Trop J Phytochem Pharm Sci, May 2025; 4(5): 216 - 224 **ISSN 2955-1226 (Print)**
ISSN 2955-123(Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

Effect of Blanching, and Solvents on the Phytochemical Composition, Cytotoxicity and Digestive Enzyme Inhibition of Conventional and Wild Mango Kernels

Godwin A Audu*, Sunday S. Arogba

Department of Biochemistry, Faculty of Natural Sciences, Prince Abubakar Audu University, PMB 1008 Anyigba, Kogi State, Nigeria.

ABSTRACT

Natural tropical vegetation provides its peculiar varieties of plant sources with possible alternative efficacy to orthodox medicine in the management of some nutritional disorders, diabetes type II and obesity inclusive. Conventional mango (*Mangifera indica*) and wild mango (*Irvingia* species) kernels are still optimally under-utilized in nutraceutical applications. Effects of blanching temperature, blanching water, and other solvent extracts of the blanched kernels on cytotoxicity of brine shrimp larvae and levels of inhibition of α-amylase, α-glucosidase, and lipase were investigated *ex-vivo*. The blanching water extracts were rich in both phenolic acids and saponins in the range of 11-63 mg/g dry wt. kernels, while flavonoids, tannins, glycosides and alkaloid contents were very low. Only alkaloids available as 6 to 10 mg/g dry wt. exhibited relatively high hydrophobicity, compared with the other phytoconstituents, and had no apparent toxic effects on brine shrimp larvae ($p > 0.05$). Blanching water and ethanol extracts from the three blanched kernel types (*Mangifera indica*, *Irvingia wombolu* and *Irvingia gabonensis*) studies exhibited both cytotoxicity (LC_{50} 375.3 to 624.2 µg/mL) and digestive enzyme inhibitory effect (IC_{50} 66.0 to 269.4 µg/mL). Filtered 'off-the-shelf' yeast tablets purchased, and employed in inhibitory study exhibited similar α-glucosidase activity as the expensive reference source. Conclusively, use of blanching water was the most efficient solvent among options, for intended medicinal formulations because its ready availability, cost and high hydrophilicity of bioactive phytochemicals of *Mi, Iw, and Ig* kernels. Residual constituents in the blanched kernels would be good functional ingredients in food processing for their antioxidant capability.

Keywords: *Mangifera*/*Irvingia* Kernels, Phytoconstituents, Digestive enzymes, Cytotoxicity, Inhibition, Yeast α-glucosidase.

Received 01 May 2025
Revised 27 May 2025
Accepted 31 May 2025
Published online 01 June 2025

Introduction

Metabolic disorders such as diabetes and obesity are rapidly increasing worldwide, necessitating the search for safer and more effective natural therapeutic alternatives to conventional drugs. Conventional mango (*Mangifera indica*) belongs to the family of *Anarcadiaceae* while wild mango species (*Irvingia wombolu* and *Irvingia gabonensis*) popularly called African mango, bush mango, dika nut or ogbono belongs to the *Irvingiacaea* family. They are tropical plants that bare fruits which are widely consumed for their nutritional benefits.[1] However, their kernels remain largely underutilized despite being rich in bioactive compounds, such as soluble phenolics, flavonoids, tannins, saponins, glycosides, and alkaloids, which have been associated with antioxidant, anti-inflammatory, and anti-diabetic properties.[2,3] Despite these medicinal properties, the kernels are often discarded as agricultural waste,[4] highlighting the need for research into their potential as functional food items and nutraceuticals.[5,6]

*Corresponding author. Email: audu.ga@ksu.edu.ng

Tel: +2347016884122

Citation: Audu GA, Arogba SS. Effect of Blanching, and Solvents on the Phytochemical Composition, Cytotoxicity and Digestive Enzyme Inhibition of Conventional and Wild Mango Kernels. Trop J Phytochem Pharm Sci. 2025; 4 (5): 216 – 224 http://www.doi.org/10.26538/tjpps/v4i5.4

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The bioactivity of plant extracts is highly dependent on the method and solvent used for extraction, which influences the yield and composition of the phytochemicals obtained.[7] Blanching and solvent extraction techniques,[8] can enhance the concentration of bioactive compounds. A preliminary study on the safety of the kernel extracts is recommended. Hence, cytotoxicity assay using brine shrimp larvae[9] could be conducted. Efforts to balance therapeutic efficacy with safety is fundamental for the development of natural enzyme inhibitors as potential pharmaceutical or nutraceutical agents. One of the key therapeutic potentials of these kernel extracts lies in their ability to inhibit digestive enzymes such as α-amylase, α-glucosidase, and lipase, which play crucial roles in carbohydrate and lipid metabolism. The inhibition of these enzymes can help regulate postprandial glucose levels and fat absorption, providing a promising strategy for managing metabolic disorders such as type 2 diabetes and obesity.[10]

Bioactive compounds in fruits and seeds have shown modulatory effects on key digestive enzymes—namely, α-amylase, α-glucosidase, and lipase—which are essential for carbohydrate and lipid digestion. Agada *et al*.[11] demonstrated that hexane and ethyl acetate extracts from *Carica papaya* (pawpaw) seeds significantly inhibited α-amylase and α-glucosidase. However, studies on the extracts from the kernels of *M. indica, I. wombolu* and *I. gabonensis* have not been quantified for their phyto-constituents, cytotoxicity, and their enzyme inhibitory activity on selected digestive enzymes which this report addresses. Poovitha and Parani[12] reported that protein extracts from *Momordica charantia* (bitter gourd) effectively reduced the activities of these enzymes, suggesting potential benefits in controlling postprandial blood glucose levels. In addition, studies have shown that extracts from grape seeds, green tea, and white tea, which are rich in catechins, exhibit potent inhibition of these enzymes, with grape seed extract displaying activity comparable to acarbose[13]. Islam *et al*.[14] further revealed that by-products from various fruits, including peels and seeds, possess strong

antioxidant properties and significant α-glucosidase inhibitory activity, sometimes even outperforming the edible portions of the fruits.

Beyond the inhibition of carbohydrate-digesting enzymes, natural products from fruits and seeds also impact lipase activity, which is crucial for lipid digestion. Sosnowska *et al*.[15] evaluated 31 fruit extracts and found that extracts from chokeberry (*Aronia melanocarpa*), red gooseberry (*Ribes uva-crispa*), and red currant (*Ribes rubrum*) substantially reduced pancreatic lipase activity, thereby potentially lowering fat absorption and aiding in obesity management. Furthermore, Hadrich *et al*.[16] reported that pomegranate (*Punica granatum*) peel extracts, particularly the ethanol fraction, completely inhibited pancreatic lipase at 1 mg/mL after 30 minutes of incubation. Research by De Pradhan *et al*.[17] on *Mangifera indica* pulp extracts identified metabolites such as gallic acid and certain amino acids that inhibit lipase activity *in-vitro*, with gallic acid showing comparable effects to the commercial inhibitor orlistat. Furthermore, Arogba[18] had demonstrated that polyphenolic compounds in the testa of the intact wild mango (*Irvingia gabonensis*) kernel had significant inhibitory property in delaying enzymic browning by 64% caused by polyphenol oxidase (PPO) system. Collectively, these studies highlighted the diverse sources and significant inhibitory potential of natural products from fruits and seeds on digestive enzymes, offering promising avenues for the development of alternative therapeutic strategies for metabolic disorders.

