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Comparative study of physicochemical properties, fatty acid composition, antioxidant and toxicological potential of *Citrullus lanatus* and *Citrullus colocynthis* seeds oils

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ABSTRACT

Citrullus colocynthis (*C. colocynthis*) seed is a common and expensive cuisine in West African diet unlike the seeds from *Citrullus lanatus* (*C. lanatus*) that are agro waste. The oils from these seeds were comparatively profiled using their physicochemical properties, fatty acids and phenolic contents, *in-vitro* antioxidant capacity (DPPH, TEAC, FRAP, ORAC), inhibitions of lipid peroxidation and tyrosinase. Toxicities of the oils in mice were also assessed using plasma AST, ALT, cholesterol, HDL-C, liver, heart and kidney (GSH, GPx and SOD) and clastogenic effects in mouse bone marrow. *Citrullus lanatus* oil contains higher peroxides, saponification, saturated fatty acids and stearic acid (6-fold) levels, with a lower acid value when compared to *C. colocynthis*. Strong antioxidant capacity, inhibition of lipid peroxidation and tyrosinase by *C. lanatus* oil extract is proposed to be related to the presence of catechins that are absent in *C. colocynthis*. The *in-vivo* study indicated that *C. lanatus* induced significant increase in ALT while a significant increase of AST was observed in the *C. colocynthis* treated group. *Citrullus lanatus* induced a significantly ($p<0.05$) higher heart GPx activity when compared with *C. colocynthis* and the control groups. Both oils did not affect SOD activity and showed no clastogenic effect in mouse bone marrow ($p>0.05$). This work reports for the first time the presence of stearic acid and catechin in *C. lanatus* oil which is important for its compelling *in vitro* activity. High doses of both oils could evidently induce toxicological indices in mice.

1. Introduction

Free radicals are highly reactive, acting as oxidants and when in excess causing induced oxidative damage to macromolecules such as protein, lipids, DNA and amino acids leading to disruption of major cell functions (Chainy and Sahoo, 2020; Di Meo and Venditti, 2020). A free radical product of normal metabolic process can only induce oxidative tendency due to low cell antioxidant capacity (Akbari et al., 2019). Fatty acids and oils with natural antioxidant properties are of growing interest in recent years to the food, cosmetic and pharmaceutical industries because of their known health promoting benefits (Segura Munoz et al., 2020; Huang et al., 2018). The assessment of their antioxidant value provides an alternative to the use of synthetic antioxidants mainly because of their relative lesser toxicity (Amorati et al., 2013). Antioxidants of plant origin have shown to not only delay development of rancidity but also stabilize auto-oxidation due to the presence of phenolic compounds (Conde et al., 2008; Matumoto-Pintro et al., 2017). Synthetic antioxidant compounds such as butylhydroquinone and butylated hydroxyanisole are extensively used in foods and food products to prevent its oxidation, but are frowned upon as certain health challenges like toxicosis and carcinogenesis promotions have been documented (Andrade et al., 2016). Results of scientific studies have positively correlated plant antioxidant potential with oxidative stress defense and consequently protecting humans from different disease types, including cancer, atherosclerosis and the aging process (Griffiths et al., 2016; Karimian et al., 2017). Plant derived oils have multi-varied purposes such as an additive and stabilizer in foods, drinks, cosmetic creams, antimicrobial and anti-fungal agents (Bardají et al., 2016, Komane et al., 2017). Oils obtained from seeds have been shown to be good sources of antioxidant components (Ishtiaque et al., 2013; Ribeiro et al., 2017). The search for oils rich in bio-active components has been a new trend of research focus in recent years. This is because

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they are important as additives for lipid-containing meals as stabilizers and modulators of oxidative rancidity (Schmidt et al., 2003).

The fruits of *Citrullus lanatus* (Thunb.) are widely eaten as snacks without due regards to the seeds, mainly ascribed to the lack of enough knowledge on the nutritional composition of the seeds which are simply thrown away. This is in spite of the fact that it is of the same family as *Citrullus colocynthis* (L.) which seeds have several applications in the southern part of Nigeria. Oil of *Citrullus colocynthis* seeds is one of the most expensive cooking oils one can find in Nigeria. However, the seed of *Citrullus lanatus* does not enjoy this. This research is therefore designed to comparatively study the fatty acids contents, physicochemical property, *in-vitro* antioxidant capacity and *in-vivo* effects of *Citrullus lanatus* and *Citrullus colocynthis L.* seed oils. Assessment of potential components and biochemical influence of *Citrullus lanatus* could provide an exciting alternative for the sustainable agricultural product for food security.

2. Materials and methods

2.1. Reagents

Petroleum ether, methanol, N-hexane, hydrogen sulphate, potassium hydroxide, ethanol were purchased from British Drug House (BDH) Chemicals Limited (Poole, England). Rutin, gallic acid, quercetin, catechin, caffeic acid, chlorogenic acid, quercetin, trolox (6-hydroxyl-2, 5, 7, 8- tetramethylchroman-2-carboxylic acid), EGCG (Epigallocatechin gallate), and other reagents such as fluorescein sodium salt, potassium peroxodisulphate, ABTS (2,2- azino-bis (3-ethylbenzo thiazoline-6-sulfonic acid) diammonium salt), AAPH (2,2-Azobis (2-methylpropionamidine) dihydrochloride, iron (III) chloride hexahydrate, TPTZ (2,4,6-tri[2-pyridyl]-s-triazine reduced glutathione (GSH), epinephrine and May-Grunewald stain were bought from Sigma-Aldrich (Cape Town, South Africa). Aspartate Aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, protein kits were purchased from RANDOX (Randox Laboratories, Crumlin, County Antrim, UK). Fatty acid compositions of the oils were determined using Gas Chromatography Flame Ionization Detector (GC-FID) (7820A GCFID, Agilent Technologies, Santa Clara, CA 95051, USA). Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA 95051, USA) was used to identify polyphenolic compounds in the oils.

2.2. Seeds

Citrullus lanatus (Water melon) and *Citrullus colocynthis* (melon) used for this study were purchased from a local market in Osogbo, Osun State, Nigeria. *Citrullus lanatus* seeds and *C. colocynthis* seeds were manually removed from their fruits washed and air dried and kept in an airtight container until ready for use.

