Antidiabetic Antioxidant and Phytochemical Profile of Yellow-Fleshed Seeded Watermelon 
(*Citrullus Lanatus*) Extracts

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Received November 24, 2018; Revised January 12, 2019; Accepted January 26, 2019

Abstract

Watermelon (*Citrullus lanatus*) consumption as shown by different studies is attributed to many health benefits, like in prevention of hypertension, cancer, cardiovascular diseases, and even type 2 diabetes due to its phytochemical constituents. The anti-diabetic effects of the seed, flesh, rind and leaf of yellow flesh watermelon extracts were evaluated through the inhibition of alpha-amylase and alpha-glucosidase enzymes activities by standard methods. The total phenolics content (TPC) and the total antioxidant capacities of the extracts were also evaluated using 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1,1-pycrylhydrazine (DPPH) assay methods. Metabolites of the 70% aqueous extract were profiled by liquid chromatographic mass spectrometry. Result of the study showed that the 70% aqueous ethanol flesh extract showed the highest α-amylase inhibition, followed by ethanol leaf extract. The 70% aqueous ethanol leaf extract had the highest α-glucosidase inhibition potential than the other studied extracts. The highest and lowest TPC were observed in 80% and 50% aqueous ethanol leaf and flesh extracts respectively. Ethanolic leaf extract showed the highest antioxidant activity in terms of FRAP, been higher than that of standard ascorbic acid. Based on ABTS radical scavenging and FRAP, 70% aqueous ethanolic leaf extract had the highest antioxidant activity. The 90% aqueous ethanol gave the highest extraction yield for seed, 60% for flesh and rind, and 100% ethanol for leaf extract. Among the metabolites identified in watermelon extracts are curcumenol, curcubitacin E, citrulline, 6-gingerol, citric acid, leucine, arginine, palmitic acid, arjunolic acid, glucose, fructose, sucrose, naringenin 5,7-dimethyl ether 4'-O-xylosyl-(1->4)-arabinoside, 4'-apo-beta,psi-caroten-4'-al, caffeic acid 3-glucoside, luteolin 7-rhamnosyl (1->6) galactoside, apigenin 7-(4'',6''-diacetylalloside)-4'-alloside among others. Therefore, this result indicates that *C. lanatus* has antidiabetic and antioxidant potentials. The leaf having the best α-glucosidase inhibition ability and the best antioxidant potentials, could be regarded a good raw material to explore lead molecule(s) against diabetes mellitus and antioxidant.

Keywords: yellow fleshed seeded watermelon, citrulline, watermelon extracts, extraction yield, *Citrullus lanatus*, LC-ESI-QTOF-MS, α-amylase inhibition


1. Introduction

Uncontrolled hyperglycaemia over a long period has been linked to the cause of micro and macro vascular diseases, failure of vital organs including the heart, kidneys, eyes, blood vessels and the nerve cells due to a long-term damage and dysfunction caused by oxidative stress and metabolic syndrome in diabetes mellitus disease condition [1]. Diabetes mellitus, a metabolic disorder characterised by sustained hyperglycaemia, hyperinsulinemia and insulin resistance, results from either non-responsiveness of cells to insulin, insufficient insulin and or lack of insulin production [2]. A human population of 415 million adults (aged 18-79) have diabetes worldwide, about 44% of the diabetes burden is attributed to overweight and obesity [3]. Type two diabetes mellitus is the most
common type of diabetes, it affects over 90% of diabetics’ population. It has overweight, obesity, insulin resistance, impaired glucose intake among others as risk factors [4,5,6].

Plants as food to man have always been a point of call, since food has a link to health and disease, and has contributed beneficially to man under the concept of “food and medicine” [7,8]. Plant materials are known to have some substances which when ingested, could be potent and affect human health positively [8]. It therefore, suffices to say that the relationship between man and plant in terms of health is as old as the advent of man. Fruits are said to be a rich source of antioxidants, fiber, and other phytoconstituents that benefits human health. Increasing fruit consumption has been recommended for the primary prevention of oxidative stress, many chronic diseases, including type 2 diabetes mellitus [9].

Watermelon is the largest cultivated member of the Cucurbitaceae family and one of the widely cultivated crop in the world [10]. It is highly consumed daily around the world, either eaten as fresh fruit or in mixed fruit salad. It is also made into jam, fruit juice, pickled rind, and others. It contains amino acids, citrulline and arginine specifically in high concentrations, carotenoids (especially high lycopene content in red fleshed type), β-carotene (in yellow fleshed type) and phenolic compounds [11-15]. In addition to minerals like Ca, K, Mg and Fe, watermelon is also a good source of lipophilic vitamins, A and E, and hydrophilic vitamins, B and C [16,17]. Watermelon is said to have low caloric value and its consumption is attached to numerous health benefits. It has been used in traditional medicine as anthelmintic, antibacterial, antifungal, diuretic, etc. it has also been used as antihypertensive, anti diabetic, and antioxidant among others [18,19].

The anti-diabetic, antioxidant potentials, and the biochemical profiles of some watermelon cultivars have been documented [11,12,13,17,20-24]. However, this information on other cultivars of watermelon are still limited especially, the yellow fleshed watermelon. Furthermore, data on the suitable solvent and the ratio of aqueous solvents for the extraction of different parts of the plant are still not readily available. In this work, the in vitro anti-diabetic and antioxidant capacity of the seed, flesh, rind and leaf extracts of yellow fleshed seeded watermelon extracted with water and different ratios of water/ethanol mixtures were evaluated. The possible phytochemical constituents of the extracts were also profiled using LC-ESI-QTOF-MS.

2. Materials and Methods

2.1. Sample Collection and Preparation

Fresh whole watermelon (C. lanatus) plants were harvested from a farm located at Felder Fikir Hilir, Bander Seri Jompol, Negeri Sembilan, Malaysia. The leaves were collected from the stem, washed and dried under air. The fruits were washed and peeled to separate the rind (the exocarp and the mesocarp) from the flesh (the endocarp). The seeds were gently separated from the flesh, washed and also air dried. The flesh and the rind were immediately freezed in a -20 refrigerator. The flesh and the rind were then ground into fine powder using a pistil and mortar. The seed and leaf were respectively ground into powder after air drying. All powdered samples were stored in dry plastic containers and kept in cold room before use.

Plant parts were respectively extracted using water, absolute ethanol, 90%, 80%, 70%, 60% and 50% ethanol. The solute to solvent ration used was 1:40 for the extraction processes. The solute and solvent mixture was first sonicated for 20 minutes at 29°C, and then left in a laboratory shaker for 40 minutes at 120 revolutions per minute and room temperature. All extracts were concentrated using rotary evaporator at 40°C. Samples were dried in oven at 40°C; dried samples were stored in air tight glass tubes and stored at 4°C.

2.2. Pancreatic Alpha-amylase Inhibition Assay

The method as described by Adisakwattana and others [25] was used to evaluate the inhibitory effects of C. lanatus extracts on pancreatic α-amylase. A working solution of porcine pancreatic α-amylase was prepared containing 3 Units/mL in 0.1 molar phosphate buffer saline, pH 6.9. C. lanatus extracts were prepared in 5 different concentrations (3, 2, 1, 0.75 and 0.5 mg/mL), 10 µL of the extracts was added to a solution of 1 g/L starch in phosphate buffer (165 µL), 75 µL of the enzyme solution was added to this mixture, the reaction was initiated and was incubated for 10 minutes at room temperature. After 10 minutes, 250 µL of dinitro salicylic acid (DNS) reagent was added to the reaction mixture and heated for 10 minutes at 100°C to stop the reaction. After stopping the reaction, 40 g/dL of sodium potassium tartrate solution (250 µL) was added to stabilise the colour of the reaction mixture. The mixture was cooled to room temperature and the absorbance of the mixture was measured at 540 nm wavelength in a microplate reader. The percent inhibition was calculated using the following formula:

\[
\% \text{Inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100 \quad (1)
\]

The IC₅₀ of C. lanatus extracts for α-amylase inhibition were calculated from a standard curve of % inhibition against concentrations of C. lanatus (µg/mL), and extrapolation from the equation of the curve, \(y = mx + c\). Where \(y\) is IC₅₀ = 50, \(m\) is the gradient of the curve, \(x\) is the concentration and \(c\) is the intercept at \(y\) axis.

