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Antioxidant Effect and Characterization of Metabolites in *Citrullus lanatus* Seed Extract

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Abstract

Watermelon (*Citrullus lanatus*) seeds contain a complex matrix of phenolic and lipid-derived metabolites capable of modulating oxidative processes at the molecular level. In this study, the antioxidant effect of *C. lanatus* seed extract was evaluated via *in vitro* and *in vivo* assays, and the bioactive were characterized using FTIR and GC–MS. The seeds exhibited a higher total phenolic content (584 ± 26.1 mg/g) than flavonoid content (14.04 ± 0.20 mg/g), indicating a phenolic-rich antioxidant system. DPPH assays showed strong radical-scavenging activity, with the aqueous fraction displaying the lowest IC_{50} (1.65 ± 0.13 µg/mL) compared to other fractions, while total antioxidant capacity was highest in the ethyl-acetate (355.50 ± 5.20 µg/mL) and n-hexane (316.30 ± 0.76 µg/mL) fractions. FTIR analysis revealed characteristic O–H stretching (3286 cm^{-1}), C–H bending (1400–1346 cm^{-1}), and C–O/C–N stretching (1033 cm^{-1}), confirming the presence of alcohols, water, and complex organic functional groups associated with redox activity. GC–MS profiling of the aqueous fraction identified several antioxidant-associated metabolites, including dodecanoic acid ethyl ester, decanoic acid ethyl ester, tetradecanoic acid ethyl ester, trilaurin, oleic acid (two peaks), and dodecanoic acid hydroxy-ethyl ester. Evaluating the antioxidant activity *in vivo* demonstrated modulation of oxidative stress biomarkers, with extract-treated groups showing reductions in malondialdehyde (e.g., 0.47 ± 0.085 nmol/mg at 250 mg/kg) relative to diabetic controls (0.79 ± 0.038 nmol/mg), alongside dose-dependent alterations in catalase and superoxide dismutase activities. These biochemical shifts indicate attenuation of lipid peroxidation and partial restoration of endogenous antioxidant defenses. These findings demonstrate that *C. lanatus* seeds possess a distinct antioxidant signature driven by phenolic–lipid interactions and enzymatic modulation, supporting their relevance as a natural source of redox-active compounds.

INTRODUCTION

An imbalance between the levels of reactive oxygen species and the natural antioxidants is the root cause of oxidative stress, which serves as an important factor for the development and progression of some chronic diseases, such as diabetes, cardiovascular diseases, and inflammatory diseases.

Watermelon (*C. lanatus*) seeds which belong to the *Cucurbitaceae* family, contain a complex matrix of phenolic and lipid-derived metabolites capable of modulating oxidative processes were considered as potential sources of natural antioxidants. There are various reports available that show high levels of free radical scavenging and reductive capacities for *C. lanatus* seed extracts. Recent work by [1] pinned down some

major phytologically active compounds through GC-MS and confirmed high levels of antioxidant activity for the seed extracts. On similar lines, the aqueous and methanol extracts of *C. lanatus* seed were shown to possess high levels of DPPH, FRAP, nitric oxide scavenging, and reductive potential by [2,3] proving it to be an effective radical scavenger. Additionally, studies have confirmed that the antioxidant properties of the seeds are closely associated with their phenolic and lipid components, which contribute to their free-radical scavenging behavior and overall reducing capacity.

In the recent time, there has been increasing interest for the search of plant-based antioxidants as safe and available alternatives. Despite the growing body of evidence on the antioxidant properties of *C. lanatus* seed, watermelon (*C. lanatus*) seeds are often neglected and thrown away. Similarly, most findings on its antioxidant effect are limited to *in vitro*. Thus, this study, explores the *in vitro* and *in vivo* antioxidant properties and characterize the bioactive compounds in *C. lanatus* seed extract.

MATERIALS AND METHODS

Collection and Sample Pretreatment
Mature watermelons were procured fresh from local fruit vendors around Kano metropolis in December 2024. The seeds were removed from the flesh, properly rinsed with water, and air-dried at room temperature for three days. The dried seeds were then pulverized into a fine powder using a laboratory blender, sieved, and kept in suitable containers for further examination [4].

Crude Extraction of *C. lanatus* Seeds
The method of Odebisi and Sofowora (1998) was adopted with slight modifications. In this procedure, 800 mL of absolute methanol was added to 400 g of powdered seed sample in a 2000 mL conical flask and placed in an ultrasonic bath (40 kHz), allowed to soak for an hour at room temperature, with agitation carried out at least twice during the period. After washing, the mixture was filtered using a muslin cloth, and the resulting extract was placed in a water bath at 50 °C to evaporate the methanol and recover the concentrated extract.

Fractionation of the Methanolic Extract of *C. lanatus* Seeds
To fractionate the seed bioactive, column chromatography and a solvent gradient system from non-polar to highly polar solvents were used as follows:

n-hexane → chloroform → ethyl acetate → ethanol → water.

Procedure

Sample Preparation
The crude methanolic extract (about 20 g) of *Citrullus lanatus* seeds was dried and mixed with an equivalent quantity of silica gel in a 1:1 (w/w) ratio to generate a free-flowing dry powder. This procedure ensured the consistent application of the sample onto the column.

