BAJOPAS Volume 16 Number 1, June, 2023

http://dx.doi.org/10.4314/bajopas.v16i1.1

Bayero Journal of Pure and Applied Sciences, 16(1): 1 - 15
Received: February, 2023
Accepted: April, 2023
ISSN 2006 – 6996

NUTRACEUTICAL AND FUNCTIONAL FOOD POTENTIALS OF AFRICAN EBONY (*Diospyros mespiliformis*) TREE FRUIT PULP

Jibril, M. M*[1]., Wasila, T. M[1]., Kamaladdeen, I.,[1] and Hamisu, M. U[1].
[1]Biochemistry Department Bayero University Kano
*Corresponding author: Department of Biochemistry, Bayero University, Kano.
mmjibril.bch@buk.edu.ng

ABSTRACT
Diospyros mespiliformis is an underutilized plant found in Northern Nigeria, reported for treatment of diseases. This study aimed to evaluate the antidiabetic and antioxidant potentials of Kano grown D. mespiliformis fruit pulp and its phytochemical profiles. The fruits were air dried, grounded into fine powder and proximate composition was determined. Extracts of aqueous, 50%, 60%, 70%, 80%, 90% and 100% ethanol were prepared. Alpha-glucosidase and α-amylase inhibition assays, total phenolics content (TPC), total flavonoids content (TFC) and DPPH radical scavenging assays were evaluated using standard methods. Phytochemical profiles of extracts were analyzed using liquid chromatographic mass spectrometry. The ash, crude fiber, protein, fat, total carbohydrate and moisture contents on dry weight basis were 6.3 ± 0.20%, 4.1 ± 0.72%, 6.9 ± 0.08%, 7.7 ± 0.02%, 66.2 ± 0.80% and 8.8 ± 0.23% respectively. The 60% ethanol gave the highest extraction yield, while the aqueous extract was the lowest. The inhibition assays indicated that 50% ethanol extract with IC_{50} of 149.4 mg/mL had the highest inhibition against α-glucosidase activity while that of standard acarbose was 144.3 mg/mL. The 100% ethanol extract with the IC_{50} value 299.58 mg/mL showed the highest inhibition against α-amylase activity, while that of standard acarbose was 228.9 mg/mL. The DPPH assay indicated that both 50% and 60% ethanol extracts, IC_{50} values 150.1 ± 0.58 mg/mL and 150.6 ± 0.32 mg/mL respectively, had the highest antioxidant capacity, while that of standard ascorbic was 88.24 ± 0.21 mg/mL. Also, 50% ethanol gave the highest TPC, 167.22 ± 1.11 mg GAE/g, but the 60% ethanol gave the highest TFC (40.2 ± 1.60 mg GAE/g). Bioactive phytochemicals belonging to flavonoids, terpenoids, shogaols, allicin, linalool, and unsaturated fatty acids with health promoting effects were identified with their suggested names. Therefore, D. mespiliformis fruit pulp may be a good raw material for functional food and nutraceuticals against diabetes mellitus, oxidative stress and other chronic diseases.
Key Words: Alpha-glucosidase and alpha-amylase, functional food, phytochemical profiles, antioxidant, antidiabetic.

INTRODUCTION

The human race (nearly two third) have since believed in the healing powers of the active compounds in plants due to reasons that includes relative safety, affordability and availability among others. This have triggered the exploration of functional and medicinal benefits of food over thousands of years back, and the birth of the modern day nutraceutical industry during the 1980s (Muredzi, 2013; Rajasekaran & Xavier, 2015). Depending on the chemical compounds they are made of, adequate intakes of fruits serves in the protection and reduction of damages caused by free radicals and chronic diseases to our body cells (Ahmad Wani et al., 2023). Fruits are known to be very rich in vitamins and minerals, high fiber contents and are naturally endowed with secondary metabolites (phytochemicals), thus serving as functional food and important source of nutraceuticals. They hold health benefits that includes decreased coronary heart disease, decreased blood pressure, antioxidant, anti-inflammation, and antidiabetic activities among others when consumed by man (Ahmad Wani et al., 2023; Vattem & Maitin, 2016). Wild fruits are underutilized fruits rich in phytochemicals, such as anthocyanins, proanthocyanidins, flavonoids, phenolics and other polyphenols.

Numerous studies pointed out that wild fruits possess various bioactivities as mentioned earlier, thus, they have untapped potentials to be developed into functional foods and nutraceuticals for the prevention and treatment of quite a great deal of chronic diseases (Fu et al., 2010; Li et al., 2016; Xia et al., 2010). African Ebony tree (*D. mespiliformis*) fruit is a wild fruit native to Africa and ubiquitously found in northern Nigeria. The genus *Diospyros* has been used against different disease conditions including diarrhea, hemorrhage, insomnia, hiccough, antibacterial, etc. Phytochemical constituents such as gallic acid, terpenoids, tannins, hydrocarbons, lipids, and other polyphenols, have been reported in different species of this genus (Adu et al., 2022; Ebbo et al., 2019; Rauf et al., 2017). This study aimed to evaluate the antidiabetic and antioxidant activities, and the phytochemical profiles of the fruit pulp of *D. mespiliformis* cultivated in Kano State.

MATERIALS AND METHODS

Materials

Water bath sonicator (WUC-D10H, Wised Clean sonicator, Seoul, Korea), beaker, cuvette, and laboratory shaker (WSB-45, Digital shaking water bath, Seoul, Korea). Rotary evaporator (R-100, Buchi Services, Switzerland), funnel, Whatman filter paper no 1, conical flasks, beaker, measuring cylinder, aluminium foil, plastic rubber, and oven drier (MINO/100, Serial No, 14H030, Tanhouse Lane Cheshire). Furnace (S33 6RB, Hope valley, England), weighing balance, Soxhlet apparatus, spectrophotometer (MODEL 752S, New life medical instrument England), weighing balance, incubator, LC-MS machine, and thermometer.

Methods

Extract preparation

Fresh *Diospyros mespiliformis* fruit locally known as *Kanya* in Hausa language, were collected from Kurmi market Kano. The plant was identified by a plant taxonomist at the Department of Plant Biology Bayero University, Kano. A voucher specimen was deposited for future reference. Collected samples were immediately washed under running tap water and air dried. The dried and grounded *D. mespiliformis* fruit pulp sample was extracted using absolute ethanol, water, and 90%, 80%, 70%, 60%, and 50% ethanol:water (v/v). This solvent combinations were used considering health safety issues. The solute to solvent ratio used was 1:40, which is a modification of the methods of ultrasound assisted extractions previously reported (Wang et al., 2008; Wong et al., 2014). The mixture of dried/grounded *D. mespiliformis* fruit pulp sample and solvent was sonicated first for 20 minutes at 30 ± 2 °C, and placed in a laboratory shaker for 40 minutes at 120 revolutions per minute and room temperature. Extracts were concentrated using rotary evaporator at 40 °C. Excess solvents in extracts were allowed to evaporate by air drying at laboratory temperature. Dried samples were stored in air tight glass tubes and stored at 4°C.

Determination of Total moisture

The AOAC International method (Suzanne, 2014) was adopted in moisture determination. Empty crucible and lid were placed in the oven at 105 ^{o}C for 3 hours and transferred to desiccator to cool. The empty crucible was weighed and about 5.0g of sample was weighed into the dish, and spread to uniformity. The dish containing 5.0 g sample was placed in the oven and dried for 3 hours at 105 ^{o}C. After drying, the dish with covered lid was transferred to the desiccator to cool. The crucible containing the dried sample was weighed repeatedly and returned into the desiccator after each weighing.

Calculation: % of Moisture Content = $\frac{W_1 - W_2}{W_1} \times 100$

Where: W_1 = Weight (g) of sample before drying, W_2 = Weight (g) of sample after drying, W3 = Weight (g) of crucible and dried sample.

