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Antioxidant and modulatory properties of kernel flour extracts of three Nigerian local mango varieties on enzymes relevant to metabolic syndrome

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ABSTRACT

Aim: Recent studies have shown that flour of mango kernel is a rich natural source of biologically active phytochemicals with important health benefits, such as phenolic compounds. Hence, this study evaluated antioxidant and modulatory properties of methanol extracts of kernel flours of three Nigerian local mango varieties (*Sherri*, *Ogbomoso*, and *Elemi*) on some enzymes relevant to the development of metabolic syndrome (MS).

Methods: Enzymes [pancreatic lipase (PL), α-amylase, α-glucosidase, angiotensin I-converting enzyme (ACE), and xanthine oxidase (XO)] inhibitory assays, antioxidant activities, and antioxidant phytochemicals contents of extracts were carried out using spectrophotometer.

Results: All the three mango varieties strongly inhibited PL, α-amylase, α-glucosidase, ACE, and XO. Generally, *Elemi* had the strongest inhibitory effect on the enzymes, followed by *Sherri* and *Ogbomoso*. It also had the highest total phenols, total flavonoids, tannins, and total saponins contents, and displayed the strongest [2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) radical cation] $ABTS^{*+}$ and (2,2-diphenyl-2-picrylhydrazyl radical) $DPPH^{*}$ scavenging activities and reducing power, compared with *Sherri* and *Ogbomoso*.

Conclusions: By inhibiting PL, α-amylase, α-glucosidase, ACE, and XO, kernel flour extracts of the three Nigerian local mango varieties may be beneficial for retarding the production of fatty acids, glucose, angiotensin II, and uric acid, representing an important approach for managing obesity, T2D, hypertension, and hyperuricemia, respectively. The mango kernel flours, especially *Elemi*, may, therefore, be harnessed to develop low-cost nutraceuticals for managing MS.

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Introduction

Metabolic syndrome (MS), a group of related metabolic disorders that increases the risk of an individual to develop type 2 diabetes (T2D) and cardiovascular disease, is known to have dyslipidemia, abdominal obesity, hypertension, loss of β-cells, glucose intolerance, insulin resistance, and low-grade chronic inflammation as some of its components [1,2]. Recently, hyperuricemia, a diagnostic biomarker of gout caused by over-activity of xanthine oxidase [3], has been identified as a component of MS [4]. It also serves as a key predictor of the development of the other components of MS including obesity, T2D, and hypertension [4,5]. Furthermore, oxidative stress is a well-known denominator of MS and its various components [6].

The involvement of enzyme catalysis in the production of the metabolites whose excessive accumulation predisposes to the various components of MS (fatty acids for obesity, sugars for T2D, angiotensin II for hypertension, and uric acid for hyperuricemia/gout), makes the inhibition of the relevant enzymes a therapeutic strategy for treating MS. Some of such enzymes include pancreatic lipase (PL) that catalyzes the digestion of dietary fats to release fatty acids [7]; α-amylase and α-glucosidase that breakdown starch to sugars [8]; angiotensin I-converting enzyme (ACE)

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that cleave angiotensin I to angiotensin II [9], and xanthine oxidase (XO) that oxidizes hypoxanthine to xanthine and eventually to uric acid [10]. Thus, synthetic inhibitors of these enzymes, such as orlistat for PL, acarbose for α-amylase and α-glucosidase, captopril for ACE, and allopurinol for XO have been developed. These synthetic inhibitors have some adverse effects such as gastrointestinal tract disturbances hepatic dysfunction due to orlistat [11]; abdominal distention and flatulence due to acarbose [12]; proteinuria and hypotension due to captopril [13]; and kidneys impairment and liver dysfunction due to allopurinol [14], which undermine their therapeutic efficacy. However, it is heart-warming to know that some bioactive secondary metabolites found in functional foods and nutraceuticals are efficacious in inhibiting these enzymes without adverse effects [15,16], and therefore, may be beneficial for managing MS.

Mango (*Mangifera indica* L.), belonging to the *Anacardiaceae* family, is one of the major fruits in the tropical regions of the world, with global annual production of about 42 million tons, being next to annual production of banana globally [17]. Approximately, there are 1,000 varieties of mango worldwide [18]. In the western part of Nigeria, three common varieties are locally known as *Sherri*, *Ogbomoso*, and *Elemi*. Mango is prominent for its food and medicinal uses. Some studies have focused on its kernel flour as a natural source of biologically active compounds with important health benefits. For instance, extract of the kernel flour was reported to contain various phenolic compounds including phenolic acids and flavonoids, and to inhibit carbohydrate-metabolizing enzymes [19]. Also, diet supplemented with mango kernel flour was reported to display anti-diabetic effects in type 2 diabetic Wistar rats [20].

Despite the reported pharmacological benefits of mango kernel, its potential as a functional food and nutraceutical source has not been unleashed, as over 1 million tons of mango seeds are turned out yearly as waste product [21]. Therefore, to further explore the potential of mango kernel flour as a source of functional foods and nutraceuticals, this study evaluated the antioxidant and modulatory properties of kernel flour extracts of three Nigerian local mango varieties (*Sherri*, *Ogbomoso*, and *Elemi*) on some enzymes (PL, α-amylase, α-glucosidase, ACE, and XO) relevant to the development of metabolic syndrome.

