

ANTI-HYPERGLYCAEMIC, ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITIES OF *CARICA PAPAYA* AND *CITRULLUS LANATUS* SEEDS

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ABSTRACT

The study investigated the anti-hyperglycaemic, anti-inflammatory and antioxidant potentials of methanolic seed extracts of *Carica papaya* and *Citrullus lanatus*. The inhibitory effects of the extracts (100, 200 and 400 mg/kg, orally) on α -amylase and α -glucosidase were assessed in glucose and sucrose-induced hyperglycaemic rats using glimepiride (25 mg/kg) and acarbose (50 mg/kg), as positive controls. The anti-inflammatory activities were evaluated by membrane stability, xanthine oxidase inhibition and inhibition of denaturation of albumin models. Their antioxidant potentials were determined using standard methods and their total phenolic and flavonoid contents were also estimated. The extracts gave a comparable ($p > 0.05$) hyperglycaemia lowering and α -glucosidase inhibitory activities to glimepiride (25 mg/kg) and acarbose (50 mg/kg), respectively. However, *C. papaya* gave a significantly ($p < 0.05$) higher α -amylase inhibitory activity than *C. lanatus* at all concentrations. The seed extract of *C. papaya* was significantly more active in red blood cell membrane stabilizing activity at all concentrations than *C. lanatus* and ibuprofen while their order of xanthine oxidase inhibitory activities was: allopurinol $>$ *C. papaya* $>$ *C. lanatus*. In albumin denaturation assay, *C. papaya* gave a comparable activity to the positive control at 0.25 – 1.00 μ g/ml and significantly higher effect at 0.0625-0.125 μ g/ml while in the 1, 1, diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, total antioxidant capacity (TAC) and ferric reducing antioxidant power (FRAP) assays, the order of antioxidant activities was, ascorbic acid (positive control) $>$ *C. papaya* $>$ *C. lanatus*. The total phenolic and flavonoid contents of *C. papaya* expressed as gallic acid and quercetin equivalents were found to be 82.00 and 35.00 mg/g respectively; while those of *C. lanatus* were 40.00 and 20.00 mg/g, respectively. The results showed the seed extract of *C. papaya* to be a better anti-hyperglycaemic, anti-inflammatory and antioxidant agent than *C. lanatus* and suggested that their high flavonoid and phenolic contents could be responsible for these activities.

Keywords: Hyperglycaemia lowering, Antioxidant, Anti-inflammatory, *C. papaya*, *C. lanatus* seeds.

INTRODUCTION

Free radicals are generated as byproducts of normal aerobic metabolism and also from reactions with xenobiotics (Singh-Deveraj and Jialal, 2005). Reactive oxygen species (ROS) are formed as necessary intermediates in a variety of enzymatic reactions. Exogenous ROS can be produced from external sources such as pollutants, tobacco smoke, drugs, xenobiotics or radiation (Bagchi and Puri, 1998). Excessive production of these free radicals however, results in alteration in the balance between ROS and endogenous antioxidants and creates oxidative stress which reflects an imbalance between the systemic manifestations of ROS and the biological system's ability to readily repair the resulting damage. Excess ROS then oxidize biological molecules such as proteins, DNA and lipids. Oxidative damage to DNA, proteins, and

other macromolecules has been implicated in the pathogenesis of a wide variety of diseases, including diabetes, cardio vascular disease, cancer, Alzheimer's disease, inflammatory diseases, ageing etc (Halliwell, 1994).

Antioxidants are molecules that inhibit the oxidation of other molecules or protect cells from damages that result from the formation of free radicals and they do so by terminating the chain of reactions or by removing free radical intermediates and inhibit other oxidation reactions and they are oxidized in the process (Sies, 1991). Antioxidant functions are associated with decreased DNA damage, diminished lipid peroxidation, maintained immune function and inhibited malignant transformation of cells (Gropper *et al.*, 2009).

Plant derived antioxidant compounds have received considerable attention because of their physiological effects like, anti-diabetic, anti-inflammatory, antitumor activities and low toxicity compared with those of synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate (Kumar *et al.*, 2008).