Column Packing
A glass chromatographic column was packed with silica gel (60–120 mesh) using the wet packing method: A slurry of silica gel in n-hexane (a non-polar solvent) was created. The slurry was poured carefully into the column while gently tapping it to dislodge trapped air bubbles. Layers of cotton wool and sand were put at the base and top of the silica gel bed to maintain the stationary phase and avoid disruption during elution.

Loading the Sample
The resulting crude extract-silica combination was carefully poured on top of the packed column. A small amount of n-hexane was then injected to rinse off any leftover material and to prevent disturbance of the column bed surface.

Gradient Elution
Gradient elution was performed using solvents in order of increasing polarity. Fractions (e.g., 50–100 mL each) were collected at each solvent stage. Each solvent was run separately to ensure progressive elution based on polarity. The eluted fractions were collected in numbered test tubes for adequate identification and tracking.

Concentration of Fractions
The pooled fractions were concentrated using a rotary evaporator under decreased pressure at solvent-specific temperatures: n-hexane and chloroform fractions were concentrated at about 40 °C. Ethyl acetate and ethanol fractions were evaporated at roughly 50–55 °C. The aqueous fraction was concentrated in a water bath at ~60 °C.

Determination of *In vitro* Antioxidant Activity
Various standard assays were used to assess the antioxidant capacity of the watermelon seeds fractionated extracts:

Total Phenolic Content (TPC):
The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method [5]. The crude extract (1 mg/mL) was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of sodium carbonate. The mixtures were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development, and absorbance was then measured at 765 nm using a spectrophotometer. Total phenolic content was expressed as mg/mL tannic acid equivalent using the following equation based on the calibration curve: y = 0.1216x, where x was the absorbance and y was the tannic acid equivalent (mg/mL).

Total Flavonoid Content (TFC):
The TFC was measured using the aluminum chloride colorimetric technique, where flavonoids form a stable combination with aluminum chloride, generating a yellow color detectable at 415 nm.

Procedure
Sample Preparation: Each solvent portion (e.g., 1 mg/mL) was dissolved in methanol to prepare the test samples. Color Development: A volume of 0.5 mL of each fraction or standard solution was pipetted into separate test tubes. Then, 0.3 mL of 5% sodium nitrite ($NaNO_2$) was added to each tube. After 5 minutes, 0.3 mL of 10% aluminum chloride ($AlCl_3$) was added. Following another 6 minutes of incubation, 2 mL of 1 M sodium hydroxide (NaOH) was added to the mixture. The volume was then brought up to 5 mL with distilled water. Each tube was mixed completely and left to stand at room temperature for 15 minutes to achieve full color development. The absorbance of each reaction mixture was measured at 415 nm using a UV–Visible spectrophotometer.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay
DPPH solution was added to 1 mL of each extract/fraction. A blank was also prepared using only DPPH in methanol. The mixture was incubated for 30 minutes and measured at 517 nm using a spectrophotometer. The percentage of DPPH radical inhibition was calculated using the following formula [6]:

Inhibition (%) = $A_o - A_1 \div A_o \times 100$

Where;
A_o is the absorbance of the control, and
A_1 is the absorbance of the sample [6].

Total Antioxidant Capacity (TAC)
TAC was evaluated using the phosphomolybdate method, which was based on the reduction of Mo(VI) to Mo(V) by the antioxidants, forming a green phosphate/Mo(V) complex measurable at 695 nm. To create the reagent solution, mix sulfuric acid, sodium phosphate, and ammonium molybdate in distilled water. In a test tube, 0.3 mL of each fraction (1 mg/mL) was mixed with 3 mL of reagent solution, the reaction mixtures were incubated at 95 °C in a water bath for 90 minutes. After incubation, the samples were allowed to cool to room temperature. The absorbance of each sample was measured at 695 nm using a spectrophotometer against a blank of reagent solution and solvent. All tests were performed in triplicate, and the results were presented as mean ± standard deviation (SD) [7].

FTIR Spectroscopy
The protocol of [8] with modification, was used to identify the functional groups in the fractionated extracts of *C. lanatus* by Fourier Transform Infrared (FTIR) spectroscopy. Approximately 1 mg of a finely ground dried extract was mixed with 100 mg of potassium bromide (KBr) in an agate mortar to obtain a uniform powder. The mixture was then compressed into a translucent disc using a hydraulic press under vacuum. The prepared KBr pellets were scanned in the mid-infrared region using an FTIR spectrometer (Shimadzu FTIR-8400S or equivalent) over the wavelength range of 400–4000 cm^{-1}. The resolution was set to 4 cm^{-1} with 32 scans averaged per spectrum to improve the signal-to-noise ratio. The spectra obtained were compared with standard functional group frequencies to identify the characteristic peaks and the associated classes of compounds [8].

GC-MS Assay
The sample was prepared using the method described by the National Institute of Standards and Technology (NIST) [9]. The concentrated extract (1 µL) was injected into the GC-MS using a capillary column (HP-5MS: 30 m × 0.25 mm, film thickness 0.25 µm). The initial oven temperature was set at 60 °C and maintained for 2 minutes, followed by a temperature ramp of 10 °C per minute up to 280 °C, which was then held for 10 minutes. Helium served as the carrier gas at a flow rate of 1 mL/min. Ionization was performed using electron impact (EI) at 70 eV, with the mass range set from 50 to 600 m/z. The ion source temperature was maintained at 230 °C, while the interface temperature was set at 250 °C. The total run time was approximately 35–40 min. The compounds were identified by comparing the mass spectra with the NIST (National Institute of Standards and Technology) library or the Wiley library. The retention time, molecular formula, molecular weight, and peak area for each identified compound were recorded.