Digestive enzymes such as α-amylase, α-glucosidase, and lipase are crucial for breaking down macronutrients into absorbable units, supporting digestion and nutrient assimilation. While these enzymes are traditionally derived from animal tissues, alternative sources have been identified in plants, microorganisms, and insects. α-amylase primarily hydrolyzes starch into simpler sugars. It is conventionally found in mammalian pancreatic secretions and saliva.[19] However, it is also present in plants, animals, and microorganisms.[20] Microbial sources, particularly *Aspergillus oryzae, A. niger,* and *A. awamori*, are widely utilized for commercial production due to their high yield and stability.[21] α-glucosidase facilitates carbohydrate digestion by breaking down disaccharides into glucose. While typically sourced from mammalian intestinal tissues, it is also found in yeast, fungi, bacteria, plants, archaea, and animals.[22] Moreover, some edible insects contain α-glucosidase inhibitory peptides, highlighting their potential for novel enzyme inhibitors.[23] Lipase catalyzes fat hydrolysis into glycerol and free fatty acids. Although the pancreas is the primary source, this enzyme is also expressed in liver tissue. Microbial lipases from filamentous fungi, yeasts, and bacteria are particularly valued in industrial applications due to their catalytic versatility and stability.[24] Additionally, plant seeds serve as an emerging source of lipases, with potential applications in food and agriculture. [25]

Biochemical characteristics of enzymes include Michaelis–Menten constant (Km) and maximum reaction velocities (Vmax) as kinetic parameters for better understanding of enzyme-substrate interactions. These parameters can be determined using graphical methods like the Lineweaver–Burk plot, which helps analyze enzyme kinetics and assess catalytic efficiency.[26] Other characterizations include optimum pH, temperature, and incubation time determinations. Orthodox research studies employ use of expensive commercial reagents as enzyme kits, which might not be readily available at time of need. On the contrary, studies on the alternative sources could provide viable and effective similar functions.

Efforts to Balance therapeutic efficacy with safety is fundamental for the development of natural enzyme inhibitors as potential pharmaceutical or nutraceutical agents.

In this study, a commercial yeast source was investigated as a possible, viable and cheaper source than a reference enzyme kit source for α-glucosidase activity. This alternative source was also for the first time characterized in respect of Vmax, Km, optimum pH, time, temperature, substrate and enzyme concentrations, using the crude and purified extracts of the yeast. In addition, the effects of blanching temperature and solvent polarity on the extracts of *Mangifera indica (Mi), Irvingia wombolu (Iw), and Irvingia gabonensis (Ig)* kernel were analyzed for their phytochemical constituents, cytotoxicity and inhibitory effects on selected digestive enzymes (α-amylase, α-glucosidase and lipase). The findings could provide baseline information for the utilization of these kernels in developing functional food and plant-derived enzyme inhibitors for managing metabolic disorders, as well as on-site application of yeast α-glucosidase in laboratory studies.

Materials and Methods

Sample Collection and Preparation: Mature kernels of *Mi*, *Iw*, and *Ig* were plucked directly from trees in Anyigba (7º 28' 51.39" N and 7º 11' 14.86" E) Kogi State, Nigeria. The plants of *Mi, Iw, and Ig* were authenticated with voucher numbers (KSU-PT-B-201), (PAAU/PSB/HNO/2172) and (KSU-PT-B-177) respectively at the department of Plant Science and Biotechnology, Faculty of Natural Sciences, Prince Abubakar Audu University (formerly, Kogi State University), Anyigba, Nigeria. The seeds were dissected using a stainless-steel knife to obtain the kernels and soaked immediately in sulphited water according to the method of Arogba.[5]

Effect of temperature on phytochemical yield

a. Blanching of the kernels: For clarity, the adhering testa on the wild mango kernels (*Iw*, *Ig*) were left intact during blanching, unlike the conventional mango kernel. The methodology of Arogba[5] with some modification, was adopted for blanching. For each kernel type, 100 g of the kernels were blanched at 95 ºC in 250 mL of sulphited water (potassium metabisulphite) in a stainless-steel bowl for 4 min. after which the blanched kernels were cooled immediately in ice. The blanching water was collected, de-solventized and stored as sample at 4 ºC for further analysis, while the blanched kernels were pulverized and preserved for further extraction of residual bioactive compounds.
b. Ambient Temperature Extraction: Similar treatments as above, was employed at ambient temperature (28º ± 2 ºC).

Effect of solvents on phytochemical yield: The Soxhlet extraction technique, as described by Dai & Mumper,[27] was utilized for the solvent extraction of the blanched kernels of *Mi*, *Iw* and *Ig*. Each kernel powder (100 g) was extracted with 300 mL of a solvent type (aqueous, ethanol, or n-hexane) in a 500 mL round-bottom flask. The extraction was conducted at 95 °C of water, 78 ºC of 99% ethanol or 68 °C of n-hexane for 6 h. The extracts were concentrated on regulated water-bath and the dried extracts were kept in amber glass bottles at 4 °C until subsequent analyses.

Phytoconstituent quantitation: The extracts of blanched kernels of *Mi*, *Iw*, *Ig*, and the extracts from blanching water were labelled as 'test' samples against 'standard' samples in all determinations conducted below.

a. Total soluble phenolic content: The Folin-Ciocalteu assay method was adopted[1]. All analyses were performed in triplicate. The total phenolic content of the samples was calculated from a calibration curve derived using gallic acid standard and expressed thus (equation 1):

$$Gallic\ acid\ eqv.(mg/g\ dry\ wt.) = cV/m \ldots\ldots\ldots\ldots\ldots (1)$$

Where: c = concentration of gallic acid extrapolated from the calibration curve (mg/ml), V = volume of sample extract (mL), m = weight of the sample taken.

b. Total flavonoid content: It was determined using the aluminum chloride colorimetric method[28] and was modified.[1] The total flavonoid content was calculated similarly from the equation 2:

$$F = qV/w \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where: F = total content of flavonoid compounds in quercetin equivalent, q = concentration of quercetin extrapolated from the reference curve, V= volume of extract (mL), w = weight of the sample.

c. Total tannin content: The vanillin method was employed[29] and the total tannin content was calculated as equation 3:

$$T = cV/w \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where: T = total content of tannin compounds in catechin equivalent (mg CaE/g dry wt), c = concentration of catechin extrapolated from the reference curve, V= volume of extract (mL) and w = weight of the sample.

d. Saponin content: Saponins were quantified using vanillin-sulfuric acid method,[30] with diosgenin as the reference standard, using the formula (equation 4):

$$S = cV/w \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where: S = total content of saponin compounds in diosgenin equivalent, c = concentration of diosgenin extrapolated from the reference curve, V= volume of extract (mL), w = weight of the sample

e. Glycoside content: Glycosides quantitation (equation 5) was conducted using a colorimetric method of Harborne.[31]

Given:

$$G = cV/w \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where: G = total content of glycosides compounds in linamarin equivalent, c = concentration of linamarin extrapolated from the reference curve, V= volume of extract (ml), w = weight of the sample.

