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Fatty Acid Composition, Steroid Content, *In Vitro* Antioxidant and Antidiabetic Potentials of *Citrullus lanatus* and *Carica papaya* Seed Oils

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Abstract

Plant-derived oils rich in bioactive constituents have attracted attention as potential antioxidant and antidiabetic agents. The present study assessed the phytochemical profile, antioxidant activity, enzyme inhibitory activity, and fatty acid profile of pawpaw (*Carica papaya*) and watermelon (*Citrullus lanatus*) seed oils. Antioxidant activity was assessed by DPPH radical scavenging and FRAP assays, while antidiabetic activity was evaluated through *in vitro* α-amylase and α-glucosidase inhibition. Phytochemical analysis revealed triterpenoids, steroids, and cardiac glycosides in both oils. In the DPPH assay, pawpaw and watermelon seed oils showed maximum inhibitions of 13.32% and 15.91% at 200 µg/mL, respectively; while the FRAP values were low (9.06 and 14.51 µM Fe^{2+}/mg). Enzyme inhibition assays indicated differential activity: pawpaw seed oil strongly inhibited α-glucosidase (84.69% at 1000 µg/mL) while watermelon seed oil strongly inhibited α-amylase (81.81%). GC-MS analysis of the oils revealed the most abundant fatty acids as cis-10-heptadecenoic acid (895.57 mg/L) arachidonic acid (263.49 mg/L), 5,8,11,14,17-eicosapentaenoic acid (241.80 mg/L). Essential fatty acids such as γ-linolenic acid and docosahexaenoic acid (DHA) were also present. In conclusion, the seed oils exhibit moderate antioxidant activity and complementary enzyme inhibitory effects. Their rich unsaturated fatty acid profiles support potential use as functional dietary oils for diabetes management.

Keywords: *Carica papaya*; *Citrullus lanatus*; diabetes; fatty acids; GC-MS; seed oils.

INTRODUCTION

Many human diseases arise from oxidative stress, which happens when reactive oxygen species are produced in amounts greater than the body's ability to neutralize them with its antioxidant defenses (Sies, 2020). Reactive radicals, including hydrogen peroxide, hydroxyl, nitric oxide, and superoxide anions exert a significant impairment on various cellular macromolecules. This damage can contribute to the development of a range of diseases, including atherosclerosis, neurodegenerative disorders, cancer formation, and diabetes mellitus (Chaudhary *et al.,* 2023).

Diabetes mellitus is a prevalent global public health concern, characterized as a complex metabolic disorder arising from multiple etiologies. It is marked by impaired glucose homeostasis and associated disturbances in carbohydrate, fat, and protein metabolism, resulting from abnormalities in insulin production, insulin activity, or both (Tafesse *et al.,* 2017). The widespread occurrence of diabetes mellitus renders it a highly feared metabolic condition prevalent in the population. Medicinal herbs have traditionally been utilized for centuries in the treatment of diabetes, offering a wide range of bioactive constituents possessing potential antidiabetic properties. Research indicates that certain plant-derived substances can significantly reduce blood glucose levels and enhance insulin sensitivity, offering a promising approach for managing diabetes. Moreover, these plant-based treatments are often easier to access and more affordable, particularly in developing and emerging economies (Usai *et al.,* 2022). Notable among plants exhibiting these positive attributes are *Carica papaya* (pawpaw) and *Citrullus lanatus* (watermelon). Growing evidence indicates that consumption of watermelon and pawpaw seeds can markedly decrease the risk of heart-related diseases and reduce the occurrence of diabetes (Cena & Calder, 2020; Oaikhenan, 2023).

Carica papaya Linn. (Caricaceae), popularly referred to as pawpaw or papaya, is a widely cultivated fruit, with its major production occurring in tropical and subtropical areas. It is consumed globally in both fresh and processed forms (Jiao *et al.,* 2022; Saba and Pattan, 2022). *Carica papaya* fruits and seeds have demonstrated anti-amoebic and anti-helminthic properties in scientific studies (Faijan and Maheshwari, 2023).

Citrullus lanatus (family Cucurbitaceae) is another important tropical fruit (Nkoana *et al.,* 2022). The

watermelon fruit is characterized by a thick outer shell and a succulent, water-rich center, making it an excellent thirst quencher. Nutritional studies reveal that watermelon fruits and seeds are rich in proteins, oils, citrulline, carotenoids, lycopene, B vitamins, niacin, and dietary fiber, which contribute to their high nutritional significance (Lakhe *et al.,* 2022). While the bioactive potential of fruit seeds such as watermelon and pawpaw is gaining recognition, there is still an absence of detailed scientific studies investigating the chemical profile and biological properties of their oils extracts.

Therefore, the current study aims to explore the *in vitro* antidiabetic and antioxidant potentials and the fatty acid constituents of pawpaw and watermelon seed oils.