Determination of ash content

The ash content was measured using the method of AOAC International (Suzanne, 2014). Crucible with lid were placed in the Muffle furnace at 550 ^{o}C overnight to burn off impurities on the surface of crucible. The crucible was cooled in desiccator for 30 mins and weighed as W_1. About 5.0g dried sample was weighed into the empty crucible weighed and recorded as W_2. The lid was removed and crucible without lid was placed in the furnace at 550 ^{o}C overnight. After overnight heating, the lid was placed back to prevent loss of fluffy ash, Cooled in desiccator and weigh when the ash turned gray and recorded as W_3.

% of Ash content = $\frac{W_3 - W_1}{W_2} \times 100$

Where: W_1 = Weight of empty dried crucible, W_2 = Weight of crucible and sample, W_3 = Weight of crucible and dried sample

Determination of Crude Protein

Kjeldahl method as described by AOAC International (Suzanne, 2014) was used for crude protein determination. About 1.0 g of sample was placed in the digestion flask of Kjeldahl apparatus, followed by 5.0g of catalyst and 200 ml concentrated H_2SO_4. Another flask containing the above mixture except sample as blank was prepared.

Flasks were placed in inclined position and heated gently until frothing ceases and boiled briskly until clear solutions were obtained. All flasks were cooled and 60 ml distilled water was cautiously added to each flask. Flasks were immediately connected to digestion bulk on the condenser and with tip of condenser immersed in standard acid and 5-6 drops of mix indicator in receiver. Flasks were rotated to mix content thoroughly, heated until all NH_3 is distilled. Receiver was removed, tip of condenser washed and excess standard acid titrated with distilled standard NaOH solution.

Calculation: % of Crude Protein Content = $\frac{(S- B) X N X 14.007 X 6.25}{W (g)} X 100$

Where: S = volume (mL) of 0.2N HCl used for sample titration, B = volume (mL) of 0.2N HCl used in blank titration, N = normality of HCl, W = weight (g) of sample, 14.007 = atomic weight of nitrogen, 6.25 = the protein-nitrogen conversion factor.

Determination of crude fiber

The method described by AOAC International (Suzanne, 2014) was adopted for crude fiber determination. About 3.0 g (W_1) of the finely ground sample was weighed into a round bottom flask, 100 ml of 0.23M H_2SO_4 solution was added. The mixture was boiled under reflux for 30 min and quickly filtered under suction. The insoluble matter (residue) was washed several times with 50 ml hot water until it was acids free. It was quantitatively transferred into the flask and 100 ml of 0.31M NaOH solution was added. The saw mixture boiled again under reflux for 30 min and quickly filtered under suction. The insoluble residue was washed with boiling water and acetone until it was base free. It was transferred to a weighed crucible and then dried to constant weight in the oven at 105°C, cooled in the desiccators and weighed (W_1). It was then incinerated in a muffle furnace at 550°C for 3 hours, cooled in the desiccators and reweighed (W_2).The loss in weight on incineration was calculated as:

$W_2 – W_3$. % Crude fiber = $\frac{W_2 – W_3}{W_1} X 100$

Determination of crude fat (lipid) content

Crude fat was determined by method of AOAC International (Suzanne, 2014). Round bottom flask was place the bottle and the lid in the incubator at 105^0C overnight to ensure that weight of the bottle is stable. Weigh about 5.0g of sample to filter paper and wrap. Take the sample into extraction thimble and transfer into Soxhlet apparatus. Fill petroleum ether about 250ml into the bottle and take it on the heating mantle. Connect the Soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle. Heat the sample and Allowed the process to continue for 4 hours before the defatted sample was removed Evaporate the solvent by using the vacuum condenser. Incubate the bottle at 80-90 ^{0}C until solvent completely evaporate and bottle is completely dry. Reweigh the bottle and its dried content.

Calculation: % of Fat Content = $\frac{W_3 - W_1}{W_1} X 100$

Where: W_1 = weight of extraction bottle, W_2 = weight of sample, W_3 = weight of bottle and dried lipid.

Determination of total carbohydrate

The total carbohydrate content was determined by difference as described by AOAC International (Suzanne, 2014). The sum of the percentage moisture, ash, crude lipid, crude protein and crude fiber was subtracted from 100. It was calculated thus:

% Total soluble carbohydrate 100 – (% Moisture + % crude fat + % crude protein + % fiber + ash).

Alpha-glucosidase inhibition assay

This assay was carried out according to the method described by (Kim et al., 2005). Using α-glucosidase from *Saccharomyces cerevisiae*. A total of 50µL of extract of varied concentrations, 10, 15 and 20 mg/mL was placed in a tube and 100µL of α-glucosidase (1.0 mg/mL) in 100 mM sodium phosphate buffer (pH 6.9) was added. The mixture was incubated at room temperature (25°C) for 10 min, after which 50µL of substrate solution, p-nitrophenyl glucopyranoside (p-NPG) (3.0 mM) in 0.02 M sodium phosphate buffer (pH 6.9) was added. This reaction mixture was again incubated at 37°C for 20 min. The reaction was finally quenched by 2 mL of 0.1 M Na_2CO_3. The absorbance of the yellow colored p-nitrophenol, released from p-NPG, (A_{Sample}) was measured at 405 nm. Absorbance of control ($A_{Control}$) was also measured. The assay was carried out in triplicate. The results were expressed as percent inhibition of α-glucosidase activity using the following formula:

Inhibition (%) = $\frac{(A_{Control} - A_{Sample})}{(A_{Control})} \times 100$

A control is the absorbance of α-glucosidase and p-NPG without the extract and the blank is the test sample without enzyme. The IC_{50} of the extracts for α-glucosidase inhibition were calculated from linear regression curves of the form y = mx + c obtained by plotting % inhibition against concentration of extracts. The IC_{50} of the extracts were expressed as mg/mL.

Alpha-amylase inhibition assay

This assay was carried out using a modified procedure (McCue & Shetty, 2004). A total of 250 µL of extract of varied concentrations, 2, 4, 6, 8 and 10 mg/mL was placed in a tube and 250 µL of pancreatic α-amylase solution (0.5 mg/mL) in 0.02 M sodium phosphate buffer (pH 6.9) was added. The mixture was incubated at 25°C for 10 min, after which 250 µL of starch solution (1 %) in 0.02 M sodium phosphate buffer (pH 6.9) was added. This reaction mixture was again incubated at 25°C for 10 min. The reaction was finally quenched by adding 500 µL DNS reagent (96 mM 3,5-dinitrosalicylic acid), and further incubated in boiling water for 5 min and then cooled to room temperature. The content of each test tube was diluted with 5 mL distilled water and the absorbance (A_{Sample}) was measured at 540 nm in a spectrophotometer. The absorbance of control ($A_{Control}$) was also measured. The assay was carried out in triplicate. The results were expressed as percent inhibition of α--amylase activity using the following formula:

$$\text{Inhibition (\%)} = \frac{(A_{Control} - A_{Sample})}{(A_{Control})} \times 100$$

A control is the absorbance of α-amylase and its substrate without the extract and the blank is the test sample without enzyme. The IC_{50} of the extracts for α-amylase inhibition were calculated from linear regression curves of the form y = mx + c obtained by plotting % inhibition against concentration of extracts. The IC_{50} of the extracts were expressed as mg/mL.

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

The free radical scavenging activity of the extracts ware analyzed by DPPH assay method (Brand-Williams et al., 1995). Each of the extracts (2 mL) at different concentrations (25, 50, 100, 200, and 400 µg/mL) ware mixed with 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark at room temperature. The concentrations were prepared in triplicates and the percentage antioxidant activity calculated as follows:

$$\text{\% DPPH radical inhibition} = \frac{(A_{Control} - A_{Sample})}{(A_{Control})} \times 100$$

One milliliter of methanol plus 2.0 mL of the extract was used as the blank, while1.0 mL of the 0.5 mM DPPH solution plus 2.0 mL of methanol was used as the negative control. Ascorbic acid was used as reference standard. The IC_{50} of the extracts for DPPH radical inhibition were calculated from linear regression curves of the form y = mx + c obtained by plotting % inhibition of DPPH radical against concentration of extracts. The IC_{50} of the extracts were expressed as mg/mL.