Phenols and phenolic compounds such as flavonoids are secondary metabolites that are commonly found in both edible and non-edible plants and have been reported to have a broad spectrum of biochemical activities such as antioxidant, antidiabetic, anti-inflammatory, antimutagenic and anticarcinogenic abilities (Marinova *et al.*, 2005, Ajayi *et al.*, 2016). Flavonoids have been found to display a crucial role in scavenging free radicals (Mahadev *et al.*, 2016) and previous studies reported that antioxidant activities of plant materials are very well correlated with their phenolic contents (Velioglu *et al.*, 1998). Generally, the mechanisms of phenolic compounds for antioxidant activity involve neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals (Li *et al.*, 2006).

Carica papaya L. (Caricaceae) commonly known as pawpaw is one of the most popular, cheapest, economically important fruit tree grown and consumed for its nutritional content (Baiyewu and Amusa, 2005). Traditionally, different parts of papaya plant are used in the treatment of various ailments such as asthma, ulcers, eczema, diabetes and fever (Nguyen *et al.*, 2013). Its leaves have been reported for antimalarial and antifungal activities (Kisangau *et al.*, 2007) while its seeds have been shown to have antimicrobial properties and other pharmacological uses (Adebiyi *et al.*, 2003; Lohiya *et al.*, 2006; Akujobi *et al.*, 2010; Ikeyi *et al.*, 2013; Peter *et al.*, 2014; Rahmani and Aldebasi, 2016).

Citrullus lanatus L. (Cucurbitaceae) is a prostrate or climbing annual plant with several herbaceous, firm and stout stems up to 3 m long. Its fruits are eaten as a febrifuge and drastic purgative when fully ripe, while the seeds are used in the treatment of urinary tract infections, dropsy and renal stones (Yusuf *et al.*, 2015). Despite the wide and historical use of *C. papaya* and *C. lanatus* in the traditional

management of many diseases (Ali *et al.*, 2011), the scientific validation of the use of their seeds as antioxidant, anti-inflammatory and antidiabetic agents is scarce. This work was therefore designed to investigate their seed extracts for their antidiabetic, antioxidant and anti-inflammatory activities and also to assess their phenolic and flavonoid contents.

MATERIALS AND METHODS

Reagents and Chemicals

Folin-Ciocalteu's phenol reagent, gallic acid and anhydrous sodium carbonate used were products of Fluka (Buchs, Switzerland). Starch, pancreatic α -amylase, 1,1,-diphenyl-2-picrylhydrazyl radical, ascorbic acid, H₂O₂, iron (II) sulphate, xanthine, xanthine oxidase (EC 1.1.3.22) from cow's milk and allopurinol were products of Sigma-Adrich Co., St Louis, USA. Iron (III) chloride 6-hydrate and trichloroacetic acid were Fisher products. Aspirin and Ibuprofen were obtained from the drug research and production unit of Obafemi Awolowo University, Ile-Ife. Other chemicals and reagents used were of analytical grade.

Plant materials and Extraction

The seeds of *C. papaya* and *C. lanatus* were collected from fresh fruits that were obtained from Obafemi Awolowo University (OAU), Ile-Ife Campus, Osun State, Nigeria. Voucher specimens of the plants with herbarium numbers, IFE 17545 and 17567 respectively, were deposited at the Herbarium of the Department of Botany, O.A.U., Ile-Ife. The seeds were washed with distilled water, air-dried, powdered (100 g) and separately extracted with methanol. The extracts were concentrated *in-vacuo* to obtain their methanolic extracts with 6.8 and 8.9% w/w yields, respectively.

Animals

Healthy albino rats (135 \pm 2.14 g) of both sexes bred under standard conditions (temp. 27 \pm 3 °C, relative humidity 65%) at the animal house, Department of Pharmacology, Faculty of Pharmacy, O.A.U., Ile-Ife, Nigeria were used for the experiment. They were fed on a standard pellet diet (Bendel Feeds, Nigeria) and water was given *ad libitum*. All animal experiments conformed to the *Guide for the Care and Use of Laboratory Animals* published by the National Academies Press

(Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).

Anti-diabetic studies

Hyperglycaemia lowering activity of the extracts

A glucose tolerance test was performed by orally giving glucose (10 g/kg) to 24-h fasted rats and those that were hyperglycaemic (blood glucose level ≥ 7.0 mmol/l (126 mg/dl) after 0.5 h (time point 0, T_0) were divided into groups of five and orally administered with 1% Tween 80 in normal saline (negative control), extracts (100, 200, 400 mg/kg) or glimepiride (5 mg/kg). A drop of blood, taken from the tip of the tail of each rat at 0.0, 0.5, 1.0, 2.0 and 4.0 h after administration of the test agents, was dropped onto a glucometer strip and the blood glucose (bg) levels were read off directly. The blood glucose level at 0.0 h (T_0) was taken as 100%, while those at other times were expressed as percentages of these values (Adebajo *et al.*, 2013a).