MATERIALS AND METHODS

Collection and Sample Preparation

The pawpaw, **B1** (*Carica papaya*) and watermelon, **B2** (*Citrullus lanatus)* fruits used in this study were purchased from Swali Market, Yenagoa, Bayelsa State, Nigeria. The pawpaw seeds were carefully selected, washed properly with distilled water to remove all traces of dirt or contaminants, and then dried in a laboratory drying oven at 150°C for 24 hours to ensure complete moisture removal. This step is essential to prevent contamination and ensure accurate extraction of the oil. After drying, the seeds were ground using a Xnewster blender to achieve a fine, uniform powder for efficient oil extraction.

Extraction of Oil

For oil extraction, 200 g each of the dried B1 and B2 seed materials were weighed and transferred into a Soxhlet extractor. 500 mL of n-hexane (99.9% purity) served as the solvent for the extraction. The Soxhlet extraction process was performed for 6 hours at 60°C, ensuring maximum yield of oil from the seeds. Once the extraction was complete, the oil was carefully separated from the solvent utilizing a rotary evaporator under lowered pressure at 40°C to remove the hexane.

Qualitative Analysis of Phytochemicals

Test for saponin (Frothing Test)

About 0.1 g of oil was diluted with distilled water to 5 mL, boiled for 2 minutes and filtered. The filtrate was shaken vigorously in a graduated cylinder for 2 minutes. Formation of about $1cm^3$ layer of foam that persists for about 5 minutes was observed, which demonstrates the occurrence of saponins.

Test for reducing sugar (Fehling's test)

To 2 mL of prepared aqueous stock solution (10 mg/mL) extract, 1mL of Fehling's A and 1mL Fehling's B solutions were added and mixed in a test-tube, then placed in a boiling water bath and heated for 10 minutes, appearance of yellow and then brick red precipitate reveals the existence of reducing sugars.

Test for triterpenoids (Liebermann-Burchard's test)

About 0.1 g of oil was treated with 3 mL of acetic anhydride and a drop of concentrated sulfuric acid; the formation of a greenish-blue color indicated the occurrence of triterpenoids.

Test for steroids (Salkwoski's test)

5 mL of chloroform was added to about 0.1 g of the oil, shaken well and filtered using filter paper. 3 mL of concentrated sulphuric acid was slowly poured into the filtrate through the side of the test-tube. Colour change of reddish-brown at the interface indicates existence of steroids.

Test for phenolic compounds (Lead acetate test)

To 5 mL of prepared aqueous stock aqueous of the extract, 2-3 drops of lead acetate solution was added. Formation of white-yellow colour indicates the occurrence of phenolic compound.

Test for flavonoids (Shinoda test)

About 0.5 g oil was treated with 5 mL of 95% ethanol and boiled for two minutes in the water-bath and hydrolysed by addition of 0.5 mL concentrated HCl acid. Pink-to-red colour appears after adding magnesium turnings which demonstrates the occurrence of flavonoid.

Test for tannins (Ferric chloride test)

5 mL of the aqueous stock solution was treated with 2-3 drops of $FeC1_3$ solution and observed for brownish to greenish-black or a blue-black colouration which indicates that tannin is present in the extract.

Test for alkaloids

About 0.5 g of sample was boiled in 10 mL 50% ethanol dilute HCl acid in a testtube for 5 minutes over water bath and filtered. The filtrate was basified with dilute ammonia and test with litmus paper. The basified solution was purified and partitioned with 3-5 mL chloroform thrice and the chloroform fraction was collected. The fraction was concentrated over water bath to a reduced volume and about 2 mL of dilute hydrochloric acid was added to it. The acid fraction was carefully collected with pasteur pipette and kept for further tests (filtrate).

a) *Mayer's Test:* The filtrates were treated with Mayer's reagent (potassium mercuric iodide) and a yellow precipitate appearance signifies the existence of alkaloids.
b) *Wagner's Test:* Treatment of the filtrates with Wagner's reagent (iodine and potassium iodide) resulted in a brown to reddish precipitate, confirming the existence of alkaloids.

c) *Dragendroff's Test:* Treatment of filtrates with Dragendorff's reagent produced a red precipitate, indicating alkaloids.

Test for cardiac glycoside (Keller killani's test)
0.1 g of Extracts were treated with 3 mL of glacial acetic acid and a drop of dilute ferric chloride solution in a test tube. The solutions were filtered. The test tube was tilted to angel 45ºC degree and about 2mL of concentrated H_2SO_4. A brown ring was observed at the interface for existence of cardiac glycosides.

Test for anthraquinones (Borntrager's Test)
About 0.5 g of the extract was boiled with 5 mL 10% HCl and 2 mL 10% $FeCl_3$ for 5-10 minutes in a water bath. It was filtered while hot and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate, shaken and allowed to settle. The organic layer was collected and aqueous layer was discarded. About 2 mL of dilute NH_3 solution was added to the collected fraction and shaken. A pink-red coloration in the ammoniacal layer confirms the existence of anthraquinones.