Determination of total phenolics contents (TPC)

The concentration of phenolics in plant extracts was determined using Folin–Ciocalteau's method (1927). Methanolic solution of the extracts in the concentration of 1mg/mL was used in the analysis. The reaction mixture was prepared by mixing 1mL of methanolic solution of extract to 2.5 mL of 10% Folin-Ciocalteau's reagent dissolved in water and the contents were mixed. After 3 min, 2.5 mL of 7.5% NaHCO3 solution was added. These were incubated at 45°C for 45 min. The absorbance ware read in a spectrophotometer at765nm against blank (distilled water). The samples were prepared in triplicate for each analysis and the mean absorbance ware obtained. A calibration curve was constructed using gallic acid standard solutions (20, 40, 60, 80, and 100 mg/L). Then the content of phenols in each extract was expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

Determination of total flavonoid contents (TFC)

The content of flavonoids in the examined plant extracts was determined according to a modified colorimetric assay method with aluminium chloride (Mohammed et al., 2016). Approximately 1 mL of methanol solution of the extract (1mg/mL) was added to a test tube, followed by the addition of 0.3 mL of solution of NaNO2 (0.05g/ L). After 5 min, 0.3 ml of a 0.1 g/L solution of AlCl3 was added and 5 min later, 2 mL of NaOH (1 mol/L) was added to the mixture, the solution was mixed and the absorbance was measured at 415 nm against blank (distilled water). Quercetin was used as the standard for the construction of a calibration curve in different concentrations (20, 40, 60, 80, and 100 mg/L). Flavonoid content was expressed in terms of quercetin equivalents (mg QE/g extract).

LC-MS analysis and identification of compound

The phytochemical profiles of the extracts were done based on the protocol for LC-MS analysis (Generic Method) using LC Waters e2695 separation module with W2998 PDA and couple to ACQ-QDA MS. The samples were analyzed using liquid chromatography (LC) tandem mass spectrophotometer (MS) as described by (Cavaliere et al., 2018) with some modifications. The extracted samples were reconstituted in methanol and filtered through polytetrafluoroethylene (PTFE) membrane filter with 0.45 µm size.

The filtrate (10.0 µL) was injected into the LC system and allowed to separate on Sunfire C18 5.0µm 4.6mm x 150 mm column. The run was carried out at a flow rate of 1.0 mL/min, sample and column temperature at 25 °C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) with gradients as shown below:

Time	% A	% B
0	95	5
1	95	5
13	5	95
15	5	95
17	95	5
19	95	5
20	95	5

From ratio of A/B 95:5 this ratio was maintained for further 1 min, then A/B 5:95 for 13min, to 15min. then A/B 95:5 to 17min, 19min and finally 20min. the PDA detector was set at 210-400nm with resolution of 1.2nm and sampling rate at 10 points/sec. The mass spectra were acquired with a scan range from m/z 100 – 1250 after ensuring the following settings: ESI source in positive and negative ion modes; capillary voltage 0.8kv (positive) and 0.8kv (negative); probe temperature 600oC; flow rate 10 mL/min; nebulizer gas, 45 psi. MS set in automatic mode applying fragmentation voltage of 125 V. The data was processed with Empower 3. The compounds were identified on the basis of the following information, elution order, and retention time (tR), fragmentation pattern, and Base m/z.

RESULTS AND DISCUSSION

Result

The percent extraction yield remained relatively at the same value from 100% to 80% ethanol. This slightly increased with 70% ethanol, which resulted to a significant increase with 60% ethanol. The percent yield then suddenly decreased with 50% ethanol, which continued decreasing with increasing water in the extraction solvent.

Table 1. Percent yield of *D. mespiliformis* fruit pulp

Extract	**Percent (%) yield**
100%	11.48 ± 0.58^{a}
90%	11.30 ± 0.16^{a}
80%	11.45 ± 0.35^{a}
70%	11.74 ± 0.11^{ab}
60%	12.29 ± 0.37^{b}
50%	11.98 ± 0.88^{ab}
Aqueous	11.15 ± 0.46^{a}

Values are means ± standard deviation, values with different superscript are significantly different at $P < 0.05$.

The measured α-glucosidase and α-amylase activities inhibition of *D. mespiliformis* were expressed as IC_{50} and presented in Table 1.

Table 2. IC_{50} values for α-glucosidase and α-amylase activities inhibition (mg/mL)

Extract	**α-glucosidase**	**α-amylase**
100%	164.14 ± 0.01^{a}	299.58 ± 0.03^{a}
90%	156.59 ± 0.01^{b}	316.05 ± 0.04^{b}
80%	155.13 ± 0.03^{b}	338.29 ± 0.03^{c}
70%	150.42 ± 0.02^{c}	332.44 ± 0.07^{d}
60%	150.37 ± 0.01^{c}	354.35 ± 0.01^{e}
50%	149.43 ± 0.01^{c}	406.17 ± 0.02^{f}
Aqueous	157.62 ± 0.05^{b}	306.56 ± 0.02^{g}

IC_{50} value of standard acarbose for α-glucosidase activity inhibition is 144.34 ± 0.00 mg/mL while that of α-amylase activity inhibition is 228.93 ± 0.00 mg/mL. Values are ± standard deviation. Values in the same column with different superscript are significantly different at $P < 0.05$.

As presented in Table 2, the IC_{50} values for inhibition of α-glucosidase activity by *D. mespiliformis* fruit pulp extracts decreased from 100% ethanol down to 50% ethanol. This then increased in aqueous extract while 50% ethanol extract maintained the lowest IC_{50} value. The 50% ethanol extract with the lowest IC_{50} value had the most potent inhibition against the activity of α-glucosidase. So also, the IC_{50} value for standard acarbose slightly lower than that of the 50% ethanol *D. mespiliformis* fruit pulp extract.

On the other hand, Table 2 shows that the IC_{50} value of *D. mespiliformis* fruit pulp for α-amylase inhibition kept increasing from 100% to 5% ethanol extract, indicating that 100% ethanol extract had the highest inhibition against the enzyme.

The antioxidant potentials of *D. mespiliformis* extracts were measured by DPPH radical scavenging assay. The results are expressed as IC_{50} presented in Table 3.

Table 3. IC_{50} values for DPPH radical scavenging activity

Extract	IC_{50} (mg/ml)
100%	201.79 ± 2.02^{a}
90%	182.76 ± 1.53^{b}
80%	169.95 ± 0.49^{c}
70%	171.23 ± 0.41^{c}
60%	150.60 ± 0.32^{d}
50%	150.06 ± 0.58^{d}
Aqueous	336.95 ± 3.05^{e}

IC_{50} value of standard ascorbic acid for DPPH radical scavenging is 88.24 ± 0.21 mg/mL. Values are ± standard deviation. Values in three same column having different superscripts are statistically different at $P < 0.05$.

Looking at the results presented in Table 3, the IC_{50} value of ascorbic acid for DPPH radical scavenging was highest in 100% ethanol extract and lowest in both 50% and 60% ethanol extracts. These values generally decreased from 100% to 50% and 60% ethanol extracts. This later increased drastically in aqueous extract. That of standard ascorbic acid was found to be significantly lower than those of 50% and 60% extracts.

The total phenolics contents (TPC) of the extracts is highest in 50% ethanol extract followed by 60% ethanol extract, with aqueous extract having the lowest TPC value as presented in Table 4.

Table 4. Total phenolics and total flavonoid contents of extracts

Extract	TPC (mg GAE/g)	TFC (mg QE/g)
100%	121.48 ± 1.79^{a}	28.95 ± 3.11^{a}
90%	132.22 ± 1.92^{b}	29.87 ± 1.60^{b}
80%	133.33 ± 0.56^{c}	33.54 ± 2.77^{c}
70%	139.07 ± 2.1^{c}	37.18 ± 2.47^{d}
60%	143.15 ± 1.79^{b}	40.18 ± 1.60^{e}
50%	167.22 ± 1.11^{c}	38.46 ± 3.85^{f}
Aqueous	44.07 ± 1.4^{b}	9.10 ± 1.60^{g}

Values are means ± standard deviation. Values with different superscript are significantly different at $P < 0.05$.

The TPC values of the extracts increased from 90% to 50% ethanol extract, which the decreased in aqueous extract with very wide margin. Similarly, the total flavonoid contents of the extracts increased from 90% to 60% ethanol extract, which then decreased from 50% ethanol extract to aqueous extract. The 60% ethanol extract had the highest TFC and lowest in aqueous extract.