2.3. Extraction of seed oils

Water melon and melon seed oils were extracted with petroleum ether using a soxhlet extractor as described in AOAC methods (1990). The oils were obtained after evaporating the solvent at 70^{o}C. The extracted seed oils were stored at -20^{o}C until analysis.

2.4. Physico-chemical properties of oil

The physico-chemical analyses of the oils were carried out following the methods as described in AOAC (2000).

2.4.1. Determination of Saponification value

One gram of oil and 25 ml of 0.1 M alcoholic potassium hydroxide were heated under reflux for 1 h with continuous constant shaking in Erlenmeyer flask until saponification was completed. The blank sample without oil was conducted along with the sample. Thereafter, excess potassium hydroxide was titrated with standard 0.5 M hydrochloric acid (HCl) using phenolphthalein as an indicator until cloudy solution is formed. The saponification value was calculated as stated in Eq. 1 below;

$$\text{Saponification value} = \frac{56.1 \times (b - a) \times N}{W} \quad (1)$$

where 'a' is the sample and 'b' is the blank titre values, N is 0.5 M normality of HCl and W stands for the weight of 1 gram oil.

2.4.2. Determination of iodine value

Oil (5 g) was dissolved in 10 ml carbon tetrachloride and 20 ml Hanus' solution was added inside 250 ml glass stoppered flask. The content was swirled for mixing and kept in the dark for 30 min. Then, 15 ml of potassium iodide was added followed by 100 ml of distilled water and 1 ml starch. The liberated iodine was titrated with 0.1 M standard thiosulphate solution to form blue colour that disappeared after shaking. Iodine value was calculated according to the Eq. 2 below;

$$\text{Iodine value} = \frac{(b - a) \times N \times 1.269 \times 100}{W} \quad (2)$$

Where 'a' and 'b' is sample and blank titre values respectively, N is normality of thiosulphate and W stands for the weight of the sample.

2.4.3. Determination of peroxide value

Oil sample (5 g) was added to 30 ml of acetic acid and chloroform (3:2) mixture followed by swirling to dissolve the oil. Potassium iodide solution (1 ml) was added to the mixture in the dark with occasional shaking for 1 min after which 30 ml of distilled water was added. The solution was titrated with 0.01 N sodium thiosulphate solution using 1 ml starch solution as an indicator. The titration reaction was continuously shaken until blue colour permanently disappear, an indication of release of iodine (I_2) from chloromethane (CH_3Cl) layer. The result was calculated as stated in the Eq. 3 below;

$$\text{Peroxide value} = \frac{V \times N \times 100}{W} \quad (3)$$

Where, *V* is volume of sodium thiosulphate, *N* is normality used for titre, and *W* is weight of the sample.

2.4.4. Determination of specific gravity of the oil

The specific gravity of the oil samples was determined using 25 ml specific gravity bottle. The bottle was filled with the oil sample up to the 25 ml mark on the bottle. Then, the bottle containing the sample oil was weighed. The weight of the oil obtained, by removing the weight of the empty bottle from the weight of the bottle filled with oil was divided by the weight of an equal volume of water to get specific gravity of the sample oil.

2.4.5. Determination of oil refractive index

Refractometer (Hackettstown, NJ, USA) was used to determine the refractive index of oil samples. Few drops of oil sample were placed on the prism. The prism was closed and allowed for 2 min to stand. The instrument and lighting were adjusted until distinct reading was obtained. The instrument reading was converted to refractive index using Butyro refractometer reading and indices of refraction table.

2.4.6. Determination of acid value and percentage free fatty acids (FFA)

Oil sample (1 g) was dissolved in 10 ml ethanol (95%) in a conical flask. The solution was boiled for 5 min and allowed to cool. The solution was titrated with 0.1 M potassium hydroxide (KOH) using phenolphthalein as an indicator under constant shaking. The titration was stopped as pink colour of the reaction disappeared. Acid value was calculated according to the Eq. 4 and percentage free fatty acids

calculated using the Eq. 5 below;

$$\text{Acid Value} = \frac{2.82 \times V \times 100}{W \times 1000 \times 4} \quad (4)$$

$$\text{Free Fatty acid (FFA\%)} = \frac{(v - b) \times N \times 28.2}{\text{Weight of sample (g)}} \quad (5)$$

For acid value; W stands for weight of the oil (1 g), V is titre value of 0.1 M KOH and 2.82 is equivalent oleic acid while 2.00 was used for lauric equivalent.

For percentage free fatty acids; v = volume of titrant (KOH), b= blank volume (titration devoid of mixing with oil) N = Normality of KOH, 28.2 = conversion factor of oleic acid while 20.03=conversion factor of lauric acid.

2.5. Fatty acid profiling

Fatty acid methyl esters (FAMEs) of the two oils were prepared following the method described by Wang et al. (2015) for the profiling of the fatty acid constituents. The extracted oil (40 μl) was added to 700 μl, 10 M potassium hydroxide and 5.3 ml of methanol in a centrifuge tube. The contents were allowed to react for 1.5 h with constant mixing for 5 sec at every 20 min at 55°C where after it was allowed to cool to room temperature before adding 500 μl of 10 M sulphuric acid solution. It was further incubated 55°C for 1.5 h with constant mixing for 5 sec at every 20 min and again allowed to cool down when 3 ml of n-hexane was added and mixed for 5 min. The tube was centrifuged for 5 min at 150 g and the extract removed for subsequent fatty acid methyl ester analysis on the GC-FID (with J&W HP5 Column). The identification of the oils' constituents was done by matching retention time with that of GC-FID FAME standards.

2.6. In vitro antioxidant activity and lipid peroxidation inhibition

2.6.1. 2, 2-diphenyl-1-picrylhydrazl (DPPH) scavenging activity

The DPPH scavenging ability was determined by following the spectroscopic method described by Kim et al. (2002). Seed oil extracts of graded concentrations at 100 μl were added to 100 μl of 0.25 mM DPPH (in methanol) in 96-well microtitre plate. The reacting mixtures were allowed incubate in the dark for 30 min. The absorbance was read using spectrophotometry (Multiskan, Thermo Scientific, USA) at 517 nm.