Materials and Methods

Chemicals and reagents

Acarbose, xanthine, allopurinol, orlistat, captopril, hippuryl-histidyl-leucine, Trolox, diosgenin, quercetin, tannic acid, L-ascorbic acid, gallic acid, ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic), DPPH (2,2-diphenylpicrylhydrazyl), porcine pancreatic lipase, α-amylase, rabbit lung ACE, α-glucosidase, and xanthine oxidase were purchased from Sigma (St. Louis). Analytical grade of all other chemicals and solvents was used.

Collection and preparation of sample

Fresh ripe mango fruit samples of three different local varieties (*Sherri*, *Ogbomoso*, and *Elemi*) were bought from a local market in Ilorin (*Oja-oba*), Kwara State, Nigeria. The samples were taxonomically identified at the Plant Biology Department, University of Ilorin, Nigeria. Afterward, the mesocarp of the fruits was sliced off and the kernels were manually removed from the endocarp. The kernels were diced into small irregular sizes with kitchen knife, and air-dried to a constant weight at ambient room temperature for 7 days. Thereafter, the dried samples were milled into flour (0.5 mm) using a Marlex kitchen blender, and hermetically packed in amber plastic containers and stored at 4°C for further analysis.

Preparation of flour extract

Two gram of the flour of each variety was extracted by soaking in 20 ml of methanol overnight. Afterward, the mixture was centrifuged at 4,000 rpm for 5 minutes, and filtered through Whatman (No. 2) filter paper. The filtrate, subsequently referred to as extract, was concentrated using a rotary evaporator at 45°C, and later diluted to 6 ml with methanol [22].

Pancreatic lipase (PL) inhibition assay, in vitro

PL inhibition assay was performed by the method earlier described by Eom et al. [23], using *p*-nitrophenyl butyrate as substrate, and orlistat as a reference inhibitor. To prepare the enzyme solution, 30 µl (10 units) of porcine PL in 10 mM morpholinepropane sulphonic acid and 1 mM ethylenediaminetetraacetic acid (EDTA) (pH 6.8) was added to 850 µl Tris buffer (100 mM Tris-HC1 containing 5 mM $CaCl_2$, pH 7.0). Afterward, a reaction mixture containing 100 µl of different dilutions (5, 10, 15, and 20 µg/ml) of the extract (or orlistat) and the enzyme solution (880 µl) was

incubated at 37°C for 10 minutes. Next, 20 µl of 10 mM *p*-nitrophenyl butyrate solution in dimethyl formamide was added to initiate hydrolysis at 37°C for 20 minutes. Subsequently, absorbance of the *p*-nitrophenol formed from *p*-nitrophenyl butyrate hydrolysis was measure in a UV-Visible spectrophotometer at 405 nm, and the percentage PL inhibitory activity of the extract was calculated.

Alpha-amylase inhibition assay, in vitro

Alpha-amylase inhibition assay was conducted using the method described by Kwon et al. [24]. Porcine pancreas α-amylase (EC 3.2.1.1) and soluble starch (substrate) were used in this assay. Different dilutions of methanol extract totaling 500 µl, and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/ml α-amylase solution were incubated at 37°C for 10 minutes. Afterward, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer was added. Next, the reaction mixture was incubated at 37°C for 15 minutes, after which 1.0 ml of DNSA color reagent (1% 3, 5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH) was added to terminate the reaction. Afterward, the reaction mixture was incubated for 5 minutes in a boiling water bath, cooled to room temperature, and diluted with 10 ml distilled water. The absorbance was measured at 540 nm. The mean absorbance of triplicate tests was used to calculate percentage α-amylase inhibition as follows:

$$\% \text{ Inhibition} = [(A540_R - A540_S)/A540_R] \times 100;$$

where $A540_R$ is mean absorbance reading of the reference; $A540_S$ is mean absorbance reading of the sample.

Alpha-glucosidase inhibition assay, in vitro

Alpha-glucosidase inhibitory activity was conducted following the method reported by Kim et al. [25], using *Bacillus stearothermophillus* α-glucosidase (EC 3.2.1.20) and para-nitrophenylglucopyranoside (PNPG) as the substrate. Briefly, five units aliquot of α-glucosidase was incubated with 20 µg/ml of the different kernels methanol extracts for 15 minutes. Next, 3 mM PNPG dissolved in 20 mM phosphate buffer, pH 6.9 was added as a substrate to initiate the hydrolytic reaction. The hydrolytic reaction was allowed to proceed for 20 minutes at 37°C, after which it was terminated by adding 2 ml of 0.1 M Na_2CO_3. The absorbance of the yellow *p*-nitrophenol released from PNPG hydrolysis was measured at 400 nm. Each test was performed in triplicates and the mean absorbance was used to calculate percentage α-glucosidase as follows:

$$\% \text{ Inhibition} = [(A400_R - A400_S)/A400_R] \times 100;$$

where $A400_R$ is mean absorbance reading of the reference; $A400_S$ is mean absorbance reading of the sample.