Quantitative Phytochemical Analysis
Estimation of steroid
A 1 mL portion of the test oil or steroid solution was placed in a 10 mL volumetric flask, followed by the addition of 2 mL of 4 M H_2SO_4, 2 mL of 0.5% (w/v) $FeCl_3$, and 0.5 mL of 0.5% (w/v) potassium hexacyanoferrate (III). The mixture was placed in a water bath and heated at 70 ± 2°C for 30 mins with intermittent shaking and then made up to volume with distilled water. The absorbance was recorded at 780 nm against a reagent blank. Total steroids in oil was presented in terms of cholesterol equivalents (mg of CHO/g of extract).

***In Vitro* Antioxidant Activities**
DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity
The antioxidant property of each oil sample was examined by evaluating its capacity to neutralize the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, with ascorbic acid serving as the reference standard (Katalinić et al., 2006). Equal volumes (2 mL each) of DPPH solution and the test extract or ascorbic acid standard across a range of concentrations (10, 25, 50, 100, and 200 µg/mL) were mixed. After incubation in the dark for 30 minutes, the absorbance was measured at 517 nm utilizing a UV–Vis spectrophotometer (Thermo Spectronic BioMate 3, USA), taking methanol as the blank.

The antioxidant activity was expressed as:

$$\%\, Inhibition = [(Acontrol - Asample)/Acontrol] \times 100\%$$

Evaluation of Antioxidant Potential via the Ferric Reducing Antioxidant Power (FRAP) Assay
The FRAP assay was executed in accordance with the procedure outlined by Benzie and Devaki (2018). To 0.2 mL aliquots of the methanolic extract at four concentrations (0.1, 0.5, 1.0, and 2.0 mg/mL; three replicates per concentration), 3.8 mL of FRAP reagent was introduced. The mixture was then incubated at 37 °C for 30 minutes. An increase in absorbance was monitored at 593 nm utilizing a UV–Vis spectrophotometer (Thermo Spectronic BioMate 3, USA). A blank was prepared by replacing the extract together with an equivalent volume of methanol. The findings were presented as milligram $FeSO_4$ equivalents per milligram of dry sample.

Total antioxidant capacity (TAC)
The total antioxidant capacities of the oils were evaluated by the phosphomolybdenum method as described by Prieto *et al.,* (1999). Aliquots 10, 25, 50, 100 and 200 ug/mL for standard ascorbic acid were prepared. TAC reagent prepared by dissolving 0.6 M H_2SO_4 acid, 28 mM sodium phosphate, sodium phosphate dibasic (Na_2HPO_4) 4 mM ammonium molybdate. All mixed in equal proportions. To 1 mL of sample, 9 mL TAC reagent was added and incubated at 95ºC for 90 min, and then cool to room temperature. The absorbance was recorded at 765 nm with a UV–Vis spectrophotometer (Thermo Spectronic BioMate 3, USA). Antioxidant effect was expressed relative to ascorbic acid equivalents.

***In Vitro* Antidiabetic Activities**
Assessment of α-Amylase Inhibitory Activity
A starch solution of 0.1% w/v was prepared by stirring 0.1g of potato starch in 100 mL of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of α-amylase in 100 mL of distilled water. The colorimetric reagent was prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution (96 mM). Different extract concentrations (50–1000 µg/mL) were mixed with 1 mL of starch solution and allowed to stand for 10 minutes. The reaction was then initiated by adding the enzyme solution and maintained under alkaline conditions at 25°C for another 10 minutes. Subsequently, the reaction was halted through the addition of 1 mL of colorimetric reagent, followed by incubation in a boiling water bath for 5 minutes and cooling to room temperature. The test mixture was brought to a final volume with 10 mL of distilled water, and the absorbance was was read at 540 nm. The control, representing 100% enzyme effect, was prepared similarly by replacing the extract with DMSO. A parallel experiment was performed using acarbose as the standard drug.

Determination of α-glucosidase activity
The inhibitory effect was assessed by incubating 1 mL of starch substrate solution (2% w/v maltose or sucrose) with 0.2 M Tris buffer (pH 8.0) and varying concentrations of the extract (50–1000 µg/mL) for 5 minutes at 37°C. The reaction was started by adding 1

mL of α-glucosidase enzyme (1 µg/mL) and incubated at 35°C for 40 minutes. It was stopped by adding 2 mL of 6 M HCl, after which the color intensity was recorded at 540 nm. A control was prepared by substituting the extract with DMSO, and acarbose was utilized as the reference standard. The percentage inhibition was determined using the following formula:

% Inhibition = (OD value of control - OD value of samples) / OD value of control of inhibition) X 100.

Statistical Analysis

All measurements were done in three replicates, and data are presented as mean ± SEM.