2.6.2. Ferric reducing antioxidant power (FRAP) evaluation

Ferric reducing antioxidant power (FRAP) of the seed oils were evaluated using the method as reported by Benzie and Strain (1996). Seed oil samples (10 μl) were added to 300 μl FRAP reagent (consisting of 10 mM tripyridyl triazine (TPTZ) in 40 mM hydrochloric acid (HCl), acetate buffer (300 mM, pH 3.6) and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$) in ratio 1:10:1 (v/v/v)). The reacting mixture was incubated for 30 min in the dark and read at wavelength of 593 nm in the multiplate reader. Ascorbic acid was used as standard using varying concentrations of 0 to 500 μM and result presented as ascorbic acid equivalents per milligram dry weight (μM AAE/g) of the test oil samples.

2.6.3. Trolox equivalent antioxidant capacity (TEAC) evaluation

Trolox equivalent antioxidant capacity (TEAC) was evaluated following the method of Re et al. (1999). The working solution was prepared by keeping the mixture of 88 μl of 140 mM potassium peroxydisulphate ($K_2S_2O_8$) and 5 ml of 7 mM 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) in the dark for 16 h. Then, working solution was diluted with ethanol after 16 h until the absorbance at 734 nm read within 2.0 ($\pm$ 0.1). The seed oil samples or standard (25 μl) was mixed with 300 μl working solution and left to incubate for 30 min at room temperature. Trolox was used as standard with concentration range between 0 to 500 μM. The multiplate reader was used to read the absorbance at 734 nm. The results were presented as Trolox equivalents per milligram dry weight (μM TE/mgDW) of test oil samples.

2.6.4. Oxygen radical absorbance capacity (ORAC)

ORAC was assessed using the method described by Cao et al. (1997) that measures antioxidant capacity of thermal decomposition generated by peroxyl radical of 2, 2'-azobis (2-aminopropane) hydrochloride (AAPH). Oil samples (12 μl) were added to 138 μl fluorescein working solution in black walled 96-well microplate. The reaction was initiated by the addition of 50 μl AAPH (19 mM). Fluoroskan plate reader was programmed to measure the absorbance at every 2 min for 2 h after the addition of AAPH at excitation and emission wavelength set at 485 and 530 nm respectively. The ORAC data were calculated using a regression equation ($Y=a + bx + CX^2$) between Trolox concentration (Y in μM) and the area under the fluorescence decay curve (X). The values obtained were expressed as μMTE/mg of oil samples.

2.7. Ferrous ion-induced microsomal lipid peroxidation inhibition

Ferrous ion-induced microsomal lipid peroxidation was evaluated as described by Snijman et al. (2009). Oil samples stock solution in methanol was diluted in 0.01M phosphate buffer (pH 7.0) to make working solution. Working solution of oil sample (100 μl) was preincubated with 500 μl microsomes at 37 °C in shaking water bath for 30 min. Potassium chloride buffer (200 μl) followed by 200 μl ferrous sulphate (2.5 mM) were added to reacting mixture allowed to incubate for 1 h at 37 °C in shaking water bath. The reaction was terminated using 1 ml trichloroacetic acid (TCA) (10%) solution, containing 125 μl butylated hydroxytoluene (BHT, 0.01%) and 1mM ethylenediaminetetraacetic acid (EDTA). The reacting mixture was centrifuged for 15 min at 2000 rpm. Thereafter, 1 ml of supernatant was mixed with 1 ml thiobabituric acid (0.67%) solution. The reacting mixture was heated for 20 min at 90 °C, allowed to cool down and absorbance was taken using the Microplate Reader at wavelength of 532 nm. The percentage inhibition of lipid peroxidation in terms of thiobarbituric reactive species (TBARS) relative to control was calculated using the Eq. 6 below;

$$\%\ \text{TBARS Inhibition} = \frac{(\text{Acontrol} - \text{Asample}) \times 100}{\text{Acontrol}} \quad (6)$$

Where Acontrol= absorbance without sample (0.01 M phosphate buffer) and Asample= absorbance in the present of sample.

2.8. Tyrosinase inhibition

The tyrosinase enzyme activity was evaluated following the methods of Chompo et al. (2012) and Momtaz et al. (2008). The stock oil samples were dissolved in dimethyl sulfoxide and working solution was made with 50 mM phosphate buffer (pH 6.5). The oil sample or standard (Kojic acid) (70 μl) was added to 30 μl tyrosinase (500 units/ml in sodium phosphate buffer) and allowed to incubate for 5 min at 25 °C. Then, 110 μl substrate (2 mM L-tyrosine) was added to each well and left to incubate for 30 min. The absorbance was read at 490 nm using the multiplate reader. Percentage tyrosinase inhibition was calculated according to the Eq. 7 below;

$$\%\ \text{Tyrosinase inhibition} = \frac{[(A - B) - (C - D)] \times 100}{(A - B)} \quad (7)$$

Where A is the absorbance of Control in the presence of enzyme, B is absorbance of control without enzyme, C is the absorbance of test sampleS and D stands for absorbance of sample without enzyme.

2.9. Phenolic compounds identification

Polyphenolic compound profiling was done on the Agilent 1200 series HPLC using a YMC Pack-pro C18 column with 150 mm x 4.6 I. D. The mobile phase A was water containing 0.1% trifluoroacetic acid and phase B was methanol containing 0.1% trifluoroacetic acid. The gradient elution was with 100% A at 0 min changing to 100% at 25 min with flow rate at 1 ml/min and column temperature at 21°C. The wavelength detection was set at 280, 320 and 350 nm. The oils were dissolved in ethanol and identified by comparing the retention time in a chromatogram with the reference standards gallic acid, rutin, quercetin, catechin, caffeic acid, chlorogenic acids and quercetin.