α -Glucosidase inhibitory activity of the extracts

The basal blood glucose levels of five groups of 24-h fasted rats (five per group) were checked using glucometer strips inserted into the glucometer after which sucrose (10 g/kg) and extracts (100, 200, 400 mg/kg), sucrose and acarbose (50 mg/kg) (positive control), and sucrose and 1% Tween 80 in normal saline (negative control) were administered orally. A drop of blood, taken from the tip of the tail of each rat, was dropped onto a glucometer strip and the blood glucose (bg) level read off directly at 0.5, 1, 2 and 4 h (Matsumura *et al.*, 2000). All values were related to blood glucose at T_0 that was taken as 100%.

α -Amylase inhibitory activity of the extracts

This assay was carried out based on a procedure reported by Jain *et al.* (2013). The total assay mixture consisted of 1000 μ l of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 1000 μ l (0.04 units of pancreatic α -amylase solution) and 400 μ l extracts at various concentrations ranging from 100-500 μ g/ml (w/v). After pre-incubation at 37 °C for 10 min, 1000 μ l of 1% (w/v) starch solution was added to

each tube and incubated at 37 °C for additional 15 min. The reaction was terminated with 1.0 ml 3,5-dinitrosalicylic acid (DNSA) reagent, and placed in boiling water for 5 min after which it was cooled to room temperature, and the absorbance was read at 540 nm using a spectrophotometer (spectrum lab S23A). Extract was not added to the control group and it represented 100% enzyme activity.

The % inhibition of alpha amylase was calculated as follows:

$$\text{Inhibitory activity (\%)} = \frac{\text{Abs}(\text{control}) - \text{Abs}(\text{test})}{\text{Abs}(\text{control})} \times 100$$

In-vitro antioxidant assays of the extracts

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH assay was carried out following the method reported by Brand-Williams *et al.*, (1995) with slight modifications. One ml of 0.1 mM DPPH solution in methanol was mixed with 1 ml of seed extracts solution of varying concentrations (0.625, 0.125, 0.25, 0.5 and 1 mg/ml). Ascorbic acid was used as reference standard. One ml of methanol added to 1ml DPPH solution was used as control. The decrease in absorbance was measured at 517 nm after 30 min in the dark using a spectrophotometer. The percentage of DPPH radical scavenging was calculated as:

$$\% \text{ scavenging activity} = \frac{\text{Abs}(\text{control}) - \text{Abs}(\text{sample})}{\text{Abs}(\text{control})} \times 100$$

Total Antioxidant Capacity

Total antioxidant capacity of the seed extracts of *C. papaya* and *C. lanatus* was measured and expressed as ascorbic acid equivalent (AAE) according to the method of Prieto *et al.*, (1999). An aliquot of 0.1 ml of the extracts were separately added to 1 ml of reagent solution consisting of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate in an Eppendorf tube. The tubes were capped and incubated in a water bath at 95 °C for 90 min after which the tubes were cooled to room temperature and the absorbance of the reaction mixtures were measured at 695 nm against a reagent blank that contained 1 ml of methanol in place of the extracts. The antioxidant capacity was expressed as ascorbic acid equivalent.

Ferric Reducing Antioxidant Power Assay (FRAP)

FRAP assay was carried out according to the method described by Benzie and Strain, (1999). FRAP working reagent was freshly prepared from a 10:1:1 mixture of A: B: C. (A = 300 mM acetate buffer of pH 3.6, B = 10 mM of 2,4,6-tri (2-pyridyl)-1,3,5-triazine, C = 20 mM ferric chloride). To 50 µl aliquot of extracts of varying concentrations in a test tube was added 1.5 ml of FRAP reagent and their absorbance was measured at 593 nm after 10 min. Distilled water (50 µl) and ascorbic acid (50 µl) were used as reference and standard respectively. The ferric reducing antioxidant capacity of the extracts was expressed as ascorbic acid equivalent/g dry extract. All measurements were carried out in triplicate.