Table 5. Proximate Composition of Diospyros *Mespiliformis* fruit pulp

Parameter	Quantity (%)
Moisture	8.87 ± 0.23
Ash	6.30 ± 0.20
Crude fiber	4.10 ± 0.72
Crude protein	6.92 ± 0.08
Crude fat	7.66 ± 0.02
Total carbohydrate	66.15 ± 0.80

Values are mean ± standard deviation of three different experimental readings.

Table 5 presents the proximate composition of *D. mespiliformis* fruit pulp. The carbohydrate content was found to be 66.15 ± 0.80%, the fat content was 7.66 ± 0.02%, while the protein was 6.92 ± 0.08%. The recorded fiber, ash and moisture contents were 4.10 ± 0.72%, 6.30 ± 0.20% and 8.87 ± 0.23% respectively.

Phytochemicals identified in *D. mespiliformis* fruit pulp extracts

The phytochemical profiles of *D. mespiliformis* fruit pulp extracts were studied by LC-ACQ-QDA-MS and presented in Table 6, Table 7, Table 8, and 9, for 100%, 60%, 50% and aqueous extracts.

These were chosen in accordance to their strong bioactivities, total phenolics contents and total flavonoids content.

Table 6. Phytochemical profile of 100% ethanol extract of *D. mespiliformis* fruit pulp

S/N	Retention time (min)	Propose compound	Molecular formula	Maximum intensity	Peak base (m/z)
1.	2.026	n-Eicosane	$C_{17}H_{34}O_2$	2630.376563	338.882
2.	9.627	n-Eicosane	$C_{20}H_{43}$	9801.687402	367.564
3.	10.156	n-Octadecanoic acid, 2-ox-methyl ester	$C_{19}H_{36}O_3$	1958.368750	293.829
4.	10.389	Tellurium monohydride	HTe	2668.776563	338.832
5.	11.104	Diglycolic acid, ethyl-oct-4-yl ester	$C_{14}H_{26}O_5$	3935.945313	202.752
6.	11.346			4961.280763	267.434
7.	12.604	n-Tetradecane	$C_{14}H_{30}$	4701.420055	567.559
8.	12.710	2-Methylnonadecane	$C_{20}H_{42}$	4210.886003	367.769
9.	14.668	3-Tetradecyne	$C_{14}H_{26}$	5662.244391	293.293
10.	14.961	n-Eicosane	$C_{20}H_{42}$	2884.718372	118.142
11.	15.570	n-Nonadecane	$C_{19}H_{40}$	2579.176562	104.197
12.	16.390	Terpinen-4-ol	$C_{10}H_{18}O$	2809.560937	367.434

Table 7. Phytochemical profile of 60% ethanol extract of *D. mespiliformis* fruit pulp

S/N	Retention time (min)	Propose compound	Molecular formula	Maximum intensity	Peak base (m/z)
1.	1.322	Procyanidin	$C_{30}H_{26}O_{13}$	161802.663775	104.180
2.	1.538	Nonane, 2,6-dimethyl	$C_{11}H_{24}$	242788.364071	338.333
3.	2.316	Allicin	$C_6H_{10}OS_2$	139799.366119	132.198
4.	2.348	n-Tetradecane	$C_{14}H_{30}$	210809.399786	132.189
5.	3.796	Shagaol	$C_{17}H_{24}O_2$	3125.989062	118.159
6.	4.592	Linalool	$C_{10}H_{18}O$	28152.995212	328.278
7.	5.135	Pentadecane,2,6,10-trimethyl	$C_{18}H_{38}$	2734.194313	480.528
	5.558	n-Hexadecane	$C_{16}H_{34}$	7120.295864	116.149
8.	5.651	n-Tridecane	$C_{13}H_{28}$	14813.893750	466.435
9.	6.351	n-Octadecane	$C_{18}H_{38}$	8478.055330	116.328
10.	6.539	n-Hexadecane	$C_{16}H_{34}$	4138.567819	278.949
11.	6.641	Pentadecanoicacid,14-methyl-methylester	$C_{17}H_{34}O_2$	9171.308594	512.472
12.	6.827	n-Tetradecanoic acid	$C_{14}H_{28}O_2$	9365.525000	500.785
13.	7.094	9,12-Hexadecadienoic acid,methylester	$C_{17}H_{30}O_2$	7594.794293	73.689
14.	7.256	14-Octadecenoic acid,methylester	$C_{19}H_{36}O_2$	11976.366406	401.266
15.	7.613	n-Octadecanoic acid,methylester	$C_{19}H_{38}O_2$	3962.442500	280.208
16.	7.741	9-Octadecenoic acid(Z)	$C_{18}H_{34}O_2$	4661.990625	166.950
17.	7.923	n-Octadecanoicacid	$C_{18}H_{36}O_2$	3233.395312	159.926
18.	8.284	cis-9-cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	2495.799579	176.222
19.	8.426	3-Tetradecyne	$C_{14}H_{26}$	16295.544531	478.702
20.	11.304	n-Eicosane	$C_{20}H_{42}$	17352.370312	339.876
21.	14.862	n-Eicosane	$C_{19}H_{40}$	37403.276714	408.667

Table 8. Phytochemical profile of 50% ethanol extract of *D. mespiliformis* fruit pulp

S/N0	**Retention time (min)**	**Propose compound**	**Molecular formula**	**Maximum intensity**	**Peak base (m/z)**
1.	1.322	Procyanidin	$C_{30}H_{26}O_{13}$	161802.663775	104.180
2.	1.538	Nonane, 2,6-dimethyl	$C_{11}H_{24}$	242788.364071	338.333
3.	2.316	Allicin	$C_6H_{10}OS_2$	139799.366119	132.198
4.	2.348	n-Tetradecane	$C_{14}H_{30}$	210809.399786	132.189
5.	3.796	Shagaol	$C_{17}H_{24}O_2$	3125.989062	118.159
6.	4.592	Linalool	$C_{10}H_{18}O$	28152.995212	328.278
7.	5.135	Pentadecane,2,6,10-trimethyl	$C_{18}H_{38}$	2734.194313	480.528
8.	5.558	n-Hexadecane	$C_{16}H_{34}$	7120.295864	116.149
9.	5.651	n-Tridecane	$C_{13}H_{28}$	14813.893750	466.435
10.	6.351	n-Octadecane	$C_{18}H_{38}$	8478.055330	116.328
11.	3 6.539	n-Hexadecane	$C_{16}H_{34}$	4138.567819	278.949
12.	6.641	Pentadecanoic acid,14-methyl-methylester	$C_{17}H_{34}O_2$	9171.308594	512.472
13.	6.827	n-Tetradecanoic acid	$C_{14}H_{28}O_2$	9365.525000	500.785
14.	7.094	9,12-Hexadecadienoic acid, methylester	$C_{17}H_{30}O_2$	7594.794293	73.689
15.	7.256	14-Octadecenoic acid, methylester	$C_{19}H_{36}O_2$	11976.366406	401.266
16.	7.613	n-Octadecanoic acid, methylester	$C_{19}H_{38}O_2$	3962.442500	280.208
17.	7.741	9-Octadecenoic acid(Z)	$C_{18}H_{34}O_2$	4661.990625	166.950
18.	7.923	n-Octadecanoic acid	$C_{18}H_{36}O_2$	3233.395312	159.926
19.	8.284	cis-9-cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	2495.799579	176.222
20.	8.426	3-Tetradecyne	$C_{14}H_{26}$	16295.544531	478.702
21.	11.304	n-Eicosane	$C_{20}H_{42}$	17352.370312	339.876
22.	14.862	n-Eicosane	$C_{19}H_{40}$	37403.276714	408.667

Table 9. Phytochemical profile of aqueous extract of *D. mespiliformis* fruit pulp