2.10. Animals study

Male Albino mice (45) of average weight of 25 g were purchased from the Medical Laboratory Science Department, Ladoke Akintola University of Technology, Osogbo, Osun State. The study was conducted according to the recommendations in the Guide for the Care and Use of Laboratory Animals (based on ARRIVE guidelines) of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The study was approved by the Committee on the Care and Use of Laboratory Animals, Faculty of Basic Medical Sciences. The animals were kept in the Animal House of the Department of Biochemistry to acclimatize for 2 weeks before the commencement of the experiment. They were maintained at ambient condition with access to water and standard mouse chow *ad-libitum*. The animals were randomly divided into different experimental groups as stated below;

Group A: Animals were orally intubated with 5 ml/kg body weight of *C. lanatus* seed oil
Group B: Animals were orally intubated with 5 ml/kg body weight of *C. colocynthis* seed oil.
Group C: Control group were orally intubated with water only.

The animals were exposed to their respective treatments daily for 15 days consecutively. At the end of the exposure, the animals were fasted overnight and sacrificed under mild anesthesia. The blood was collected by cardiac puncture into heparinized tube while liver, kidneys and heart were excised from the animals for biochemical analyses. The blood samples were centrifuged at 5,000 rpm for 5 min to obtain plasma. The different tissues were homogenized in a cold homogenizing buffer using a Teflon head homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C to obtain the post mitochondrial supernatant fraction used for the evaluation of enzyme activity. All the tissue samples were stored at -20°C in aliquots until ready to be analyzed.

2.11. Biochemical analysis

Plasma alanine transaminase (ALT), aspartate transaminase (AST), cholesterol and protein concentrations were assessed using RANDOX (Randox Laboratories Ltd, 55 Diamond Road, Crumlin, County Antrim, UK) reagent kits according to the Manufacturer's instruction. Plasma HDL-C was also evaluated using the same commercial kit after precipitating with a heparin- manganese (II) chloride solution.

2.12. Tissue glutathione content and antioxidant enzyme activity

2.12.1. Tissue reduced glutathione (GSH) evaluation

Reduced glutathione level in the homogenates was assessed by following the Beutler's method as described by Chavan et al. (2005). Tissue homogenate (0.2 ml) was diluted in 1.8 ml normal saline (0.9% NaCl) (1 in 10 dilution; V/V). Then, 3 ml of 4% sulphosalicyclic acid was added and allowed to stand for 10 min. The reacting mixture was centrifuge for 5 min at 3000 rpm. The obtained supernatant (0.5 ml) was added to 0.5 ml 0.1 M phosphate buffer (pH 7.4). Elliman's reagent (1mM; 0.5 ml) was added to the reacting mixture and absorbance was read at 412 nm. Tissue GSH content was extrapolated using the calibration curve of standard GSH. The result is expressed as GSH unit/mg protein

2.12.2. Tissue reduced glutathione reductase (GPx) enzyme

Rostruck et al. (1973) method was employed to evaluate glutathione peroxidase (GPx). Glutathione peroxidase reacting mixture consisting of 200 μl Tris-HCl (0.04 M), 100 μl sodium azide (0.01 M), 100 μl H_2O_2 and 200 μl GSH (0.4 mM) were added to homogenate sample (200 μl). The reacting mixture was incubated for 30 min. The reaction was stopped by the addition of 200 μl 10% trichloroacetic acid (TCA) and centrifuged for 10 min at 300 rpm. Supernatant (0.5 ml) was added to 0.5 ml 0.1 M phosphate buffer (pH 7.4). Elliman's reagent (1mM; 0.5 ml) added to the reacting mixture and absorbance read at 412 nm. The result was expressed as μGSH remaining/mg protein.

2.12.3. Tissue superoxide dismutase enzyme (SOD)

An indirect method of adrenaline autooxidation to adrenochrome was employed to evaluate SOD activity at 320 nm wavelength (Sun and Zigman, 1978). Diluted tissue homogenate (1: 9 in normal saline) sample (300 μl) was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to incubate briefly. The reaction started by the addition of 300 μl of adrenaline and absorbance was taken for 2.5 min at every 30 sec at a wavelength of 360 nm. Unit of SOD was calculated according to the Eq. 8 below:

$$\%\ \textit{adrenaline to adrenochrome inhibition} = \frac{100 - (\textit{increase in absorbance of sample} \times 100}{\textit{increase in absorbance of blank}}. \quad (8)$$

1 unit of SOD activity is defined as the quantity of SOD necessary to elicit 50% inhibition of adrenaline oxidation to adrenochrome in one min. The final unit of SOD is stated as SOD unit/mg protein.

2.13. Micronucleus assay

This assay is used to assess the clastogenic effect of a compound. The method used followed the procedure as described by Schmid (1975). The bone marrow of mice in bovine serum albumin was spread on the microscopic slide and allowed to dry. The slide containing bone marrow was stained with May-Grunewald stain (1:1 distilled water). Excess stain was removed with distilled water and dried at room temperature. The nucleated cell stained dark blue while polychromatic cells stained violet-blue.

2.14. Statistical analysis

Results are expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by Tukey's test was used to analyze the results with $p < 0.05$ considered significant.

3. Results and discussion

There is growing interest in underutilized plant materials with bio-active compounds for potential uses in health and cosmetic industries (Koné et al., 2011; Hunter et al., 2019). This work evaluated potential benefit and adverse effect of the oil extracted from the underutilized *C. lanatus* (water melon) seeds in comparison with the oil from *C. colocynthis* (melon) seeds. The result of the percentage acid content of the oils (Table 1) revealed a lower % for *C. lanatus* oil when compared to *C. colocynthis* oil. Acid value is an important

Table 1.
Physicochemical Properties of CL and CC seeds oils.

Sample	*CL	*CC	p-value
FFA as oleic acid (%)	12.1±0.3	16.1±0.1	0.0001
Acid Value as oleic (%)	24.0±0.1	32.0±0.2	0.0001
FFA as lauric acid (%)	8.6±0.7	11.4±0.3	0.0021
Acid Value as lauric (%)	17.1±0.1	22.7±0.6	0.0001
Specific Gravity (gcm^{-1})	0.9±0.0	0.9±0.0	1.0000
Degree of refractive Index	1.5±0.0	1.5±0.0	1.0000
Iodine Value ($meqkg^{-1}$)	78.7±1.0	80.7±0.9	0.0643
Saponification Value ($mgKOHg^{-1}$)	95.4±1.0	88.4±1.3	0.0019
Peroxide Value ($meqkg^{-1}$)	6.6±0.1	3.4±0.4	0.0002

* Values are expressed as mean ± SD of triplicate evaluations. FFA=free fatty acid, CL= *Citrullus lanatus*, CC= *Citrullus colocynthis*.