***In vivo* Antioxidant Activity of *C. lanatus* Seed Extract**

Serum Catalase (CAT) assay
The serum was incubated with an H_2O_2 standard solution to quantify the sample's H_2O_2 concentration drop, which was used to evaluate catalase activity. Two reactions were used to run the test. Samples were incubated in reaction A with 65 mM H_2O_2, and sodium azide was used to halt the reaction. An enzyme-linked colorimetric detection approach in process B, which yields quinoneimnine dye, was used to assess the remaining H_2O_2 concentration. The dye was read at 520 nm. After adding hydrogen peroxide, formaldehyde standard wells, positive control wells, and sample wells were constructed, and the reactions began. At 540 nm, absorbance was measured and stopped when reactions took place. The catalase activity was calculated based on the rate at which H_2O_2 decomposed [10].

Serum Superoxide dismutase (SOD) assay
The serum SOD activity was measured in the sample, which was produced by superoxide radicals that reacted with a colorless substrate to produce a yellow-colored product. The absorbance of this product was measured at 450 nm. The amount of the yellow product decreases and the enzyme activity increases with increasing SOD levels in the sample. Xanthine oxidase was added after they were put into the wells. The response was measured as absorbance after 20 minutes at room temperature. The rate of inhibition was calculated to determine the SOD activity and check for appropriate blanks for accurate measurement [11].

Malondialdehyde (MDA) assay
Malondialdehyde (MDA) and thiobarbituric acid (TBA) reacted to generate an MDA-TBA_2 adduct that absorbed at 532 nm. This was then put through the MDA assay. The material was treated with EDTA and butylated hydroxytoluene (BHT) to stop artificial lipid oxidation. The temperature of the reaction mixture was regulated in order to reduce the breakdown of lipid hydroperoxide. The detection reagents were applied to the standard, blank, and sample wells. After many washes at 37 °C, the plate was incubated with the substrate solution, a blue color shift was seen, and when a stop solution was added, a yellow color shift was seen. Absorbance immediately measured at 450 nm to obtain the MDA concentration [12].

RESULTS AND DISCUSSION

Antioxidant Activity of *C. lanatus* Seeds

Total phenolic and flavonoid contents of *C. lanatus* seeds
The total phenolic and flavonoid contents of *C. lanatus* seeds expressed in mg/g as mean ± standard deviation was presented in **Fig. 1**. The seeds display significant ($p<0.05$) total phenolic content measured as 584 ± 26.1 mg/g, and the flavonoid was recorded as 14.04 ± 0.20 mg/g.

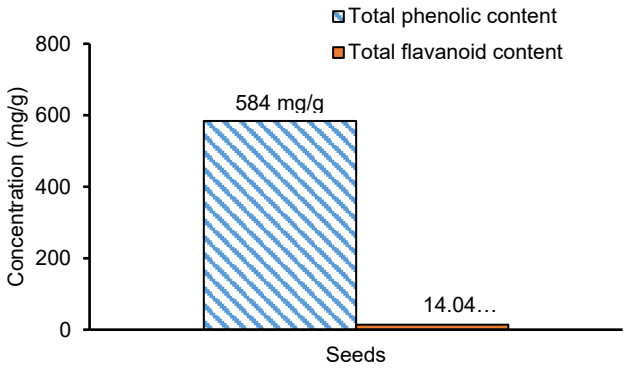

Fig. 1. Total Phenolics and Flavonoids Contents of the methanolic extract of *C. lanatus* Seeds. Results are mean ± S.D of triplicate determinations.

DPPH radical scavenging activity of *C. lanatus* seeds
The DPPH radical scavenging activity of *C. lanatus* seed extracts presented as IC_{50} (µg/mL) was presented in **Table 1**. It was found

that the aqueous fraction of the methanol extract has the least IC_{50} (1.65 ± 0.13 µg/mL), which was significantly lower ($p<0.05$) than the crude extract, but significantly higher ($p<0.05$) than the garlic acid standard (0.0001 µg/mL). Similarly, among the various solvent fractions, the ethyl acetate fraction had the highest IC_{50} value of 31.30 ± 6.22 µg/mL, which was significantly lower than the crude extract (86.58 ± 5.97 µg/mL). However, the IC_{50} of n-hexane, chloroform, and ethanol fractions varied insignificantly, but were significantly higher than the garlic acid standard, though significantly lower than the crude extract.

Table 1. DPPH Radical Scavenging Activity of *C. lanatus* Seeds.

Fraction	*Citrullus lanatus* seeds IC_{50} (µg/mL)
N-hexane	18.24 ± 0.21^{a}
Chloroform	17.73 ± 2.27^{b}
Ethyl-acetate	$31.30 \pm 6.22^{a, b}$
Ethanol	17.48 ± 3.62^{c}
Aqueous	$1.65 \pm 0.13^{a, b, c, d}$
Crude extract	$86.58 \pm 5.97^{a, b, c, d, e}$
Standard (Gallic acid)	$0.0001 \pm 0.00^{a, b, c, d, e}$

Results are mean ± S.D. of triplicates, values with same superscripts indicate significant difference at $p<0.05$.