S/N	Retention time (min)	Proposed compound	Chemical formula	Max (intensity)	Peak base
1.	1.425	n-Hexadecane	$C_{16}H_{34}$	3529.102386	294.685
2.	1.541	n-Tridecane	$C_{13}H_{28}$	120310.659479	338.281
3.	1.640	n-Hexadecane	$C_{16}H_{34}$	275539.729573	126.171
4.	2.98	n-Eicosane	$C_{20}H_{42}$	35813.315466	294.365
5.	2.339	Dianon	N_6H^{2+}	109917.770397	294.310
6.	2.42	Glutaric acid, monoamideditridecyl ester	$C_{30}H_{56}O_4$	129645.321151	294.233
7.	3.213	L-valine, N-(3-chloro-2-fluoroben-zoyl)-heptadecyl ester	$C_{29}H_{47}ClFNO_3$	5885.616958	280.261
8.	3.582	Fumaric acid	$C_{21}H_{13}ClO_4$	3957.947306	231.700
9.	3.761	Proline	$C_5H_9NO_2$	4740.141848	278.870
10.	4.576	Tetracosane	$C_{23}H_{50}$	21649.941452	328.181
11.	4.951	L-isoleucine	$C_6H_{13}NO_2$	5275.998045	278.896
12.	5.084	Isorhamnetin	$C_{16}H_{12}O_7$	8122.346651	278.924
13.	5.271	Nonane-2,6-dimethyl	$C_{11}N_{24}$	7770.708594	367.269
14.	5.548	Kaempferol	$C^{15}H^{10}O_{11}$	3298.318750	220.517
15.	5.648	L-isoleucine	$C_6H_{13}NO_2$	11844.197656	466.336
16.	5.747	n-Octadecanoic acid, 2-ox-methyl ester	$C_{19}H_{36}O_3$	2806.959093	278.970
17.	6.150	Diglycolic acid, ethyl-oct-4-yl ester	$C_{14}H_{26}O_5$	20041.289241	231.285
18.	6.336	3,8-Dimethylundecane	$C_{13}H_{28}$	2459.210903	278.920
19.	6.535	13-Docosenoicacid	$C_{22}H_{42}O_2$	4832.169176	278.960
20.	6.629	Citral	$C_{10}H_{16}O$	3343.890625	276.720
21.	6.817	6-methyladenine TMS	$C_6H_{10}ClN_3O_2$	3865.28960	260.418
22.	7.189	2,3,4-Trifluorobenzamide	C_7H4F_3NO	2674.804558	278.870
23.	11.300	Diazoxide	$C_8H_7ClN_2O_2S$	3490.318750	262.718
24.	14.857	Paradols	$C_7H_{26}O_3$	11556.00117	240.636
25.	3.582	Fumaric acid	$C_{21}H_{13}ClO_4$	3957.947306	231.700

DISCUSSION

The extraction yield of the extracts presented in Table 1 indicated that the 60% ethanol extract gave the highest yield compared to the other extracts. When compared, all the other extracts had no significant difference as they all had similar extraction yield. This is an indication that the extraction ability of the solvents used in this study are close to one another therefore, were able to cause similar mass movement from the plant matrix into the solvent system, which lead to almost the same extraction yield with the exception of 60% ethanol extract.

Inhibition of the activities of glycosidases located at the brush border cells of the intestinal lumen is one of the gold standards for the control of postprandial hyperglycemia in people living with diabetes mellitus. The decrease observed in IC_{50} values for α-glucosidase activity inhibition is an indication that increase in the ratio of water to ethanol in the binary solvent during the extraction of *D. mespiliformis* fruit pulp, may have eased the release of more bioactive compounds capable of inhibiting the activity of α-glucosidase from the plant material during extraction. Similar reports have been published using plant materials as antidiabetic agents (Madubuike et al., 2018; Mbagwu et al., 2022; Okoduwa et al., 2016). This phenomena was replicated in the TPC and TFC of the extracts as presented in Table 4. The extracted TPC increased with increased water content in the ethanol:water binary solvent from 90% to 50% ethanol, which gave the highest TPC. These finding supported previous studies which reported that binary solvent systems (i.e. ethanol/water) are better for the extraction of phenolic compounds (Jibril et al., 2019; Thoo et al., 2013; Wong et al., 2014). The nature and quantity of phytochemicals extracted from plant materials are affected by the extraction solvent used (Dirar et al., 2019), this was exhibited in the quantity and types of polyphenols extracted as seen in Table 4.

This was clear as the highest TPC was extracted by 50% ethanol while 60% ethanol extracted the highest TFC (Sasadara & Wirawan, 2021). It is worthy to mention that the possible bioactive phytochemical constituents of *D. mespiliformis* fruit pulp used in this study, that inhibited the activity of α-glucosidase are polyphenols in nature since the most potent extracts (i.e. 50% and 60% ethanol) as shown by the IC_{50} values in Table 2 also gave the highest TPC and TFC values respectively as shown in Table 4 (Sukhikh et al., 2023; Umeno et al., 2016). These findings are in consonance with previous studies (Madubuike et al., 2018; Maharaj et al., 2022; Praparatana et al., 2022). In addition, these polyphenols could be 50% hydrophilic and 50% lipophilic based on the result presented in Tables 2 and 4. The lower IC_{50} value of 50% ethanol extract for the inhibition of α-glucosidase means it has the highest inhibition against the activity of α-glucosidase. This is so because the lower the IC_{50} of an active substance, the stronger the potency of that substance in terms of pharmacological effect (Cortés et al., 2001; Rashighi & Harris, 2017; Yung-Chi & Prusoff, 1973). The IC_{50} value of the standard drug acarbose for α-glucosidase inhibition was only 1.03 times more potent than that of 50% ethanol extract, indicating that the 50% ethanol extract of *D. mespiliformis* fruit pulp may contain bioactive phytochemicals with potencies similar to that of the standard drug acarbose in terms of the inhibition of α-glucosidase activities.

As presented in Table 2, unlike the inhibition of α-glucosidase activity, the IC_{50} values for the inhibition of α-amylase activity by *D. mespiliformis* fruit pulp extracts was lowest at 100% ethanol extract and showed the strongest inhibition potential. Dissimilar from α-glucosidase activity inhibition, the IC_{50} values for α-amylase activity inhibition gradually increased with increasing water content of ethanol (i.e. binary solvent system) from 100% ethanol to 50% ethanol, and became much lower with aqueous extract than the ethanol:water binary solvent systems. This observed trend (i.e. reduction of the potency of these extracts with increased polarity of ethanol), could mean the following: 1). That increased polarity of the extraction solvent has inversely reduced the extraction of potent phytochemical constituents from the plant's fruit pulp that could inhibit the activity of α-amylase. 2). That the bioactive phytochemicals in *D. mespiliformis* fruit pulp with the strongest inhibition potentials against α-amylase activity could be less polar, and so, were less extracted by the binary mixtures of ethanol:water, which showed lower potency against the enzyme's activity. 3). It could also mean that the active phytochemical constituents of *D. mespiliformis* fruit pulp with the strongest potency for inhibiting α-amylase activity are lipophilic in nature, therefore, were easily extracted by the less polar extraction solvent (100% ethanol), thereby exhibiting the strongest inhibition potential as shown in Table 2. Previous studies have also explained that plant polyphenols of diverse nature are good inhibitors of α-amylase activity (Aleixandre et al., 2022; Nyambe-Silavwe et al., 2015; Payan, 2004; Zhao et al., 2021).

DPPH radical scavenging capacity assay is frequently used to determine the free radical scavenging ability of various food components (Dorman & Hiltunen, 2004), The antioxidant capacity of *D. mespiliformis* fruit pulp extracts measured in terms of DPPH radical inhibition, also indicated that the 50% and 60% ethanol extracts showed the strongest inhibition potential against the radical, because they both gave the lowest IC_{50} values for this assay as presented in Table 3. These two extracts also gave the highest TPC and TFC values respectively as presented in Table 4. This is a strong indication that the antioxidant compounds in both of these extracts are polyphenols in nature. It also depicts that the polyphenols contents of these bioactive extracts are both lipophilic and hydrophilic in nature (Thoo et al., 2010, 2013). It is also clear that the *D. mespiliformis* fruit pulp extracts contains phytochemicals with diverse polarity, as such, none of the solvent combinations used was able to extract all the phytochemicals of this sample at a time. This observation was also reported by (Thoo et al., 2010). This could also explain why as the polarity of 100% ethanol increases to 50%, during the extraction, both the TPC and TFC values increased and became maximum at 50% and 60% ethanol respectively, which dropped drastically with aqueous extract as seen in Table 4.