Table 2.
Fatty acids profile of CL and CC seed oils using GC-FID.

Fatty acids	Lipid value	Concentration (mlL^{-1})	
		CL oil	CC oil
Myristic acid	C14:0	9.13215e-2	4.41411e-1
Tridecanoic acid	C13:0	5.56508e-1	4.11945e-1
cyclopropanenonanoic	C12:0 (cyclo)	8.27797e-3	2.36975e-1
Palmitoleic	C16:1	1.50309e-1	3.07259e-1
Palmitic	C16:0	2.02192e-1	1.96669e-1
Hexadecenoic	C16:1Δ9	2.47594e-1	2.31887e-1
Heptadecanoic	C17:0	1.11218e-1	1.07261e-1
Linolelaidic	C18:1Δ9,12	1.32151	1.52880
Trans-13-octadecenoic	C19:0Δ2	5.19642e-1	3.71040e-1
Stearic acid	C18:0	1238.38343	264.49121
Elaidic acid	C18:1	2.08255	1.59644
Cis-10-heptadecenoic	C17:1Δ10	7.52246e-1	7.54692e-1
Arachidic acid	C20:0	7.01930e-1	8.08240e-1
Heneicosanoic acid	C21:0	6.21648e-1	6.08041e-1
Cis-11,14-eicosadienoic	C20:2Δ11,14	1.28443	1.24767
11-hexadecenoic acid	C17:0Δ11	4.19380	4.29970
Behenic	C22:0	1.52743	1.47979
Tricosanoic acid	C23:0	1.00142	1.01991
Pentadecanoic acid	C25:0	————	3.07180e-2

CL= Citrullus lanatus, CC= Citrullus cococynthis

parameter used in evaluating the quality of oil (Ogata et al., 2014; Negash et al., 2019).

Lower acid values are usually indicative of a lower free fatty acid composition, which suggests lesser susceptibility of the oil to rancidity (Li et al., 2009). Rancidity is the occurrence of oxidative degradation as a result of free radical attack on the double bonds in the oil causing an unpleasant smell or odour. Oil of low acidity has been considered to be acceptable for edible application (Ogbunugafor et al., 2011). All the acid values of *C. lanatus* were consistently lower than that of *C. colocynthis* oil. This implies that *C. lanatus* oil is less susceptible to rancidity and therefore is of better quality when compared with *C. colocynthis*. The amounts of peroxide value of oils indicate the degree of primary oxidation and therefore its likeness of becoming rancid. The higher peroxide value (Table 1). of *C. lanatus* oil (6.6 meq/kg) compared to *C. colocynthis* oil (3.4 meq/kg) is within an acceptable range of not more than 10-20 meq/kg fat to avoid a rancid flavour (Kong and Singh, 2011). The specific gravity of both seed oils was not significantly different ($p>0.05$). The result of specific gravity of *C. lanatus* obtained in this study (0.92) is comparable to the result (0.85) reported by Duduyemi et al. (2013) and Negash et al. (2019). The recommended value for biodiesel production is between 0.87-0.90 specific gravity (Ejikeme et al., 2010). The results propose that both the oils can be utilized for biodiesel production. Specific gravity of *C. colocynthis* oil obtained is (0.91) which is in agreement with the value obtained by Ogunwa et al. (2015). Also, the specific gravities of the two oils are less dense when compared with water. Low specific gravity of the oil when compared with water is an asset for skin cream production because it makes the oil to flow and spread easily on the skin (Oyeleke et al., 2012). Saponification value is important for the suitability of the oil for soap making. Saponification value for *C. lanatus* oil is higher than that obtained from *C. colocynthis* oil. The previous report on the saponification value of *C. lanatus* oil by Duduyemi et al. (2013) is higher (183.13 mgNaOH/g) than what we obtained in this study. The disparity in the value of saponification obtained in this study and the previous work of Duduyemi et al. (2013) can be related to differences in the solvent and methods used for extracting the oil. Iodine value provides details about the level of unsaturation in oil and indicates its stability towards oxidation (Knothe, 2002). There is no significant difference in the iodine values of both oils tested in the current study (Table 1), which implies that the two oils have the same level of unsaturation. There is also no significant difference ($P>0.05$) in the refractive index (Table 1) of *C. lanatus* (1.4735) and *C. colocynthis* (1.4729). However, the fatty acid profile of the oils revealed by GC-FID showed that *C. lanatus* and *C. colocynthis* present the same major oil, stearic acids (Table 2). The stearic acid content of *C. lanatus* is six (6) fold higher than that of *C. colocynthis*. *Citrullus lanatus* seed oil can, therefore, serve as a good source of stearic acid. Stearic acid is a saturated long chain fatty acid with unique health promoting potential. It has been reported to play several health beneficial roles as an anti-inflammatory lipid, induction of selective apoptosis, protecting the brain from ischemic and oxidative abuse, it increases serum oleic acid through the stimulation of hepatic enzymes and it is neutral to blood cholesterol unlike other saturated lipids like palmitic, myristic and lauric acid (Wang et al., 2007; Goradel et al., 2016).