f. Alkaloid content: A precipitation method was employed.[31] The alkaloid content was calculated with the equation 6 below:

$$\%\ Alkaloid = \frac{W2-W1}{Weight\ of\ sample}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Where W_2 = Weight of filter paper + alkaloid; W_1 = Initial weight of filter paper

Cytotoxicity bioassay: The cytotoxicity bioassay was carried out *ex-vivo* using brine shrimps (*Artemia salina*) larvae[1] after hatching the eggs. The percentage lethality for each concentration was calculated using the formula (equation 7) below while LC_{50} was derived as reported by Asomugha. [32]

$$\%\ Lethality = \frac{Number\ of\ dead\ shrimps}{Number\ of\ surviving\ shrimps\ in\ control}\ x\ 100 \ldots\ldots\ldots (7)$$

Enzyme inhibitory assay: α-amylase, α-glucosidase (commercial, yeast) and lipase activity were assessed as described below (equation 8). Absorbance was measured on spectrophotometer (Pc-Medical USA 752N) at appropriate λ max. In all instances;

$$\%\ Inhibition = \frac{\boldsymbol{Abs(control) - Abs(sample)}}{\boldsymbol{Abs(control)}}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Determination of α-amylase inhibition: The colorimetric method described by Sanni *et al.* [33]was employed, using 1% starch solution as substrate and acarbose as standard.

Determination of α-glucosidase inhibition: Commercial α-glucosidase (Sigma Chemical Co. reference commercial (EC 3.2.1.20)) and yeast α-glucosidase (Gauze Pharmaceuticals & Laboratories Ltd) were individually employed for the analysis. The yeast tablets purchased were pulverized before use.

Enzyme extraction: The method of Halvorson[34] was adopted to extract α-glucosidase from yeast tablets (Gauze Pharmaceuticals & Laboratories Ltd, Awka, Anambra State, Nigeria; each tablet weighing approximately 0.312g). The powdered yeast (1 g) in 10 mL of cold 50 mM phosphate buffer (pH 6.8), was vortexed and stirred on ice for 20 min. The homogenate was transferred into centrifuge tubes and centrifuged at 12,000 rpm for 20 min. at 4 ºC. The resulting supernatant, which contained the **α**-glucosidase enzyme was divided into two portions. One portion was adjusted to a final concentration of 1.0 U/mL while the other portion was pre-filtered through Whatman (No. 1) filter paper.

Enzyme inhibition: Each kernel extract was examined using a colorimetric method described by Agada *et al.*,[11] with pNPG (p-Nitrophenyl-α-D-glucopyranoside) as substrate and acarbose as standard.

Determination of lipase activity: The enzyme was first extracted from *wistar* rat liver[35] and inhibition analysis was conducted[36] using olive oil as substrate and orlistat as standard. Ethical approval was obtained from College of Health Sciences Research Ethics Committee (CHSREC), Prince Abubakar Audu University Anyigba. A reference protocol number, CREC-CHS/PAAU/2025/0005 was assigned for the approval of handling of experimental rats. Three (3) *wistar* rats with an average weight of 210 g were procured from the Department of Biochemistry, Prince Abubakar Audu University Anyigba for the purpose of this experiment. Procedures on animal handling outlined by the CHSREC were strictly adhered.

IC_{50} value determination: A plot of the percentage inhibition of the enzyme against the extract concentration (µg/mL) to obtain regression equation from which IC_{50} was calculated.[11]

Biochemical characterization of yeast α-glucosidase: The following optimum characteristics were evaluated using the methods described by Arogba[8]and Agada *et al.*,[11] with modification of substrates.

Optimum Substrate Concentration/Lineweaver-Burk Analysis: A fixed enzyme solution (1.0 g/10mL buffer) was mixed with varied pNPG (p-Nitrophenyl-α-D-glucopyranoside) concentration of 20, 40, 60, 80, 100, 120, 140, and 160 µg/mL, at ambient temperature, for 25 min. using 50 mM sodium phosphate buffer (pH 6.8), the reaction was halted by adding NaOH (100 µL, 0.1 M). Absorbance readings were then taken at 405nm. Reaction velocities were recorded and used to construct Michaelis–Menten curves. The data were transformed into a Lineweaver–Burk plot (1/V vs. 1/[S]) corresponding to the equation 9:

$$\frac{1}{V} = \frac{Km}{Vmax[S]} + \frac{1}{Vmax} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

Vmax and Km were calculated using the linear regression equation. [37]

Optimum Enzyme Concentration: A fixed substrate pNPG (60 µg/mL) was mixed with varied enzyme concentration of (0.4 – 3.6 g/10 mL buffer), at ambient temperature, for 25 min. using 50 mM sodium phosphate buffer (pH 6.8), the reaction was halted by adding NaOH (100 µL, 0.1 M). Absorbance readings were then taken at 405nm.

Optimum Incubation Time: The substrate, pNPG (60 µg/mL) was mixed with the enzyme concentration equivalent to 1.0 g dissolved in 10 mL of 50 mM sodium phosphate buffer (pH 6.8) at ambient temperature. The mixture was incubated for 5, 10, 15, 20, 25, 30, and 35 minutes. After each incubation period, the reaction was stopped by adding NaOH (100 µL, 0.1 M), and the absorbance was measured at 405nm to assess enzymatic activity. A graph of enzyme activity (Δ Abs) against time was plotted.

Optimal pH: Similar conditions above were employed except for pH range of 3.0 to 9.0 which were prepared using sodium phosphate buffer (pH 6.8), adjusted with 1.0 M NaOH or HCl accordingly before adding the substrate pNPG (60 µg/mL) at ambient temperature for 25 min.

Optimal Temperature: Similar conditions above were employed except for varying temperatures of 5º to 50º C using a thermostated water-bath.

Statistical analysis: Data were subjected to the standard error of mean (SEM) using a calculator at *miniwebtool.com.* Results were expressed as Mean ± SEM, and separated by test of significance at ($p < 0.05$). All measurements were done in triplicate (n=3).

Results and Discussion

The influence of blanching temperature and solvent polarity on the physical properties and phytochemical composition of *Mi*, *Iw*, and *Ig*, kernels were investigated. The different extracts were concentrated to dryness and were evaluated against standard inhibitors for their cytotoxicity levels and *in-vitro* inhibitory effects on unicellular larvae of brine shrimps and digestive enzymes, respectively. The exercise was of interest because of their modulatory bioactivity potential when engaged in nutraceutical applications.

It was observed that yields from different extracts ranged from 2.8 to 54.0 % dry matter w/w of the kernel types but their appearance in respect of colour and texture was more a definition of the kernel types. The *Mi* was brownish and powdery while the two species of *Irvingia* (*Iw*, *Ig*) were yellowish and viscous or semi-solid at ambient temperature. The differences observed were attributed to their relative lipid content. The percentage fat content of mango kernel ranged between 7.7 % and 13.5 % depending on variety[38] while *Irvingia* kernel contains between 54 and 70 % depending on species.[39,40] *Irvingia* kernels are better described as "oil-seeds" unlike the conventional mango kernel (*Mi*).