Total antioxidant capacity of *C. lanatus* Seeds

The total antioxidant capacity of *C. lanatus* seeds was expressed in µg/mL and presented in **Table 2**. The TAC value of the various solvent fractions varied significantly ($P<0.05$), with the ethyl acetate fraction have the highest TAC value of 355.5 ± 5.20 µg/mL, followed by n-hexane, ethanol, and chloroform fractions, respectively. The aqueous fraction reveals the lowest TAC, which was significantly lower than all the fractions and the crude extract.

Table 2. Total antioxidant capacity of *C. lanatus* seeds.

Fraction	*Citrullus lanatus* seeds (µg/mL)
N-hexane	316.30 ± 0.76^{a}
Chloroform	$113.30 \pm 3.21^{a, b}$
Ethyl-acetate	$355.50 \pm 5.20^{a, b, c}$
Ethanol	$136.50 \pm 4.40^{a, b, c, d}$
Aqueous	$54.30 \pm 2.46^{a, b, c, d, e.}$
Crude extract	$187.00 \pm 6.95^{a, b, c, d, e}$

Results are mean ± S.D. of triplicates, values with the same superscripts indicate significant difference at $p<0.05$.

FTIR Analysis of *Citrullus lanatus* seeds

Aqueous fraction of the seeds

Table 3 displays the functional groups identified on FTIR spectrum of the aqueous portion of *C. lanatus* seeds. A wide, strong absorption band at 3286 cm^{-1} corresponds to O–H stretching vibrations, suggestive of alcohols or water. A medium-intensity band at 1633 cm^{-1} is ascribed to H–O–H bending, indicating the existence of water. A sharp, faint band detected at 2333 cm^{-1} corresponds to the asymmetric stretch of atmospheric CO_2. Moderate bands at 1400 cm^{-1} and 1346 cm^{-1} reflect C–H bending, coupled with residues of organic molecules. A faint band at 1033 cm^{-1} is linked to C–O or C–N stretching. Additional peaks at 1514, 1266, 925, and 780 cm^{-1} occur within the fingerprint region, suggesting the presence of complex chemical compounds with weak to moderate intensities (**Fig. 2**).

Table 3. FTIR analysis aqueous fraction of the seeds.

Absorbance	Class of compound	Functional group	Intensity
3286	Alcohols / Water	O–H stretching	Broad, strong
1633	Water	H–O–H bending	Medium
2333	Atmospheric CO_2	C=O / CO_2 asymmetric stretch	Sharp, weak
1400, 1346	Organic compound traces	C–H bending	Moderate
1033	Organic compound traces	C–O or C–N stretching	Weak
1514, 1266, 925, 780	Complex organic compounds	Fingerprint region signals	Weak to moderate

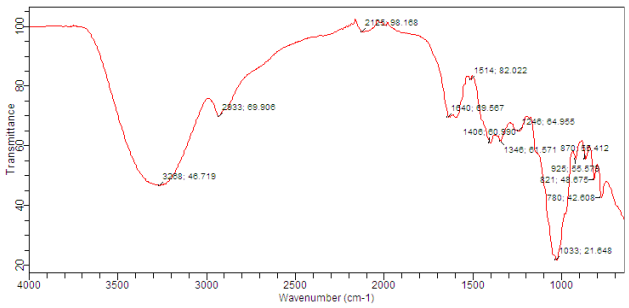

Fig. 2. FTIR Spectrum of Aqueous Fraction *C. lanatus* Seeds.

GC-MS analysis of *C. lanatus* seeds

Characterization of the aqueous seed fraction of *C. lanatus* via GC–MS, identified eight compounds previously reported to possess antioxidant activity (**Table 4**). These metabolites were detected at retention times ranging from 6.3 to 14.3 minutes and included dodecanoic acid ethyl ester, decanoic acid ethyl ester, tetradecanoic acid ethyl ester, 9-acridinamine, trilaurin, dodecanoic acid hydroxy-ethyl ester, and two peaks corresponding to oleic acid. Among these, oleic acid showed the highest peak areas at retention times of 11.9 and 14.3 minutes, followed by decanoic acid ethyl ester and dodecanoic acid derivatives (**Figs. 3** to **8**). Trilaurin and tetradecanoic acid ethyl ester appeared at moderate intensities, while 9-acridinamine was present at a lower but distinct peak area.

Table 4. Metabolites of *C. lanatus* aqueous seed fraction identified by GC-MS.