Health importance of food products is mainly a function of their antioxidant activities. Lipids are food components that are known to be vulnerable to oxidation, which compromise colour, odour and nutritive value of the food. Antioxidant components present in the oil prevent such changes by impeding the process of oxidation (or rancidity). It also stabilizes auto-oxidation due to the presence of phenolic compounds (Andrade et al., 2016). A wide variety of methods have been used to determine the antioxidant capacity of many oils. In this study, a battery of assays, ORAC, FRAP, TEAC and DPPH were used to evaluate the *in vitro* antioxidant activities of the oils while HPLC was used to identify bioactive polyphenolic compounds. Figs. 1 and 2 show the antioxidant activity of the *C. lanatus* oil is significantly ($P<0.05$) higher than *C. colocynthis* oil as shown by the ORAC, FRAP TEAC and DPPH capacities. This result implies that *C. lanatus* oil might not require addition of synthetic antioxidant riddled with safety concerns for its stability and increase shelf-life period like *C. colocynthis* (Taghvaei and Jafari, 2015). The protective ability of natural antioxidant containing foods against several oxidative related health

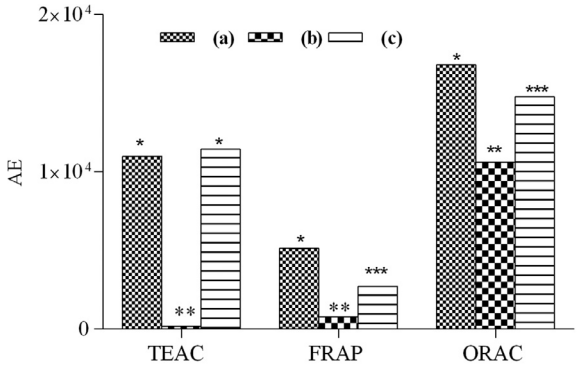

Fig. 1. Antioxidant levels of *Citrullus lanatus* oil (a), *Citrullus colocynthis*, oil (b) and EGCG (c) evaluated using Trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) assays. Each bar represents Mean ±SD of three different separate experiments. Bar with different numbers of asterisks (*) is significantly difference at $p<0.05$. Antioxidant equivalents (AE) of TEAC, FRAP and ORAC.

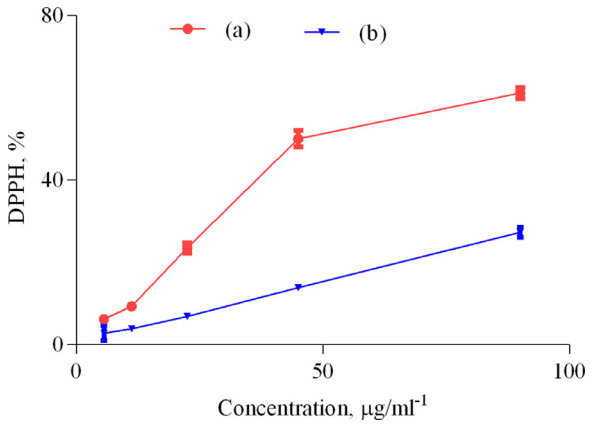

Fig. 2. Percentage 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of *Citrullus lanatus* (a) and *Citrullus colocynthis* (b) seed oils. Data are Mean ±SD of three replicate experiments.

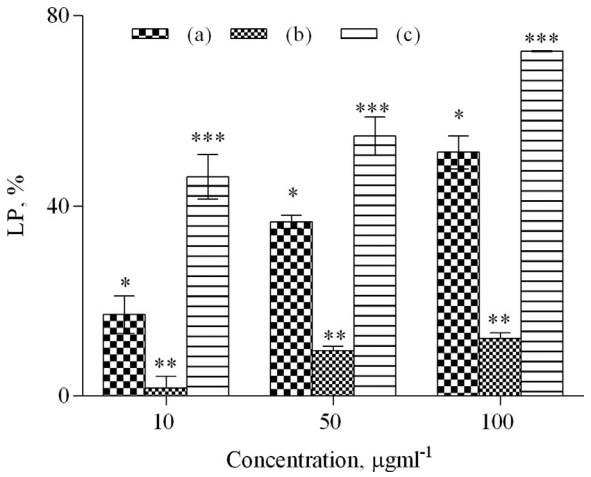

Fig. 3. Percentage inhibition of Fe2+-induced microsomal Lipid peroxidation (LP) of *Citrullus lanatus* (a) and *Citrullus colocynthis* (b) seeds oils and EGCG (c). Each bar represents Mean ±SD of three independent experiments. Each bar represents Mean ±SD of three independent experiments. Bar with different numbers of asterisks (*) is significantly difference at $p<0.05$.

challenges makes *C. lanatus* oil a better option over *C. colocynthis* oil. Several studies have related increased use of natural compounds in pharmacology, cosmetics and medicine to their antioxidant constituents (Zehiroglu and Sarikaya, 2019).

The HPLC chromatograph profile showed that *C. lanatus* contains catechins, which is absent in the *C. colocynthis* oil and likely to make a major contribution to the antioxidant activity for *C. lanatus*. Antioxidant activity is the ability of an extract/compound to donate electron so as to terminate the radical chain reaction and convert free radicals to more stable products (Park et al., 2012). Higher antioxidant activity of *C. lanatus* oil obtained is in agreement with the result of Rahman et al. (2013) who reported a high antioxidant activity of an n-hexane extract of the seed. The study concluded that *C. lanatus* n-hexane extract has the highest antioxidant capacity among all the extracts tested. As mentioned, fatty acids and oils with natural antioxidant properties are of growing interest in recent years in the food, cosmetic and pharmaceutical products because of their known health promoting benefits, and their antioxidant capacities may render them as alternatives to the use of synthetic antioxidants, mainly because of their relative lesser toxicity (Rodriguez-Garcia et al., 2016; Sweeta et al., 2019).

Inhibition of Fe^{2+}- induced microsomal lipid peroxidation and tyrosinase activity were also evaluated and results presented in Figs. 3 and 4. Inhibition of Fe^{2+}- induced microsomal lipid peroxidation is a reliable biological marker of cellular oxidative stress (Dargel, 1992). *Citrullus lanatus* oil significantly inhibited Fe^{2+}- induced microsomal lipid peroxidation. It was also observed that *C. lanatus* oil exhibits a dose dependent inhibitory activity against Fe^{2+}- induced microsomal lipid peroxidation. This was not observed for the *C. colocynthis* oil. The inhibition of Fe^{2+}-induced microsomal lipid peroxidation is related to the antioxidant potential of the *C. lanatus* oil. This shows that *C. lanatus* oil has the ability to protect the cell membrane integrity and therefore ensuring normal functioning of membrane enzymes. Tyrosinase is a copper-containing enzyme that functions in mammalian melanogenesis and in damaged fruits enzymatic browning reactions during post-harvest handling and processing (Chang, 2009; Niu and Aisa, 2017). *Citrullus lanatus* oil significantly ($P< 0.05$) inhibited tyrosinase activity in a dose dependent manner. A link has been established between the antioxidant activity of a compound and the inhibition of tyrosinase enzyme (Hong-Xin et al., 2018). Significant inhibition of tyrosinase activity exhibited by *C. lanatus* oil makes it a possible asset for the cosmetic industry.