RESULTS AND DISCUSSION

Results

Qualitative Phytochemical Screening and Quantitative Steroid Analysis

The qualitative phytochemical constituents of B1 and B2 seed oils, as presented in Table 1, reveal a selective presence of secondary metabolites that highlight the potential medicinal value of the oils. The analysis showed that triterpenoids were prominently detected in both seed oil extracts. In addition to triterpenoids, the presence of steroids was moderately confirmed in pawpaw seed oil and heavily detected in watermelon seed oil. Furthermore, cardiac glycosides were slightly detected in pawpaw seed oil and moderately in watermelon seed oil. Other phytochemicals such as alkaloids, saponins, tannins, flavonoids, and phenolic compounds were not detected.

Table 2 presents the quantitative analysis of steroids in the seed oil extracts, revealing a concentration of 46.773 ± 0.107 and 60.022 ± 0.129 mg/g respectively. The high concentration suggests that steroids are a dominant phytochemical component of the both extracts.

Table 1. Phytochemical constituents of seed oils.

Test	Method	B1	B2
Alkaloids	Mayer's Test	-	-
	Dragendroff's Test	-	-
	Wagner's Test	-	-
Saponins	Frothing Test	-	-
Reducing sugars	Fehling's test	-	-
Cardiac glycosides	Keller killani's test	+	++
Triterpenoids	Liebermann-Burchard's test	+++	+++
Steroids	Salkwoski's test	++	+++
Tannins	Ferric chloride test	-	-
Phenolic Compounds	Lead acetate test	-	-
Flavonoids	Shinoda test	-	-
Anthraquinones	Borntrager's Test	-	-

Key: Heavily detected: + + +; (>75%); Moderately detected: + +; (50%); Slightly detected: +'; Not detected: - (<50%)

Table 2. Quantitative analysis of steroids in seed oils.

Sample	Concentration (mg of CHO/g of extract)
B1	46.773 ± 0.107
B2	60.022 ± 0.129

Values are expressed as mean ± standard error of the mean (SEM) from three replicates.

DPPH radical Scavenging Activity

Figure 1 illustrates. the DPPH scavenging activity (% inhibition) of pawpaw and watermelon seed oil extract at various concentrations, compared with ascorbic acid, a standard antioxidant. In the present study, both seed oil extracts exhibited concentration-dependent radical scavenging activity, although their effects were markedly lower than the reference standard, ascorbic acid. Pawpaw seed oil extract showed minimal activity at low concentrations, ranging from 0.25% at 10 µg/mL to 13.32% at 200 µg/mL, while watermelon seed oil extract demonstrated slightly higher activity, reaching 15.91% inhibition at the highest concentration tested.

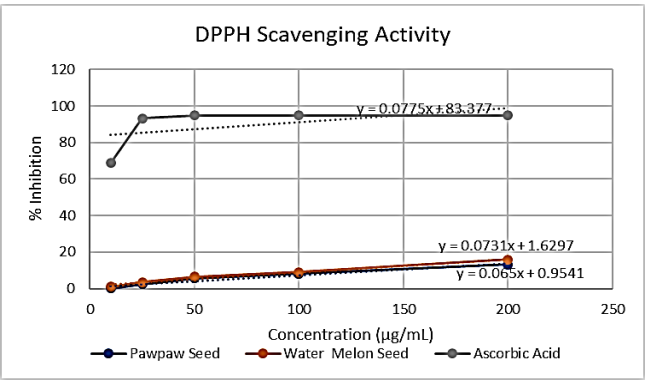

Figure 1. Dose–response curve showing the DPPH radical scavenging activity of seed oils (B1 and B2) and standard ascorbic acid with regression equations.

FRAP (Ferric Reducing Antioxidant Power)

Table 3 presents the FRAP values for pawpaw and watermelon seed oil extract and ascorbic acid. In the current study, the FRAP values of pawpaw and watermelon seed oil extracts were 9.06 ± 0.02 µM Fe^{2+}/mg and 14.505 ± 0.009 µM Fe^{2+}/mg, respectively, while the reference antioxidant, ascorbic acid, exhibited a much higher reducing potential (174.60 ± 1.33 µM Fe^{2+}/mg). The markedly higher FRAP value of watermelon seed oil relative to pawpaw seed oil suggests a stronger electron-donating capacity, in line with its superior performance in the total antioxidant capacity assay.

Table 3. Ferric reducing antioxidant power and total antioxidant capacity of seed oils.

Sample	FRAP (μM Fe^{2+} per mg of extract)	TAC (mg/g Ascorbic Acid)
B1	9.06 ± 0.02	4.734 ± 0.177
B2	14.505 ± 0.009	10.540 ± 0.187
Ascorbic Acid	174.60 ± 1.33	NA

Values are presented as MEAN ± SEM (n=3).