Peak No.	Retention Time (RT)	Area (%)	Compound
2	6.3	315828.36	Dodecanoic acid, ethyl ester
3	7.9	4143618.37	Decanoic acid, ethyl ester
4	9.4	1670434.06	Tetradecanoic acid, ethyl ester
5	10.1	1885103.81	9-Acridinamine
8	11.5	1138055.17	Trilaurin
9	11.6	1630952.74	Dodecanoic acid, hydroxy-ethyl ester
10	11.9	3368098.43	Oleic acid
12	14.3	836817.21	Oleic acid

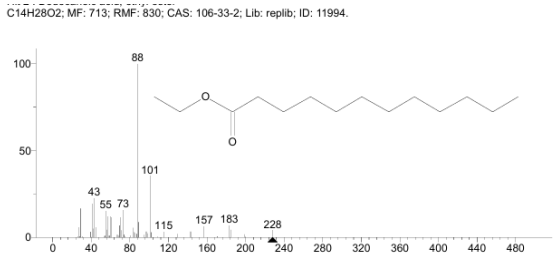

Fig 3. Dodecanoic acid, ethyl ester, RT: 6.3 and 7.9 min respectively.

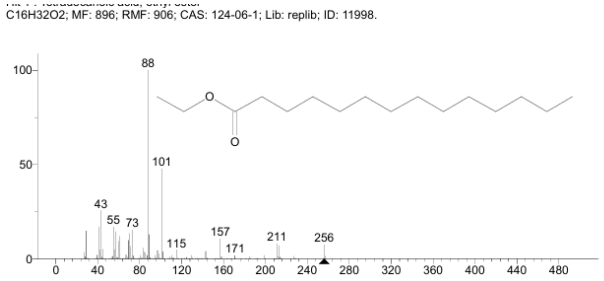

Fig. 4. Tetradecanoic acid, ethyl ester, retention time of 9.4 min.

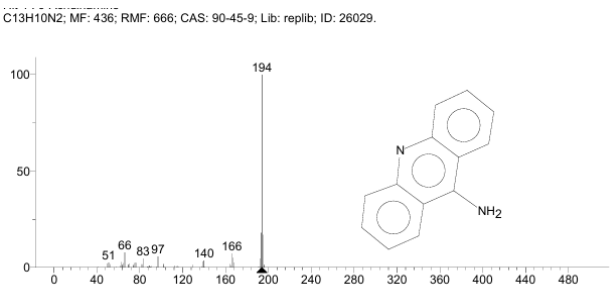

Fig. 5. 9-Acridinamine, retention time of 10.1 min.

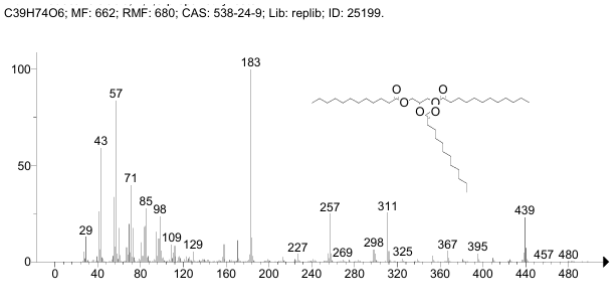

Fig. 6. Dodecanoic acid, 1,2,3-propanetriyl ester (Trilaurin), retention time of 11.5 min.

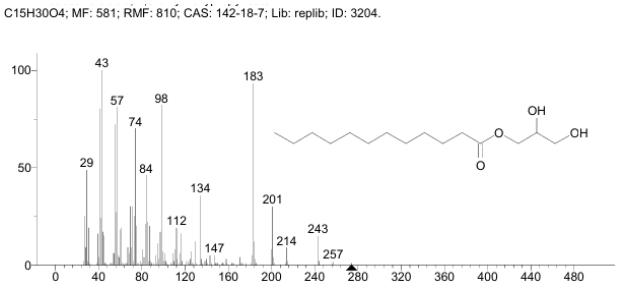

Fig. 7. Dodecanoic acid, 2,3-dihydroxypropyl ester, retention time of 11.6 min.

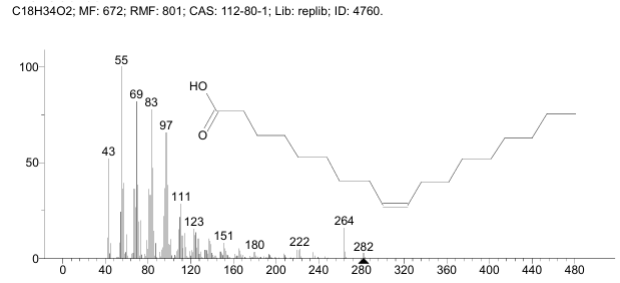

Fig. 8. Oleic acid, retention times of 11.9 and 14.3 min, respectively.

Serum malondialdehyde, superoxide dismutase activity, and catalase activity

The concentrations of serum malondialdehyde (MDA), superoxide dismutase (SOD) activity, and catalase (CAT) activities across the experimental groups illustrate the oxidative stress landscape in diabetic rat model and the modulatory effects of the aqueous fraction of *C. lanatus* seed (**Table 5**). Serum MDA levels, which reflect lipid peroxidation and cellular oxidative damage, the highest value was seen in the group III (1.49 ± 0.39 nmol/mg protein), followed closely by the group V (1.34 ± 0.64 nmol/mg protein) and group II (0.79 ± 0.375 nmol/mg protein). The normal control rats had the lowest MDA levels (0.46 ± 0.08 nmol/mg protein), consistent with minimal oxidative damage. Notably, group IV-maintained MDA levels (0.47 ± 0.085 nmol/mg protein) close to the normal baseline, while the group VI showed moderate levels (0.72 ± 0.14 nmol/mg protein), suggesting partial protection.