Angiotensin I-converting enzyme (ACE) inhibition assay, in vitro

ACE inhibition assay was carried out following the method reported by Cushman and Cheung [26], using hippuryl-histidyl-leucine as substrate and captopril as positive control. In brief, a reaction mixture containing 50 µl of different dilutions (5, 10, 15, and 20 µg/ml) of the extract (or captopril, 64 nmol/l) and 50 µl of ACE solution (4 mU/ml) was incubated at 37°C for 15 minutes. Then, 150 µl of 8.33 mM of hippuryl-histidyl-leucine in 125 mM Tris–HCl buffer (pH 8.3) was added to the mixture, and this was incubated for 30 minutes at 37°C. Subsequently, 250 µl of 1 M HCl was added to terminate the reaction, and the hippuric acid produced was extracted with ethyl acetate (1.5 ml), and separated by centrifugation. Then, 1.0 ml of the ethyl acetate layer was dispensed into a clean test tube and evaporated to dryness in an oven. The hippuric acid residue was redissolved with 1.0 ml of deionized water; following which its absorbance was measured in a UV-Visible spectrophotometer at 228 nm, and the percentage ACE inhibitory activity of the extract was calculated.

Xanthine oxidase (XO) inhibition assay, in vitro

XO inhibition assay was conducted following the method reported by Osada et al. [27], with a minor modification. Fresh 15 mM xanthine (substrate) and 0.1 mU/µl XO solutions were prepared with Tris–HCl buffer (50 mM, pH 7.4). Afterward, 40 µl of xanthine, 10 µl of XO and 1,950 µl of serial concentrations (10, 20, 30, and 40 µg/ml) of each extract were mixed and incubated at 37°C for 10 minutes. To terminate the XO-catalyzed hydrolysis of xanthine, 50 µl of perchloric acid solution in the same Tris–HCl buffer (3.2% (v/v) was added. The absorbance of the uric acid produced was then measured at 292 nm in a UV-Visible spectrophotometer, and the percentage XO inhibitory activity of the extracts was calculated.

2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) radical cation ($ABTS^{*+}$) scavenging assay

The extracts' ability to scavenge $ABTS^{*+}$ was carried out following the method reported by Re et al. [28], and expressed in Trolox equivalent antioxidant capacity (TEAC). $ABTS^{*+}$ working reagent was prepared by thoroughly mixing equal volume of aqueous solutions of $ABTS^{*+}$ (7 millimoles/l) and $K_2S_2O_8$ (2.45 millimoles/l), and incubating it in the dark at room temperature for 16 hours. Afterward, the reagent's absorbance was adjusted to 0.70 ± 0.02 with ethanol (95%) at 734 nm. Then, a reaction mixture consisting of 2.0 ml of the $ABTS^{*+}$ reagent and 0.2 ml of the extract was incubated at room temperature for 15 minutes in the dark, after which the absorbance was measured in a UV-Visible spectrophotometer at 734 nm. $ABTS^{*+}$ scavenging ability of the extract was later calculated from Trolox standard curve.

2,2-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assay*

The extracts' ability to scavenge DPPH* was determined using the method reported by Cervato et al. [29], at different concentrations (4, 8, 12, and 16 µg/ml), using ascorbic acid as a positive control. In brief, a reaction mixture containing 1.0 ml of the different concentrations of the extract (or ascorbic acid) and 3.0 ml of DPPH* solution (60 µM) was incubated at room temperature in the dark for 30 minutes. Then, the absorbance was measured at 517 nm in a UV-Visible spectrophotometer, and percentage DPPH* scavenging ability of the extract was calculated.

Determination of reducing power

The ability of the extracts to reduce $FeCl_3$ solution was tested following the method described by Oyaizu [30]. In brief, a mixture of the extract (2.5 ml), 200 mM sodium phosphate buffer (pH 6.6) (2.5 ml), and 1% potassium ferricyanide (2.5 ml) was incubated at 50°C for 20 minutes, after which 10% trichloroacetic acid (2.5 ml) was added. Next, the mixture was centrifuged at 650 × g for 10 minutes. A portion of 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride, and the absorbance was read at 700 nm. Afterward, the reducing power of the extracts was calculated and expressed in mg gallic acid equivalent per gram.

Determination of antioxidant phytochemicals (total phenolics, total flavonoids, tannins and total saponins) contents

Total phenolics content was determined using the Folin–Ciocalteu method reported by Chan et al. [31], and the result was expressed in mg gallic acid equivalent/g (mg GAE/g) sample. Total flavonoids content was determined using aluminum chloride method as reported by Meda et al. [32], and the result was expressed in mg quercetin equivalent/g (mg QE/g) sample. Tannins content was estimated using the method reported by Amorim et al. [33] and the result was expressed in mg tannic acid equivalent/g (mg TAE/g) sample. Total saponins content was estimated using the method reported by Makkar et al. [34], and the result was expressed in mg diosgenin equivalent/g (mg DE/g) sample.

Statistical analysis

The means of independent triplicate determinations were subjected to one-way analysis of variance, followed by least significant difference test, using SPSS statistical software (version 17), at $p < 0.05$. SC_{50} (concentration of the extracts that

Table 1. IC_{50} of kernel flour extracts of three Nigerian local mango varieties on PL, α-amylase, α-glucosidase, ACE, and XO activities.