Total antioxidant capacity

The results as shown in Table 3 revealed that the watermelon seed oil extract exhibited a significantly higher TAC value (10.540 ± 0.187 mg/g) compared to the pawpaw seed oil extract (4.734 ± 0.177 mg/g). This suggests that watermelon seed oil possesses a greater ability to reduce Mo (VI) to Mo (V), indicating a higher concentration of antioxidant constituents.

In Vitro Antidiabetic Investigation

The inhibitory activities of pawpaw and watermelon seed oil extracts against α-amylase and α-glucosidase enzymes are presented in Tables 4 and 5. Both extracts demonstrated a concentration-dependent inhibition of the enzymes, although their effects were considerably lower than that of acarbose, the reference inhibitor. At the lowest concentration (50 μg/mL), pawpaw seed oil extract exhibited minimal inhibition (1.70%), compared to watermelon seed oil extract (7.80%), indicating a relatively weak interaction with the enzyme at low doses. However, inhibition increased with concentration, reaching a peak of 48.07% for pawpaw and 81.81% for watermelon seed oil extracts at 500 μg/mL and 1000 μg/mL, respectively. Interestingly, pawpaw seed oil extract showed a slight decline at 1000 μg/mL, suggesting possible saturation effects or reduced stability at higher concentrations. In comparison, acarbose consistently exhibited strong inhibitory activity across all tested concentrations, with inhibition values ranging from 60.03% at 50 μg/mL to 93.68% at 1000 μg/mL.

Table 4. α-Glucosidase scavenging activity (% Inhibition) of seed oils.

Sample	50 μg/mL	100 μg/mL	250 μg/mL	500 μg/mL	1000 μg/mL
B1	24.875 ± 0.191	40.932 ± 0.110	54.992 ± 0.083	69.426 ± 0.144	84.692 ± 0.110
B2	1.913 ± 0.072	12.230 ± 0.181	18.594 ± 0.181	30.699 ± 0.083	45.549 ± 0.231
Acarbose	50.874 ± 0.083	62.022 ± 0.150	75.915 ± 0.125	87.854 ± 0.110	93.095 ± 0.110

Values are presented as MEAN ± SEM (n=3).

Table 5. α-Amylase scavenging activity (% Inhibition) of seed oils.

Sample	50 μg/mL	100 μg/mL	250 μg/mL	500 μg/mL	1000 μg/mL
B1	1.703 ± 0.119	13.754 ± 0.162	29.167 ± 0.155	48.073 ± 0.162	43.190 ± 0.119
B2	7.796 ± 0.078	23.029 ± 0.195	37.097 ± 0.135	65.681 ± 0.089	81.810 ± 0.122
Acarbose	60.036 ± 0.119	67.518 ± 0.119	81.272 ± 4.022	90.950 ± 0.081	93.683 ± 0.094

Values are presented as MEAN ± SEM (n=3).

GC-MS Analysis of Fatty Acid Composition of the Seed Oils

The fatty acid composition of B1 and B2 seed oils as presented in Table 6 while Figs. 2 and 3 show the existence of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) in varying proportions. A total of 27 fatty acids were identified by GC-MS in both oils. Among the saturated fatty acids, pentadecanoic acid methyl ester (106.53 mg/L) was the most abundant, while the least abundant SFAs were dodecanoic acid, methyl ester and capric acid, methyl ester (0.07 mg/L). The moderate levels of SFAs suggest they contribute to the oxidative stability of the oils. The MUFA group was dominated by cis-10-heptadecenoic acid (895.57 mg/L), which constituted the highest single fatty acid detected across all classes. Other notable MUFAs include cis-11-eicosenoic acid (129.32 mg/L) and oleic acid methyl ester (97.22 mg/L), while cis-10-pentadecenoic acid was only detected in trace amounts. Polyunsaturated fatty acids were also present in appreciable amounts. Arachidonic acid (263.49 mg/L) and 5,8,11,14,17-eicosapentaenoic acid (EPA) (241.80 mg/L) were predominant, followed by cis-11,14-eicosadienoic acid (198.49 mg/L). Essential fatty acids such as γ-linolenic acid (35.99 mg/L) and docosahexaenoic acid (DHA, 16.65 mg/L) were also identified.

Table 6. Fatty acids identified by GC-MS in oils.