The proximate composition of *D. mespiliformis* fruit pulp showed that this fruit is high in crude fiber, ash, fat, and protein content than those reported for the seed of *D. mespiliformis* fruit (Ilouno et al., 2018). The moisture content in this study is similar to that reported for the seed of D. mespiliformis seed by Ilouno et al. (2018), but the carbohydrate content in this study was lower compared to that reported by Ilouno et al. (2018). Due to the varied nature of plants and their chemical compositions, numerous natural compounds like polyphenols, terpenoids, saponins, tannins, mono and polyunsaturated fatty acids, etc. capable of functioning as antioxidants and antidiabetics among others have been reported in plant materials.

The phytochemical profiles *D. mespiliformis* fruit pulp extracts expressed chemical constituents of diverse classes, which are mostly polyphenols, anthocyanins, organosulfur compounds, saturated and unsaturated fatty acids, terpenes, amino and organic acids. The 50% ethanol extract had the highest TPC, and highest inhibition against α-glucosidase activity and DPPH radicals. This extract together with the 60% ethanol extract were found to contain allicin, shagaol, linalool, procyanidin cis-9-cis-12-octadecadienoic acid, 9, 12-hexadecadienoic acid, methyl-ester, 9-octadecenoic acid(Z), 14-octadecenoic acid, methyl-ester and arachidonic among others. This may indicated that the polarities of these two extracts are very close such that they both extracted similar compounds from the plant matrix, which lead to their closeness in the inhibition of α-glucosidase activity DPPH radicals. The polarity of these two extracts may have also contributed to the differences in their TPC and TFC contents, hence, the extraction of more phenolic acids by 50% ethanol but higher flavonoids by 60% ethanol. n-Hexadecane n-octadecanoic, n-nonadecane, were some of the long chain saturated fatty acids also identified in the studied extracts. A good member of the flavonoids family, kaempferol was identified in the aqueous extract of *D. mespiliformis* fruit pulp extract. In his work, Maroyi (2018) have shown that the phytochemical profiles of *Diospyros lycoides* revealed some of the chemical compounds identified in this work.

Studies have reported that shagaol, allicin and procyanidin which are some of the identified constituent of *D. mespiliformis* fruit pulp, exhibited antidiabetic, anti-inflammatory, antioxidant and anti-tumor properties (Annamalai & Suresh, 2018; Bak et al., 2012; Chung, 2006; Dasiman et al., 2022; Dugasani et al., 2010; Elkayam et al., 2003; Gong-chen et al., 2014; Gruhlke et al., 2017; Jiao et al., 2023; Nadeem et al., 2022; Rauf et al., 2019; Wei et al., 2017; Wood et al., 2002; Yi et al., 2019). Another study demonstrated that shagaol prevented oral squamous cell carcinoma by activating pro-apoptotic factors in in vitro and in vivo experimental model (Annamalai & Suresh, 2018). Linalool a triterpene alcohol is also a strong antidiabetic molecule identified in the fruit pulp of *D. mespiliformis* used in this study. It was found to facilitate glucose uptake and metabolism by cells (Al-Kury et al., 2022; More et al., 2014). These studies are strong indications that the strong antidiabetic activities exhibited by the 50% ethanol fruit pulp extract may have also been contributed by shagaol and linalool. Cis-9-cis-12-octadecadienoic acid (alpha-linoleic acid), 9, 12-hexadecadienoic acid, methyl ester, 9-octadecenoic acid(Z) and 14-octadecenoic acid, methylester were some of the essential fatty acids also identified in this study. Previous studies have also identified these compounds from other plant species and their antioxidant, antidiabetic, etc., potentials (Alqahtani et al., 2019; B. R. Kim et al., 2020; Kirmizigül et al., 2007; Mazumder et al., 2020).

CONCLUSION

The studied *D. mespiliformis* fruit pulp extracts exhibited very good antidiabetic and antioxidant potentials especially the inhibition of α-glucosidase activity and DPPH radical by the 50% ethanol extract. The extracts are also rich in polyphenols, and other compounds including allicin, shagaol, linalool, procyanidin, cis-9-cis-12-octadecadienoic acid, 9, 12-hexadecadienoic acid, methylester, 9-octadecenoic acid(Z), 14-octadecenoic acid, methylester. This study also proved that the inhibition of carbohydrate digestion, thereby delaying its absorption is one of the mechanisms by which plants phytochemicals controls hyperglycemia and diabetes mellitus. Therefore, *D. mespiliformis* fruit pulp could be an excellent raw material for functional food and nutraceutical industries.

Author's Contribution

Muhammad Mustapha Jibril conceived the whole idea of the research, wrote the proposal of and protocol of the research. He wrote the manuscript and did the editing as well. M. M. Jibril also guided and supervised the work of individual co-authors during the research work. Wasila Tanko Muhammad performed the phytochemical profiling of the extracts and data processing. Kamaladdeen Idris did the in vitro antidiabetic assays, Muhammad Hamisu Usman did the quantification of polyphenols and antioxidant assay. All the authors did the extraction and proximate composition experiments together.

REFERENCES

Adu, O. T., Naidoo, Y., Lin, J., Adu, T. S., Sivaram, V., Dewir, Y. H., & El-Banna, A. N. (2022). Phytochemical Screening and Biological Activities of Diospyros villosa (L.) De Winter Leaf and Stem-Bark Extracts. *Horticulturae*, *8*(10). https://doi.org/10.3390/horticulturae8100945

Ahmad Wani, S., Kour, J., Haq, R., & Elshikh, M. S. (2023). Fruits and Their Roles in Nutraceuticals and Functional Foods. In

Fruits and Their Roles in Nutraceuticals and Functional Foods (Issue May). https://doi.org/10.1201/9781003259213

Al Kury, L. T., Abdoh, A., Ikbariah, K., Sadek, B., & Mahgoub, M. (2022). In vitro and in vivo antidiabetic potential of monoterpenoids: An update. *Molecules*, *27*(1), 1–29. https://doi.org/10.3390/molecules27010182

Aleixandre, A., Gil, J. V., Sineiro, J., & Rosell, C. M. (2022). Understanding phenolic acids inhibition of α-amylase and α-glucosidase and influence of reaction conditions. *Food Chemistry*, *372*. https://doi.org/10.1016/j.foodchem.2021.131231

Alqahtani, F. Y., Aleanizy, F. S., Mahmoud, A. Z., Farshori, N. N., Alfaraj, R., Al-sheddi, E. S., & Alsarra, I. A. (2019). Chemical composition and antimicrobial, antioxidant, and anti-inflammatory activities of Lepidium sativum seed oil. *Saudi Journal of Biological Sciences*, *26*(5), 1089–1092. https://doi.org/10.1016/j.sjbs.2018.05.007

Annamalai, G., & Suresh, K. (2018). [6]-Shogaol attenuates inflammation, cell proliferation via modulate NF-κB and AP-1 oncogenic signaling in 7,12-dimethylbenz[a]anthracene induced oral carcinogenesis. *Biomedicine and Pharmacotherapy*, *98*(October 2017), 484–490. https://doi.org/10.1016/j.biopha.2017.12.009

Bak, M. J., Ok, S., Jun, M., & Jeong, W. S. (2012). 6-Shogaol-Rich Extract From Ginger Up-Regulates the Antioxidant Defense Systems in Cells and Mice. *Molecules*, *17*(7), 8037–8055. https://doi.org/10.3390/molecules17078037

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Cavaliere, C., Capriotti, A. L., La Barbera, G., Montone, C. M., Piovesana, S., & Laganà, A. (2018). Liquid chromatographic strategies for separation of bioactive compounds in food matrices. *Molecules*, *23*(12). https://doi.org/10.3390/molecules23123091

Chung, L. Y. (2006). The antioxidant properties of garlic compounds: Alyl cysteine, alliin, allicin, and allyl disulfide. *Journal of Medicinal Food*, *9*(2), 205–213. https://doi.org/10.1089/jmf.2006.9.205