Anti-inflammatory Assays

Membrane stabilizing assay

Preparation of bovine red blood cells

Fresh bovine blood sample was collected into anticoagulant bottle containing dextrose (2%), sodium citrate (0.8%), citric acid (0.05%) and sodium chloride (0.42%) and centrifuged at 3000 rpm on a bench centrifuge for 10 min at room temperature after which the supernatants (plasma and leucocytes) were carefully removed while the packed red blood cells were washed in fresh normal saline (0.85% w/v NaCl). The processes of washing and centrifugation were repeated five times until the supernatants were clear. Then, bovine erythrocyte (2% v/v) was prepared as reported by Oyedapo *et al.*, (2004).

Membrane stabilizing assay

The assay mixtures consisted of 1 ml of hypo saline (0.25% (w/v) sodium chloride), 0.5 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 2% (v/v) bovine erythrocyte suspension, varying concentrations (0 – 350 µg/ml) of standard and extracts and the final reaction mixtures were made up to 3 ml with isosaline. Extracts were omitted in blood control representing 100% lysis, while the negative control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 56 °C for 30 min on a water bath and centrifuged at 5000 rpm for 10 min at room temperature. The absorbance of the hemoglobin was read at 560 nm. The percentage membrane stability was

estimated using the expression:

$$\frac{100 - (\text{Abs of test drug} - \text{Abs of drug control})}{(\text{Abs of blood control})} \times 100$$

In-vitro xanthine oxidase inhibitory assay

Xanthine oxidase inhibitory activity was measured spectrophotometrically as described by Filha *et al.*, (2006) with slight modifications. The extract (1 mg/ml) was prepared in methanol and then screened for xanthine oxidase inhibitory activity. The assay mixture consisted of 150 µl phosphate buffer (250 mM, pH 7.5), 50 µl extract solution and 50 µl enzyme solution (0.28 U/ml in phosphate buffer). The reaction was initiated by adding 250 µl of substrate solution (0.15mM xanthine) and the absorbance at 295 nm was measured spectrophotometrically every 12 seconds for 2 min. Methanol solution serves as the negative control while allopurinol which is a well-known inhibitor of xanthine oxidase was used as the positive control. All experiments were carried out in triplicate and xanthine inhibitory activity was expressed as the percentage inhibition of xanthine oxidase, calculated as:

$$\% \text{ inhibition} = 1 - \frac{B}{A} \times 100$$

Where A = change in absorbance per min of the blank and

B= change in the absorbance per min of the test

Inhibition of bovine albumin denaturation

The ability of the methanolic seed extracts of *C. papaya* and *C. lanatus* to inhibit the denaturation of albumin was investigated by method of Mizushima and Kobayashi (1968) as reported by Sakat *et al.*, (2010) with slight modifications. Typically, varying concentrations of the methanolic seed extracts were prepared and the volumes were adjusted to 2.5 ml with 0.85% NaCl. This was followed by the addition of 0.5 ml of bovine albumin (1.5 mg/ml). The mixture was incubated at 37 °C for 20 min and further incubated at 57 °C for 20 min. The tubes were cooled and 2.5 ml of 0.5 M sodium phosphate buffer pH 6.3 was added. The turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate and the standard was used in place of the extract. The percentage inhibition of albumin denaturation was calculated as follows:

Percentage inhibition =

$$\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100\%$$

Total Phenolic Content of the Extracts

The total phenolic content (TPC) of the seeds of *C. papaya* and *C. lanatus* was determined by Folin-Ciocalteu method of Singleton *et al.*, (1999). Typically, 0.5 ml of the extract was pipetted into clean dried test tubes in triplicate and the volumes were adjusted to 1.0 ml with distilled water. Into each of the test tubes, 1.5 ml of Folin Ciocalteu's phenol reagent (1:10) was added. The reaction mixtures were incubated at room temperature for 85 min after which 1.5 ml of 7.5% (w/v) Na₂CO₃ was added and the reaction mixture was incubated for additional 1½ hours. The absorbance was read at 725 nm against the reagent blank and total phenolics were expressed as mg/g (GAE) gallic acid equivalent and reported as mg/gallic acid equivalent/g extract by reference to standard curve using the formula:

$$\text{TPC} = \frac{\text{Concentration of extract}}{\text{Weight of extract}} \times \text{Volume}$$

Total Flavonoid Content of the Extracts

The total flavonoid content (TFC) of the seeds of *C. papaya* and *C. lanatus* was determined by using the method of Sun *et al.*, (1999) and expressed as quercetin equivalents per gram of the seed extracts. Typically, 0.5 ml of the extract was pipetted into test tubes in triplicates and diluted to

5.0 ml with distilled water after which 0.3 ml of 5% (w/v) NaNO₂, 0.3 ml of 10% AlCl₃ and 4.0 ml of 4% (w/v) NaOH were added to each test tubes. The reaction mixtures were incubated at room temperature for 15 min and the absorbance of the product was read at 500 nm against reagent blank. The flavonoid content was expressed as mg/g (QE) quercetin equivalent.

