests. The acceptable level of significance was $p < 0.05$ for all the data.

RESULTS

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of aqueous extract of endocarp/exocarp, mesocarp and ethanol extract of seeds of citrullus lanatus

The DPPH radical scavenging activity of the endocarp, mesocarp and the seed extracts at all concentrations were higher when compared to that of the ascorbic acid which served as the control. The highest percentage DPPH radical scavenging activity in the endocarp (96.4500±.58%) was observed at 500µg/ml, followed by 94.5967±.67% at 250 µg/ml, 90.0500±.00% at 125 µg/ml, 89.196±.13% at 62.50 µg/ml, 88.89±.23% at 31.25 µg/ml and 81.02±.23% at 15.63 µg/ml while the least percentage DPPH scavenging activity was 59.2600±3.24% at 1000 µg/ml. For the mesocarp, the highest percentage of DPPH scavenging activity was 96.76±.61% at 125 µg/ml, followed by 92.67±.93% at 62.50 µg/ml, 88.43±.70% at 250µg/ml, 84.10±1.07% at 500 µg/ml, 82.33±2.91 at 31.25 µg/ml, 75.34±3.01% at 15.63 µg/ml and 73.05±.94% at 1000 µg/ml. For the seed extract, the highest percentage DPPH radical scavenging activity was 69.98±1.09% at 500 µg/ml, followed by 68.13±.27% at 250 µg/ml, 65.20±.13% at 125 µg/ml, 64.35±.00% at 62.50µg/ml, 63.58±1.06% at 31.25 µg/ml, 62.65±.27% at 15.63 µg/ml and 61.88±1.07% at 1000 µg/ml. Comparatively, the endocarp showed the highest cumulative DPPH radical scavenging ability followed by the mesocarp while the seed extract was the least as shown in table 1.

Ferric Reducing Antioxidant Power (FRAP) radical scavenging activity of aqueous extract of endocarp/ exocarp, mesocarp and ethanol extract of seeds of *C. lanatus*

Table 2 shows that the FRAP radical scavenging activity of extracts of the plant parts (endocarp/exocarp, mesocarp and the seeds) were all lower than that of gallic acid used as standard at all concentrations. For the endocarp/exocarp, the highest ferric reducing antioxidant power scavenging activities was 0.04±.00 µMfe^{2+}/g extract at 250 µg/ml, 500 µg/ml and 1000µg/ml, followed by 0.03±0.00 µMfe^{2+}/g extract at 15.63µg/ml and 31.25µg/ml while the lowest percentage FRAP scavenging activity was 0.02±.00 µMfe^{2+}/g extract at 62.50µg/ml and 125µg/ml. For mesocarp, the ferric reducing antioxidant power was 0.01±0.00 µMfe^{2+}/g extract at all concentration. The highest ferric reducing antioxidant power for the seeds extracts was 0.05±0.00 µMfe^{2+}/g extract at 1000µg/ml, followed by 0.03±0.00 µMfe^{2+}/g extract at 500µg/ml and 250 µg/ml while the least was 0.02±0.00 µMfe^{2+}/g extract at 15.63µg/ml, 31.25µg/ml and 62.50µg/ml. Comparatively, the endocarp showed the highest cumulative FRAP radical scavenging ability followed by the ethanol extract of the seeds while the mesocarp was the least.

Effect of aqueous extract of endocarp/exocarp, mesocarp and ethanol extract of seed of *citrullus lanatus* pre-treatment on Pro-oxidant and antioxidant parameters