S/N	Fatty Acid	Molecular Formula	Class	Concentration (mg/L)	
				B1	B2
1	Octanoic acid, methyl ester	$C_9H_{18}O_2$	SFA	0.23	0.14
2	Capric acid, methyl ester	$C_{11}H_{22}O_2$	SFA	0.08	0.07
3	Dodecanoic acid, methyl ester	$C_{12}H_{24}O_2$	SFA	0.07	0.10
4	Tridecanoic acid, methyl ester	$C_{13}H_{26}O_2$	SFA	0.21	0.23
5	Methyl myristoleate	$C_{15}H_{28}O_2$	SFA	0.89	13.92
6	Methyl tetradecanoate	$C_{15}H_{30}O_2$	SFA	0.37	0.40
7	cis-10-pentadecenoic acid, methyl ester	$C_{15}H_{28}O_2$	MUFA	7.92	11.22
8	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	SFA	106.53	0.16
9	9-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	MUFA	80.92	16.81
10	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	SFA	41.76	3.39
11	cis-10-heptadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	MUFA	895.57	768.25
12	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	SFA	29.82	39.20
13	γ-Linolenic acid, methyl ester	$C_{18}H_{30}O_2$	PUFA	35.99	25.80
14	Oleic acid methyl ester	$C_{19}H_{36}O_2$	MUFA	97.22	8.29
15	Methyl stearate	$C_{19}H_{38}O_2$	SFA	22.45	1.76
16	Arachidonic acid, methyl ester	$C_{20}H_{32}O_2$	PUFA	263.49	40.88
17	5,8,11,14,17-Eicosapentaenoic acid (EPA)	$C_{20}H_{30}O_2$	PUFA	241.80	38.69
18	cis-11,14-Eicosadienoic acid, methyl ester	$C_{20}H_{36}O_2$	PUFA	198.49	86.79
19	cis-11-Eicosenoic acid, methyl ester	$C_{20}H_{38}O_2$	MUFA	129.32	69.49
20	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	SFA	4.69	0.32
21	Cis-12-Heneicosenoic acid, methyl ester	$C_{22}H_{42}O_2$	MUFA	1.39	0.51
22	4,7,10,13,16,19-Docosahexaenoic acid (DHA)	$C_{22}H_{32}O_2$	PUFA	16.65	6.54
23	13-Docosenoic acid, methyl ester	$C_{23}H_{44}O_2$	MUFA	7.72	2.79
24	Docosanoic acid, methyl ester	$C_{22}H_{44}O_2$	SFA	10.75	0.17
25	Tricosanoic acid, methyl ester	$C_{24}H_{48}O_2$	SFA	26.32	6.85
26	15-Tetracosenoic acid, methyl ester	$C_{24}H_{46}O_2$	MUFA	14.08	6.91
27	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	SFA	0.42	0.46

B1 = Pawpaw seed oil; **B2** = Watermelon seed oil; SFA = Saturated Fatty Acid; **MUFA** = Monounsaturated Fatty Acid; **PUFA** = Polyunsaturated Fatty Acid.

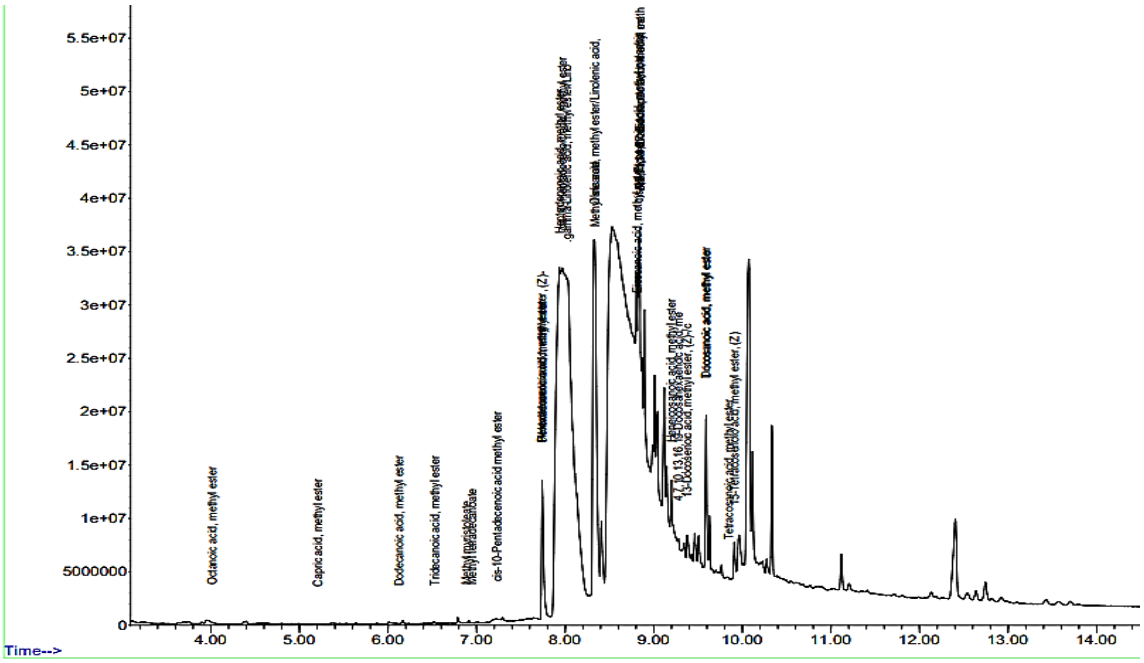

Figure 2. GC-MS Chromatogram of fatty acids in pawpaw seed oil (B1).