CORTÉS, A., CASCANTE, M., CÁRDENAS, M. L., & CORNISH-BOWDEN, A. (2001). Relationships between inhibition constants, inhibitor concentrations for 50% inhibition and types of inhibition: new ways of analysing data. *Biochemical Journal*, *357*(1), 263–268. https://doi.org/10.1042/bj3570263

Dasiman, R., Nor, N. M., Eshak, Z., Mutalip, S. S. M., Suwandi, N. R., & Bidin, H. (2022). A review of procyanidin: Updates on current bioactivities and potential health benefits. *Biointerface Research in Applied Chemistry*, *12*(5), 5918–5940. https://doi.org/10.33263/BRIAC125.59185940

Dirar, A. I., Alsaadi, D. H. M., Wada, M., Mohamed, M. A., Watanabe, T., & Devkota, H. P. (2019). Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *South African Journal of Botany*, *120*, 261–267. https://doi.org/10.1016/j.sajb.2018.07.003

Dorman, H. J. D., & Hiltunen, R. (2004). Fe(III) reductive and free radical-scavenging properties of summer savory (Satureja hortensis L.) extract and subfractions. *Food Chemistry*, *88*(2), 193–199. https://doi.org/10.1016/j.foodchem.2003.12.039

Dugasani, S., Pichika, M. R., Nadarajah, V. D., Balijepalli, M. K., Tandra, S., & Korlakunta, J. N. (2010). Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. *Journal of Ethnopharmacology*, *127*(2), 515–520. https://doi.org/10.1016/j.jep.2009.10.004

Ebbo, A. A., Sani, D., Suleiman, M. M., Ahmed, A., & Hassan, A. Z. (2019). Phytochemical composition, proximate analysis and antimicrobial screening of the methanolic extract of diospyros mespiliformis Hochst ex a. DC (Ebenaceae). *Pharmacognosy Journal*, *11*(2), 362–368. https://doi.org/10.5530/pj.2019.11.55

Elkayam, A., Mirelman, D., Peleg, E., Wilchek, M., Miron, T., Rabinkov, A., Oron-Herman, M., & Rosenthal, T. (2003). The Effects of Allicin on Weight in Fructose-Induced Hyperinsulinemic, Hyperlipidemic, Hypertensive Rats. *American Journal of Hypertension*, *16*(12), 1053–1056. https://doi.org/10.1016/j.amjhyper.2003.07.011

Folin, O. and Ciocalteu, V. (1927). On Tyrosine and Tryptophan Determinations in Proteins. The Journal of Biological Chemistry, 73, 627-650.

The Journal of Biological Chemistry, 73, 627-650. Gong-chen, W., Lu-lu, H., Jing, W., Wan-nan, L., Chuan-yi, P., & Yan-fei, L. (2014). Effects of Allicin on Lipid Metabolism and Antioxidant Activity in Chickens. *Journal of Northeast Agricultural University (English Edition)*, *21*(3), 46–49. https://doi.org/10.1016/s1006-8104(14)60068-4

Gruhlke, M. C. H., Nicco, C., Batteux, F., & Slusarenko, A. J. (2017). The effects of allicin, a reactive sulfur species from garlic, on a selection of mammalian cell lines. *Antioxidants*, *6*(1), 1–16. https://doi.org/10.3390/antiox6010001

Ilouno, E. L., Omaji, O. G., & Anthony, O. E. (2018). Proximate, mineral and Anti-Nutritional Composition of Jackal Berry (Diospyros Mespiliformis) Seeds. *FUDMA Journal of Sciences (FJS) ISSN: 2616-1370 Vol. 2 No. 1, April, 2018, Pp 75-79 PROXIMATE, 2*(1), 75–79.

Jiao, W., Sang, Y., Wang, X., & Wang, S. (2023). Effects of 6-Shogaol on Glucose Uptake and Intestinal Barrier Integrity in Caco-2 Cells. *Foods*, *12*(3). https://doi.org/10.3390/foods12030503

Jibril, M. M., Abdul-Hamid, A., Ghazali, H. M., Dek, M. S. P., Ramli, N. S., Jaafar, A. H., Karrupan, J., & Mohammed, A. S. (2019). Antidiabetic Antioxidant and Phytochemical Profile of Yellow-Fleshed Seeded Watermelon (*Citrullus Lanatus*) Extracts. *Journal of Food and Nutrition Research, Vol. 7, 2019, Pages 82-95*, *7*(1), 82–95. https://doi.org/10.12691/JFNR-7-1-10

Kim, B. R., Kim, H. M., Jin, C. H., Kang, S. Y., Kim, J. B., Jeon, Y. G., Park, K. Y., Lee, I. S., & Han, A. R. (2020). Composition and antioxidant activities of volatile organic compounds in radiation-bred coreopsis cultivars. *Plants*, *9*(6). https://doi.org/10.3390/plants9060717

Kim, Y. M., Jeong, Y. K., Wang, M. H., Lee, W. Y., & Rhee, H. I. (2005). Inhibitory effect of pine extract on α-glucosidase activity and postprandial hyperglycemia. *Nutrition*, *21*(6), 756–761. https://doi.org/10.1016/j.nut.2004.10.014

Kirmizigül, S., Böke, N., Sümbül, H., Göktürk, R. S., & Arda, N. (2007). Essential fatty acid components and antioxidant activities of eight Cephalaria species from southwestern Anatolia. *Pure and Applied Chemistry*, *79*(12), 2297–2304. https://doi.org/10.1351/pac200779122297

Li Fu, Bo-Tao Xu, Xiang-Rong Xu, Xin-Sheng Qin, R.-Y. G. and H.-B. L. (2010). Antioxidant Capacities and Total Phenolic Contents of 56 Wild Fruits from South China. *Molecules*, *15*, 8602–8617. https://doi.org/10.3390/molecules15128602

Li, Y., Zhang, J., Xu, D., Zhou, T., Zhou, Y., Li, S., & Li, H. (2016). *Bioactivities and Health Benefits of Wild Fruits*. https://doi.org/10.3390/ijms17081258

Madubuike, K. G., Nnadi, C. O., Anaga, A. O., & Asuzu, I. U. (2018). *Bioactivity-guided isolation of the antidiabetic principle in Pterocarpus Santalinoides leaf extract*. 1–11.

Maharaj, V., Ezeofor, C. C., Naidoo Maharaj, D., Muller, C. J. F., & Obonye, N. J. (2022). Identification of Antidiabetic Compounds from the Aqueous Extract of Sclerocarya birrea Leaves. *Molecules*, *27*(22), 1–19. https://doi.org/10.3390/molecules27228095

Maroyi, A. (2018). Diospyros lycioides Desf.: Review of its botany, medicinal uses, pharmacological activities and phytochemistry. *Asian Pacific Journal of Tropical Biomedicine*, *8*(2), 130–136. https://doi.org/10.4103/2221-1691.225617

Mazumder, K., Nabila, A., Aktar, A., & Farahnaky, A. (2020). Bioactive variability and in vitro and in vivo antioxidant activity of unprocessed and processed flour of nine cultivars of Australian lupin species: A comprehensive substantiation. *Antioxidants*, *9*(4). https://doi.org/10.3390/antiox9040282

Mbagwu, I. S., Mbanefo, M., Orji, U. H., Idokoja, L. O., & Ajaghaku, D. L. (2022). Extraction solvent polarity affects the antidiabetic activity of Dioscorea bulbifera L. (Dioscoreaceae) Tuber. *Journal of Pharmacognosy and*

Phytochemistry, *11*(4), 202–207. https://doi.org/10.22271/phyto.2022.v11.i4c.14465

McCue, P., & Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. *Asia Pacific Journal of Clinical Nutrition*. http://www.apjcn.org/update/pdf/2004/1/101-106/101.pdf

Mohammed, A. A., Ahmed, K. S., Sulimam S, I., Amna, A., Omer, S. and Mohammed, G. (2016).

In vitro Antioxidant Activity, Phytochemical analysis and Cytotoxicity of *Diospyros mespiliformis*. *International Journal of Botany Studies*, 1(1): 23-28.