Table 3 shows that the pro-oxidant parameter, Malondialdehyde (MDA) concentrations in the negative control group (group 2) increased significantly ($p < 0.05$) when compared to normal control group (group 1), The values of the pro-oxidant marker (MDA) increased in group 2 (5.95±0.64) compared to group 1 (3.63±0.31), indicating the expected effect of acetaminophen toxicity. However, administration of the extracts in the test groups 4,5,6,7,8 and 9 (3.03±0.28, 3.07±0.14, 2.75±0.15, 2.46±0.38, 2.62±0.54 and 2.81±0.04 respectively) decreased significantly ($p < 0.05$) the MDA concentration when compared to group 2 (5.95±0.64), implying that the extracts could ameliorate the degenerating effect on cell lipid membrane induced by actaminophen. The activities of catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) in group 2 (13.77±1.49, 21.29±1.90 and 11.17±0.25 respectively) decreased significantly ($p < 0.05$) when compared to group 1 (22.56±0.98, 27.45±1.73 and 11.50±0.02 respectively). The administration of the extracts in the test groups 4 and 5 (20.55±0.99 and 26.03±1.08) increased significantly ($p < 0.05$) the catalase (CAT) activities when compared to group 2 (13.77±1.49)but the catalase activities in the test groups 6,8 and 9 (11.87±0.64, 11.21±0.67 and 11.64±1.54 respectively) decreased significantly ($p < 0.05$) when compared to group 2 (13.77±1.49).The GPx activity was significantly higher in groups 6,7 8 and 9 (25.21±0.43, 24.23±2.19, 24.845±0.25 and 9 respectively) compared to group 2 (21.29±1.90).

Table 1. DPPH radical scavenging activity of aqueous extract of endocarp/exocarp, mesocarp and ethanol extract of seeds of *Citrullus lanatus*

Concentration (µg/ml)	Endocarp (% inhibition)	Mesocarp (% inhibition)	Seeds (% inhibition)	Ascorbic Acid (% inhibition)
15.63	81.02±0.23[a]	75.34±3.01[e]	62.65±0.27[j]	60.70±0.00
31.25	88.89±0.23[b]	82.33±2.91[f]	63.58±1.06[jk]	63.15±0.00
62.50	89.196±0.13[b]	92.67±0.93[g]	64.35±0.00[k]	63.92±0.00
125	90.0500±0.00[b]	96.76±0.61[h]	65.20±0.13[k]	63.81±0.44
250	94.5967±0.67[c]	88.43±0.70[i]	68.13±0.27[l]	64.41±0.28
500	96.4500±0.58[c]	84.10±1.07[f]	69.98±1.09[l]	63.33±0.03
1000	59.2600±3.24[d]	73.05±0.94[e]	61.88±1.07[j]	63.87±0.00

Results are expressed in Means ± SD (n = 3)
Mean values with different letters as superscripts down the groups are considered significant at $p < 0.05$

Table 2. FRAP activity of aqueous extracts of endocarp/exocarp, mesocarp and ethanol extract of seed of *Citrullus lanatus*

Concentration (µg/ml)	Endocarp µMfe^{2+}/g extract	Mesocarp µMfe^{2+}/g extract	Seeds µMfe^{2+}/g extract	Gallic Acid µMfe^{2+}/g extract
15.63	0.03±0.00[a]	0.01±0.00[c]	0.02±0.00[d]	0.05±0.00
31.25	0.03±0.00[a]	0.01±0.00[c]	0.02±0.00[d]	0.08±0.00
62.50	0.02±0.00[a]	0.01±0.00[c]	0.02±0.00[d]	0.09±0.00
125	0.02±0.00[a]	0.01±0.00[c]	0.02±0.00[d]	0.09±0.00
250	0.04±0.00[b]	0.01±0.00[c]	0.03±0.01[e]	0.15±0.00
500	0.04±0.00[b]	0.01±0.00[c]	0.03±0.01[e]	0.13±0.00
1000	0.04±0.00[b]	0.01±0.00[c]	0.05±0.00[f]	0.18±0.00

Results are expressed in Means ± SD (n = 3)
Mean values with different letters as superscripts down the groups are considered significant at $p < 0.05$

Table 3. Effect of aqueous extracts of endocarp/exocarp, mesocarp and ethanol extract of seeds of *Citrullus lanatus* pre-treatment on Malondialdehyde (MDA) and antioxidant parameters in acetaminophen intoxicated rats