Inhibitor	PL IC_{50} (µg/ml)	α-amylase IC_{50} (µg/ml)	α-glucosidase IC_{50} (µg/ml)	ACE IC_{50} (µg/ml)	XO IC_{50} (µg/ml)
Sherri	15.68 ± 1.06^b	13.34 ± 1.26^b	8.59 ± 0.96^c	18.01 ± 1.29^a	26.52 ± 2.13^a
Ogbomoso	16.26 ± 1.63^a	14.06 ± 1.13^a	9.42 ± 1.08^b	18.98 ± 1.36^a	25.50 ± 2.11^b
Elemi	12.94 ± 1.01^c	11.72 ± 1.83^c	7.94 ± 1.06^d	16.13 ± 1.14^b	23.85 ± 2.07^c
Orlistat	0.19 ± 0.01^d	-	-	-	-
Acarbose	-	8.50 ± 0.94^d	15.47 ± 1.37^a	-	-
Captopril	-	-	-	5.72 ± 0.46^c	-
Allopurinol	-	-	-	-	6.86 ± 0.36^d

Results are means ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

scavenged DPPH* by 50%) and IC_{50} (concentration of the extracts that inhibited enzymes activity by 50%) were calculated using version 4.0 of Graphpad Prism® (Sandiego, CA).

Results

The concentrations of kernel flours extracts of the three Nigerian local mango varieties causing 50% inhibition (IC_{50} µg/ml) of PL, α-amylase, α-glucosidase, ACE, and XO activities are presented in Table 1. The IC_{50} values of the various mango varieties on PL were consistently in the order of *Elemi* < *Sherri* < *Ogbomoso*, indicating that *Elemi* had the strongest inhibitory effect on PL, followed by *Sherri* and *Ogbomoso*. Orlistat, a reference PL inhibitor with the least IC_{50} (0.19 ± 0.01 µg/ml), had a stronger inhibitory effect on PL than the three mango varieties. Similarly, the IC_{50} values of the three mango varieties on α-amylase and α-glucosidase, were consistently in the order of *Elemi* < *Sherri* < *Ogbomoso*, implying that *Elemi* had the strongest inhibitory effect on these enzymes, followed by *Sherri* and *Ogbomoso*. The result further shows that the three mango varieties had stronger inhibitory effect on α-glucosidase than on α-amylase. In contrast, acarbose (reference α-amylase and α-glucosidase inhibitor) had stronger inhibitory effect on α-amylase (IC_{50}: 8.50 ± 0.94 µg/ml) than on α-glucosidase (15.47 ± 1.37 µg/ml).

The three mango varieties also inhibited ACE, with IC_{50} (µg/ml) values in the order of *Elemi* < *Sherri* < *Ogbomoso* (Table 1); inversely indicating ACE inhibitory strengths in the order of *Elemi* > *Sherri* > *Ogbomoso*. However, captopril (reference ACE inhibitor) with the least IC_{50} (5.72 ± 0.46 µg/ml) had the strongest inhibitory effect. Similarly, the XO inhibitory potencies of the three mango varieties were in the order of *Elemi* < *Ogbomoso* < *Sherri* (Table 1); indicating that *Elemi* displayed the most potent XO inhibitory activity, followed by *Ogbomoso* and *Sherri*. Allopurinol, a reference inhibitor of XO with IC_{50} of 6.86 ± 0.36 µg/ml, was more potent in inhibiting XO than the three mango varieties.

The antioxidant activities of extracts of the three mango varieties are presented in Table 2. The result shows that there was significant difference ($p < 0.05$) in the extracts' ability to scavenge $ABTS^{*+}$ and reduce ferric ion, such that *Elemi* > *Sherri* > *Ogbomoso*. Their $ABTS^{*+}$ scavenging ability, expressed as TEAC, ranged from 5.93 ± 0.81 in *Ogbomoso* to 7.68 ± 0.63 mmol TE/g DW in *Elemi*. Their reducing powers were significantly ($p < 0.05$) different, with *Elemi* having the highest reducing power (101.89 ± 2.02 mg GAE/g), followed by *Sherri* (85.93 ± 1.61 mg GAE/g) and *Ogbomoso* (79.23 ± 1.01 mg GAE/g). The result further shows that the

Table 2. DPPH* SC_{50}, $ABTS^{*+}$ scavenging ability and reducing power of kernel flour extracts of three local varieties of mango on dry weight basis.

Antioxidant	DPPH* SC_{50} (µg/ml)	$ABTS^{*+}$ scavenging ability (mmol TEAC/g)	Reducing power (mg GAE/g)
Sherri	10.31 ± 0.46[a]	6.74 ± 0.57[b]	85.93 ± 1.61[b]
Ogbomoso	10.98 ± 0.65[a]	5.93 ± 0.81[c]	79.23 ± 1.01[c]
Elemi	9.76 ± 0.74[a]	7.68 ± 0.63[a]	101.89 ± 2.02[a]
Ascorbic acid	6.83 ± 0.47[b]	-	-

Results are means ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$). SC_{50}: extract concentration that scavenged 50% of DPPH*; TEAC: Trolox equivalent antioxidant capacity; GAE: Gallic acid equivalent.

Table 3. Antioxidant phytochemical composition of kernel flour extracts of three local varieties of mango on dry weight basis.

Mango variety	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)	Tannins (mg TAE/g)	Total saponins (mg DE/g)
Sherri	121.21 ± 0.83[b]	18.96 ± 0.41[b]	87.48 ± 0.98[b]	83.61 ± 0.45[b]
Ogbomoso	113.39 ± 1.17[c]	15.21 ± 0.38[c]	70.86 ± 1.07[c]	76.79 ± 1.12[c]
Elemi	137.09 ± 0.50[a]	24.41 ± 0.35[a]	97.14 ± 0.99[a]	111.62 ± 1.89[a]

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

concentrations of *Elemi* (9.76 ± 0.74 µg/ml), *Sherri* (10.31 ± 0.46 µg/ml), and *Ogbomoso* (10.98 ± 0.65 µg/ml) extracts that scavenged 50% of DPPH* (SC_{50}) were comparable ($p > 0.05$). However, ascorbic acid, a standard antioxidant (SC_{50}: 6.83 ± 0.47 µg/ml), was more effective in scavenging DPPH* than the extracts of the three mango varieties.