2.3. Alpha-glucosidase Inhibition Assay

The inhibitory effect of C. lanatus extracts on α-glucose was conducted using the method explained by Jabeen and others [26], with modification. A volume of 70 µL of 100 mM potassium phosphate buffer (pH 6.8) was added to 10 µL C. lanatus extracts (5 different concentrations, 3, 2, 1, 0.75 and 0.5 mg/L) in a 96 well microplate, followed by 10 µL solution of α-glucosidase enzyme (1 Unit/mL). The mixture was vortexed for 10 seconds and pre-incubated for 10 minutes at 37°C. The
reaction was initiated by adding 10 μL of 5 mM para-nitrophenyl glucose after pre-incubation and the 100 μL mixture was incubated for 30 minutes at the same temperature after mixing. The absorbance of the mixture was then read at 405 nm wavelength by ELISA 96-well microplate reader (BioTek Model EL800, BioTek Instruments, USA), with the buffer solution used as the blank. The whole process was repeated for Acarbose, a standard drug used for lowering hyperglycaemia and management of type 2 diabetes mellitus. The per cent enzyme inhibition was calculated using the formula:

\[
\% \text{ Inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100 (1)
\]

The IC\(_{50}\) of \(C. \text{lanatus}\) extracts for α-glucosidase inhibition were calculated from a linear regression curve of % inhibition against concentrations of \(C. \text{lanatus}\) extracts (µg/mL), and extrapolation from the equation of the curve, \(y = mx + c\). Where \(y\) is IC\(_{50}\) = 50, \(m\) is the gradient of the curve, \(x\) is the concentration and \(c\) is the intercept on \(y\) axis.

### 2.4. Antioxidant Assays

The ABTS radical scavenging assay of \(C. \text{lanatus}\) extracts were conducted using the method as modified by Kim and others [27]. Reaction mixtures of 100 µL volume were formed in the wells of 96 well microplate, by mixing 10 µL of \(C. \text{lanatus}\) extract or standard (in methanol) with 90 µL diluted ABTS working reagent. The ABTS reagent was prepared by mixing freshly prepared 10 mL 2.45 mM potassium peroxydisulfate and 10 mL 7 mM ABTS in distilled water, and allowed to react in the dark for 16 hours at room temperature, to release the ABTS- radical. This was further diluted with distilled water to get a spectrophotometric absorbance of 0.700 ± 0.005, as the working solution. The reaction mixtures were read at 734 nm wavelength by ELISA 96-well microplate reader (BioTek Model EL800, BioTek Instruments, USA) and ascorbic acid was used as the standard. The ABTS radical scavenging was calculated as percent disappearance of the deep blue colour (i.e. % reduction in absorbance) as follows:

\[
\% \text{ Inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100 (1)
\]

The DPPH radical scavenging activity of \(C. \text{lanatus}\) extracts were evaluated as described by Brand-Williams and others [28]. About 50 µL of \(C. \text{lanatus}\) extracts (3, 2, 1, 0.75, and 0.5 mg/mL) were reacted with 195 µL of 0.2 mM DPPH in methanol in the wells of a microplate, with swirling for 1 minute. The mixtures were then incubated for 60 minutes in the dark at 29°C and their absorbance were read in ELISA 96-well microplate reader (BioTek Model EL800, BioTek Instruments, USA) at 540 nm wavelength. Tocopherol was used as the standard (positive control), while methanol was used as the negative control. The % inhibition of DPPH radical were calculated as follows:

\[
\% \text{ Inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100 (1)
\]

A linear regression curve was obtained by plotting % inhibition against the concentrations of \(C. \text{lanatus}\) extracts. The linear regression equation was used to extrapolate IC\(_{50}\) of the extracts for DPPH radical scavenging by inversely relating IC\(_{50}\) with antioxidant activity [29].

The ferric reducing antioxidant power (FRAP) of \(C. \text{lanatus}\) extracts were evaluated using the method as described by Benzie and Strain [30]. Each of \(C. \text{lanatus}\) extracts solution (3, 2, 1, 0.75 and 0.5 mg/mL) 10 μL and ascorbic acid (standard) were mixed with 200 μL of freshly prepared FRAP working reagent. This was prepared by mixing 0.3 M sodium acetate buffer pH 3.6, 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl acid and 20 mM ferric chloride solution in 10:1:1 ratio in 96-well microplate. The mixtures were vortexed for few seconds and allowed to stand for 4 minutes; the absorbance were then read at 593 nm wavelength using an ELISA 96-well microplate reader (BioTek Model EL800, BioTek Instruments, USA).

An absorbance (Xo) at time zero obtained by mixing FRAP reagent and the solvent used for \(C. \text{lanatus}\) extraction was subtracted from those of \(C. \text{lanatus}\) and ascorbic acid (XCL and XAA respectively). The absorbance of iron (II) sulphate solutions (Fe\(_2\)SO\(_4\)_7H\(_2\)O) with concentration range of 0.1 to 1.0 mM were equally obtained as above. These were plotted against the concentrations of Fe\(_2\)SO\(_4\)_7H\(_2\)O to get a standard calibration curve. Based on the absorbance obtained, the FRAP values of \(C. \text{lanatus}\) extracts and that of ascorbic acid were calculated using the regression equation in the form of \(y = mx + c\) obtained from the standard curve. The results were expressed as µM Fe\(_{2+}\)/g weight of extract or ascorbic acid.

Total phenolics content of \(C. \text{lanatus}\) extracts was measured by Folin-Ciocalteau’s method as described by Yuan and his others [31]. Equal volumes of \(C. \text{lanatus}\) extracts (100 μL) were added to 2.0 ml of 2% Na\(_2\)CO\(_3\) in a 96-well microplate and incubated at 29°C for 2 minutes. This was followed by the addition of 100 μL of 50% Folin–Ciocalteau’s phenol reagent. The reaction mixture was re-incubated for 30 minutes at 29°C and the absorbance was finally read at 720 nm. The process was repeated with standard Gallic acid solution.

### 2.5. Statistical Analysis

All data obtained were analysed using SPSS version 22 and statistical differences between plant parts and extracts were performed by two-way ANOVA. Results are presented as means of three different experimental results ± standard deviation (SD). Means are considered significantly different at P < 0.05.

### 2.6. Liquid Chromatography Separation Conditions

Phytochemicals present in the \(C. \text{lanatus}\) extracts were profiled using Agilent 1290 infinity LC system coupled to Agilent 6520 Accurate-Mass Quadruple Time of Flight (Q-TOF) mass spectrometer (MS), with dual ESI source (i.e. C) (Agilent Technologies, Palo Alto, CA, USA). It is equipped with a vacuum degasser, an auto sampler and binary pump. The column used was Agilent Zorbax...
Eclipse XDB-C18, Narrow-Bore, 2.1 X 150 mm, 3.5 micron (P/N:930990-902) (Agilent Technologies Palo Alto, CA, USA). The column temperature was 25 °C, auto sampler temperature 4 °C, and flow rate, 0.5 mL/minute. Solvent A, which contained 0.1% formic acid in water and B, containing 0.1% formic acid in acetonitrile were used as the mobile phase. Injection volume of the mobile phase was 1 µL and sample analysis were done in negative (U-ESI-XDB-C18-NB-MS-25(30)-Neg- and positive (U-ESI-XDB-C18-NB-MS-25(30)-Pos-ionization modes. The gradient elution was set at time 0, 5% B; 5 minutes, 5% B; 20 minutes, 1100% B; and 25 minutes, 100% B. the post run time was 5 minutes therefore, the total run time was 30 minutes.

2.7. Mass Spectrometry Parameters

The LC was coupled to a QTOF MS (micrOTOF Bruker Daltonics GmbH, Bremen, Germany, Agilent Technologies) operating in both negative (3500 V) and positive (4000 V) modes MS scan only. Other source parameters are fragmentor voltage, 125 V; drying gas temperature, 300°C; drying gas flow rate, 10 L/minute; skimmer, 65 V; OCT 1 RF Vpp, 750 V; nebulizer pressure, 45 psig; mass range, (m/z) minimum 100, maximum 3200; acquisition rate (spectra/sec) 1.03 L/minutes; acquisition time (ms/spectrum) 970.9 seconds and transient/spectrum is 9611.