Statistical analysis

Data represent mean ± SEM and n = 5 for animals in the group. They were analysed with one way analysis of variance (ANOVA), followed by Bonferroni t-test or Student-Newman-Keuls post-hoc tests. P < 0.05 was considered significant.

RESULTS AND DISCUSSION

There was significant time dependent reduction in the blood glucose levels (bgl) up to the fourth hour in the glucose-induced hyperglycaemic rats administered with normal saline (negative control) due to homeostatic regulatory mechanism (Table 1). This confirmed that the rats' pancreases were functioning well (Kar *et al.*, 1999; Adebajo *et al.*, 2009, 2013a). Glimpiride (2.5 mg/kg), *C. papaya* extract (100-400 mg/kg) and *C. lanatus* (200-400 mg/kg) had similar profile of activity that was time-dependent (Table 1). Hence, they may have the same extrapancreatic and high insulinotropic activities like glimepiride.

Table1: Dose-related anti- hyperglycaemic activity of methanolic seed extracts of *C. papaya* and *C. lanatus*

Extract/Drug (mg/kg)	Blood glucose levels as percentages of T ₀ (% reduction in blood glucose relative to negative control at T ₀)				
	0 h	0.5 h	1 h	2 h	4 h
Glu (10g/kg)	100.00	94.2±1.0 ^b	83.7±6.2 ^b	73.3±4.2 ^b	59.6±5.0 ^b
CPS (100)	100.00	75.5±4.0 ^a (19.9 %)	61.2±5.6 ^a (26.9 %)	48.4±4.8 ^a (34.0 %)	37.6±1.7 ^a (36.9 %)
CPS (200)	100.00	78.4±2.0 ^a (16.8 %)	65.3±3.8 ^a (22.0 %)	52.5±3.0 ^a (28.4 %)	36.9±2.4 ^a (38.0 %)
CPS (400)	100.00	75.3±0.1 ^a (20.1 %)	63.5±3.6 ^a (24.2 %)	53.1±2.6 ^a (27.7 %)	33.9±3.5 ^a (43.1 %)
CLS (100)	100.00	72.4±2.7 ^a (23.2 %)	55.0±2.1 ^a (34.3 %)	49.8±2.3 ^a (32.0 %)	39.1±1.4 ^a (34.4 %)
CLS (200)	100.00	79.8±4.0 ^a (15.3 %)	59.4±4.2 ^a (29.0 %)	46.5±1.1 ^a (36.6%)	37.5±1.9 ^a (37.1%)
CLS (400)	100.00	77.7±1.9 ^a (17.5 %)	61.1±2.4 ^a (25.4 %)	52.4±3.8 ^a (28.6 %)	39.7±3.3 ^a (33.7 %)
Glim (2.5)	100.00	80.9±5.0 ^a (14.2 %)	58.8±3.6 ^a (29.7 %)	55.7±6.3 ^a (24.8 %)	40.4±0.9 ^a (32.2 %)

Data show the mean \pm SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T₀), $n = 5$. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test). **Glu:** Glucose (negative control); **CPS:** *Carica papaya* seed; **CLS:** *Citrillus lanatus* seed; **Glim:** Glimpiride (positive control).

Also, their highest hyperglycaemia lowering activity at 4 h may suggest insulin release as their main mechanism of action (Adebajo *et al.*, 2013a, b). Similarly, extracts of *G. latifolium*, *E. uniflora*, *Jatropha tanjorensis* and *C. lansium* have been reported to have insulin stimulation as their mechanism of action (Olayiwola *et al.*, 2004; Adebajo *et al.*, 2007, 2009; 2013a,b). The extracts gave comparable ($p > 0.05$) activity at all-time points and at the tested doses indicating a non-dose dependent activity. However, 400 mg/kg of *C. papaya* seed extract with 43% blood glucose level reduction at 4 h could be said to be the most active (Table 1).