Table 1 indicated that the three kernel types contained highest concentrations of water-soluble phenolic acids (gallic acid eqv.) and

Table 1: Water-soluble phytoconstituents (mg/g dry wt) of kernel types at different temperatures

Temperature	Kernel type	Total Soluble Phenols (GAE)	Flavonoids (QE)	Tannins (CaE)	Saponins (DE)	Glycosides (LE)	Alkaloids (%)
Water (95 ºC)	*Mi*	47.15 ± 0.47^{b}	1.64 ± 0.00^{b}	1.48 ± 0.02^{b}	63.33 ± 0.33^{c}	4.86± 0.01^{b}	0.95 ± 1.2^{b}
(Blanching, Bw)	*Iw*	28.10 ± 0.30^{a}	0.43 ± 0.00^{a}	1.05 ± 0.01^{a}	27.67 ± 0.33^{b}	2.04 ± 0.02^{a}	0.10 ± 1.5^{a}
	Ig	23.85 ± 0.17^{a}	0.25 ± 0.01^{a}	0.96 ± 0.01^{a}	11.00 ± 0.58^{a}	1.14 ± 0.01^{a}	0.10 ± 2.0^{a}
(SEM)		7.164	0.436	0.160	15.434	1.121	0.283
Water (28 ºC)	*Mi*	9.32 ± 0.25^{c}	0.22 ± 0.01^{b}	0.91 ± 0.03^{b}	12.67 ± 0.55^{c}	0.90 ± 0.21^{c}	0.62 ± 0.8^{b}
(Ambient)	*Iw*	5.65 ± 0.18^{b}	0.12 ± 0.03^{a}	0.63 ± 0.01^{a}	5.67 ± 0.21^{b}	0.60 ± 0.32^{b}	0.10 ± 1.0^{a}
	Ig	3.78 ± 0.12^{a}	0.10 ± 0.02^{a}	0.51 ± 0.01^{a}	1.0 ± 0.32^{a}	0.34 ± 0.10^{a}	0.10 ± 1.0^{a}
(SEM)		1.627	0.037	0.119	3.391	0.162	0.173

Where: *Mi* = *Mangifera indica*; *Iw* = *Irvingia wombolu*, *Ig* = *Irvingia gabonensis*, GAE = Gallic Acid Eqv., QE = Quercetin Eqv., CaE = Catechin Eqv., DE = Diosgenin Eqv. and LE = Linamarin Eqv. Values are expressed as mean ± standard error (SE) of three determinations. Values with the same letter in the same column and temperature are not significantly different at $p < 0.05$

saponins (diosgenin eqv.) compared with flavonoids, tannins, glycosides and alkaloids.

Furthermore, significant effect of temperature on the extractability of these constituents was also observed, which Wang *et al*.[41] attributed to effective disruption of cell membrane. Blanching by immersion in water at 95 ºC compared with soaking at ambient temperature of 28 ºC extracted from all kernel types (*Mi*, *Iw*, *Ig*) not less than 5 times of phenolic acids and saponins, and 1.5 times of tannins. On the contrary, alkaloids were least extracted by water and also not influenced by temperature. However, compositional differences in kernel types could explain why the blanching temperature extracted 3 times more flavonoids and glycosides from *Irvingia* species (*Iw*, *Ig*), while 8 times flavonoids and 5 times glycosides were extracted from *Mangifera indica* (*Mi*).

Effect of solvent polarity on further extraction of phytoconstituents of blanched kernels at ambient temperature (28 ºC) is presented in Table 2. In support of the earlier observation of the poor effect of water for extracting alkaloids, the non-polar constitution of alkaloids in these kernels was evident by their highest solubility in n-Hexane employed, which were significantly higher in concentration in the *Irvingia* species than the *Mangifera indica*, *Mi* ($p < 0.05$). Except for the alkaloid content, the blanched *Mi* still contained relatively and significantly higher phytoconstituents ($p < 0.05$), irrespective of solvents employed in the extraction process.

Table 2: Phytoconstituents (mg/g dry wt) of blanched kernel types in different solvents at ambient temperature

Solvent type	Blanched Kernel extract	Total Soluble Phenols (GAE)	Flavonoids (QE)	Tannins (CaE)	Saponins (DE)	Glycosides (LE)	Alkaloids (%)
Water, W	*Mi*	36.60 ± 0.24^{b}	0.33 ± 0.00^{b}	1.13 ± 0.00^{b}	17.67 ± 0.88^{c}	2.00 ± 0.02^{b}	0.95 ± 0.8^{b}
	Iw	13.75 ± 0.14^{a}	0.36 ± 0.01^{b}	0.94 ± 0.01^{a}	7.67 ± 0.33^{b}	0.80 ± 0.03^{a}	1.20 ± 1.0^{c}
	Ig	7.12 ± 0.14^{a}	0.23 ± 0.00^{a}	0.91 ± 0.01^{a}	2.0 ± 0.58^{a}	0.68 ± 0.01^{a}	0.50 ± 1.2^{a}
(SEM)		8.929	0.039	0.068	4.581	0.421	0.205
Ethanol, E	*Mi*	21.47 ± 0.24^{b}	1.05 ± 0.01^{b}	1.27 ± 0.01^{b}	18.14 ± 0.23^{c}	4.20 ± 0.01^{b}	1.45 ± 1.5^{b}
	Iw	9.15 ± 0.30^{a}	0.21 ± 0.01^{a}	0.99 ± 0.01^{a}	15.23 ± 0.13^{b}	1.21 ± 0.02^{a}	0.35 ± 2.0^{a}
	Ig	5.16 ± 0.30^{a}	0.25 ± 0.01^{a}	0.95 ± 0.01^{a}	9.21 ± 0.33^{a}	1.21 ± 0.01^{a}	0.35 ± 2.5^{a}
(SEM)		4.909	0.274	0.101	2.629	0.997	0.367
n-Hexane, n-H	*Mi*	15.34 ± 0.14^{b}	0.39 ± 0.01^{b}	0.94 ± 0.01^{b}	13.0 ± 0.00^{c}	2.34 ± 0.01^{b}	6.40 ± 1.5^{a}
	Iw	2.46 ± 0.15^{a}	0.19 ± 0.00^{a}	0.91 ± 0.01^{a}	8.13 ± 1.33^{b}	2.30 ± 0.02^{b}	9.24 ± 2.0^{b}
	Ig	1.40 ± 0.14^{a}	0.19 ± 0.01^{a}	0.92 ± 0.01^{a}	5.0 ± 1.15^{a}	0.90 ± 0.01^{a}	10.10 ± 2.5^{b}
(SEM)		4.480	0.067	0.009	2.328	0.473	1.118

Where: *Mi* = *Mangifera indica*; *Iw* = *Irvingia wombolu*, *Ig* = *Irvingia gabonensis*, GAE = Gallic Acid Eqv., QE = Quercetin Eqv., CaE = Catechin Eqv., DE = Diosgenin Eqv. and LE = Linamarin Eqv. Values are expressed as the mean ± standard error (SE) of three determinations. Values with the same letter in the same column and solvent are not significantly different at $p < 0.05$.

The observation supports the ready acceptability of *Irvingia* kernels even with intact testa as thickening agent in traditional soup making,[42] unlike the necessity for processing *Mi* kernel for similar culinary use. Arogba[5] had reported the bitter principle in raw *Mi* kernel without testa as 4.5% (w/w) tannin. However, the residual phytoconstituents recorded in this study could now present a positive potential for its nutraceutical applications. Further study of the kernels' levels of inhibition on digestive enzymes is hereby reported.

 ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

The results of cytotoxicity assay (Table 3) showed that potassium dichromate ($K_2Cr_2O_7$), and kernel extracts using blanching water, and absolute ethanol were most toxic to brine shrimp larvae ($p < 0.05$).

Table 3: Brine shrimp lethality bioassay of concentrated extracts of blanched kernels.

	LC_{50} (µg/mL)				
	Extracts				Standard
Kernel type	Bw	W	E	n-H	($K_2Cr_2O_7$)
Mi (SEM = 242.22)	375.3[a]	625.3[b]	455.9[a]	1606.9[c]	268.9[a]
Iw (SEM = 331.76)	500.0[a]	870.6[c]	555.6[a]	2136.6[d]	268.9[a]
Ig (SEM = 364.34)	624.2[a]	1059.5[b]	823.6[b]	2396.6[c]	268.9[a]

Where: *Mi* = *Mangifera indica*; *Iw* = *Irvingia wombolu*, *Ig* = *Irvingia gabonensis*, Bw = Blanching water, W = Water, E = Ethanol and n-H = n-Hexane. Values were derived from the mean ± standard error (SE) of three determinations. Values with different superscripts on the same row are significantly different at $p < 0.05$

The observation reflected the significant effects of temperature, and high degree of hydrophilicity of the phenolic acid and saponin constituents in the three kernel types. In medicinal formulations, Tables 1 and 2 indicated that their synergistic concentrations in the ranged of 11 – 63 mg/g dry wt/kernels from blanching water or 5 – 21 mg/g equivalents from ethanol extraction of blanched kernels would be recommendable dosage for application. Chirumbolo[43] and Ohiagu *et al*.[44] reported that such phytochemical content could act as pro-oxidants to induce apoptosis.

The significant contribution of saponin to cytotoxicity assessment was evident when its lower concentrations in blanching water and ethanol extracts (Table 2) and the higher LC_{50} (Table 3) were compared between those of *Iw* and *Ig*.

However, for application of the three kernel types in food processing, water blanching by immersion of the kernels appeared to remain the viable option. Compared with the blanching water extract, Bw, (Table 3), the residual concentrations of phytoconstituents in the blanched kernel water, W, were less toxic as evident in the LC_{50} value recorded. Furthermore, the n-hexane extracts which largely contained alkaloids (Table 2) had least toxicity effect on brine shrimp larvae. The very high LC_{50} values from the blanched kernel extracts reflected a range of 6 - 10 mg/g dry wt. It implied that *Mi*, *Iw* and *Ig* kernels are poor sources of alkaloids with no apparent adverse nutritional potential, irrespective of solvent choice for analysis. On this basis, also, the n-hexane extracts were excluded from the enzyme inhibition investigation that proceeded.

Inhibitory activity of blanching water (Bw) or absolute ethanol (E) extracts of *Mi*, *Iw* and *Ig* were compared with reference samples of acarbose on α-amylase, α-glucosidase (commercial), yeast α-glucosidase (test sample), and orlistat on lipase (test sample) (Table 4)

Table 4: Inhibitory effects of *Mi*, *Iw* and *Ig* extracts, acarbose or orlistat on α-amylase, α-glucosidase or lipase

		IC_{50} (µg/mL)			
		Extracts		Standard	
Enzyme	Kernel type	Bw	E	Acarbose	Orlistat
α-amylase (commercial)	*Mi* (SEM = 26.41)	66.0[b]	101.8[c]	11.0[a]	NA
	Iw (SEM = 42.36)	108.9[b]	154.6[c]	11.0[a]	NA
	Ig (SEM = 58.89)	121.8[b]	214.7[c]	11.0[a]	NA
α-glucosidase (commercial)	*Mi* (SEM = 25.32)	69.2[b]	109.2[c]	21.6[a]	NA
	Iw (SEM = 43.55)	117.4[b]	170.4[c]	21.6[a]	NA
	Ig (SEM = 60.65)	132.5[a]	231.6[c]	21.6[a]	NA
Yeast α-glucosidase (test sample)	*Mi* (SEM = 29.84)	82.3[b]	124.4[c]	21.6[a]	NA
	Iw (SEM = 47.61)	127.9[b]	184.5[c]	21.6[a]	NA
	Ig (SEM = 71.54)	146.6[b]	269.4[c]	21.6[a]	NA
Filtered Yeast α-glucosidase (test sample)	*Mi* (SEM = 29.84)	77.0[b]	117.2[c]	21.6[a]	NA
	Iw (SEM = 48.45)	125.1[b]	184.5[c]	21.6[a]	NA
	Ig (SEM = 71.54)	142.3[b]	249.9[c]	21.6[a]	NA
Lipase (test sample)	*Mi* (SEM = 28.26)	70.6[b]	107.9[c]	NA	10.8[a]
	Iw (SEM = 45.27)	112.1[b]	165.2[c]	NA	10.8[a]
	Ig (SEM = 63.19)	128.7[b]	229.5[c]	NA	10.8[a]

Where: *Mi* = *Mangifera indica*; *Iw* = *Irvingia wombolu*, *Ig* = *Irvingia gabonensis*, Bw = Blanching water, W = Water, E = Ethanol and NA = Not Applicable. Values were derived from the mean ± standard error (SE) of three determinations. Values with different superscripts on the same row are significantly different at $p < 0.05$

The IC_{50} values were derived from linear regression curves of percentage enzyme inhibition against kernel extract concentrations.

Enzyme inhibition assays revealed that the kernel extracts of Bw possessed more significant inhibitory activity against the selected digestive enzymes than E. In all cases, however, the commercial acarbose and orlistat samples were the most potent inhibitors ($p < 0.05$). However, *Mi* extract showed the most potent inhibition among the kernel types which could be attributed to its phytochemical composition by structure and function. Its IC_{50} (µg/mL) on the digestive enzymes ranged from 66 - 82 for Bw and 102 - 124 for E extracts. The enhanced inhibitory activity in the *Mi* extracts could be attributed to its higher concentration of phenolic compounds and saponins, which are known

to interfere with enzyme function by complexing and altering its conformation, thereby reducing substrate accessibility.[45,46] For example, there would be delay in carbohydrate digestion and subsequent glucose absorption, a mechanism beneficial for glycemic control in type 2 diabetes.[10] These findings support the potential use of *Mi* kernel extracts as natural agents for managing obesity and modulating lipid digestion, while also emphasizing the importance of extraction methods in optimizing the yield of key inhibitory phytochemicals. [47,48]The beneficial effect of blanching in delaying enzymic browning has its usefulness in food processing to retain the nutritional integrity of food items.