2.8. MS Data Processing and Analysis

LC-MS data was analysed with Agilent Mass Hunter Qualitative Analysis B.07.00 by employing Metabolomics-171107-00100 method. Raw data was processed with Molecular Feature Extraction (MFE) with the following settings: i) Extraction algorithm: Small molecule (chromatographic); ii) Peak filters: Use peaks with height ≥ 100 counts; iii) Input data range: 400-500m/z; iv) Compound Filters: Only look for compound with absolute height ≥ 5000 counts and relative height ≥ 2.5%. Quality score ≥ 80.0; v) Ion species: Allow positive ions: +H, +Na, +K, +NH4. Allow negative ions: -H; vi) Isotope grouping: Peak spacing tolerance 0.0025 m/z plus 7.0ppm, isotope model - common organic molecules; vii) Limit assigned charge states to a maximum of 2.

2.9. Compounds Identification

In order to identify and assign names to compounds from chromatogram peaks, Metlin_AM_PCDL-N-170502.cdb, database search was conducted with the following parameters: i) Value to match: Mass; ii) Match tolerance: 5 ppm; iii) Spectrum peak searches: Maximum number of peaks to search when peaks are not specified graphically: 5; iv) Positive Ions: +H, +Na, +NH4; v) Negative Ions: -H; vi) Charge state range: 1-2; vii) Limit Results to the best 10 hits.

In the case of compounds that are not available in the database, the commands below were employed to generate formulas of unknown compounds, i) allowed species: C (minimum of 3 and maximum of 60); H (minimum 0 and maximum 120); O (minimum 0 and maximum 30); N (minimum 0 and maximum 120); S (minimum 0 and maximum 5) and Cl (minimum 0 and maximum 3). ii) Maximum Neutral mass for which formulas should be calculated: 1000.0000; iii) Limits on result: Minimum overall score of 35.00; iv) Isotope grouping: peak spacing tolerance, 0.0025 m/z plus 7.0ppm; isotope model: Common organic molecules and limit assigned charge states to a maximum of 2.

3. Results and Discussions

3.1. Results

3.1.1. Extraction Yield of Yellow Fleshed Seeded

\textit{C. lanatus}

The extraction yield for water and different ratios of aqueous ethanol extracts of \textit{C. lanatus} parts are presented in Table 1. The yield ranged between 1.83 ± 0.00 to 91.90 ± 0.00 % in the seed (lowest) and flesh (highest) extracts respectively. In the seed, 90% aqueous ethanol extract had the highest yield, 60% aqueous ethanol gave the highest yield for flesh and rind extracts, while absolute ethanol gave the highest yield for leaf extracts. The effect of concentration of ethanol started to manifest on the yield of seed extracts, as the yield decreased from 90% aqueous ethanol down to the aqueous extract. The yield of flesh extracts increased with decrease in the concentration of ethanol down to 60% aqueous ethanol, which decreased at 50% aqueous ethanol but higher again with aqueous extract. Moving from absolute ethanol, yield increased in the rind extracts at 90% aqueous ethanol, decreased at 80% aqueous ethanol but subsequently increased between 70% and 50% aqueous ethanol, and lowest yield was observed in aqueous extract. In the leaf extracts, the extraction yield was highest in the absolute ethanol extract and suddenly the lowest in the 90% aqueous extract. However, the extraction yield subsequently increased with decrease in the concentration of ethanol.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Seed</th>
<th>Flesh</th>
<th>Rind</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol Extract</td>
<td>1.83 ± 0.00E-4</td>
<td>27.32 ± 0.047b</td>
<td>4.88 ± 0.02E-2</td>
<td>59.37 ± 0.05E-5</td>
</tr>
<tr>
<td>90% Ethanol</td>
<td>17.33 ± 0.04E-5</td>
<td>68.86 ± 0.01E-4</td>
<td>23.11 ± 0.01E-2</td>
<td>10.35 ± 0.03E-4</td>
</tr>
<tr>
<td>80% Ethanol</td>
<td>15.10 ± 0.01E-6</td>
<td>73.10 ± 0.01E-2</td>
<td>7.90 ± 0.00E-6</td>
<td>19.50 ± 0.3E-5</td>
</tr>
<tr>
<td>70% Ethanol</td>
<td>14.01 ± 0.02E-6</td>
<td>89.51 ± 0.07E-3</td>
<td>33.85 ± 0.04E-3</td>
<td>24.59 ± 0.01E-3</td>
</tr>
<tr>
<td>60% Ethanol</td>
<td>10.70 ± 0.00E-6</td>
<td>91.90 ± 0.00E-4</td>
<td>35.99 ± 0.00E-4</td>
<td>23.16 ± 0.00E-4</td>
</tr>
<tr>
<td>50% Ethanol</td>
<td>11.51 ± 0.00E-6</td>
<td>87.03 ± 0.00E-4</td>
<td>34.80 ± 0.00E-4</td>
<td>24.77 ± 0.00E-6</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>9.04 ± 0.00E-6</td>
<td>90.91 ± 0.00E-2</td>
<td>3.98 ± 0.00E-2</td>
<td>23.99 ± 0.00E-4</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of three different experimental results. Means within columns that do not share capital letter, and within rows that do not share small letter, are significantly different (P < 0.05).
3.1.2. Inhibition of Alpha-amylase Activity

The IC₅₀ of yellow fleshed seeded *C. lanatus* extracts for α-amylase inhibition are presented in Figure 1. These values in the seed extracts, ranged between 58.51 ± 0.30 µg/mL for absolute ethanol and 214.04 ± 1.42 µg/mL for 50% aqueous ethanol. In the flesh extracts, they are between 31.83 ± 0.54 µg/mL for 70% aqueous ethanol and 57.80 ± 1.07 µg/mL for aqueous extract. In the rind extracts, they range between 42.13 ± 0.49 µg/mL for 70% aqueous ethanol and 176.99 ± 1.51 µg/mL for 50% aqueous ethanol.

While in the leaf extracts, the IC₅₀ falls within the range of 36.75 ± 3.47 µg/mL for absolute ethanol and 131.26 ± 0.54 µg/mL for aqueous extract. Absolute ethanol and 70% aqueous ethanol gave the best α-amylase inhibition in the seed and leaf extracts, and flesh and rind extract respectively. In the seed and rind extracts, the IC₅₀ values increased when the concentration of ethanol decreased to 90% but, decreased at 80 and 70%. The IC₅₀ increased again as the concentration of ethanol further decreased. The IC₅₀ for α-amylase inhibition decreased as the extraction solvent was diluted from absolute ethanol down to 50% aqueous ethanol in the flesh extracts except, at 60% aqueous ethanol. This is not the case in the leaf extracts, as the IC₅₀ value increased with increase of water in the ethanol from 0 to over 50%. However, there was a decrease in IC₅₀ at 70% aqueous ethanol in the leaf extracts. Looking at α-amylase inhibition among *C. lanatus* plant parts, the IC₅₀ values ranges from 31.83 ± 0.54 to 214.04 ± 1.42 µg/mL for flesh (lowest) and seed (highest), respectively. The order of decrease in the inhibition of α-amylase by *C. lanatus* plant parts is flesh > leaf > rind > seed.

3.1.3. Inhibition of Alpha-glucosidase Activity

Figure 2 presents the IC₅₀ values of yellow fleshed seeded *C. lanatus* extracts for α-glucosidase inhibition. In the seed extracts, the IC₅₀ range from 32.50 ± 0.36 to 313 ± 1.36 µg/mL for 70% aqueous ethanol and aqueous extracts respectively; in the flesh, the values are between 41.38 ± 1.04 and 178.35 µg/mL for 70% aqueous ethanol and aqueous extracts respectively; in the rind, it is between 45.44 ± 0.18 and 120.61 ± 0.40 µg/mL for 70% aqueous ethanol and aqueous extracts respectively; while the IC₅₀ values ranges between 26.26 ± 0.29 and 180.33 ± 1.31 µg/mL for 70% aqueous ethanol and aqueous extracts of the leaf respectively, the values all have statistical significant difference at P < 0.05. The 70% aqueous ethanol extract gave the lowest IC₅₀ value for α-glucosidase inhibition across all the studied *C. lanatus* extracts. Within the extracts, the IC₅₀ value decreased in the seed and leaf as the concentration of the solvent changes from absolute ethanol to 70% aqueous ethanol, and increased as the concentration of ethanol further decreased. The extraction behaviour in the case of the flesh and rind shows that, the IC₅₀ values increased initially with increase of water in the extraction solvent up to 80% aqueous ethanol but decreased at 70% aqueous ethanol.