Further to the *in vitro* evaluation of the oils, *in vivo* effects of the oils at a high dose were also assessed to determine possible toxicological potential in a mouse model. The animals were exposed to oils through oral intubation for 15-day at 5 mL/kg body weight (BW) per day. The biomarkers related to liver function, antioxidant enzymes, lipid peroxidation and clastogenic effects were evaluated.

The measurement of transaminases activities in serum/plasma is frequently used as a diagnostic tool in human and animals for the assessment of damage to vital organs when exposed to toxic substances (Yousef et al., 2002; Sookoian and Pirola, 2015). During cellular damage, these enzymes are leaked into the serum/plasma and hence evaluation of the activities of these enzymes in serum/plasma is considered as a marker of cell damage (Barse et al., 2006; Giannini et al., 1999). The *C. lanatus* oil treated group showed a significant ($p<0.05$) increase in plasma ALT activity when compared with the control group. The increased plasma ALT level recorded in the *C. colocynthis* oil treated group was not significant when compared with the control group. Contrarily to plasma ALT, *C. colocynthis* oil induced a significant ($P<0.05$) elevation of AST when compared with the control while *C. lanatus* oil induced induction of AST was not significant. The substantial increase of the plasma ALT level is an indication of liver damage while the plasma AST is not specific to any tissue of the body. This result implies that exposure of the mice to the high dose of these oils have potential toxic risk effects.

High levels of cholesterol in the serum over a long time period (years) promote human cardiovascular diseases such as coronary heart disease and stroke (Csaba et al., 2016). The build-up of

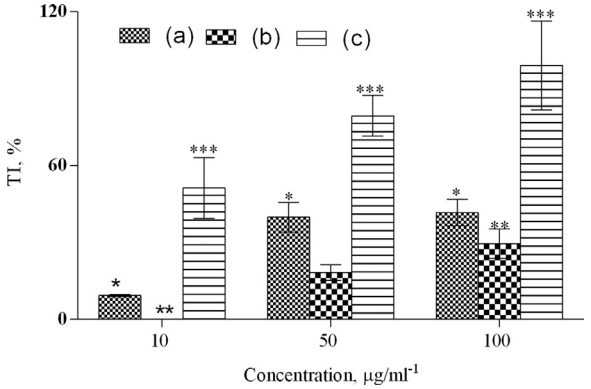

Fig. 4. Tyrosinase Inhibition of *Citrullus lanatus* and *Citrullus colocynthis* seeds oils and Kojic acid (Standard). Each bar represents Mean ±SD of three independent experiments. Bar with different alphabet of each concentration is significantly difference at $p<0.05$.

Table 3.
Plasma Cholesterol, HDL-C, Total protein, ALT, AST and MPCE of Mice Treated with *C. lanatus* and *C. colocynthis* Seeds oils.

Groups	Treatments	Cholesterol (mmol/l	HDL-C (mmol/l)	ALT (U/L)	AST (U/L)	MPCE/1000PCE
A	*C. lanatus*	3.80 ± 0.42^{a}	2.22 ± 0.55^{a}	115.80 ± 1.82^{a}	$112.67 \pm 1.39^{a, b}$	2.34 ± 0.78^{a}
B	*C. colocynthis*	4.50 ± 0.75^{a}	1.14 ± 0.70^{a}	$105.52 \pm 5.73^{a, b}$	113.05 ± 4.62^{a}	3.59 ± 1.20^{a}
C	water (control)	2.27 ± 0.26^{a}	0.56 ± 0.04^{a}	98.94 ± 4.69^{b}	100.52 ± 8.55^{b}	3.79 ± 0.83^{a}

Values are expressed as mean ± SD. Values with different superscripts are significantly different at $P<0.05$. High density lipoprotein-cholesterol (HDL-C), Alanine amino transferase (ALT), Aspartate amino transferase (AST), Micronucleated polychromatic erythrocytes (MPCE), polychromatic erythrocytes (PCE).

cholesterol in blood vessel walls can lead to atherosclerosis, which increases with age (Blesso, 2018; Wiegman, 2018). High total cholesterol and low levels of HDL-cholesterol are correlated with an increased risk of coronary heart diseases with the onset of atherosclerosis and hypertension as the major manifestations (NCEP, 2002). No significant difference was observed in the plasma cholesterol level of mice treated with *C. lanatus* oil when compared with *C. colocynthis* oil treated group ($p>0.05$) (Table 3). The oils from both seeds induced a non-significant ($p>0.05$) increase of plasma cholesterol when compared with the control group. The increase plasma cholesterol observed in both groups fed the oil can be linked to the presence of palmitic and myristic saturated fatty acids in the oils (Wang et al., 2007). The sustained increase in plasma cholesterol, although not significant in this study could lead to several heart complications such as cardiac contractile dysfunction, mycordial infarction and oxidative/nitrative stress induction (Csaba et al., 2016). *Citrullus lanatus* and *C. colocynthis* oils induced a non-significant increase in plasma HDL-cholesterol when compared with the control group ($p>0.05$). No significant differences between the *C. colocynthis* oil treated group and the Control group were observed in this study. The increase in plasma HDL-cholesterol observed in the *C. lanatus* treated group might be due to the adaptive response to increased plasma cholesterol as observed in this study. The fold increase in plasma HDL-C in both oils when compared with the control is significantly higher than the fold increase in plasma cholesterol (Table 3). This implies that the induced plasma HDL-C increase will clear from the system the plasma cholesterol and lower the risk of cholesterol induced negative impact on the heart. The increase of the HDL-C is important for removal of excess cholesterol in the circulation and reduces its potential risks to induce coronary heart disease (Donovan et al., 2011).