The SOD activity was highest in the normal control group (0.59 ± 0.01 U/mL), which was expected in healthy physiological states. The group II displayed a slight reduction (0.59 ± 0.01 U/mL), while all extract-treated groups exhibited a marked decrease, especially at 250 mg/kg and 1000 mg/kg (group IV and VI) doses (0.39 ± 0.24 and 0.39 ± 0.01 U/mL respectively). Interestingly, the group V and III maintained near-normal SOD levels (0.58 ± 0.004 and 0.58 ± 0.07 U/mL respectively), indicating robust enzymatic defense at these doses.

Catalase activity, which serves as another key antioxidant enzyme protecting cells from hydrogen peroxide toxicity, was lowest in the group I (12.09 ± 1.35 kU). Diabetes markedly elevated catalase activity (20.55 ± 0.37 kU) in the group II, presumably as a compensatory response to oxidative stress. The highest CAT activity was recorded in group V (22.63 ± 0.89 kU), followed by group IV (17.69 ± 0.23). While the group III and VI showed intermediate levels (12.63 ± 2.13 and 16.72 ± 2.41 kU respectively), they were notably lower than the diabetic control group.

Table 5. Serum malondialdehyde, superoxide dismutase and catalase activities.

Group	Malondialdehyde (nmol/mg prot.)	Superoxide Dismutase (U/mL)	Catalase (CAT) (kU)
I (Normal Cont.)	0.46 ± 0.08^{a}	0.59 ± 0.01^{a}	12.09 ± 1.35^{a}
II (Diabetic Cont.)	0.79 ± 0.038$^{a, b}$	0.59 ± 0.01^{a}	20.55 ± 0.37$^{a, b}$
III (Drug Cont.)	1.49 ± 0.39$^{b, c}$	0.58 ± 0.07^{a}	12.63 ± 2.13^{a}
IV (250 mg/kg)	0.47 ± 0.085^{a}	0.39 ± 0.024$^{a, b}$	17.69 ± 0.23$^{b, c}$
V (500 mg/kg)	1.34 ± 0.064$^{c, d}$	0.58 ± 0.004^{a}	22.63 ± 0.89$^{b, d}$
VI (1000 mg/kg)	0.72 ± 0.14$^{a, b}$	0.39 ± 0.01$^{b, c}$	16.72 ± 2.41$^{c, e}$

Values are presented as mean ± standard deviation of triplicate readings (n=3). Values within the same column bearing different superscript letters are statistically $p<0.05$ different.

DISCUSSION

Total phenolic and Total flavonoid content of the seeds

The results show the presence of high amounts of total phenolic content (584 ± 26.1 mg/g) and moderate levels of flavonoids (14.04 ± 0.2 mg/g) in *C. lanatus* seeds. These results highlight the high antioxidant activity in these seeds, owing to the well-documented ability of phenols and flavonoids in eliminating free radicals and reducing oxidative stress. The high amounts of phenols, in particular, show high ability in reducing reactive oxygen species (ROS) associated with many chronic diseases, especially in cases of cancer, diabetes, and cardiovascular diseases. The result for the analysis of total phenols (584 mg/g) is higher than in many studies conducted. In this regard, studies had ranged between 70 mg/g and 150 mg/g for *C. lanatus* seeds [13], while others, as in [14], had approximated results close to

180 mg/g. These differences in amounts can be attributed to differences in solvents used in the separation, genetic types, regions, or pre-processing treatments. In a similar context, the result for flavonoid content (14.04 mg/g) falls in line with [15], who had established the content between 12 mg/g and 15 mg/g, evidencing the presence of many bioactive molecules in these seeds possessing health benefits. The rising concentration of phenolics in this study may have been due to the ultrasonic-assisted method employed for bioactive compound extraction.

Total phenolic content (TPC) and total flavonoid content (TFC) are fundamental in evaluating the antioxidant potential of plant-derived materials. Phenolics, particularly phenolic acids, tannins, and polyphenols, exhibit free radical scavenging, metal chelating, and enzyme modulation properties. They play a major role in combating oxidative stress and inflammation, thus assisting in disease prevention. Flavonoids, a particular class of polyphenols, possess diverse bioactive properties such as antibacterial, anti-inflammatory, anti-diabetic, and cardio protective functions. Thus, the assessment of such properties assumes significance in nutraceutical and therapeutic evaluations of *C. lanatus* seeds.

The presence of these phytochemicals indicates the significance of watermelon seeds in functional food development and their potential use in managing oxidative stress-related diseases. The significance of watermelon seeds in health-enriched foods and their potential use in health and pharmaceutical formulations is well supported by these findings.

Total Antioxidant Capacity of *C. lanatus* Seeds

Table 1 shows the total antioxidant activity of *C. lanatus* seed extracts. It is clear that the aqueous extracts showed the greatest antioxidant activity, measured as the lowest IC50 (54.3 ± 2.46 µg/mL) followed by ethanol (136.5 ± 4.4 µg/mL), chloroform (113.3 ± 3.21 µg/mL) extracts, followed by the crude extract (187 ± 69.5 µg/mL) with n-hexane extracts (316.3 ± 0.76 µg/mL) and finally the ethyl acetate (355.5 ± 5.2 µg/mL) extracts. The standard gallic acid had an IC50 of 0.0001 ± 0 µg/mL, used as the reference standard for highly active antioxidants. This data suggests that the antioxidants present in *C. lanatus* seeds are mostly polar in nature with much higher activity in aqueous and ethanol extracts likely due to higher water-soluble phenolic and flavonoid compounds.