The antioxidant phytochemicals composition (mg/g DW) of flours of the kernel mango varieties is presented in Table 3. Consistently, *Elemi* significantly ($p < 0.05$) had the highest levels of total phenols, total flavonoids, tannins, and total saponins, followed by *Sherri* and *Ogbomoso*. Their levels of total phenolics ranged from 113.39 ± 1.17 to 137.09 ± 0.50 mg GAE/g; total flavonoids ranged from 15.21 ± 0.38 to 24.41 ± 0.35 mg QE/g; tannins ranged from 70.86 ± 1.07 to 97.14 ± 0.99 mg TAE/g; while total saponins ranged from 76.79 ± 1.12 to 111.62 ± 1.89 mg DE/g in *Ogbomoso*, and *Elemi*, respectively.

Discussion

Inhibition of enzymes that catalyze the production of metabolites whose accumulation predisposes to metabolic diseases is an important index for assessing the therapeutic potential of natural products. In this study, extracts of the three Nigerian local mango varieties inhibited PL, α-amylase, α-glucosidase, ACE, and XO activities. Comparing their PL inhibitory strengths (IC_{50} range: 12.94–16.26 µg/ml) with a few other plants that have been recently reported to inhibit PL shows that the three mango varieties displayed stronger PL inhibitory effect than various parts of *Ficus carica*, with IC_{50} range of 230.475–276,085.1 µg/ml [35]; but weaker PL inhibitory strength than the leaf of tropical almond, with IC_{50} of 10.82 µg/ml [15]. As the most active enzyme involved in the digestion of dietary fats to produce fatty acids and stimulation of their uptake into the lumen of the small intestine [7], PL inhibition is one of the strategic regulatory targets in the treatment of obesity [35]. Thus, its inhibition by the three mango varieties may therefore decelerate the release of fatty acids from dietary fats and their assimilation into the intestinal lumen, thereby preventing their subsequent accumulation in the cells.

When compared with the IC_{50} values of mango kernel flour extract on α-amylase (71 µg/ml) and α-glucosidase (34 µg/ml) earlier reported by Irondi et al. [19], the three mango varieties in this present study all exhibited more potent inhibitory effect on α-amylase (IC_{50} values: 13.34 ± 1.26, 14.06 ± 1.13, and 11.72 ± 1.83 µg/ml for *Sherri*, *Ogbomoso*, and *Elemi*, respectively) and α-glucosidase (IC_{50}: 8.59 ± 0.96, 9.42 ± 1.08, and 7.94 ± 1.06 µg/ml for *Sherri*, *Ogbomoso*, and *Elemi*, respectively). The stronger inhibitory effect of the three mango varieties on α-glucosidase than on α-amylase, relative to acarbose is of therapeutic importance, and agrees with some previous reports that showed similar trend [19,36]. Since the side effects, such as abdominal distention and flatulence, associated with acarbose are due to its stronger inhibition of α-amylase than α-glucosidase [12], this suggests that the extracts of the three mango varieties may not cause such side effects. Alpha-amylase and α-glucosidase are involved in the digestion of dietary carbohydrates. Whereas pancreatic α-amylase in the small intestine hydrolyzes starch α-1,4 bonds, producing disaccharides and oligosaccharides such as maltose and dextrins; α-glucosidase in the brush border of the small intestine completes the digestion by hydrolyzing the disaccharides and oligosaccharides to produce absorbable simple monosaccharides including glucose and fructose [8]. Thus, their inhibition is a well-established clinical strategy for controlling postprandial hyperglycemia in the management of T2D, and a key mechanism of action of many anti-diabetic agents [25], including drugs, natural products, and functional foods.

The catalytic role ACE plays in the rennin-angiotensin system (RAS), where it catalyzes the hydrolytic cleavage of angiotensin I to produce angiotensin II, a physiologically active vasoconstrictor, makes its inhibition a therapeutic target for controlling the blood pressure [37]. When produced in excess, angiotensin II is known to induce a rise in the blood pressure by stimulating the inactivation of a vasodilator and hypotensive peptide, bradykinin, while activating the secretion of aldosterone [9]. Relative to some plants that have been recently reported to inhibit ACE, the three mango varieties had stronger ACE inhibitory effect than *Ocimum gratissimum* (IC_{50}: 56.63 µg/ml) [38] and raw *Brachystegia eurycoma* seed (IC_{50}: 29.08 ± 1.74 µg/ml) [39]; but weaker effect than tropical almond leaf (IC_{50}: 14.98 µg/ml) [15].

A comparison of the XO inhibition result obtained in this study with a recent finding by Esatbeyoglu et al. [40] shows that the three mango varieties exhibited stronger XO inhibitory effect than different fractions of purple sweet potato, with IC_{50} range of 0.2 to 0.3 g/l [40]. XO catalyzes the two terminal reactions of purine nucleotides degradation, in which hypoxanthine is first oxidized to xanthine,

which is ultimately oxidized to uric acid; with concomitant generation of reactive oxygen species [10]. The uric acid so-produced, when in excess (hyperuricemia), can stimulate acute constriction of blood vessels by activating the RAS, and plummeting circulating nitric oxide level; thereby promoting the onset of hypertension [41]. In addition to this direct association with hypertension, hyperuricemia is as an important predictor of obesity, T2D, and hypertension [4,5]. Thus, the ability of the three mango varieties to inhibit XO suggests it may have a far-reaching beneficial effect for the prevention and management of MS.