Aerobic organisms are imbued with endogenous GSH, which protects oxidation of cellular macromolecules by free radicals generated either through noxious foreign materials or natural metabolic process (Mirończuk-Chodakowska et al., 2018). It has been established that glutathione acts by donating a proton to free radicals so as to stabilize/ "inactivate" them. The ability to donate a proton to quench rampaging free radicals is due to nucleophilicity of thiol constituent of GSH (Subramani et al., 2019). The GSH contents were found to be reduced in the heart and liver of all the treated groups. However, the decrease occasioned by the oil treatments was found to be significant ($P<0.05$) only in the liver of the group treated with *C. colocynthis* (Table 4). The GSH concentration in the kidney of mice treated with *C. lanatus* was significantly ($P<0.05$) elevated when compared with the control group. Contrarily, there was a non-significant decrease in the kidney of the animals treated with *C. colocynthis*. The reductions in the levels of GSH might be due to utilization by the enzymes such as GR, G6PD, GPx and GST that are responsible for its enzymatic maintenance (Subramani et al., 2019). Glutathione peroxidase (GPx) protects cells against oxidative damage by catalyzing the reduction of hydrogen peroxide using GSH. Metabolism of GPx is one of the most crucial antioxidant defense mechanisms that is utilized by the cell (Grazioli and Schiaro, 1998). The *C. lanatus* oil treated group showed a significantly ($p<0.05$) increased activity of GPx in heart tissue when compared with the control (Table 4).

Table 4.
Protein, glutathione GSH (unit/mg protein), SOD (unit/mg protein) and GPx (μGSH remaining/mg protein) activities of various tissues of mice treated with CL and CC seeds oils.

Groups	*CL	*CC	*Water (control)
Heart Protein (mgdl $^{-1}$)	18.3 ± 1.2^{a}	16.5 ± 1.2^{a}	19.4 ± 1.1^{a}
Liver Protein (mgdl $^{-1}$)	28.2 ± 6.3^{a}	31.7 ± 2.4^{a}	32.7 ± 3.7^{a}
Kidney Protein (mgdl $^{-1}$)	25.2 ± 4.5^{a}	23.4 ± 4.2^{a}	27.9 ± 4.3^{a}
Heart GSH (unit/mg protein)	2.3 ± 0.09^{a}	2.4 ± 0.17^{a}	2.6 ± 0.14^{a}
Liver GSH (unit/mg protein)	$2.21 \pm 0.20^{a,b}$	2.13 ± 0.10^{a}	2.48 ± 0.48^{b}
Kidney GSH (unit/mg protein)	2.6 ± 0.18^{a}	2.2 ± 0.10^{b}	2.3 ± 0.14^{b}
Heart SOD (unit/mg protein)	99.5 ± 0.40^{a}	99.2 ± 0.15^{a}	99.1 ± 0.15^{a}
Liver SOD (unit/mg protein)	99.8 ± 0.10^{a}	99.9 ± 0.06^{a}	99.9 ± 0.09^{a}
Kidney SOD (unit/mg protein)	99.7 ± 0.26^{a}	99.5 ± 0.11^{a}	99.6 ± 0.07^{a}
Heart GPx (μGSH remaining/mg protein)	4.8 ± 1.21^{a}	3.6 ± 2.26^{b}	2.5 ± 0.36^{b}
Liver GPx (μGSH remaining/mg protein)	4.0 ± 0.28^{a}	3.6 ± 0.90^{a}	3.1 ± 0.72^{a}
Kidney GPx (μGSH remaining/mg protein)	2.4 ± 0.48^{a}	1.8 ± 0.31^{a}	2.9 ± 1.11^{a}

* Values are expressed as mean ± SD. Values of each tissue with different superscripts are significantly different at $P<0.05$. CC= *Citrullus colocynthis*, CL= *Citrullus lanatus*, Reduced Glutathione (GSH), Superoxide dismutase (SOD), Glutathione peroxidase (GPx).

The increase in the activities of the enzymes in the liver of the *C. lanatus* treated group and in the heart and liver of the *C. colocynthis* treated group showed no significant difference ($P>0.05$) when compared with the control group. The GPx activities in the kidney of treated groups showed a non-significant ($P>0.05$) decrease when compared with the control group. It is essential to note that the increased activities of GPx in the heart and liver of treated groups corresponds with the decrease observed in GSH contents of these tissues. This implies that reductions of GSH contents in these tissues were due to its utilization by the increase in the activities of the GPx enzymes. Superoxide dismutases (SOD) are important antioxidant enzymes that protect the cell system by catalyzing the dismutation of superoxide anion into oxygen and hydrogen peroxide (Qiao et al., 2020). All the treated groups showed no significant change in the activity of SOD when compared with the control group (Table 4) showing that consumption of these oils did not impact the SOD activities in any of the tissues examined in this study.

The Micronucleus assay is the most reliable method for detecting *in vivo* chromosomal damage/loss and an attractive tool for the evaluation of clastogenicity of compounds in several cell types (Andrade et al., 2016; Kishino et al., 2019; Brandsma et al., 2020). The *C. lanatus* and *C. colocynthis* oils did not induce chromosomal aberration, as confirmed by the non-significant increase in the frequency of micronucleated cells among the normal erythrocyte and bone marrow when compared with the control group (Table 3). This implies that both oils do not possess clastogenic potential at the tested dose.

4. Conclusion

The *Citrullus lanatus* oil showed a higher *in vitro* antioxidant potential when compared to the *C. colocynthis* oil and is proposed to

be due to the presence of the phenolic compound, catechin. This is the first work that report fatty acid and phenolic constituents of *C. lanatus* and *C. colocynthis* seeds oils. The Exposure of high or unregulated levels of the *C. lanatus* and *C. colocynthis* oils could be toxic to multicellular organisms. Results from this study clearly indicate that *C. lanatus* seed oil may have nutraceutical potential and could serve as an important agent for use in the food, pharmaceutical and cosmetic industries. The possibility to be utilized as a sustainable agricultural product should be further explored.

Declaration of Competing Interest

The authors declare no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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