When compared to previously available data, this finding matches previous studies done by [16,17], whereby the data depicted higher activity in the extracts processed using more polar solvents due to their higher solubility for antioxidant compounds found in plants. Similarly, [18] emphasized the importance of using more polar solvents in the efficient isolation of antioxidants particularly those of phenolic nature, hence justifying this data. Also, gallic acid's activity validates the accuracy of the test used and establishes a standard measure for extract intensity. The IC50 parameter used in this test is an important defining factor for intensity of antioxidant activity, whereby extracts with lower concentrations have higher activity. Statistical variation at $p < 0.05$ between various extracts as shown by different superscripts confirms that the intensity of antioxidants also varies as per their respective polarity.

DPPH radical scavenging activity of *Citrullus lanatus* seeds

The result from the DPPH free radical scavenging activity shows that *C. lanatus* seed solvent fractions are highly diverse in their level of antioxidants. The aqueous fraction was highly active as determined by its low IC50 value (1.652 ± 0.13 µg/mL), which nearly approached that of Gallic acid (0.0001 ± 0 µg/mL). The ethanol, chloroform, and n-hexane solvent fractions had moderate levels of activity with IC50 values between 17–18 µg/mL, while ethyl acetate was less active (31.30 ± 6.22 µg/mL). The highest IC50 value was by the crude extract (86.57 ± 5.97 µg/mL), indicating low levels of activity relative to all the solvent fractions. Results like these have shown that *C. lanatus* seed antioxidants are highly polar and water-soluble, and as such, aqueous solvents are most efficient at extracting these compounds.

These results were consistent with those from previous publications by [13,19], which showed that watermelon seed aqueous fractions had higher levels of activity relative to their non-polar solvent counterparts. Additionally, their relative high performances against those from ethanol and chloroform solvent fractions suggested that *C. lanatus* seed contained hydrophilic antioxidants such as phenolic or flavonoid compounds [15]. On their part, results from the crude *C. lanatus* seed fractions nearly supported those publications by showing extremely low levels of activity. Similarly, all experiments involving seed and plant samples suggested that solvent polarity directly affected their potential and efficacy in producing and extracting their respective antioxidant compounds. The IC50 value calculated using the DPPH assay represents the extract concentration needed to neutralize 50% of the total DPPH free radicals. This value is an important indicator of antioxidant activity, where lower IC_{50} values indicate higher free radical scavenging activity.

Therefore, the importance of this value lies in its application for the assessment of therapeutic usage of natural resources for combating oxidative stress, an emerging factor in the development of chronic diseases like cancer, cardiovascular diseases, and neurological disorders. Furthermore, the large variation between the different extracts suggests the importance of selecting solvents for the measurement of antioxidants and serves as an aid in the development of nutraceuticals. The radical-scavenging activity of the aqueous extracts of *Citrullus lanatus* seeds shows promising antioxidant activity, with values close to the standard antioxidant gallic acid. This observation further emphasizes that hydrophilic antioxidants play an important role in antioxidant activity. These findings correlate with previous studies [20] and provide further evidence supporting the importance of *Citrullus lanatus* seeds as an important natural source of antioxidants.

FTIR Analysis of Aqueous fraction of *C. lanatus* seeds

The Fourier-transform infrared (FTIR) spectrum of the aqueous extract of Citrullus lanatus seeds shows the presence of various functional groups, signifying the varied range of bioactive compounds present. The wide and strong absorbance peak at 3286 cm^{-1} due to O–H stretching is characteristic of alcohols or phenolic compounds, suggesting the presence of polar plant compounds with possible antioxidant properties. The peak at 1633 cm^{-1} is due to H–O–H bending vibrations of water molecules, thereby confirming that the extracted material is aqueous.

The low-intensity peak at 2333 cm^{-1} is due to CO_2 asymmetric stretching, possibly due to exposure of the sample to atmospheric CO_2 during processing. The moderate peak at 1400 and 1346 cm^{-1} is due to C–H bending vibrations, suggesting the presence of aliphatic or aromatic hydrocarbon moieties. The peak at 1033 cm^{-1} corresponds to stretching of C–O or C–N, possibly due to compounds like sugars, ethers, or amines, signifying the presence of saccharides or compounds containing amino groups. The fingerprint region (1514, 1266, 925, and 780 cm^{-1}) corresponds to complex vibrational bands signifying compounds like

proteins, polysaccharides, or other second metabolites. These results are in agreement with the FTIR analyses of the extracts of watermelon seeds performed by [19], who has reported similar bands of O–H and C-O stretching vibrations, which confirm the presence of phenolic compounds, alcohols, as well as other oxygen functional groups. The fingerprint spectral bands as well as the bands of C-H bending are supported by the research conducted by [21], which indicates the existence of varied biological molecules in the seeds of *C. lanatus* in the forms of proteins as well as complex lipids

Absorbance bands and related functional group patterns are core in the FTIR analysis and provide information necessary for the identification of different types of chemical bonds in substances and subsequently the presence of different molecules. Absorbance band characteristics, including intensity and band width, are indicative of the molecular environment in which different substances exist. For example, broad and strong absorption in the O-H stretching region detects high hydroxyl species such as phenolics or alcohols, usually responsible for antioxidant properties. C-H bending and C-O/C-N stretching provide confirmatory information on aliphatic compounds, carbohydrates, and proteins. The fingerprint region is very informative in assessing differentiation in substances and identification at a molecular scale, but in some cases, it may be difficult to rely on FTIR alone for identification and additional information from chromatographic techniques may be necessary.