The $ABTS^{*+}$ and $DPPH^{*}$ scavenging abilities of the three mango varieties observed in this study are greater than the ranges (TEAC: 0.80 ± 0.07–3.10 ± 0.07 mmol trolox/g DW; $DPPH^{*}$ IC_{50}: 2.45–9.31 µg/ml) Khammuang and Sarnthima [42] reported for the seed kernel extracts of four Thai mango varieties. Oxidative stress, resulting when the free radicals and reactive oxygen species (oxidants) burden of the cell overwhelms its antioxidant defence system, is a common feature of MS. Thus, through their antioxidant activities, the three mango varieties may prevent the initiation and progression of oxidative stress in MS, thereby complementing their enzymes inhibitory activities. This is supported by a previous report that indicated that improving cellular antioxidant strength is vital for a successful management of the various components of MS [15].

The total phenolics contents observed in this study fall within the range of total phenolics (89.49–200.05 mg/g) Barreto et al. [43] reported for the kernel of various mango cultivars grown in Brazil. The total phenolics contents of the three mango varieties are, however, much higher than the 23.90 mg GAE/g (DW) reported for mango kernel powder by Ashoush and Gadallah [44]. The range of total flavonoids contents of the three mango varieties is lower than the 12.2% DW (i.e., 122 mg/g) Dakare et al. [45] reported for raw mango kernel flour. However, it is higher than the range (0.057–1.3 g catechin equivalents/100 g DW, that is, 0.57–13 mg/g) reported by Dorta et al. [46] for mango seed extracts obtained using different solvents and temperatures. The variations in the total phenolics, total flavonoids and antioxidant activities observed in this study relative to the values previously reported could be due to differences in sample collection period and extraction method; as well as differences in abiotic and biotic factors that are all known to influence the levels of secondary metabolites in plants and their bioactivities [47,48]. The tannins contents of the three local mango varieties are within the range (14–130 mg tannic acid equivalents/g DW) Dorta et al. [46] reported for mango seed extracts. The range of the saponins contents agrees with the value (105 mg/g DW) Dakare et al. [45] reported for raw mango kernel flour.

Phenolic compounds such as flavonoids and tannins are prominent for their various health benefits including antioxidant, anti-diabetic [49], anti-obesity, anti-hypertensive, and anti-hyperuricemia [15] activities. Saponins have also been shown to exhibit these health benefits, including anti-obesity [50], anti-diabetics [51], anti-hypertensive [52], anti-hyperuricemic [53], and antioxidant [54] activities. The phenolic compounds generally have been reported to inactivate digestive enzymes including PL, α-amylase, and α-glucosidase via non-specific binding to the individual enzymes [55]. They are also known for their high affinity for proteins via hydrogen and hydrophobic bonding, enabling them to inhibit enzymes such as PL, α-amylase, α-glucosidase, ACE, and XO by denaturation of protein [16]. Thus, the bioactivities of the three mango varieties may be attributed to the combined effects of the polyphenolics and the saponins present in them. This may also account for why *Elemi* with the highest levels of total phenols, total flavonoids, tannins, and total saponins, exhibited the strongest bioactivities.

In conclusion, the three Nigerian local mango varieties (*Sherri*, *Ogbomoso*, and *Elemi*) strongly inhibited pancreatic lipase, α-amylase, α-glucosidase, angiotensin I-converting enzyme, and xanthine oxidase. They also effectively scavenged ABTS and DPPH radicals, reduced ferric ion and had high levels of total phenolics, total flavonoids, tannins, and saponins (antioxidant phytochemicals). Generally, *Elemi* consistently had the strongest enzymes inhibitory effects and antioxidant activities, as well as highest levels of antioxidant phytochemicals, followed by *Sherri* and *Ogbomoso*. The enzymes inhibitory and antioxidant effects of the three mango varieties, which could be due to their phytochemicals, suggest that they may be beneficial for lowering the levels of fatty acids, sugars, angiotensin II, uric acid, and oxidants, which are essential for the prevention and management of metabolic syndrome.

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Conflict of interest

There is no conflict of interest to declare.

References

[1] Osakabe N, Hoshi J, Kudo N, Shibata M. The flavan-3-ol fraction of cocoa powder suppressed changes associated with early-stage metabolic syndrome in high-fat diet-fed rats. Life Sci 2014; 114:51–6.

[2] Strat KM, Rowley IV TJ, Smithson AT, Tessem JS, Hulver MW, Liu D, et al. Mechanisms by which cocoa flavanols improve metabolic syndrome and related disorders. J Nutri Biochem 2016; 35:1–21.

[3] Aggarwal R, Ringold S, Khanna D, Neogi T, Johnson SR, Miller A, et al. Distinctions between diagnostic and classification criteria?. Arthritis Care Res 2015; 67:891–7.