In the α -glucosidase inhibitory assay, there was an increase in blood glucose level of rats from 100 to 220 and 180% after 0.5 h and 1 h of oral sucrose (10 g/kg) administration in the negative control

group (Table 2), showing the breaking down of sucrose to glucose by the α -glucosidases in the rats intestine that was later absorbed into the blood stream leading to the observed hyperglycaemia (Deshpande *et al.*, 2009). However, oral co-administration of acarbose (50 mg/kg) and sucrose inhibited increase in blood glucose level and caused 53% decrease in hyperglycaemia compared to the negative control at 0.5 and 1 h. This showed that acarbose inhibited α -glucosidase enzymes which resulted into little rise in plasma glucose concentration (Table 2). The extract of *C. papaya* (100 and 400 mg/kg) gave maximum α -glucosidase inhibitory activity of 45 and 46% at 0.5 h, respectively that was comparable ($p > 0.05$) to that of acarbose while the activity of *C. lanatus* (100-400 mg/kg), was comparable to acarbose at 0.5-1 h (Table 2).

Table 2: Dose related hyperglycaemia-preventing activity of methanolic seed extracts of *C. papaya* and *C. lanatus*

Extract/Drug (mg/kg)	Blood glucose levels as percentages of T ₀ (% reduction in blood glucose relative to negative control at T _i)				
	0 h	0.5 h	1 h	2 h	4 h
Suc (10 g/kg)	100.00	220.0±26.6 ^c	179.5±13.3 ^c	149.6±10.5 ^c	124.8±6.8 ^c
CPS (100)	100.00	121.9±6.9 ^{ab} (44.6 %)	120.1±6.5 ^b (33.1%)	110.6±17.4 ^{ab,c} (26.1%)	91.5±14.2 ^{ab,c} (26.7%)
CPS (200)	100.00	132.0±4.1 ^b (40.0%)	121.8±6.3 ^b (32.2%)	116.4±5.7 ^b (22.2%)	103.5±2.9 ^b (17.1%)
CPS (400)	100.00	119.7±1.1 ^a (45.6%)	122.3±4.2 ^b (31.9%)	115.5±4.5 ^b 22.8%)	109.2±2.7 ^{bc} 12.5%)
CLS (100)	100.00	115.0±3.1 ^a (47.7%)	97.6±4.6 ^a (45.7%)	94.1±4.0 ^a (37.1%)	82.8±2.4 ^a (33.7%)
CLS (200)	100.00	127.5±9.3 ^{ab} (42.1%)	115.9±17.5 ^a (35.5%)	105.4±10.6 ^{ab} (29.6%)	86.8±11.8 ^a (30.5%)
CLS (400)	100.00	125.6±6.9 ^{ab} (42.9%)	103.4±5.3 ^b (43.4%)	107.5±8.8 ^{ab} (28.1%)	95.3±6.9 ^b (23.6%)
Acar (50)	100.00	102.6±8.1 ^a (53.4%)	83.6±4.1 ^a (53.4%)	85.6±4.1 ^a (42.8%)	73.6±4.3 ^a (41.0%)

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h (T₀), *n* = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different (*p* < 0.05, one-way analysis of variance followed by the Student–Newman–Keuls' test). **Suc:** Sucrose (negative control); **CPS:** *Carica papaya* seed; **CLS:** *Citrillus lanatus* seed; **Acar:** Acarbose (positive control).

Both *C. papaya* and *C. lanatus* seed extracts gave a non-dose dependent α-amylase inhibitory activity at the tested doses (100-500 µg/ml) that was significantly less active than acarbose. However, *C. papaya* seed extract gave a significantly (*p* < 0.05) higher activity than *C. lanatus* at all concentrations (Table 3).

Table 3: Alpha- amylase inhibitory activity of the methanolic seed extracts of *C. papaya* and *C. lanatus*

Concentrations of the extracts (µg/ml)	% α-amylase Inhibition		
	CPS	CLS	Acarbose
100	67.70±0.1 ^c	49.9±0.1 ^b	23.1±1.2 ^a
200	60.70±0.1 ^c	44.2±0.3 ^a	48.0±0.9 ^b
300	66.40±0.1 ^b	45.8±0.1 ^a	70.3±1.5 ^c
400	67.62±0.3 ^b	50.0±0.2 ^a	95.1±2.1 ^c
500	69.60±0.1 ^b	48.7±0.1 ^a	98.2±0.1 ^c

Data show the mean ± SEM (*n* = 6). Values with different superscripts within columns are significantly different (*p* < 0.05, one-way analysis of variance followed by the Student–Newman–Keuls' test); **CSP:** *C. papaya* seed; **CSC:** *C. lanatus* seed.