From FTIR analysis of *C. lanatus* (watermelon seed oil) aqueous extracts, various functional groups responsible for phenolics, alcohols, carbohydrates, and proteins are detected. The high O-H and C-O/C-N absorption content in this extract demonstrates that it is rich in polar bioactive compounds that are responsible for detected antioxidant and anti-inflammatory properties associated with its use. The FTIR analysis illustrates and validates bioactive properties associated with *C. lanatus* and represents its potential role in being used as a rich dietary supplement at different heights to provide different functionalities at distinct altitudes in diverse nutritional compositions.

GC-MS Analysis of Aqueous Fraction of *C. lanatus* Seeds

Gas chromatography–mass spectrometry (GC–MS) analysis of the aqueous seed fraction of *C. lanatus* revealed eight metabolites that were previously reported to show antioxidant activity: dodecanoic acid ethyl ester, decanoic acid ethyl ester, tetradecanoic acid ethyl ester, 9-acridinamine, trilaurin, dodecanoic acid hydroxyethyl ester, and two peaks representing oleic acid. The preponderance of fatty acid derivatives among the identified compounds correlates well with the fact that vegetable oils high in medium-chain and long-chain fatty acids were shown to express antioxidant activity, as measured by standard laboratory cell culture assays [22]. Findings involving plant and vegetable oils reveal that the total antioxidant activity of an oil bears a high degree of relationship to the oil's component fatty acids, particularly the proportions of saturates and unsaturates [23].

In this study, oleic acid appears as two peaks with high area values. Research has shown that oleic acid is one of the most important monounsaturated acids present in vegetables, as well as olive, and which was previously shown to exert antioxidant and protective roles [23]. Lauric acid lipids, such as trilaurin and the dodecanoic acid metabolites, are well-studied compounds present mainly in coconut oil and similar vegetable products, where the antioxidant activity of the lauric acids was confirmed by the DPPH and ABTS assays, and it was shown that lauric acid is involved in the radical scavenging activity of the mixture [24]. The oxidative stability emphasize that the original constituent FA, including the medium-chain saturated FAs like decanoic and particularly the MDA, dodecanoic acid, and their esters, play important roles as modulators of the resistance against oxidation and interaction of the lipids with the naturally occurring and exogenously applied antioxidants [25].

Serum antioxidant enzymes and oxidative stress markers

Oxidative stress plays a central role in the pathophysiology of diabetes, particularly through elevated production of reactive oxygen species (ROS) and impaired antioxidant defenses. Malondialdehyde, a well-known biomarker of lipid peroxidation, tends to rise under high oxidative conditions. The significantly elevated MDA level in the drug control group is unexpected and may point to drug-induced mitochondrial or hepatic stress, an observation previously noted with certain antidiabetic agents in chronic exposure settings [26].

The modest MDA levels in group IV, almost identical to the normal control, suggest that *C. lanatus* seed extract at low dosage confers substantial antioxidant protection, possibly through its rich phenolic and flavonoid content. These compounds are known for their free radical scavenging capabilities and their ability to inhibit lipid peroxidation [27]. However, the spike in MDA at 500 mg/kg (group V) and in the drug-treated group indicates that higher doses may not linearly enhance protection and could even exacerbate oxidative damage via metabolic overload or pro-oxidant behavior, a phenomenon also documented in other polyphenol-heavy extracts [28].

Superoxide dismutase activity, surprisingly stable in the normal and diabetic control groups, was considerably reduced in the extract-treated groups, particularly at low and high doses. This suggests that while the extract may quench peroxides (as seen in elevated catalase), its impact on dismutation of superoxide radicals may be dose-dependent or selectively modulated. Prior work has shown that certain phytocompounds can inhibit SOD expression under specific conditions, potentially due to feedback inhibition or enzyme inactivation [29].

Catalase activity tells an even more interesting story. While diabetes triggered a robust increase, likely as a stress response, the highest level appeared in group V. This suggests that *C. lanatus* seed extract not only supports endogenous enzymatic defense but may also upregulate catalase activity, thus, boosting the breakdown of hydrogen peroxide [30]. The lower catalase levels in the drug control and normal rats also hint that the extract offers a more dynamic modulation of oxidative balance than conventional pharmacotherapy.

CONCLUSION

The findings of this study demonstrate that watermelon (*C. lanatus*) seeds possess a distinct antioxidant profile supported by both compositional richness and functional activity. The extract exhibited strong radical-scavenging capacity, high phenolic content, and clear modulation of oxidative stress biomarkers, consistent with the presence of redox-active metabolites identified through GC-MS and FTIR analyses. Together, these results establish *C. lanatus* seeds as a credible natural source of antioxidant compounds and provide a biochemical basis for their potential application in oxidative stress management.

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