[4] Billiet L, Doaty S, Katz JD, Velasquez MT. Review of hyperuricemia as new marker for metabolic syndrome. ISRN Rheumatol 2014; 852954; doi:10.1155/2014/852954

[5] Chen Y-Y, Kao T-W, Yang H-F, Chou C-W, Wu C-J, Lai C-H, et al. The association of uric acid with the risk of metabolic syndrome, arterial hypertension or diabetes in young subjects—an observational study. Clin Chimica Acta 2018; 478:68–73.

[6] Rani V, Deep G, Singh RK, Palle K, Yadav UCS. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. Life Sci 2016; 148:183–93.

[7] Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov Today 2007; 12:879–89.

[8] Tucci SA, Boyland EJ, Halford JCG. The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: a review of current and emerging therapeutic agents. Diabetes Metab Syndr Obes 2010; 3:125–43.

[9] Eriksson U, Danilczyk U, Penninger JM. Just the beginning: novel functions for angiotensin converting enzymes. Curr Biol 2002; 12:R745–52.

[10] Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: Molecular mechanisms and pathophysiological implications. J Physiol 2004; 555:589–606.

[11] Filippatos T, Derdemezis C, Gazi I, Nakou E, Mikhailidis D, Elisaf M. Orlistat-associated adverse effects and drug interactions: a critical review. Drug Saf 2008; 31:53–65.

[12] Dalar A, Konczak I. Phenolic contents, antioxidant capacities and inhibitory activities against key metabolic syndrome relevant enzymes of herbal teas from Eastern Anatolia. Indus Crop Prod 2013; 4:383–90.

[13] Vyssoulis GP, Karpanou EA, Papavassiliou MV. Side effects of antihypertensive treatment with ACE inhibitors. Am J Hypertens 2001; 14:114–5.

[14] Khoo BP, Leow LH. A review of inpatients with adverse drug reactions to allopurinol. Singapore Med J 2000; 41:156–60.

[15] Irondi EA, Agboola SO, Boligon AA. Inhibitory effects of tropical almond leaf extract on xanthine oxidase, pancreatic lipase, and angiotensin I-converting enzyme, *in vitro*. J Food Biochem 2018; 42(4):e12481; doi:10.1111/jfbc.12481

[16] Villiger A, Sala F, Suter A, Butterweck V. *In vitro* inhibitory potential of *Cynara scolymus*, *Silybum marianum*, *Taraxacum officinale*, and *Peumus boldus* on key enzymes relevant to metabolic syndrome. Phytomed 2015; 22:138–44.

[17] Ediriweera MK, Tennekoon KH, Samarakoon SR. A review on ethnopharmacological applications, pharmacological activities, and bioactive compounds of *Mangifera indica* (Mango). Evid Based Complement Altern Med 2017; 24, 6949835; doi:10.1155/2017/6949835

[18] Torres-Leon C, Rojas R, Contreras-Esquivel JC, Serna-Cock L, Belmares-Cerda RE, Aguilar CN. Mango seed: functional and nutritional properties. Trends Food Sci Technol 2016; 55:109–17.

[19] Irondi EA, Oboh G, Akindahunsi AA, Boligon AA, Athayde ML. Phenolic composition and inhibitory activity of *Mangifera indica* and *Mucuna urens* seeds extracts against key enzymes linked to the pathology and complications of type 2 diabetes. Asian Pac J Trop Biomed 2014; 4:903–10.

[20] Irondi EA, Oboh G, Akindahunsi AA. Antidiabetic effects of *Mangifera indica* kernel flour-supplemented diet in streptozotocin- induced type 2 diabetes in rats. Food Sci Nutri 2016; 4:828–39.

[21] Abdalla AEM, Darwish SM, Ayad EHE, El-Hamahmy RM. Egyptian mango by-product 1. Compositional quality of mango seed kernel. Food Chem 2007; 103:1134–40.

[22] Engida AM, Kasim NS, Tsigie YA, Ismadji S, Huynh LH, Ju Y. Extraction, identification and quantitative HPLC analysis of flavonoids from sarang semut (*Myrmecodia pendan*), Indus Crops Prod 2013; 41:392–6.

[23] Eom SH, Lee MS, Lee EW, Kim YM, Kim TH. Pancreatic lipase inhibitory activity of phlorotannins isolated from *Eisenia bicyclis*. Phytother Res 2013; 27:148–51.

[24] Kwon YI, Apostolidis E, Shetty K. Inhibitory potential of wine and tea against α-amylase and a-glucosidase for management of hyperglycemia linked to type 2 diabetes. J Food Biochem 2008; 32:15–31.

[25] Kim YM, Jeong YK, Wang MH, Lee YH, Rhee HI. Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycaemia. Nutr 2005; 21:756–61.

[26] Cushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin I-converting enzyme of rabbit lung. Biochem Pharmacol 1971; 20:1637–48.