Red blood cell membrane stabilizing ability of the methanolic seed extracts of *C. papaya*, *C. lanatus* and the positive control increases with increase in concentration giving the highest activity at 0.5 mg/ml. The seed extract of *C. papaya* was significantly more active at all concentrations than those of *C. lanatus* and ibuprofen while *C. lanatus*

gave a significantly better activity than ibuprofen at 0.25-0.5 mg/ml (Table 4). This effect could be attributed to flavonoids content in these extracts since flavonoids have been reported to possess anti-inflammatory and anti-oxidative activities (Akinpelu *et al.*, 2012).

Table 4: Red blood cell membrane stability of the methanolic seed extracts of *C. papaya* and *C. lanatus*

(mg/mL) Concentrations (mg/ml)	% Red Blood Cell Membrane Stability			
	0.125	0.250	0.375	0.500
CPS	16.87±0.12 ^b	25.37±1.43 ^c	32.99±0.41 ^c	39.40±0.27 ^c
CLS	6.09±0.43 ^a	13.14±0.89 ^b	20.07±0.20 ^b	21.32±0.53 ^b
IBP	4.59±0.34 ^a	7.40±0.15 ^a	11.48±0.61 ^a	18.37±0.36 ^a

Data show the mean ± SEM ($n = 6$). Values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test); **CSP**: *C. papaya* seed; **CSC**: *C. lanatus* seed; **IBP**: Ibuprofen (positive control).

Xanthine oxidase inhibitory activities of the methanolic seed extracts of *C. papaya*, *C. lanatus* and the positive control were dose-dependent (Table 5). *C. papaya* exhibited a higher xanthine oxidase inhibition than *C. lanatus*. Xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and then to uric acid, the final reactions in the metabolism of purine bases (Borges *et al.*, 2002). Xanthine oxidase has been reported as a very important enzyme that increases during oxidative stress. Gout is a clinical metabolic disorder of xanthine oxidase, which is associated

with increased level of uric acid in blood (Liu *et al.*, 2008). Compounds that enhance the excretion of uric acid or inhibit uric acid biosynthesis, or have anti-inflammatory actions are generally used for treatment of gout. Flavonoids and polyphenols have been reported to have xanthine oxidase inhibitory activities (Costantino *et al.*, 1992). Hence, the phenolic and flavonoid content in the seed extracts of *C. papaya* and *C. lanatus* could be responsible for their observed xanthine oxidase inhibitory activities.

Table 5: Xanthine oxidase inhibitory activities of the methanolic seed extracts of *C. papaya* and *C. lanatus*

Concentration (µg/ml)	% Xanthine Oxidase Inhibition					IC ₅₀
	5	10	25	50	100	
CPS	40.00 ± 0.10 ^b	54.50 ± 1.00 ^b	60.10±0.03 ^b	82.40±0.50 ^b	87.0 ± 0.02 ^b	8.40±0.13 ^b
CLS	23.00± 0.05 ^a	38.0 ± 0.04 ^a	54.00 ± 0.12 ^a	67.00± 0.05 ^a	73.0 ± 0.06 ^a	21.30±0.04 ^c
ALLO	44.91 ± 0.18 ^c	66.91± 0.80 ^c	75.00± 1.40 ^c	85.46±0.80 ^c	92.25±0.50 ^c	5.70± 0.30 ^a

Data show the mean ± SEM ($n = 6$). **IC₅₀**: Concentration needed to inhibit the activity of the enzyme by 50%, values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test); **CSP**: *C. papaya* seed; **CSC**: *C. lanatus* seed; **ALLO**: Allopurinol (positive control).

Protein denaturation is a process in which proteins lose their secondary and tertiary structures by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most proteins lose their biological functions when denatured. Denaturation of proteins is a well-documented cause of inflammation (Govindappa *et al.*, 2011). The seed extracts of *C. papaya*, *C. lanatus* and the positive control (Aspirin) in this study inhibited

albumin denaturation in a dose-dependent manner with *C. papaya* giving a comparable activity to the positive control at 0.25 – 1 µg/ml and significantly higher effect at 0.0625- 0.125 µg/ml (Table 6). Papaya seed extracts (methanol and aqueous) have also been reported for anti-inflammatory activities in *in vivo* studies (Rahmani and Aldebisi, 2016; Amazu *et al.*, 2010; Umana *et al.*, 2014).