Group	MDA (mg/ml)	CAT (U/mg)	GPx (U/mg)	SOD (U/mg)
Group 1 (NC)	3.63±0.31[a]	22.56±0.98[e]	27.45±1.73[k]	11.50±0.02[m]
Group 2(NeC) ([+ acetaminophen	5.95±0.64[b]	13.77±1.49[f]	21.29±1.90[l]	11.17±0.25[m]
Group 3 [acetaminophen + silymarin)	4.25±0.54[c]	15.98±0.72[g]	24.08±4.30[l]	11.62±0.07[m]
Group 4 [(200mg/kg of Mesocarp)	3.03±0.28[a]	20.55±0.99[h]	24.51±1.42[l]	11.49±0.01[m]
Group 5 [(400mg/kg of Mesocarp)	3.07±0.14[a]	26.03±1.08[i]	23.90±1.39[l]	11.53±0.98[m]
Group 6 [(200mg/kg of Endocarp)	2.75±0.15[d]	11.87±0.64[j]	25.21±0.43[k]	11.48±0.02[m]
Group 7 [(400mg/kg of Endocarp)	2.46±0.38[d]	14.15±0.90[f]	24.23±2.19[l]	11.49±0.02[m]
Group 8 [(200mg/kg of Seeds)	2.62±0.54[d]	11.21±0.67[j]	24.845±0.25[l]	11.48±0.01[m]
Group 9 [(400mg/kg of Seeds)	2.81±0.04[d]	11.64±1.54[j]	25.91±0.85[k]	11.44±0.00[m]

Results are expressed in Means ± SD (n = 5)
Mean values with different letters as superscripts down the groups are considered significant at $p < 0.05$

The activities of SOD showed no significant difference in the extract administered groups when compared to the control groups.

DISCUSSION

This research evaluated the antioxidant capacity (*in vitro* and in *vivo*) of the aqueous extracts of mesocarp, endocarp and the ethanol extract of the seeds of *C. lanatus*, also known as watermelon. For the *in vitro* study, DPPH scavenging ability and FRAP inhibition ability as well as IC_{50}were used as test models while serum samples from albino rats was used for the *in vivo* studies. Results showed a sample concentration-dependent increase in the DPPH radical scavenging activity of the extracts from the 15.63µg/ml to 500µg/ml sample concentrations. The highest percentage DPPH radical scavenging activity in the endocarp (96.4500±.58%) was observed at 500µg/ml, followed by 94.5967±.67% at 250 µg/ml, 90.0500±.00% at 125 µg/ml, 89.196±.13% at 62.50 µg/ml, 88.89±.23% at 31.25 µg/ml and 81.02±.23% at 15.63 µg/ml while the least percentage DPPH scavenging activity was 59.2600±3.24% at 1000 µg/ml. For the mesocarp, the highest percentage of DPPH scavenging activity was 96.76±.61% at 125 µg/ml, followed by 92.67±.93% at 62.50 µg/ml, 88.43±.70% at 250µg/ml, 84.10±1.07% at 500 µg/ml, 82.33±2.91 at 31.25 µg/ml, 75.34±3.01% at 15.63 µg/ml and 73.05±.94% at 1000 µg/ml.