Biochemical characteristics of the extract and filtered yeast α-glucosidase are presented in Figs. 1-6. Kinetic studies employing Lineweaver–Burk analysis provided valuable insights into the effect of enzyme purification on the enzyme activity (Figs. 1 and 2). In Fig. 1 showing the onset of optimum substrate concentration at 100 µg/mL, the reference commercial α-glucosidase showed superior enzyme activity by 4.2% compared with the filtered yeast source. However, the difference was annulled at 160 µg/mL substrate concentration. It implied that further addition of substrate beyond 100 µg/mL had no significant effect on enzyme activity. Filtration step employed in this study is an improvement over existing literature protocol of decanting[34] for extraction of α-glucosidase. From Fig. 2 and Table 5, the Km and Vo of the filtered yeast α-glucosidase activity compared with the crude source improved by 67% and 56% respectively. However, the reference commercial α-glucosidase showed superiority by 27% and 16% respectively. This difference could be attributed to further purification process(es) beyond filtration, such as ion-exchange chromatography. It implied that interfering substances present in the extract could hinder enzyme-substrate interaction.[36]

Other optimum reaction conditions for the yeast α-glucosidase were established by systematically varying enzyme concentration, incubation time, pH, and temperature (Figs. 3 - 6). To achieve the first order reaction rate described above, fig. 3 indicated that not more than 3 g/10mL would be required. Time-course studies revealed that optimum enzyme activity was achieved at 25 min. with a slight decline observed after 30 min. which was likely due to enzyme-substrate saturation even over prolonged incubation. The optimum pH was observed to be 6.8, in consonance with the physiological pH as well as several literature reports[22,34,49] However, temperature optimization revealed that yeast tablets of unknown *Saccharomyces* species purchased for this study had its optimum between 25 and 30 °C. Temperatures above this range could lead to reduced activity, inactivation and denaturation of enzyme proteins, a phenomenon well-documented in enzymology. [49]

Repeated determinations provided our record with only an optimum peak of 28 ± 2 ºC in correlation with ambient temperature range at time of analysis. On the contrary, literature showed wide range of 37 – 65 ºC[50 - 52] for different *saccharomyces* species, under varied conditions of experimentation.

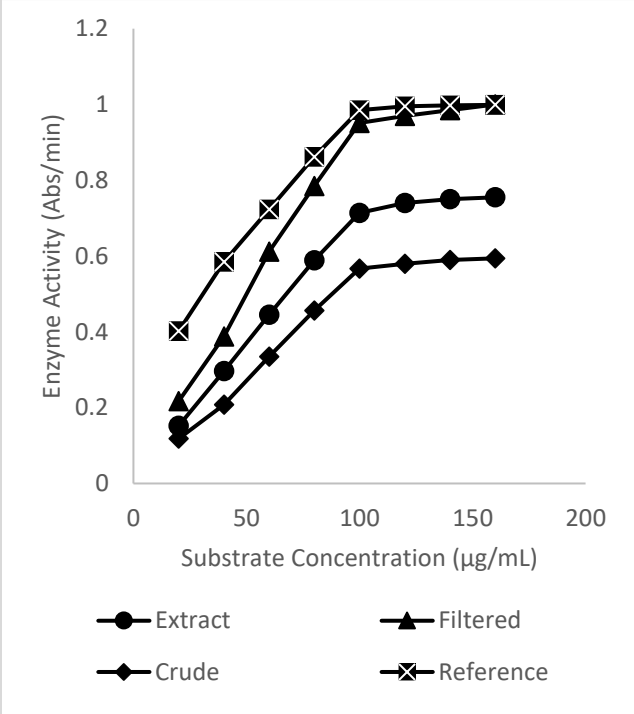

Fig. 1: Optimum Substrate Concentration Curves of Crude, Extract, Filtered and Reference yeast α-glucosidase

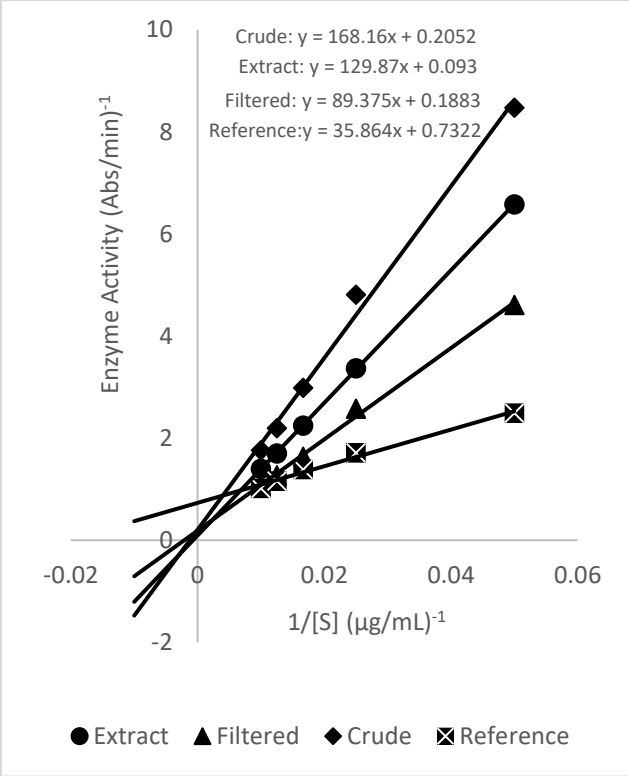

Fig. 2: Lineweaver-Burk plot of Crude, Extract, Filtered and Reference yeast α-glucosidase.

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

Table 5: Comparative Km and Vo of yeast and reference commercial α-glucosidase.

Enzyme Source	Km (µg/mL)	Vo (Abs/min)	% Difference Km (µg/mL)			Vo (Abs/min)		
Crude	80.944	2.462						
Extract (D)	40.078	1.618			50			34
Filtered (D + F)	27.078	1.078		67			56	
Reference	4.899	0.683	94			72		

Where: D = Decanted enzyme, and D + F = Decanted and filtered enzyme.
$Vo = \frac{1}{2} Vmax$ at Km.

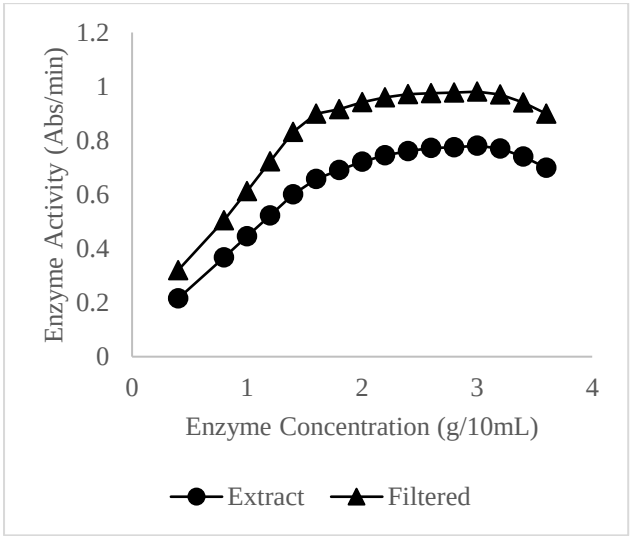

Fig. 3: Optimum Enzyme Concentration Curves of Extract and Filtered α-glucosidase.