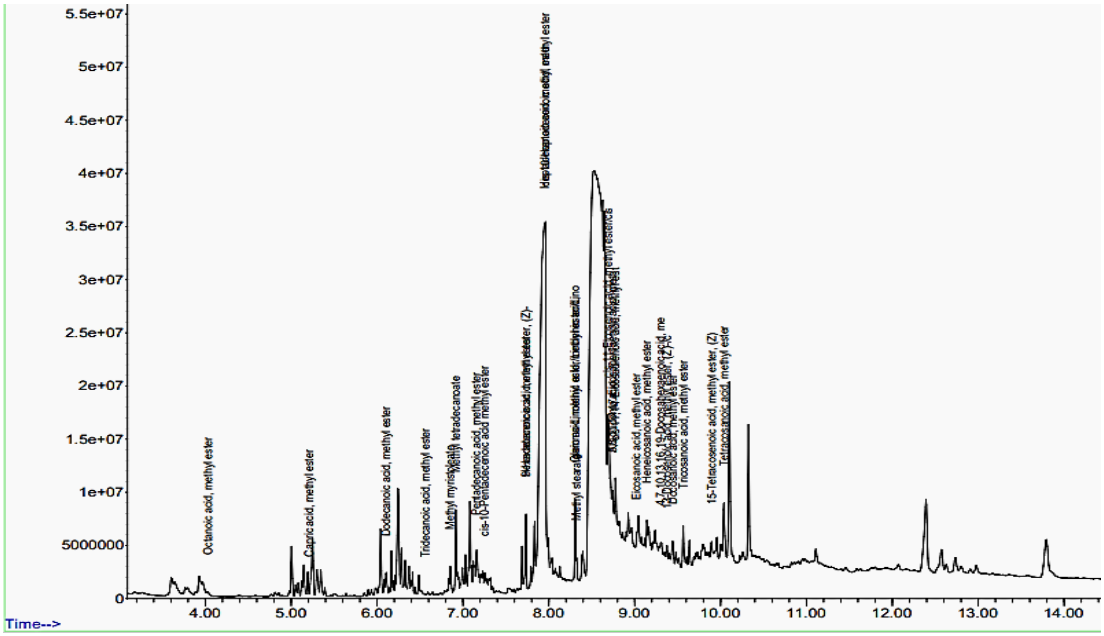

Figure 3. GC-MS Chromatogram of fatty acids in watermelon seed oil (B2).

Discussion

Pharmacological Activity of Phytochemicals

Triterpenoids are a diverse class of natural products known for their broad spectrum of pharmacological properties, including anti-inflammatory, hepatoprotective, antimicrobial, and anticancer activities (Almeida *et al.,* 2020). Their dominance suggests that the seed oils may possess significant therapeutic relevance, particularly in formulations aimed at managing inflammation and oxidative stress-related conditions. Steroids are characterized by their pharmacological significance, including anti-inflammatory, antimicrobial, and immunomodulatory properties which may contribute to the biological activity of the oils (Devi *et al.,* 2023). They play vital roles in maintaining cell membrane integrity and act as precursors for hormone synthesis (Devi *et al.,* 2023). These compounds are noted for their potent cardiotonic effects and have been traditionally utilized in treating heart failure and irregular heart rhythms (Ponce *et al.,* 2025).

The absence of alkaloids, which are commonly associated with strong bioactivity and, in some cases, toxicity, might indicate a relatively safer profile for the oil in terms of toxicity risk (Reynolds & Sofowora, 1984). Similarly, the non-detection of saponins, phenolics and flavonoids, both of which are renowned for their antioxidant, anti-inflammatory, and cholesterol-lowering effects (Mustafa *et al.,* 2022), may suggest limited antioxidant potential in the oil. Nevertheless, the dominance of triterpenoids and the existence of steroids and cardiac glycosides highlight that the seed oil extracts still possesses considerable pharmacological potentials.

Antioxidant Activity

The total antioxidant capacity (TAC) of plant-based oils is generally associated with their content of polyphenols, flavonoids, carotenoids, tocopherols, and other phytochemicals capable of donating electrons or hydrogen atoms to neutralize free radicals (Shahidi & Ambigaipalan, 2015). The content and concentration of these compounds can differ significantly between different plants (Ally-Charles *et al.,* 2024). The differences in TAC between pawpaw and watermelon seed oils can be explained by variations in their phytochemical composition. Although phenolics and flavonoids which are widely recognized as major contributors to antioxidant activity were absent in both extracts, low levels of antioxidant activity were still observed. This activity is most likely attributable to non-phenolic constituents, particularly triterpenoids and steroids, which were present in both oils and are known to exert modest radical-quenching effects in lipophilic systems (Gülçin, 2020).

In summary, the absence of phenolics and flavonoids, which are typically associated with strong antioxidant activity, may explain the relatively low radical scavenging and reducing power observed in the DPPH, FRAP, and TAC assays.