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**Figure 1.** IC₅₀ of *C. lanatus* extracts for alpha-amylase inhibition Different small letters indicate significant differences (P<0.05) between sample extracts, while different capital letters indicate significant difference between solvent systems

**Figure 2.** IC₅₀ of *C. lanatus* extracts for alpha-glucosidase inhibition Different small letters indicate significant difference (P<0.05) between sample extracts, while different capital letters indicate significant difference between solvent systems
While, there is a continuous increase from 60% aqueous ethanol as the concentration of ethanol further decreased. Across the C. lanatus plant parts, the IC₅₀ values ranged between 26.26 ± 0.29 and 313 ± 1.36 µg/mL in the leaf and seed respectively. The decreasing order of α-glucosidase inhibition by C. lanatus parts is leaf > seed > flesh > rind.

3.1.4. Phenolics Content (TPC) of C. lanatus Extracts

In this study, the total phenolics content (TPC) of C. lanatus extracts were also evaluated. As presented in Figure 3, the values for the TPC of C. lanatus extracts were between 18.22 ± 0.35 and 5292.96 ± 1.47 µg GAE/g in the flesh (lowest) and leaf (highest) respectively. In the seed and flesh extracts 70% aqueous ethanol extract gave the highest TPC value, the rind had the highest TPC value in absolute ethanol extract, while 80% aqueous ethanol extract gave the highest TPC value for the leaf extracts. The TPC of the seed and flesh extracts initially decrease as the water content of the extraction solvent increased from absolute ethanol to 80% ethanol. A sharp increase in the values of TPC were suddenly observed at 70% aqueous extract, which also continued to decrease with decrease in the concentration of ethanol. The TPC values of the rind extracts decreased initially from absolute ethanol to 70% aqueous ethanol, then from 60% aqueous ethanol, the TPC increases with increase in water content of the extraction solvent. While in the leaf extracts, the TPC values increased from absolute ethanol to 80% aqueous ethanol and a fluctuation in the values was observed from 60% aqueous ethanol. The leaf extracts of C. lanatus gave the highest TPC values when compared to the seed, flesh and rind extracts, followed by the rind extracts, while the flesh extracts had the lowest values for TPC. The order of increase in the TPC of C. lanatus extracts from the findings of this work is flesh < seed < rind < leaf.

3.1.5. Total Antioxidant Effect of C. lanatus Extracts

The total antioxidant effects of C. lanatus extracts were evaluated using ABTS, DPPH, and FRAP assay methods.

3.1.5.1. ABTS Radical Scavenging of C. lanatus Extracts

The ABTS radical scavenging effects of C. lanatus extracts are presented in Figure 4. The ABTS values ranged from 49.20 ± 0.05 to 1408.25 ± 0.31 µmol TEAC/100 g for the seed (lowest) and leaf (highest) respectively. In the seed, flesh and leaf extracts, 70% aqueous ethanol extract has the highest ABTS radical scavenging value, aqueous extract is the highest in the rind.

Generally, the ABTS radical scavenging values increased with increase in concentration of water in the extraction solvent. The leaf extracts gave the highest ABTS radical scavenging values compared to the seed, flesh and rind extracts and the lowest value is obtained in the seed extracts. Though, the leaf extracts display very high values for ABTS radical scavenging, that of the reference standard, ascorbic acid is much higher. The order of increase in the value of ABTS radical scavenging in C. lanatus parts is seed < rind < leaf.

3.1.5.2. DPPH Radical Scavenging Effects of C. lanatus Extracts

Figure 5 presents the IC₅₀ for DPPH radical scavenging activity of C. lanatus extracts. The values ranged from 16.46 ± 0.39 to 1266.14 ± 1.23 µg/mL for 70% aqueous ethanol leaf (lowest) and aqueous extracts (highest) respectively. In the seed, flesh and leaf, 70% aqueous ethanol extract gave the lowest IC₅₀ value, while the rind has 80% aqueous ethanol extract with the lowest IC₅₀ value for DPPH radical scavenging.

The IC₅₀ values in the seed, rind and leaf extracts decreased from absolute ethanol to 70% aqueous ethanol and increased from 60% aqueous ethanol downwards. This was not the same in the flesh extracts, as the IC₅₀ increased with decrease in the concentration of ethanol up to 80% aqueous ethanol. A sudden decrease was observed in the IC₅₀ of 70% aqueous ethanol flesh extract, which however increased again from 60% aqueous ethanol extract with increase in polarity of ethanol. The leaf extracts generally had the lowest IC₅₀ values for the scavenging of DPPH radical, followed by the rind, while the flesh had the highest IC₅₀ values. The IC₅₀ value for DPPH radical scavenging of the reference standard, tocopherol, is 3.44 ± 0.22 µg/mL, which was significantly lower than that of the lowest C. lanatus leaf extract.

Figure 3. Total phenolics content of yellow fleshed seeded C. lanatus extracts Different small letters indicate significant differences (P<0.05) between sample extracts, while different capital letters indicate significant difference between solvent systems
**Figure 4.** ABTS radical inhibition of *C. lanatus* extracts. Different small letters indicate significant differences (P<0.05) between sample extracts, while different capital letters indicate significant difference between solvent systems.

**Figure 5.** IC$_{50}$ of *C. lanatus* extracts for DPPH radical inhibition. Different small letters indicate significant difference (P<0.05) between sample extracts, while different capital letters indicate significant difference between solvent systems.

**Figure 6.** Ferric reducing antioxidant power of yellow fleshed seeded *C. lanatus* extracts. Different small letters indicate significant differences (P<0.05) between sample extracts, while different capital letters indicate significant difference between solvent systems.
3.1.5.3. FRAP of *C. lanatus* Extracts

Ferric reducing antioxidant power is another method used to evaluate the antioxidant effects of *C. lanatus* extracts in this study. As presented in Figure 6, the FRAP values of *C. lanatus* extracts are between 58.53 ± 1.35 and 762.10 ± 0.70 μM/g in the flesh (lowest) and leaf (highest) respectively. Absolute ethanol, 50% aqueous ethanol and aqueous extracts gave the highest FRAP values for the seed, flesh, rind and leaf extracts respectively. The FRAP values increased in the seed extracts from 90% aqueous ethanol but, decreased from 60% aqueous ethanol as the concentration of ethanol decreases.

Table 2. Phytochemical compounds detected and identified in yellow fleshed seeded *Citrus lanatus* leaf extract using LC-QTOF-MS in positive and negative ionization modes