For the seed extract, the highest percentage DPPH radical scavenging activity was 69.98±1.09% at 500 µg/ml, followed by 68.13±.27% at 250 µg/ml, 65.20±.13% at 125 µg/ml, 64.35±.00% at 62.50µg/ml, 63.58±1.06% at 31.25 µg/ml, 62.65±.27% at 15.63 µg/ml and 61.88±1.07% at 1000 µg/ml as shown in table 1. At all sample concentrations, the percentage DPPH radical scavenging activity of *C. lanatus* extracts were higher when compared to that of ascorbic acid which served as standard. The scavenging activity of endocarp/exocarp and seeds of the extracts got to their scavenging peak at 250µg/ml with scavenging activity of 94.59±0.67% and 68.13±0.27 % inhibition respectively, while mesocarp got to its scavenging peak at 125µg/ml concentration with 96.76±0.61 % inhibition. At above 500µg/ml there was a decrease in % inhibition in all the extracts (endocarp/exocarp, mesocarp and seeds). The high percentage of DPPH radical scavenging activity demonstrated by the various parts of the fruit (endocarp, mesocarp and seeds) could be due to the presence of these phytochemicals; tannins, steroids, terpenoids, glycosides and phenolics acting either individually or synergistically thereby conferring on them possible antioxidant properties. This is in agreement with (Oseni *et al*., 2013) whose work also revealed their high percentage composition in the fruit of *C. lanatus* parts. From the results above, it can be seen that Endocarp/exocarp extract of *C. lanatus* has more DPPH radical scavenging activity, followed by Mesocarp and the least radical scavenging activity was seen in the seeds extract. All the extracts of *C. lanatus*

(endocarp/exocarp, mesocarp and the seeds) were more powerful than Ascorbic acid which served as standard. This justified the presumed nutritional and health benefits ascribed to the consumption of *C. lanatus* fruit. The Ferric radical antioxidant power (FRAP) test assesses the reducing power of a substance based on its ability to reduce Fe^{3+} to Fe^{2+}. The FRAP radical scavenging activity of extracts of the plant parts (endocarp/exocarp, mesocarp and seeds) were all lower than that of Gallic acid at all sample concentrations used in this work. For the endocarp, the highest percentage FRAP radical scavenging was same; 0.04±.00%, at 250 µg/ml, 500 µg/ml and 1000µg/ml while the lowest percentage FRAP scavenging activity was 0.02±.00% at 62.50µg/ml and 125µg/ml. The ferric reuducing antioxidant power for mesocarp was observed to be 0.01±0.00 at all concentrations, while the peak FRAP scavenging activity of the seeds extract was 0.05±0.00 at 1000µg/ml. This means a low ferric reducing antioxidant power at the tested concentrations. Comparatively, the endocarp showed the highest cumulative FRAP radical scavenging ability followed by the ethanol extract of the seeds while the mesocarp was the least which is a slight deviation from(Oseni *et al*., 2013), who observed a higher percentage FRAP activity by the mesocarp and seeds. The high FRAP activity observed in this study could also be attributed to the high presence of the phytochemical compounds known to exhibit antioxidant properties and also justifies the consumption of the fruit for the purpose of preventing and curing oxidative stress-related diseases.

The values of the pro-oxidant marker (MDA) increased in group 2 (5.95±0.64)compared to group 1 (3.63±0.31), indicating the expected effect of acetaminophen toxicity. However, administration of the extracts in the test groups 4,5,6,7,8 and 9 (3.03±0.28, 3.07±0.14, 2.75±0.15, 2.46±0.38, 2.62±0.54 and 2.81±0.04 respectively) decreased significantly ($p < 0.05$) the MDA concentration when compared to group 2 (5.95±0.64), implying that the extract could ameliorate lipid peroxidation and restore normal tissue integrity. Lipid peroxidation is a chain reaction process involving the formation and propagation of lipid radicals, the uptake and propagation of lipid radicals, the uptake of molecular oxygen and arrangement of double bonds in the unsaturated lipid which constitutes the cell membrane. This may eventually leading to cell death. Malondialdehyde (MDA) is a reliable and commonly used marker of overall lipid peroxidation levels and the presence of oxidative stress (Kaya *et al*., 2015). Lipid peroxidation product accumulation in human tissues is a major cause of tissue and cellular dysfunction that plays a major role in ageing and most age-related and oxidative stress-related diseases (Anne *et al*., 2010). While the activities of antioxidant enzymes CAT, GPx and SOD decreased in group 2 (13.77±1.49, 21.29±1.90, 11.17±0.25 respectively) compared to group 1(22.56±0.98, 27.45±1.73, 11.50±0.02 respectively), also indicating the obvious inflammatory effect of the acetaminophen. This is likely because the available enzymes in the tissue could have been mobilized to cushion the ravaging effect of the acetaminophen thereby reducing in their original activities. The administration of the extracts in the test groups 4 and 5 (20.55±0.99 and 26.03±1.08) increased significantly ($p < 0.05$) the catalase (CAT) activities when compared to group 2 (13.77±1.49) implying that the mesocarp could not decompose the Hydrogen peroxide (H_2O_2) produced in the system but the catalase activities in the test groups 6,8 and 9 (11.87±0.64, 11.21±0.67 and 11.64±1.54 respectively) decreased significantly ($p < 0.05$) when compared to group 2 (13.77±1.49) implying that the hydrogen peroxide produced in the system could have been decomposed by the extracts. The GPx activity was significantly higher in groups 6,7 8 and 9 (25.21±0.43, 24.23±2.19, 24.845±0.25 and 9 respectively) compared to group 2 (21.29±1.90), implying a low redox reaction between reduced glutathione (GSH) and the oxidized form which could balance the ionic state. This scenario could lead to a poor ability to quench the effect of free radicals produced in the system by the extracts in those groups, hence a diminished antioxidant property. The activities of SOD showed no significant difference in the extract administered groups when compared to the control groups. The reason could be that not much superoxide ion was produced in the system hence effect of the extracts on it could not be significantly observed.