Antidabetic Activity

Inhibition of α-glucosidase slows the hydrolysis of dietary carbohydrates in the small intestine, thereby reducing postprandial glucose spikes in individuals with diabetes. Targeting carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase is therefore a well-recognized therapeutic approach in the management of diabetes mellitus, as they delay gastrointestinal glucose absorption and consequently lower postprandial blood glucose levels (Kazeem *et al.,* 2013).

In this study, pawpaw and watermelon seed oil extracts demonstrated inhibitory effects against α-amylase and α-glucosidase, with watermelon seed oil

exhibiting superior activity across all concentrations. The higher steroid content in watermelon seed oil may explain its stronger enzyme inhibitory activity compared to pawpaw seed oil, highlighting the potential role of these compounds in supporting the oils' antidiabetic properties. These findings align with earlier *in vivo* investigations where both oils significantly reduced fasting blood glucose in alloxan-induced diabetic rats, with watermelon seed oil achieving a 50.5% reduction, closely approaching the 71.67% observed for metformin (Oaikhenan, 2023). Taken together, the present enzyme inhibition results provide a plausible mechanistic basis for the previously reported antidiabetic effects, supporting the view that the bioactivity of these oils may partly result from their ability to interfere with carbohydrate digestion and delay glucose absorption in the gastrointestinal tract.

The high MUFA content, especially oleic acid derivatives, indicates potential nutritional value since MUFAs are associated with cholesterol regulation and cardiovascular protection. The presence of γ-linolenic acid and DHA, highlight the oils as potential dietary sources of omega-3 and omega-6 fatty acids. These fatty acids are physiologically important in preventing inflammatory and metabolic disorders.

According to several researchers, the fatty acids identified in seed oils have been known to possess diverse biological activities ranging from anti-inflammatory, cardioprotective, neuroprotective to antidiabetic effects. The most abundant fatty acid was cis-10-heptadecenoic acid. Reports in literature suggest that this uncommon MUFA exerts hypolipidemic and cardioprotective activities by reducing serum triglycerides and LDL cholesterol (Kharazmi-Khorassani *et al.,* 2021). Heptadecanoic acid is another odd-chain fatty acid that has been highlighted for its beneficial roles. According to Jenkins *et al.* (2015), it is inversely correlated with metabolic syndrome and supports improved insulin sensitivity.

Eicosapentaenoic acid (EPA) has been widely reported to exhibit cardioprotective, anti-inflammatory, and neuroprotective effects, particularly in reducing triglycerides and protecting against cardiovascular disease (Troesch *et al.,* 2020). More importantly, PUFAs such as 5,8,11,14,17-eicosapentaenoic acid (EPA), 4,7,10,13,16,19-docosahexaenoic acid (DHA), the principal omega-3 fatty acids, are well-documented for their antioxidant, anti-inflammatory, and antidiabetic properties. These fatty acids protect pancreatic β-cells, improve insulin sensitivity, reduce postprandial glucose spikes, and enhance cellular antioxidant defenses (Swanson *et al.,* 2012; Calder, 2017).

It has been found that arachidonic acid is essential for brain development, immunity, and cell signaling, although excessive levels may promote pro-inflammatory eicosanoids (Calder, 2015). Linolenic acid (PUFA), a precursor to EPA and DHA has been reported to have been involved in fundamental biological activities such as antioxidant effects, modulating the inflammatory and immune response (Fratianni *et al.,* 2021). γ-linolenic acid has been shown to reduce oxidative stress markers, enhance glucose uptake, and mitigate diabetic complications such as neuropathy (Sardesai, 2020). Its detection in these seed oils further supports their potential role in combating oxidative damage associated with diabetes.

CONCLUSION

The present study demonstrated that seed oils from *Carica papaya* and *Citrullus lanatus* are rich in triterpenoids, steroids, and cardiac glycosides but devoid of phenolics and flavonoids, which are traditionally linked to antioxidant potential. This phytochemical profile explains the relatively low antioxidant activity observed in both DPPH and FRAP assays. However, both oils exhibited pronounced inhibitory effects against carbohydrate-hydrolyzing enzymes, with pawpaw seed oil showing greater activity against α-glucosidase and watermelon seed oil against α-amylase. These complementary inhibitory patterns suggest potential for combined use in modulating postprandial hyperglycemia. Although their antioxidant contributions are minimal, the enzyme inhibition results highlight these underutilized seed oils as promising natural sources of antidiabetic agents. GC-MS analysis further revealed the presence of bioactive fatty acids, oleic acid methyl ester, cis-10-pentadecenoic acid, γ-linolenic acid, arachidonic acid, EPA, DHA, some of which are reported to exert antioxidant and antidiabetic effects. It is recommended that further studies, including *in vivo* validation and compound isolation, are warranted to fully elucidate their therapeutic potential.

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