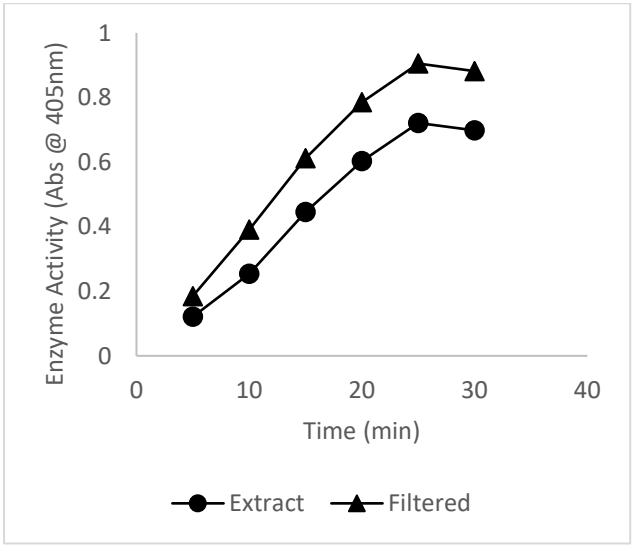

Fig. 4: Optimum Time curves of Extract and Filtered yeast α-glucosidase

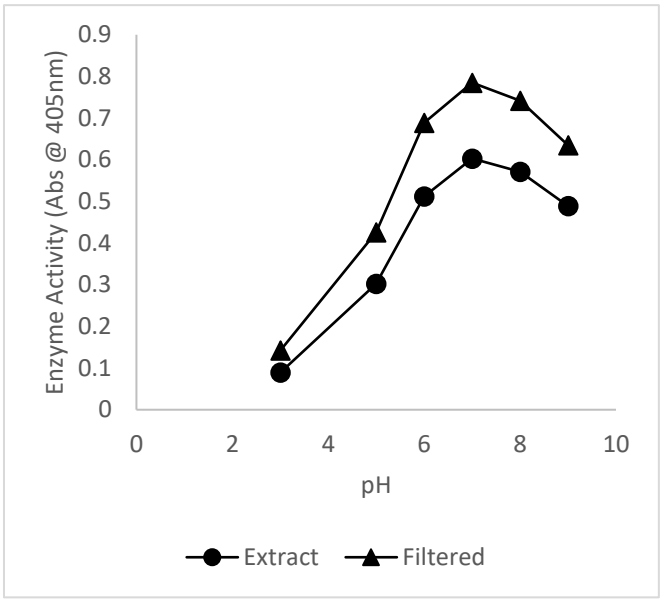

Fig. 5: Optimum pH curves of Extract and Filtered yeast α-glucosidase

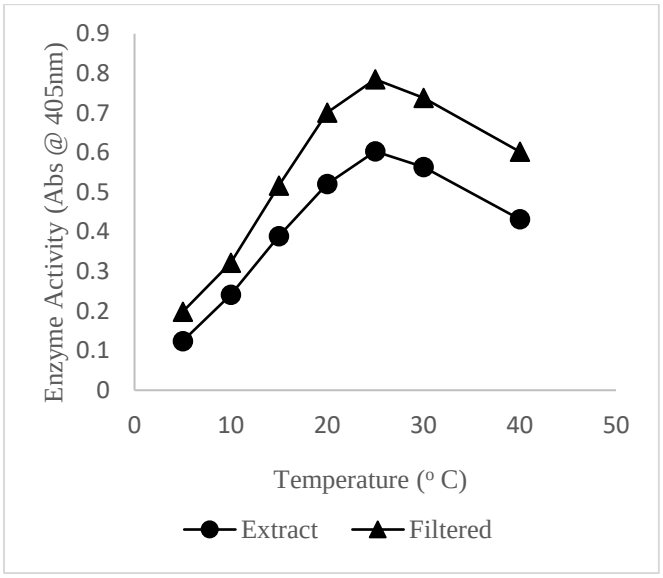

Figure 6: Optimum Temperature Curves of Extract and Filtered yeast α-glucosidase

Conclusion

The study has shown that blanching temperature effectively extracted phenolic acids and saponins in their large proportions from *Mi*, *Iw*, and *Ig* kernels in water. Quercetin and catechin equivalents, glycosides and alkaloids were available in low concentrations in these kernels. Polar solvents (water, ethanol) employed left very low concentrations of the phenolic acids and saponins in the blanched kernels while n-hexane extracted the maximally available alkaloids (6–10 mg/g dry wt). Extracts of the polar solvents were more cytotoxic than n-hexane extracts to brine shrimp larvae, and more inhibitory to α-amylase, α-glucosidase, and lipase. The choice of α-glucosidase extracted/filtered from yeast provided viable, reproducible and reliable alternative results compared with the expensive and rarely available commercial α-glucosidase. The study further showed that water blanching remains the viable option for these kernels in food processing while the kernel extracts using polar solvents are recommended for medicinal formulations in the management of metabolic disorders, particularly diabetes and obesity.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Arogba SS. Phenolics, antiradical assay and cytotoxicity of processed mango (*Mangifera indica*) and bush mango (*Irvingia gabonensis*) kernels. Niger Food J. 2014;32(1):62–72.
2. Akinmoladun AC, Oladejo CO, Josiah SS, Famusiwa CD, Ojo OB, Olaleye MT. Catechin, quercetin and taxifolin improve redox and biochemical imbalances in rotenone-induced hepatocellular dysfunction: Relevance for therapy in pesticide-induced liver toxicity? Pathophysiology. 2018;25(4):365–71.
3. Manivannan V, Johnson M. Total phenolic, tannin, triterpenoid, flavonoid and sterol contents, anti-diabetic, anti-inflammatory and cytotoxic activities of *Tectaria paradoxa* (Fee.) Sledge. Toxicol Reports. 2020;7:1465–8.
4. Mwaurah PW, Kumar S, Kumar N, Panghal A, Attkan AK, Singh VK, Garg MK. Physicochemical characteristics, bioactive compounds and industrial applications of mango kernel and its products: A review. Compr Rev Food Sci Food Saf. 2020;19(5):2421–46.
5. Arogba SS. Physical, chemical and functional properties of Nigerian mango (*Mangifera indica*) kernel and its processed flour. J Sci Food Agric. 1997;73(3):321–8.
6. Ngondi JL, Oben JE, Minka SR. The effect of *Irvingia gabonensis* seeds on body weight and blood lipids of obese subjects in Cameroon. Lipids Health Dis. 2005;4:1–4.
7. Nawaz H, Shad MA, Rehman N, Andaleeb H, Ullah N. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. Brazilian J Pharm Sci. 2020;56:e17129.
8. Arogba SS. The performance of processed mango (*Mangifera indica*) kernel flour in a model food system. Bioresour Technol. 1999;70(3):277–81.
9. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DEJ, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982;45(05):31–4.
10. Ajila CM, Naidu KA, Bhat SG, Rao UJSP. Bioactive compounds and antioxidant potential of mango peel extract. Food Chem. 2007;105(3):982–8.
11. Agada R, Usman WA, Shehu S, Thagariki D. *In-vitro* and *in-vivo* inhibitory effects of *Carica papaya* seed on α-amylase and α-glucosidase enzymes. Heliyon. 2020;6(3):e03618.
12. Poovitha S, Parani M. *In-vitro* and *in-vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC Complement Altern Med. 2016;16:1–8.
13. Yilmazer-Musa M, Griffith AM, Michels AJ, Schneider E, Frei B. Inhibition of α-amylase and α-glucosidase activity by tea and grape seed extracts and their constituent catechins. J Agric Food Chem. 2012;60(36):230 - 242.
14. Islam MR, Haque AR, Kabir MR, Hasan MM, Khushe KJ, Hasan SMK. Fruit by-products: the potential natural sources of antioxidants and α-glucosidase inhibitors. J Food Sci Technol. 2021;58:1715–26.
15. Sosnowska D, Podsędek A, Redzynia M, Żyżelewicz D. Effects of fruit extracts on pancreatic lipase activity in lipid emulsions. Plant Foods Hum Nutr. 2015;70:344–50.
16. Hadrich F, Cher S, Gargouri YT, Adel S. Antioxidant and lipase inhibitory activities and essential oil composition of pomegranate peel extracts. J Oleo Sci. 2014;63(5):515–25.
17. De Pradhan I, Dutta M, Choudhury K, De B. Metabolic diversity and *in-vitro* pancreatic lipase inhibition activity of some varieties of *Mangifera indica* L. fruits. Int J Food Prop. 2017;20(sup3):S3212–23.
18. Arogba, S. S. The effect of Testa on Physico-Chemical Properties, Browning Index, and Functional Properties of Wild Mango (*Irvingia gabonensis Var. gabonensis*) Kernel. Inter-World J. of Sci. And Techno. 2001;1(1), 159-168.
19. Akinfemiwa O, Zubair M, Muniraj T. Amylase. StatPearls. 2023;
20. Souza PM de. Application of microbial α-amylase in industry-A review. Brazilian J Microbiol. 2010;41:850–61.