<table>
<thead>
<tr>
<th><em>Proposed Compounds</em></th>
<th>Formula</th>
<th>Calculated m/z (% relative abundance)</th>
<th>MS fragments</th>
<th><strong>Polarity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>C6H14N2O2</td>
<td>174.1111 (45.84)</td>
<td>175.1184</td>
<td>+ve</td>
</tr>
<tr>
<td>Chalconaringenin 2'-rhamnosyl-(1→4)-glucoside</td>
<td>C17H16O4</td>
<td>580.1804 (96.79)</td>
<td>598.2142</td>
<td>+ve</td>
</tr>
<tr>
<td>Naringenin 5,7'-dimethyl ether 4'-O-xylosyl-(1→4)-arabinoside</td>
<td>C27H20O13</td>
<td>564.1848 (99.52)</td>
<td>582.2187</td>
<td>+ve</td>
</tr>
<tr>
<td>Cucurbitacin E</td>
<td></td>
<td>556.3025 (96.63)</td>
<td>579.2915</td>
<td>+ve</td>
</tr>
<tr>
<td>Acetyl tributyl citrate</td>
<td></td>
<td>402.2258 (99.26)</td>
<td>403.2331</td>
<td>+ve</td>
</tr>
<tr>
<td>4'-Apo-beta-caroten-4'-al</td>
<td></td>
<td>482.3563 (87.93)</td>
<td>500.3903</td>
<td>+ve</td>
</tr>
<tr>
<td>Citrulline</td>
<td>C6H12N2O2</td>
<td>175.0958 (97.63)</td>
<td>174.0887</td>
<td>-ve</td>
</tr>
<tr>
<td>Caffeic acid 3-glucoside</td>
<td>C16H10O5</td>
<td>342.0949 (99.76)</td>
<td>341.0877</td>
<td>-ve</td>
</tr>
<tr>
<td>Robinetin 3-rutinoside</td>
<td>C17H16O4</td>
<td>610.1518 (94.87)</td>
<td>609.1443</td>
<td>-ve</td>
</tr>
<tr>
<td>Lutolizin 7-rhamnosyl(1→6) galactoside</td>
<td>C27H20O15</td>
<td>594.1574 (97.74)</td>
<td>593.1502</td>
<td>-ve</td>
</tr>
<tr>
<td>Zizybose I</td>
<td>C26H16O11</td>
<td>432.1632 (92.17)</td>
<td>431.1554</td>
<td>-ve</td>
</tr>
<tr>
<td>Scutellaren-7-glucoside</td>
<td>C27H20O11</td>
<td>448.1002 (98.83)</td>
<td>447.0927</td>
<td>-ve</td>
</tr>
<tr>
<td>Naringenin 7-O-(2',6'-di-O-alpha-rhamnopyranosyl)-beta-glucopyranoside</td>
<td>C27H20O13</td>
<td>726.2345 (91.22)</td>
<td>725.2275</td>
<td>-ve</td>
</tr>
<tr>
<td>Chalconaringenin 2'-rhamnosyl-(1→4)-glucoside</td>
<td></td>
<td>580.1818 (98.31)</td>
<td>579.1729</td>
<td>-ve</td>
</tr>
<tr>
<td>Apigenin 7-(6',6'-diacetyllactoside)-4'-alloside</td>
<td>C27H20O17</td>
<td>678.1778 (94.34)</td>
<td>677.1705</td>
<td>-ve</td>
</tr>
<tr>
<td>Naringenin 5,7'-dimethyl ether 4'-O-xylosyl-(1→4)-arabinoside</td>
<td>C27H20O13</td>
<td>564.1846 (99.13)</td>
<td>563.1776</td>
<td>-ve</td>
</tr>
<tr>
<td>Platycarpentin 7-O-glucoside</td>
<td>C26H20O12</td>
<td>504.1267 (96.66)</td>
<td>503.1195</td>
<td>-ve</td>
</tr>
<tr>
<td>Isoiscutellaren-7-(6'-acetyllactosyl)-(1-2)-6'-acetylglucoside</td>
<td>C27H20O13</td>
<td>694.1717 (88.11)</td>
<td>693.1641</td>
<td>-ve</td>
</tr>
<tr>
<td>Podorhizol beta-D-glucoside</td>
<td>C26H20O13</td>
<td>578.1983 (71.33)</td>
<td>577.191</td>
<td>-ve</td>
</tr>
<tr>
<td>Fumonotonarinin</td>
<td>C26H20O14</td>
<td>594.1952 (99.21)</td>
<td>593.1888</td>
<td>-ve</td>
</tr>
<tr>
<td>Patuletin 3,7-bis(3-acetyllhamnoside)</td>
<td>C26H20O13</td>
<td>708.1883 (92.29)</td>
<td>707.1809</td>
<td>-ve</td>
</tr>
<tr>
<td>Medicarpin 3-O-(6'-malonyl)glucoside</td>
<td>C26H20O12</td>
<td>518.1414 (95.61)</td>
<td>517.1338</td>
<td>-ve</td>
</tr>
<tr>
<td>6-Methoxykaempferol 3,7-bis(3-acetyllhamnoside)</td>
<td>C26H20O13</td>
<td>692.1928 (90.99)</td>
<td>691.1855</td>
<td>-ve</td>
</tr>
<tr>
<td>Licorice glycoside C1</td>
<td>C26H20O13</td>
<td>726.2157 (99.26)</td>
<td>725.2087</td>
<td>-ve</td>
</tr>
<tr>
<td>6-Gingerol</td>
<td>C27H20O14</td>
<td>294.1826 (99.01)</td>
<td>293.1754</td>
<td>-ve</td>
</tr>
</tbody>
</table>

*All data were retrieved https://metlin.scripps.edu/landing on 13/11/2017. **+ve = Positive -ve = Negative.*

3.1.6. Phytochemicals Identified in *C. lanatus* Extracts

The phytochemical profiles from different parts of watermelon extracts were studied by LC-ESI-QTOF-MS and presented in Table 2, Table 3, Table 4 and Table 5 for leaf, rind, seed and flesh, respectively. The phytochemicals identified were from 70% aqueous ethanol extracts of the seed, flesh, rind and leaf. This is because, 70% aqueous ethanol extract gave the most active bioactivity in the anti-diabetic and antioxidant results above. About 265, 105, 154 and 194 compounds were detected by positive and negative ionisation mode MS scan in the seed, flesh rind and leaf respectively. Out of these, 125, 35, 73 and 104 compounds were tentatively identified in the seed, flesh, rind and leaf from the LC-MS library database and ChemSpider (http://www.chemspider.com).

Table 3. Phytochemical compounds detected and identified in yellow fleshed seeded *Citrus lanatus* rind extract using LC-QTOF-MS in positive and negative ionization modes

<table>
<thead>
<tr>
<th><em>Proposed compounds</em></th>
<th>Formula</th>
<th>Calculated m/z (% relative abundance)</th>
<th>MS fragments</th>
<th><strong>Polarity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>(S)-dihydroxypropic acid</td>
<td>C6H8O2S2</td>
<td>208.0586 (63.95)</td>
<td>207.0512</td>
<td>-ve</td>
</tr>
<tr>
<td>Dihydroferulic acid 4-sulfate</td>
<td>C16H12O4S</td>
<td>276.0294 (72.59)</td>
<td>275.0221</td>
<td>-ve</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>C7H10O3</td>
<td>192.0273 (99.46)</td>
<td>191.02</td>
<td>-ve</td>
</tr>
<tr>
<td>Isolacreresinol 9-O-beta-D-glucoside</td>
<td>C18H14O11</td>
<td>522.2091 (96.92)</td>
<td>521.2021</td>
<td>-ve</td>
</tr>
<tr>
<td>Gingerol</td>
<td>C7H7O4</td>
<td>294.1823 (97.43)</td>
<td>293.1751</td>
<td>-ve</td>
</tr>
<tr>
<td>Curcumenol</td>
<td>C15H8O2</td>
<td>234.1614 (97.71)</td>
<td>233.1541</td>
<td>-ve</td>
</tr>
<tr>
<td>Acetyl tributyl citrate</td>
<td>C20H18O8</td>
<td>402.2253 (99.82)</td>
<td>403.2327</td>
<td>+ve</td>
</tr>
</tbody>
</table>

*All data were retrieved https://metlin.scripps.edu/landing on 13/11/2017. **+ve = Positive -ve = Negative.*
Table 4. Phytochemical compounds detected and identified in yellow fleshed seeded C. lanatus seed extract using LC-QTOF-MS in positive and negative ionization modes