Conclusion

The results obtained from this study revealed that the aqueous extract of *C. lanatus* endocarp had the highest DPPH scavenging activities followed by the mesocarp, for FRAP, the aqueous extract of the endocarp showed the highest activity followed by the ethanol seed extract. The mesocarp, endocarp and seed extracts showed significant degrees of endogenous antioxidant activities by reducing MDA concentrations and stabilizing the endogenous enzyme activities.

Acknowledgement: The authors are grateful to Emzor Pharmaceuticals Ltd. Lagos, Nigeria which through the instrumentality of Mr. Bonaventure Enemali, provided the acetaminophen used for the research.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author's contributions: Authors 1,2,3 and 5 conceived the research, author 2 designed the experiment, author 1 handled the experimental materials and conducted the laboratory analysis, author 4 analysed the data and wrote the initial manuscript.

REFERENCES

Aebi HE, 1983. Catalase. In: Methods of Enzymatic Analysis. 3rd ed. (Bergmeyer, H.U., et al. Ed). Weinheim, Deerfield Beach, Florida. pp. 273-285.

Ahmed M, Saeed F, 2013. Evaluation of Insecticidal and Antioxidant activity of selected Medicinal plants, *Journal of Pharmacognosy and Phytochemistry,* 2(3), 153-158.

Akashi K, Nishimura N, Ishida Y, Yokota A, 2004. Potent hydroxyl radical-scavenging activity of drought-induced type-2 metallothionein in wild watermelon. *Biochem. Biophys. Res. Commun.,* 323(1):72–78.

Altaş S, Kızıl G, Kızıl M, Ketani A, Haris P.I, 2011. Protective effect of Diyarbakır watermelon juice on carbon tetrachloride-induced toxicity in rats. *Food Chem. Toxicol,* 49(9):2433–2438.

Anne N, S, Auge N, Ayala V, Basaga H, Boada J, Brenke R, Chapple S, Cohen G, Feher J, Grune T, Lengyel G, Mann E.G, Pamplona R, Poli G, Porterootin M, Riahi Y, Salvayre R, Sasson, S, Serrano J, Shamni O, Siems W, Siow R. C. M, Wiswedel I, Zarkovic K. Zarkovic N, (2010). Pathological aspects of lipid peroxidation. *Free Radical Research*, 44(10): 1125–1171

Bartsch H, Nair J, 2000. Ultrasensitive and specific detection methods for egzocyclic DNA adducts: Marker for lipid peroxidation and oxidative stress. *Toxicology,*153:105–14

Benzie IF, Strain JJ, 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol,* 299:15-27.