21. Sundarram A, Murthy TPK. α-amylase production and applications: a review. J Appl Environ Microbiol. 2014;2(4):166–75.
22. Yonekura L, Hisada H, Intravichakul J. Human α-glucosidase inhibition and phytochemical profile of natural and shinzuke treated olives: implications from the processing method. Food Prod Process Nutr. 2024;6(1):46-57.
23. Lu H, Xie T, Wu Q, Hu Z, Luo Y, Luo F. Alpha-Glucosidase inhibitory peptides: Sources, preparations, identifications, and action mechanisms. Nutrients. 2023;15(19):4267- 91.
24. Ali S, Khan SA, Hamayun M, Lee IJ. The recent advances in the utility of microbial lipases: A review. Microorganisms. 2023;11(2):510.
25. Guerrand D. Lipases industrial applications: focus on food and agroindustries. OCL Oilseeds fats Crop lipids. 2017;24(4):D403.
26. Rowe GE, Margaritis A. Enzyme kinetic properties of α-1, 4-glucosidase in Bacillus thuringiensis. Biochem Eng J. 2004;17(2):121–8.
27. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313–52.
28. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999;64(4):555–9.
29. Sundang M, Nasir SNS, Sipaut CS, Othman H. Antioxidant activity, phenolic, flavonoid and tannin content of Piper betle and Leucosyke capitella. Malaysian J Fundam Appl Sci. 2012;8(1) 1-6.
30. Hiai S, Oura H, Nakajima T. Color reaction of some sapogenins and saponins with vanillin and sulfur1c acid. Planta Med. 1976;29(02):116–22.
31. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer science & Business media; London, UK 1998;503.
32. Asomugha R. Acute and Cytotoxicity Studies of Aqueous and Ethanolic Leaf Extracts of. Pakistan J Biol Sci. 2015;18(1):46–9.
33. Sanni O, Erukainure OL, Oyebode OA, Koorbanally NA, Islam MS. Concentrated hot water-infusion of *Phragmanthera incana* improves muscle glucose uptake, inhibits carbohydrate digesting enzymes and abates Fe^{2+}-induced oxidative stress in hepatic tissues. Biomed Pharmacother. 2018;108(September):417–23.
34. Halvorson H. α-Glucosidase from yeast. In: Methods in enzymology. Elsevier; Academic Press, Oxford, UK; 1966. p. 559–62.
35. Claycomb WC, Kilsheimer GS. Purification and properties of a lipase from rat liver mitochondria. J Biol Chem. 1971;246(23):7139–43.
36. Bustanji Y, Al-Masri IM, Mohammad M, Hudaib M, Tawaha K, Tarazi H, Alkhatib HS. Pancreatic lipase inhibition activity of trilactone terpenes of *Ginkgo biloba*. J Enzyme Inhib Med Chem. 2011;26(4):453–9.
37. Lineweaver H, Burk D. The determination of enzyme dissociation constants. J Am Chem Soc. 1934;56(3):658–66.

38. Kittiphoom S, Sutasinee S. Mango seed kernel oil and its physicochemical properties. Int Food Res J. 2013;20(3):1145-1149.
39. Ekpe OO, Umoh IB, Eka OU. Effect of a typical rural processing method on the proximate composition and amino acid profile of bush mango seeds (*Irvingia gabonensis*). African J Food, Agric Nutr Dev. 2007;7(1):1-12.
40. Mateus-Reguengo L, Barbosa-Pereira L, Rembangouet W, Bertolino M, Giordano M, Rojo-Poveda O, Zeppa G. Food applications of *Irvingia gabonensis* (Aubry-Lecomte ex. O'Rorke) Baill., the 'bush mango': A review. Crit Rev Food Sci Nutr. 2020;60(14):2446–59.
41. Wang C, Han J, Pu Y, Wang X. Tea (*Camellia sinensis*): a review of nutritional composition, potential applications, and intake: Current status and future possibilities. Int J Mol Sci. 2020;21(16):5642-65.
46. Jeepipalli SPK, Du B, Sabitaliyevich UY, Xu B. New insights into potential nutritional effects of dietary saponins in protecting against the development of obesity. Food Chem. 2020;318:126474-87.
47. Hara Y, Honda M. The inhibition of α-amylase by tea polyphenols. Agric Biol Chem. 1990;54(8):1939–45.
48. Zhang X, Liang J, Lin X, Chen J, Luo X. A comprehensive review on the composition, processing methods, and sustainable utilization of tropical fruit seeds in food industry. Food Front. 2024;644-669
Omics Research. Appl Sci. 2022;12(12):5874-93.
42. Idowu MA, Omoniyi SA, Henshaw FO, Olayiwola IO. Sensory acceptability of partially defatted dikanut (*Irvingia gabonensis*) flour in ogbono soup. J Culin Sci Technol. 2013;11(4):346–55.
43. Chirumbolo S. The role of quercetin, flavonols and flavones in modulating inflammatory cell function. Inflamm allergy-drug targets (formerly Curr drug targets-inflammation allergy)(discontinued). 2010;9(4):263–285.
44. Ohiagu FO, Chikezie PC, Chikezie CM, Enyoh CE. Anticancer activity of Nigerian medicinal plants: a review. Futur J Pharm Sci. 2021;7:1–21.
45. Boccellino M, D'Angelo S. Anti-obesity effects of polyphenol
49. Suzuki H. How enzymes work: from structure to function. Jenny Stanford Publishing; United Square, Singapore. 2019;p. 284-92
50. Fleet GH. Yeast interactions and wine flavour. Int J Food Microbiol. 2003;86(1–2):11–22.
51. Deng X, Petitjean M, Teste MA, Kooli W, Tranier S, François JM, Parrou JL. Similarities and differences in the biochemical and enzymological properties of the four isomaltases from *Saccharomyces cerevisiae*. FEBS Open Bio. 2014;4:200–12.
52. Yadav AN, Rastegari AA, Yadav N. Microbiomes of extreme environments. CRC Press, Taylor and Francis Group,New York, USA; 2020;p.322.