<table>
<thead>
<tr>
<th>*Proposed compounds</th>
<th>Formula</th>
<th>Calculated m/z (% relative abundance)</th>
<th>MS fragments</th>
<th>** Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,9-Dimethylurea</td>
<td>C₈H₁₃N₂O₂</td>
<td>196.06 (95.11)</td>
<td>195.0527</td>
<td>+ve</td>
</tr>
<tr>
<td>Citric acid</td>
<td>C₆H₈O₇</td>
<td>192.0273 (99.39)</td>
<td>191.02</td>
<td>-ve</td>
</tr>
<tr>
<td>(6S)-dehydrovomifoliol</td>
<td>C₁₇H₁₄O₁₀</td>
<td>222.1254 (99.68)</td>
<td>221.1181</td>
<td>-ve</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>C₄H₆N₂O₂</td>
<td>174.1111 (86.17)</td>
<td>175.1184</td>
<td>-ve</td>
</tr>
<tr>
<td>3-Epihydroxy-2'-deoxymugineic acid</td>
<td>C₁₀H₁₄N₂O₂</td>
<td>320.1214 (99.07)</td>
<td>338.1553</td>
<td>+ve</td>
</tr>
<tr>
<td>Dihydrocaffeic acid 3-O-glucuronide</td>
<td>C₁₉H₁₄O₁₁</td>
<td>358.0899 (79.37)</td>
<td>381.0794</td>
<td>+ve</td>
</tr>
<tr>
<td>6-Hydroxyelutedin 7-rhamnoside</td>
<td>C₂₁H₂₀O₁₁</td>
<td>448.1008 (97.82)</td>
<td>449.1078</td>
<td>+ve</td>
</tr>
<tr>
<td>Macrophylline</td>
<td>C₁₇H₁₈N₂O₂</td>
<td>239.1532 (90.10)</td>
<td>240.1606</td>
<td>+ve</td>
</tr>
<tr>
<td>Curcumenol</td>
<td>C₁₁H₁₂O₂</td>
<td>234.1621 (86.69)</td>
<td>235.1694</td>
<td>+ve</td>
</tr>
<tr>
<td>Acetyl tributyl citrate</td>
<td>C₂₀H₁₄O₃</td>
<td>402.226 (98.75)</td>
<td>403.2333</td>
<td>+ve</td>
</tr>
<tr>
<td>Arjunolic acid</td>
<td>C₁₆H₁₄O₄</td>
<td>488.3477 (75.57)</td>
<td>506.3826</td>
<td>+ve</td>
</tr>
</tbody>
</table>

All data were retrieved https://metlin.scripps.edu/landing on 13/11/2017. **+ve = Positive -ve = Negative.

Table 5. Phytochemical compounds detected and identified in yellow fleshed seeded C. lanatus flesh extract using LC-QTOF-MS in positive and negative ionization modes

<table>
<thead>
<tr>
<th>*Proposed compounds</th>
<th>Formula</th>
<th>Calculated m/z (% relative abundance)</th>
<th>MS fragments</th>
<th>** Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>C₆H₈O₇</td>
<td>192.0273 (99.39)</td>
<td>191.02</td>
<td>-ve</td>
</tr>
<tr>
<td>(6S)-dehydrovomifoliol</td>
<td>C₁₇H₁₄O₁₀</td>
<td>222.1251 (98.85)</td>
<td>221.1179</td>
<td>-ve</td>
</tr>
<tr>
<td>3-Butyliden-7-hydroxyphthalide</td>
<td>C₁₀H₁₄O₃</td>
<td>204.0793 (97.63)</td>
<td>205.0865</td>
<td>+ve</td>
</tr>
<tr>
<td>Acetyl tributyl citrate</td>
<td>C₂₀H₁₄O₃</td>
<td>402.2253 (99.88)</td>
<td>403.2326</td>
<td>+ve</td>
</tr>
<tr>
<td>Docosanedioic acid</td>
<td>C₂₂H₂₄O₄</td>
<td>370.3093 (97.21)</td>
<td>371.3166</td>
<td>+ve</td>
</tr>
</tbody>
</table>

All data were retrieved https://metlin.scripps.edu/landing on 13/11/2017. **+ve = Positive -ve = Negative.

3.2. Discussion

3.2.1. Effects of Extraction Solvent on Extracts Yield of Yellow Flesheed Seed C. lanatus

The separation and recovery of bioactive compounds from the matrix keeping them intact in plant materials is the first step toward the utilisation of plant arsenal in health conditions [32]. This is thought to be influenced by the extraction method employed, the particle size of the sample, nature of phytoconstituents of the plant material and the solvent used among others [33,34]. The chemical constituent, temperature, time, pH, and the polarity of solvent used, are factors that control the extraction yield of a plant material. Keeping temperature and time of extraction constant, extraction yield is most importantly dependent on the sample composition and solvent [32,33].

In this study, it could be observed that the yield in aqueous medium (more polar) by the seed and flesh are higher than that of absolute ethanol (less polar), while in the leaf which has different chemical constituents from the seed and flesh, absolute ethanol extract gave the highest yield. Also observed, is the increase and decrease in extraction yield as the water/ethanol ratio increased in the different plant matrices, indicating that increase in solvent polarity and chemical composition, can increase or decrease extraction yield. On the average, this result showed that increase in solvent polarity also increased extraction yield. This observed increase could be due to increase in the solubility of phytoconstituents of the plant materials in the water/ethanol solvent interface. Hence, depicting the dependence of extraction yield on polarity of solvent used and the chemical constituents of the plant material. In addition, the increase in extraction yield could also be due to the high solubility of primary metabolites (carbohydrates and proteins) in the solvent systems, which may have been extracted along with the secondary metabolites. These findings are similar to the extraction yields in the work of other researchers [33,35,36].

3.2.2. Effect of C. lanatus Extracts on Alpha-amylase and Alpha-glucosidase Activities

Inhibition of the activities of carbohydrate metabolising enzymes, especially that of the α-amylase and α-glucosidase, is regarded as one of the mechanisms for the control of postprandial hyperglycaemia in diabetic condition as reviewed [37]. It is believed that the inhibition of any of these two enzymes especially the α-glucosidase, found in the brush border surface membrane cells of the intestine, could prevent the quick release of glucose form sucrose and maltose, thereby delaying the time for glucose surge experienced after taking a carbohydrate rich meal. Hence, reducing hyperglycaemia as a result of food intake in type 2 diabetic individuals. Postprandial hyperglycaemia can independently cause micro vascular and macro vascular complications of diabetes mellitus when prolonged [38,39].

Scientists have revealed the possible anti-hyperglycaemia properties of medicinal herbs in terms of the inhibition of pancreatic α-amylase and intestinal α-glucosidase activities [40-47]. In this study, it was discovered that, all the C. lanatus extracts inhibited the activity of α-amylase in vitro in different proportions as shown in Figure 1. The 70% aqueous ethanol flesh extract gave the lowest IC50 value, followed by that of absolute ethanol leaf extract and 70% aqueous ethanol rind extract. While, the 100% ethanol seed extract which gave the lowest IC50 value among the seed extracts, is higher than that of 70% aqueous ethanol rind extract (Figure 1). The IC50 for an enzyme inhibition is the concentration of an inhibitor required to inhibit the activity of an enzyme by 50% at optimum reaction conditions [48,49,50]. It is used to quantify the potency of an enzyme inhibition, the lower
the IC\textsubscript{50} value of the inhibitor, the stronger the enzyme inhibition.

Compared to other studies, the extracts of \textit{C. lanatus} seed, flesh, rind and leaf could be considered as more potent \(\alpha\)-amylase inhibitors than those reported for dried roselle, chrysanthemum, mulberry, bael and butterfly pea, 3.52 ± 0.15, >5.00, >5.00 and 4.05 ± 0.32 mg/mL respectively [42]. The extracts of \textit{C. lanatus} in this study could also be better inhibitors against \(\alpha\)-amylase when compared to those reported values, 1.77 ± 0.05, 1.23 ± 0.02, >4.00 and 1.77 ± 0.02 mg/mL for Chinese cinnamon, Ceylon cinnamon, Saigon cinnamon and Thai cinnamon respectively [40].