More, T. A., Kulkarni, B. R., Nalawade, M. L., & Arvindekar, A. U. (2014). Antidiabetic activity of linalool and limonene in streptozotocin- induced diabetic rat: A combinatorial therapy approach. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(8), 159–163.

Muredzi, P. (2013). Nutraceuticals and Disease. In P. Muredzi (Ed.), *Food is Medicine - An Introduction to Nutraceuticals* (pp. 29–47). LAP Lambert Academic Publishing.

Nadeem, M. S., Kazmi, I., Ullah, I., Muhammad, K., & Anwar, F. (2022). Allicin, an antioxidant and neuroprotective agent, ameliorates cognitive impairment. *Antioxidants*, *11*(1). https://doi.org/10.3390/antiox11010087

Nielsen, S. Suzanne (Purdue University West Lafayette, IN, U. (2014). *Food Analysis* (U. Nielsen, S. Suzanne (Purdue University West Lafayette, IN (ed.); Fourth). Springer Science+Business Media. https://doi.org/10.1007/978-1-4419-1478-1

Nyambe-Silavwe, H., Villa-Rodriguez, J. A., Ifie, I., Holmes, M., Aydin, E., Jensen, J. M., & Williamson, G. (2015). Inhibition of human α-amylase by dietary polyphenols. *Journal of Functional Foods*, *19*, 723–732. https://doi.org/10.1016/j.jff.2015.10.003

Okoduwa, S. I. R., Umar, I. A., James, D. B., Inuwa, H. M., & Habila, J. D. (2016). Evaluation of extraction protocols for anti-diabetic phytochemical substances from medicinal plants. *World Journal of Diabetes*, *7*(20), 605. https://doi.org/10.4239/wjd.v7.i20.605

Payan, F. (2004). Structural basis for the inhibition of mammalian and insect alpha-amylases by plant protein inhibitors. *Biochimica et Biophysica Acta*, *1696*(2), 171–180. https://doi.org/10.1016/j.bbapap.2003.10.012

Praparatana, R., Maliyam, P., Barrows, L. R., & Puttarak, P. (2022). Flavonoids and Phenols, the Potential Anti-Diabetic Compounds from Bauhinia strychnifolia Craib. Stem. *Molecules*, *27*(8), 1–16. https://doi.org/10.3390/molecules27082393

Rajasekaran, A., & Xavier, R. (2015). *Nutraceuticals as therapeutic agents : A Review Nutraceuticals as therapeutic agents : A Review*. *January 2008*.

Rashighi, M., & Harris, J. E. (2017). 乳鼠心肌提取 HHS Public Access. *Physiology & Behavior*, *176*(3), 139–148. https://doi.org/10.1053/j.gastro.2016.08.014.CagY

Rauf, A., Imran, M., Abu-Izneid, T., Iahtisham-Ul-Haq, Patel, S., Pan, X., Naz, S., Sanches Silva, A., Saeed, F., & Rasul Suleria, H. A. (2019). Proanthocyanidins: A comprehensive review. *Biomedicine and Pharmacotherapy*, *116*(May). https://doi.org/10.1016/j.biopha.2019.108999

Rauf, A., Uddin, G., Patel, S., Khan, A., Halim, S. A., Bawazeer, S., Ahmad, K., Muhammad, N., & Mubarak, M. S. (2017). Diospyros, an under-utilized, multi-purpose plant genus: A review. *Biomedicine and Pharmacotherapy*, *91*, 714–730. https://doi.org/10.1016/j.biopha.2017.05.012

Sasadara, M. M. V., & Wirawan, I. G. P. (2021). Effect of extraction solvent on total phenolic content, total flavonoid content, and antioxidant activity of Bulung Sangu (Gracilaria sp.) Seaweed. *IOP Conference Series: Earth and Environmental Science*, *712*(1). https://doi.org/10.1088/1755-1315/712/1/012005

Sukhikh, S., Babich, O., Prosekov, A., Kalashnikova, O., Noskova, S., Bakhtiyarova, A., Krol, O., Tsvetkova, E., & Ivanova, S. (2023). Antidiabetic Properties of Plant Secondary Metabolites. *Metabolites*, *13*(4). https://doi.org/10.3390/metabo13040513

Thoo, Y. Y., Ho, S. K., Abas, F., Lai, O. M., Ho, C. W., & Tan, C. P. (2013). Optimal binary solvent extraction system for phenolic antioxidants from mengkudu (morinda citrifolia) fruit. *Molecules*, *18*(6), 7004–7022. https://doi.org/10.3390/molecules18067004

Thoo, Y. Y., Ho, S. K., Liang, J. Y., Ho, C. W., & Tan, C. P. (2010). Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (Morinda citrifolia). *Food Chemistry*, *120*(1), 290–295. https://doi.org/10.1016/j.foodchem.2009.09.064

Umeno, A., Horie, M., Murotomi, K., Nakajima, Y., & Yoshida, Y. (2016). Antioxidative and antidiabetic effects of natural polyphenols and isoflavones. *Molecules*, *21*(6). https://doi.org/10.3390/molecules21060708

Vattem, D. A., & Maitin, V. (2016). Modulation of Redox Homeostasis by Lamiaceae Herbs. In P. D. T. S. U. Professor Dhiraj A. Vattem, Ph.D. (TEXAS STATE UNIVERSITY) AND Vatsala Maitin (Ed.), *FUNCTIONAL FOODS, NUTRACEUTICALS AND NATURAL PRODUCTS - CONCEPTS AND APPLICATIONS* (FIRST, pp. 287–295). DEStec h Publications , Inc.

Wang, J., Sun, B., Cao, Y., Tian, Y., & Li, X. (2008). Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry*, *106*(2), 804–810. https://doi.org/10.1016/j.foodchem.2007.06.062

Wei, C. K., Tsai, Y. H., Korinek, M., Hung, P. H., El-Shazly, M., Cheng, Y. Bin, Wu, Y. C., Hsieh, T. J., & Chang, F. R. (2017). 6-Paradol and 6-Shogaol, the Pungent Compounds of Ginger, Promote Glucose Utilization in Adipocytes and Myotubes, and 6-Paradol Reduces Blood Glucose in High-Fat Diet-Fed Mice. *International Journal of Molecular Sciences*, *18*(1), 1–18. https://doi.org/10.3390/ijms18010168

Wong, Y. H., Lau, H. W., Tan, C. P., Long, K., & Nyam, K. L. (2014). Binary solvent extraction system and extraction time effects on phenolic antioxidants from kenaf seeds (Hibiscus cannabinus L.) extracted by a pulsed ultrasonic-assisted extraction. *The Scientific World Journal*, *2014*(January). https://doi.org/10.1155/2014/789346

Wood, J. E., Senthilmohan, S. T., & Peskin, A. V. (2002). Antioxidant activity of procyanidin-containing plant extracts at different pHs. *Food Chemistry*, *77*(2), 155–161. https://doi.org/10.1016/S0308-8146(01)00329-6

Xia, E. Q., Deng, G. F., Guo, Y. J., & Li, H. Bin. (2010). Biological activities of polyphenols from grapes. *International Journal of Molecular Sciences*, *11*(2), 622–646. https://doi.org/10.3390/ijms11020622

Yi, J. K., Ryoo, Z. Y., Ha, J. J., Oh, D. Y., Kim, M. O., & Kim, S. H. (2019). Beneficial effects of 6-shogaol on hyperglycemia, islet morphology and apoptosis in some tissues of streptozotocin-induced diabetic mice. *Diabetology and Metabolic Syndrome*, *11*(1), 1–14. https://doi.org/10.1186/s13098-019-0407-0

Yung-Chi, C., & Prusoff, W. H. (1973). Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochemical Pharmacology*, *22*(23), 3099–3108. https://doi.org/10.1016/0006-2952(73)90196-2

Zhao, Y., Wang, M., & Huang, G. (2021). Structure-activity relationship and interaction mechanism of nine structurally similar flavonoids and α-amylase. *Journal of Functional Foods*, *86*(July), 104739. https://doi.org/10.1016/j.jff.2021.104739