Table 6: Albumin denaturation inhibitory activities of the methanolic seed extracts of *C. papaya* and *C. lanatus*

Concentration(µg/ml)	% inhibition of albumin denaturation				
	0.0625	0.125	0.25	0.5	1.0
CPS	44.00± 0.61 ^c	52.39± 0.31 ^c	64.41± 2.30 ^b	72.10± 5.81 ^a	84.00± 0.61 ^b
CLS	20.14± 0.10 ^a	32.37± 0.10 ^a	60.0± 0.10 ^a	66.67± 0.10 ^a	73.33± 0.17 ^a
ASP	40.23± 0.26 ^b	50.34±0.52 ^b	70.00± 0.10 ^b	74.02± 0.85 ^a	84.12± 0.72 ^b

Data show the mean ± SEM (*n* = 6). Values with different superscripts within columns are significantly different (*p* < 0.05, one-way analysis of variance followed by the Student–Newman–Keuls' test); **CSP:** *C. papaya* seed; **CSC:** *C. lanatus* seed; **ASP:** Aspirin (positive control).

In the DPPH radical scavenging assay, the order of activity was ascorbic acid (positive control) > *C. papaya* > *C. lanatus*. Using TAC and FRAP assays, the order of their antioxidant capacities were in agreement with that of DPPH with *C. papaya* showing significantly higher free radical scavenging activities than *C. lanatus* (Table 7).

The seed extract of *C. papaya* contained a significantly higher amount of phenols and flavonoids than *C. lanatus* (Table 8).The flavonoid

contents of these extracts were in agreement with the reported values in literature (Ayodele and Olabode, 2015; Rahman *et al.*, 2013) while there was variation in that of phenolic content. Several researchers (Braide *et al.*, 2012, Jain and Josh, 2012; Maisarah *et al.*, 2013) have reported different phenolic concentrations for the two seed extracts. This variation could be due to age, species of the plant, soil and weather condition (Scalbert *et al.*, 2005).

Table 7: 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, total antioxidant capacity and ferric reducing antioxidant power of the methanolic seed extracts of *C. papaya* and *C.lanatus*

Extracts/Drug	IC ₅₀ (mg/ml)	(µgAAEq/ml)	(µgAAEq/ml)
	DPPH	TAC	FRAP
CPS	25.75 ± 0.12 ^b	50.01 ± 0.015 ^b	73.37± 3.10 ^b
CLS	42.62 ± 0.23 ^c	19.57 ± 0.038 ^a	45.12± 1.20 ^a
Vit. C	17.25 ± 0.15 ^a	NA	NA

Data show the mean ± SEM (*n* = 6). **IC₅₀:** Concentration needed to give 50% DPPH radical scavenging activity; µgAAEq/ml: µg ascorbic acid equivalent per ml; **DPPH:** 1,1-diphenyl-2-picrylhydrazyl assay; **TAC:** total antioxidant capacity; **FRAP:** ferric reducing antioxidant power. Values with different superscripts within columns are significantly different (*p* < 0.05, one-way analysis of variance followed by the Student–Newman–Keuls' test);**CSP:** *C. papaya* seed extract; **CSC:** *C. lanatus* seed extract; **Vit. C:** vitamin C (positive control).

Table 8: Total phenolic and flavonoid contents of *C. papaya* and *C. lanatus* seed extracts

Extracts	Total Phenolic Content (mg/g GAE)	Total Flavonoid Content (mg/g QE)
CPS	82 ± 0.02 ^b	35 ± 0.07 ^b
CLS	40 ± 0.01 ^a	20 ± 0.03 ^a

Data show the mean ± SEM (*n* = 6). Values with different superscripts within columns are significantly different (*p* < 0.05, one-way analysis of variance followed by the Student–Newman–Keuls' test); **CSP:** *C. papaya* seed extract; **CSC:** *C. lanatus* seed extract.

CONCLUSION

This study concluded that the seed extracts of *C. papaya* and *C. lanatus* were good anti-hyperglycaemic, anti-inflammatory and antioxidant agents. These properties could be adduced to their high phenolic and flavonoid contents. It also showed that the seed extract of *C. papaya* was significantly more active than *C. lanatus* and could be useful in preventing or slowing down the progress of diabetes mellitus, gout and other related inflammatory disorders.

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