It was also discovered that \textit{C. lanatus} extracts under this study exhibited a remarkable \(\alpha\)-glucosidase inhibitory activity (Figure 2). The IC\textsubscript{50} of 70% aqueous ethanol leaf extract was found to be 123.8, 157.6 and 173.0% lower than those of the seed, flesh and rind extracts respectively, while the IC\textsubscript{50} of acarbose, 18.57 ± 3.71 µg/mL was only 1.41 times as better as that of the leaf, 26.26 ± 0.29 µg/mL. This indicates that the 70% aqueous ethanol leaf extract has the highest \(\alpha\)-glucosidase inhibition potential than the other \textit{C. lanatus} extracts studied. Being a crude extract, the IC\textsubscript{50} of 70% aqueous ethanol extract 26.26 ± 0.29 µg/mL, was significantly higher (\(P<0.05\)) than that of acarbose, 18.17 ± 3.78 µg/mL, a standard anti-diabetic molecule. This IC\textsubscript{50} value for \textit{C. lanatus} leaf extracts was lower than that reported for acetonate extract of Ficus lutea, 290 ± 111 µg/mL against \(\alpha\)-glucosidase activity [41]. The IC\textsubscript{50} values of \(\alpha\)-glucosidase inhibition reported for two varieties of Ficus deltoidea fruit extracts were also higher than the those reported in this work for \textit{C. lanatus} fruit extracts [51].

The IC\textsubscript{50} of 90, 80 and 70% aqueous ethanol leaf extracts of \textit{C. lanatus}, 27.64 ± 0.52, 26.02 ± 2.66 and 26.26 ± 0.29 µg/mL respectively for \(\alpha\)-glucosidase inhibition, could be more potent than the 39.0, 37.0 and 41.0 µg/mL, reported for 80, 60 and 40% ethanolic extracts respectively of Melicope lunu-ankender leaves [52]. Here, the studied \textit{C. lanatus} flesh and rind extracts had the IC\textsubscript{50} values of 41.38 ± 1.04 and 45.44 ± 0.18 µg/mL respectively. These were much lower than 0.19 and 1.22 mg/mL reported for peer peel and pulp respectively [53]. This indicates that \textit{C. lanatus} extracts could have more potent \(\alpha\)-glucosidase inhibitor(s) than Melicope lunu-ankender leaves peer.

These potent \(\alpha\)-amylase and \(\alpha\)-glucosidase inhibitory activity of \textit{C. lanatus} extracts could be attributed to the vast phytochemical content of the plant especially the polyphenols, which their extraction was maximised by the different water/ethanol ratios. It has been reported that the anti-diabetic effects of medicinal plants are partly due to their high contents of phenolic compounds [54-60], and partly due to efficiency of their extraction solvent, that the most suitable solvents for extraction of phenolics compounds are polar solvents in aqueous solution, ethanol used in this study has been known to be suitable for the extraction of polyphenols [32,33].

In addition, it could also be possible that some polyphenol-glycosides (i.e. possible analogues of the \(\alpha\)-amylase substrate) present in \textit{C. lanatus} extracts may have formed dead-end complex with the enzyme, thereby preventing it from further action and hence, its activity is inhibited. One of the suggested mechanisms through which inhibitors inhibits \(\alpha\)-amylase is mimicking the enzyme’s substrate [61]. It is also possible that the polyphenols in \textit{C. lanatus} extracts acted as electrophiles and abstracted the free H\(^+\) ion in the active site of \(\alpha\)-glucosidase enzyme, needed for the binding of its substrate, thereby preventing reaction to occur between \(\alpha\)-glucosidase and its substrates (i.e. oligosaccharides and disaccharides). Therefore, the enzyme activity is disrupted and the release of free glucose is not possible. Usually, H\(^+\) ion is needed for the catalysis of the cleavage of \(\alpha\)-(1,4)-glycosidic bond, which is supplied by the active site of \(\alpha\)-glucosidase enzyme catalysing the forward reaction. Removal of this H\(^+\) ion from the active site of the enzyme is one of the ways it is inhibited by inhibitors [61,62,63].

### 3.2.3. Polyphenols Contents of Yellow Flesched Seeded \textit{C. lanatus} Extracts and Their Antioxidant Effects

The diverse nature of plants and their phytochemical constituents have made available natural antioxidants, proven to be remedies to both animals and human health problems. In humans, continued intakes of fruits and vegetables, known to be rich in antioxidants, has been implicated in the prevention and treatment of degenerative diseases, such as Alzheimer’s disease, certain cancers, cardiovascular diseases, etc. [18,67-72]. Polyphenols have been reported to be the major contributing compounds to the hydrophilic antioxidant activity of watermelon [23] and partly attributed to the laxative properties of watermelon fruit [73].

The polyphenol content of \textit{C. lanatus} according to this study were found to be highest in the leaf extracts, followed by the rind, seed and the flesh extracts (Figure 3). Except for the rind which gave highest TPC value in absolute ethanol extracts, TPC values increased as the polarity of ethanol increased midway (i.e. 70% and 80% aqueous ethanol for seed and flesh, and leaf extracts respectively). In the aqueous ethanol medium, it could be possible that most of the phenolic constituents of the seed, flesh and leaf were soluble in the water/ethanol interphase, thereby extracted in high amount as found in this study. A mixture of polar solvents with appropriate amounts of water are very suitable for polyphenols extraction especially water/ethanol mixtures, since ethanol itself is regarded as one of the best solvents for this purpose and safe for humans [33,34,36].

In the case of the leaf extracts, it is possible that it has higher amounts of polyphenols content than the rind, hence more of it were extracted from the leaf as seen in Table 1. While in the case of the seed and flesh which presented lower polyphenols content, this could be as a result of their high carbohydrate and protein contents, with an additional high fat contents in the seed [58,74]. Increase in water/ethanol ratio does not cause corresponding increase of TPC in the rind extracts. This could be that increase in solvent polarity does not affect polyphenols extraction in the rind due to the nature of the phenolics compounds present in it. So, the nature of polyphenols (i.e. chemical nature) present in the rind played a significant role in their extraction from the matrix of the rind.

The natural antioxidant endowment of herbs is widely evaluated by free radical scavenging methods. Neutralization
of free radicals generated is the common ground for accessing the antioxidant power of biological substances employed by these antioxidant assay methods [70, 75, 76]. Authors have recommended the use of more than method in evaluating the antioxidant effects of fruits and vegetables [23, 77]. This study examined the antioxidant strength of extracts from four different parts of C. lanatus plant, by ABTS, DPPH, and FRAP antioxidant assay methods (Figure 4, Figure 5 and Figure 6).

The ABTS radical scavenging effect of C. lanatus extracts expressed as Trolox equivalence antioxidant capacity (TEAC), indicated that the leaf extracts were able to at least scavenge out the generated ABTS+. cation radicals 227.6%, 324.0% and 610% more than the rind, seed and flesh extracts respectively (Figure 4). This indicates that the leaf extracts had the highest antioxidant strength compared to the seed, flesh and rind, which could be attributed to high phenolics contents of the leaf seen in Table 1. The presence of phenolic compounds in C. lanatus extracts were implicated in their hydrophilic and lipophilic antioxidant activities, and also, their presence in plant materials is generally attributed to strong antioxidant activities [14, 78]. Though the leaf extracts of C. lanatus had the highest ABTS radical inhibition, it is about 6.7 times less than that of the standard ascorbic acid used in this study. When compared to other studies, the ABTS+ cation radical inhibition of C. lanatus leaf extracts have more antioxidant potentials than those reported by [41, 45].

It was observed in this study that the ABTS radical scavenging activity of C. lanatus extracts generally increased with increase in water content of the extraction solvent except for the flesh extracts, which initially increased and decreased after 70% aqueous ethanol extract. These increases could be due to the increase in the solubility of antioxidant phytochemical constituents in the water/ethanol interphase, thereby exposing them to the neutralization of the generated ABTS+. cation radicals. The extraction efficiency of active phytochemicals in plant materials have been attributed to the polarity of extraction solvent and the chemical nature of phytochemical contents [33, 34].

The IC50 for DPPH radical scavenging by C. lanatus extracts presented in Figure 5 also indicated a strong antioxidant potential displayed by the leaf extracts. The IC50 of antioxidant compounds in terms of biological radicals’ inhibition, is proportional to antioxidant strength in an inverse manner, since it is the concentration of a compound required to neutralise or scavenge 50% of a radical (DPPH in this case) that could pose danger to other biological molecules. The IC50 value leaf extract (70% aqueous ethanol) was significantly lower (P<0.05) than the rest, which was 17.2, 27.4 and 31.1 times lower than those of the seed, flesh and rind respectively (Figure 5). When the lowest IC50 of leaf extracts was compared with that of the pure tocopherol standard, it was found to be significantly higher (P<0.05). That is 4.8 times higher than that of the standard tocopherol, indicating that in terms of DPPH radical scavenging, tocopherol is more effective than the leaf extracts of C. lanatus.