Charoensiri R, Kongkachuichai R, Suknicom S, Sungpuag P, 2009. Beta-carotene, lycoene, and alpha-tocopherol contents of selected Thai fruits. *Food Chem,* 113(1):202–207.

Collins JK, Wu G, Perkins-Veazie P, Spears K, Claypool P.L, Baker R.A, Clevidence B.A, 2007. Watermelon consumption increases plasma arginine concentrations in adults. *Nutrition,* 23(3):261–266.

Erhirhie EO. Ekene NE, 2013. Medicinal values on *Citrullus lanatus* (watermelon): pharmacological review. *Int. J. Res. Pharm. Biomed. Sci,* 4(4):1305–1312.

Hancock JT, Desikan R, Neill SJ, 2001. Role of Reactive Oxygen Species in Cell Signaling Pathways. *Biochemical and Biomedical Aspects of Oxidative Modification,* 29 (2):345-350.

Kaya H, Aydın F, Gürkan M, Yılmaz S, Ates M, Demir V, Arslan Z, 2015. Effects of zinc oxide nanoparticles on bioaccumulation and oxidative stress in different organs of tilapia (Oreochromis niloticus), Environ Toxicol Pharmacol 40:936–947. https://doi.org/10.1016/j.etap.2015.10.001

Koleva II, Beek TAV, et al, 2002. "Screening of Plant Extracts for Antioxidant Activity: a Comparative Study on Three Testing Methods" *Phytochemical analysis,* vol-13, 8-17.

Małgorzata S, Jolanta G, Wojciech W, 2005. Roles of reactive oxygen species and selected antioxidants in regulation of cellular metabolism. *International Journal of Occupational Medicine and Environmental Health*, 18(1):15-26

Mark P, 1998. Antioxidants. Clinical Nutrition Insights, *Advanced Nutrition Publications*, Inc., Revised 19.

Maynard DM, Donald N, 2012. "Cucumbers, melons and watermelons". In Kiple, Kenneth F.; Ornelas, Kriemhild Coneè. The Cambridge World History of Food, Part 2. Medical History. 46. Cambridge University Press. pp. 267–270. doi:10.1017/CHOL9780521402156. ISBN 978-0-521-40215-6. PMC 1044500. PMID 16562324.

National Plant Data Center, NRCS, USDA, 2000. Baton Rouge, LA 70874-4490 USA. *Citrulluslanatus* var. *lanatus,* The Plants Database, database (version 5.1.1)http://plants.usda.gov

Oseni OA, Okoye VI, 2013. Studies of phytochemical and antioxidant properties of the fruits of watermelons (*Citrullus lanatus*). *Journal of Pharmaceutical and Biomedical Sciences*, 27:508-514.

Paglia DE, Valentine W.N, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Lab Clin Med,* 70, 158-169.

Patel R.M, Patel N.J, 2011. "In vitro antioxidant activity of coumarin compounds by DPPH, superoxide and nitric oxide free radical scavenging methods" *Journal of Advanced Pharmacy Education and Research,* 1, pp 52-68.

Perkins-Veazie, P, Collins J.K., Pair S.D, Roberts W, 2001. Lycopene content differs among red-fleshed watermelon cultivars. *J. Sci. Food Agric,* 81(10):983–987.

Sies H, 1997. "Oxidative stress: oxidants and antioxidants" *Experimental Physiology,* 82(2): 291–5.

Vaidyaratnam, V, 2002. Indian Medicinal Plants-A Compendium of 500 species, I, Orient longman publishing house, Kottakkal-India, 146.

Wallin B, Rosengren B, Shertzer HG, Camejo G, 1993. Lipoprotein oxidation and measurement of TBARS formation in single microlitre plate; it's use for evaluation of antioxidants. *Analytical Biochemistry,* 208: 10-15.

Xin Z, Waterman DF, Henken RM, Harmon RJ, 1991. Effects of copper status on neutrophil function, superoxide dismutase and copper distribution in steers. *Journal of Diary Sciences*, 74: 3078-3082.

****