[27] Osada Y, Tsuchimoto M, Fukushima H, Takahashi K, Kondo S, Hasegawa M, et al. Hypouricemic effect of the novel xanthine oxidase inhibitor, TEI-6720, in rodents. Eur J Pharmacol 1993; 241:183–8.
[28] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 1999; 26:1231–7.
[29] Cervato G, Carabelli M, Gervasio S, Cittera A, Cazzola R, Cestaro B. Antioxidant properties of oregano (*Origanum vulgare*) leaf extracts. J Food Biochem 2000; 24:453–65.
[30] Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986; 44:307–15.
[31] Chan EWC, Lim YY, Chew YL. Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. J Food Chem 2007; 102:1214–22.
[32] Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 2005; 91:571–7.
[33] Amorim ELC, Nascimento JE, Monteiro JM, Sobrinho P, Araujo TAS, Albuquerque UAP. A simple and accurate procedure for the determination of tannins and flavonoid levels and some applications in ethnobotany and ethnopharmacology. Funct Ecosyst Commun 2008; 2:88–94.
[34] Makkar HPS, Siddhuraju P, Becker K. Plant secondary metabolites. Humana Press Inc., Totowa, NJ, , 2007.
[35] Mopuri R, Ganjayi M, Meriga B, Koorbanally NA, Islam MS. The effects of *Ficus carica* on the activity of enzymes related to metabolic syndrome. J Food Drug Analy 2018; 26:201–10.
[36] Figueiredo-Gonzalez M, Grosso C, Valentao P, Andrade PB. α-Glucosidase and α-amylase inhibitors from *Myrcia* spp.: a stronger alternative to acarbose?. J Pharm Biomed Anal 2016; 118:322-327; doi:10.1016/j.jpba.2015.10.042
[37] Ma TK, Kam KK, Yan BP, Lam YY. Renin-angiotensin-aldosterone system blockade for cardiovascular diseases: Current status. Br J Pharmacol 2010; 160:1273–92.
[38] Shaw H-M, Wu J-L, Wang M-S. Antihypertensive effects of *Ocimum gratissimum* extract: Angiotensin-converting enzyme inhibitor *in vitro* and *in vivo* investigation. J Funct Foods 2017; 35:68–73.
[39] Irondi EA, Adebara OO, Olateju A, Boligon AA. Phenolic constituents, anti-radicals, and enzymes inhibitory potentials of *Brachystegia eurycoma* seeds: effects of processing methods. Int J Food Prop 2018; 20:S3004–14.
[40] Esatbeyoglu T, Rodríguez-Werner M, Schlösser A, Winterhalter P, Rimbach G. Fractionation, enzyme inhibitory and cellular antioxidant activity of bioactives from purple sweet potato (*Ipomoea batatas*). Food Chem 2017; 221:447–56.
[41] Feig DI. Hyperuricemia and hypertension. Adv Chronic Kidney Dis. 2012; 19:377–85.
[42] Khammuang S, Sarnthima R. Antioxidant and antibacterial activities of selected varieties of Thai mango seed extract. Pak J Pharm Sci 2011; 24:37–42.
[43] Barreto JC, Trevisan MTS, Hull WE, Erben G, De Brito ES, Pfundstein B, et al. Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). J Agric Food Chem 2008; 56:5599–610.
[44] Ashoush IS, Gadallah MGE. Utilization of mango peels and seed kernels powders as sources of phytochemicals in biscuit. World J Dairy Food Sci 2011; 6:35–42.
[45] Dakare MA, Danladi AA, Abel SA, Sunday EA. Effects of processing techniques on the nutritional and antinutritional contents of mango (*Mangifera indica*) seed kernel. World J Young Res 2012; 2:55–9.
[46] Dorta E, Lobo MG, Gonzalez M. Reutilization of mango byproducts: study of the effect of extraction solvent and temperature on their antioxidant properties. J Food Sci 2012; 77:C80–8.
[47] Braga TV, Dores RG, Ramos CS, Evangelista FCG, Tinoco LM, Varotti F, et al. Antioxidant, antibacterial and antitumor activity of ethanolic extract of the *Psidium guajava* leaves. Am J Plant Sci 2014; 5:3492–500.
[48] Mpofu A, Sapirstein HD, Beta T. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. J Agric Food Chem 2006; 54:1265–70.
[49] Bai Y, Xu Y, Wang B, Li S, Guo F, Hua H, et al. Comparison of phenolic compounds, antioxidant and antidiabetic activities between selected edible beans and their different growth periods leaves. J Funct Foods 2017; 35:694–702.
[50] Chen G, Li H, Zhao Y, Zhu H, Cai E, Gao Y, et al. Saponins from stems and leaves of *Panax ginseng* prevent obesity via regulating thermogenesis, lipogenesis and lipolysis in high-fat diet-induced obese C57BL/6 mice. Food Chem Toxicol 2017; 106:393–403.
[51] Lu J, Wang Y, Yan H, Lin P, Gu W, Yu J. Antidiabetic effect of total saponins from *Polygonatum kingianum* in streptozotocin-induced daibetic rats. J Ethnopharmacol 2016; 179:291–300.

[52] Lee KH, Bae IY, Park SI, Park J-D, Lee HG. Antihypertensive effect of Korean Red Ginseng by enrichment of ginsenoside Rg3 and arginine-fructose. J Ginseng Res 2016; 40:237–44.

[53] Zhu L, Dong Y, Na S, Han R, Wei C, Chen G. Saponins extracted from *Dioscorea collettii* rhizomes regulate the expression of urate transporters in chronic hyperuricemia rats. Biomed Pharmacother 2017; 93:88–94.

[54] Guo N, Tong T, Ren N, Tu Y, Li B. Saponins from seeds of Genus *Camellia*: Phytochemistry and bioactivity. Phytochem 2018; 149:42–55.

[55] Tan Y, Chang SKC. Digestive enzyme inhibition activity of the phenolic substances in selected fruits, vegetables and tea as compared to black legumes. J Funct Foods 2017; 38:644-655; doi:10.1016/j.jff.2017.04.005