However, the IC50 values of aqueous ethanol leaf extracts for DPPH radical inhibition in this study, were lower than the value, 37.12 µg/mL, reported for methanol leaf extract of C. lanatus [79]. There were also more potent than those reported for Limnophila aromatic [33], aqueous extract of Derris reticulata [45], methanol extracts of Melocope lunu-ankenda leaves [52], Phylanthus nurini and Phylanthus urinary [80]. The IC50 values of aqueous ethanol extracts for DPPH radical scavenging, were also lower than those reported for Ficus deltoidea fruit extracts, 547.83 ± 18.11 and 975.20 ± 20.79 µg/mL. The DPPH radical inhibition of the seed, rind and leaf showed increase as water content of the extraction solvent increased. This indicated a possible increase in the extraction of proton donating antioxidant compounds from the plant materials, which could easily donate their H+ ions to DPPH radical and quenched it [33]. This is the principle behind the use of DPPH radical scavenging system [81]. Furthermore, except for 50% aqueous ethanol rind extract, all the IC50 values of the rind extracts of this study for DPPH radical inhibition, were lower than those, 0.64 mg/mL and 11.578 mg/mL reported for watermelon rind polysaccharides and water-soluble polysaccharides of potatoes peel [17].

The FRAP antioxidant power of C. lanatus extracts presented in Figure 6 also indicated that the leaf extracts have more promising antioxidant potentials than the other extracts studied. The highest FRAP value for 100% ethanol leaf extract, was found to be 10.1, 7.7 and 5.5 times higher than the FRAP values of the flesh, seed and rind respectively. This FRAP value of leaf extract was also significantly higher (P<0.05) than that of standard ascorbic acid used in this study. This suggests that, C. lanatus leaf extracts could be a more potent antioxidant material if a suitable extraction solvent and method are used, since it could contain phytoconstituents with better antioxidant attributes. The FRAP values for flesh extracts in this study were higher than those reported by another study of watermelon cultivars [14].

3.2.4. Phytochemical Constituents of C. lanatus Extracts

As mentioned earlier, watermelon use in traditional medicine was dated back to ancient times, and still in use as antidiuretic, antibacterial, antifungal, and antioxidant and as anti-diabetic till date. It is believed that its phytochemical endowment gave it these potential health benefits yet to be fully explored [54-60]. However, detailed information on these phytoconstituents of watermelon is still scares in the literature. In this research work, we detected 296, 108, 167, and 204 compounds from the 70% aqueous ethanol extracts of seed, flesh, rind and leaf respectively of C. lanatus, using the MS data obtained from LC-ESI-QTOF-MS analysis and Metlin library software attached to the LC. Out of these, 265, 154 and 194 compounds of the seed, rind and leaf extracts were identified tentatively also, all those detected in the flesh were identified.

The tentatively identified compounds were mainly phenolic acids and glyco-conjugates, flavonoids and glyco-conjugates, metabolites of phenylpropanoid pathway, such as gingerol, cinnamic acid, p-coumaric acid, etc.; organic acids such as citric acid, iso-citrate, fumarate, etc.; carotenoids such as beta-carotene metabolism products, curcuminoids; curcubitacin E, monosaccharaides and oligosaccharides, amino acids such as Citrulline,
L-Arginine, Histidine, Tryptophan, etc.; saturated mono and polyunsaturated fatty acids, and compound lipids such as Ceramide and Sphinganine; nucleotide bases such as guanosine, uridine, etc. among others. However, those included in this article were thought to have played significant role in the anti-diabetic and antioxidant properties displayed by the studied extracts.

L-Citrulline, citrate, arginine, fructose, sucrose, valine, ascorbic acid and lysine have been identified in watermelon flesh by other researchers [82]. These metabolites were also identified in the studied C. lanatus extracts. Polyphenols such as pelargonidin, hesperatin, catechin, kaempferol, and stilbilibin, have shown potent hypoglycaemic effects. This was achieved through the inhibition of α-amylase activities as demonstrated by molecular docking [83]. Chin-Hung and his colleagues also used molecular docking to confirm that curcumin, catechin, docosanol, berberin, tetracosanol, quercetin, and 16-hydroxy-cleroda-3,13-diene-16,15-olide, (16-H), lowered hyperglycaemia by inhibition of the activities of α-amylase enzyme [84]. Curcuminoid, docosanediol acid, precursor of docosanol and tetracosanol, caffeic acid and rutin metabolites, were identified in the studied C. lanatus extracts. Using molecular docking, quercetin, epicatechin, cyanidin, curcumin, daidzein, caffeic acid, ferulic acid, syringic acid, erydicitol, narenginin, pinoresinol, resveratrol, and hesperatin, have also lowered hyperglycaemia. However, α-glucosidase activity was hindered in the process. Some of these metabolites and their derivatives especially rutin, narenginin, caffeic acid and were identified in the extracts of this study, especially caffeic acid, curcuminol, gingerol, narenginin, and curcubitacin E [83]. Molecular docking have also been used to show that actinodaphnine, 16-H, catechin, curcumin, antroquinonol, [83]. Molecular docking have also been used to show that curcuminol, 6-gingerol, 4’-apo-beta-psi-caroten-4’-al, apigenin 7-(4’,6’-diacetylallo)-4’-alloside, chalconaringenin 2’-rhamnosyl-(1-4)-glucose, Narenginin 7-O-(2’,6’-di-O-alpha-rhamnopyranosyl)-beta-glucoypyranoaside, among others.

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Some of these metabolites (polyphenols) can induce insulin secretion directly from the pancreatic beta cells, cause the release of insulin, or increase insulin sensitivity [85]. These physiological effects could be directly related to the structure of the polyphenol involve. It could be due to the number of hydroxyl (OH) groups attached to the A, B, and C rings of the polyphenol involved, the nature of other substituents attached to and the degree of unsaturation of any of the A, B, and C rings of the polyphenol in question, or glycosylation of any of the flavonoid rings. Structurally, polyhydroxylation of flavonoids rings increased their potency for inhibiting carbohydrate hydrolysing enzymes. This is also the case when there is presence of double bond and carbonyl group on the C ring of flavonoids, and α=β double bonds in stilbenes, addition of galloyl group to catechin, cyanidin-3-glucoside, et. The addition of hydroxyl groups to A ring of isoflavones and flavones, on the other hand, addition of galactose to cyanidin, methoxylution and methylation of any of flavonoid rings, non-galloylated flavonoids, etc. lowers their inhibitory effects on carbohydrate metabolising enzymes. These structural characteristics may have contributed to the α-amylase and α-glucosidase inhibitory activities displayed by C. lanatus extracts evaluated in this study.

4. Conclusion

The studied watermelon extracts have demonstrated strong anti-diabetic potential, for the flesh and leaf extracts to exhibit an independent inhibition of α-amylase and α-glucosidase inhibition respectively, C. lanatus could be a raw material for lead compounds against type 2 diabetes mellitus. The leaf extracts which demonstrated the strongest and potent antioxidant effects better than that of ascorbic acid as seen in the FRAP assay, could also be a good source of antioxidants. This may be beneficial as a functional food for promoting good health and for preservation of other food materials. Beside simple sugars, amino acids, fatty acids, organic acids identified in C. lanatus extracts, LC-MS was a useful tool in identifying secondary metabolites with strong antioxidant potentials like curcubitacin E, curcuminol, 6-gingerol, 4’-apo-beta-psi-caroten-4’-al, apigenin 7-(4’,6’-diacetylallo)-4’-alloside, chalconaringenin 2’-rhamnosyl-(1-4)-glucose, Narenginin 7-O-(2’,6’-di-O-alpha-rhamnopyranosyl)-beta-glucoypyranoaside, among others.

Acknowledgements

We thank Universiti Putra Malaysia and Ministry of Education, Malaysia for funding the research. This work was supported by the UPM Putra Grant IPS [9670900]. We also would like to acknowledge Bayero University Kano-Nigeria for the fellowship given to Muhammad Mustapha Jibril through Tertiary Education Trust Fund (TETFund).

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