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Comparative inhibitory effects of cocoa bean and cocoa pod husk extracts on enzymes associated with hyperuricemia and hypertension *in vitro*

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Abstract

Cocoa (*Theobroma cacao* L.) bean and its products have numerous health benefits. However, the pod husk is generally regarded as waste. In the present work, the inhibitory effects of cocoa bean powder (CBP) and cocoa pod husk powder (CPHP) extracts on enzymes associated with hyperuricemia (xanthine oxidase, XO) and hypertension (angiotensin 1-converting enzyme, ACE) were comparatively investigated *in vitro*. Free radicals scavenging abilities and phenolic constituents of the extracts were also determined. CBP significantly inhibited XO and ACE, and scavenged 2,2-diphenyl-2-picrylhydrazyl radical (DPPH*) more than the CPHP; but their 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) radical cation ($ABTS^{*+}$) scavenging potentials were insignificantly different. Phenolic acids (gallic, chlorogenic and caffeic acids) and flavonoids (quercitrin, quercetin and apigenin) were detected in both extracts. The levels of gallic and chlorogenic acids, and apigenin were significantly higher in the CBP than in the CPHP; whereas their levels of caffeic acids, quercitrin and quercetin were insignificantly different. Hence, both CBP and CPHP might be useful for the treatment of hyperuricemia and hypertension, through the inhibition of XO and ACE, and scavenging of free radicals. However, CBP might be more effective than CPHP due to its stronger enzymes inhibitory potentials and higher levels of certain phenolic compounds.

Introduction

Studies have shown that extracts of plants and their products rich in bioactive compounds such as polyphenolics can inhibit xanthine oxidase (XO) (EC 1.1.3.22) and angiotensin 1-converting enzyme (ACE) (EC: 3.4.15.1) (Villiger *et al*., 2015; Irondi *et al*., 2017), which is an important therapeutic approach for the treatment of hyperuricemia (elevated blood uric acid level) and hypertension, respectively. XO catalyses the two terminal reactions in the breakdown of purine nucleotides, during which hypoxanthine is converted first to xanthine, and eventually to uric acid. During these reactions, superoxide, a reactive oxygen species (ROS), is generated from the reduction of molecular oxygen (Berry and Hare, 2004). Over-production of uric acid due to the over-activity of XO leads to hyperuricemia, and the subsequent deposition of monosodium urate monohydrate crystals in body tissues, especially joints, which eventually leads to gout (Kramer and Curhan, 2002). ACE catalyses the conversion of angiotensin I to angiotensin II in the rennin/angiotensin pathway. The angiotensin II so produced is a vasoconstrictor that elevates blood pressure by stimulating the secretion of aldosterone, and at the same time, inactivating the vasodilator and hypotensive peptide, bradykinin (Eriksson *et al*., 2002). Thus, the over-activity of ACE and the concomitant over-production of angiotensin II induce the development of hypertension and some other cardiovascular diseases (Ferrario and Strawn, 2006).

Hyperuricemia and hypertension have a relationship, which has been described as bidirectional (Pan *et al*., 2015). The ROS generated by XO-catalysed reactions precipitate cardiovascular diseases, including hypertension (Weseler and Bast,

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2010), by activating the renin/angiotensin pathway (Mazzali *et al*., 2001). Hence, hyperuricemia is a well-established independent risk factor for hypertension (Feig *et al*., 2008; Perez-Pozo *et al*., 2010). Currently, drugs that inhibit XO (such as allopurinol) and ACE (such as captopril and ramipril), which are used clinically for the treatment of hyperuricemia and hypertension, respectively, (Emmerson, 1996; Thurman and Schrier, 2003), have some adverse effects. For examples, clinical use of XO inhibitors is associated with renal impairment and hepatic dysfunction (Khoo and Leow, 2000); while ACE inhibitors are associated with hypotension, proteinuria, coughing and skin rashes (Vyssoulis *et al*., 2001). The obvious adverse effects of these drugs and their high cost have necessitated the search for natural inhibitors of these enzymes. Several recent studies have shown that polyphenolics-rich extracts of some food and medicinal plants, including guava and cocoa leaves, were able to inhibit XO and ACE (Irondi *et al*., 2016; Irondi *et al*., 2017).

Cocoa (*Theobroma cacao* L.), from the Malvaceae family, is an important economic crop with many food, medicinal and industrial uses in many parts of the world. The cocoa bean in particular, and its various food products, such as chocolate, are well-known for their high levels of polyphenolic compounds, and their ability to prevent cardiovascular diseases (Ding *et al*., 2006; Galleano *et al*., 2009). In contrast, much is not known about the potential health benefits of the cocoa pod husk. Instead, it is often regarded as waste, and is therefore generally underexploited (Donkoh *et al*., 1991; Vriesmann *et al*., 2012). However, several recent studies have shown that the cocoa pod husk has antioxidant, anti-wrinkles and tyrosinase inhibitory activities (Abdul Karim *et al*., 2014, 2016). The present work was therefore designed to comparatively evaluate the inhibitory effects of cocoa bean powder (CBP) and cocoa pod husk powder (CPHP) extracts on enzymes associated with hyperuricemia (XO) and hypertension (ACE) *in vitro*, with a view to further exploring their potential health benefits.

Materials and methods

Chemicals and reagents

All chemicals were of analytical grade. Methanol, formic acid, gallic acid, caffeic acid and chlorogenic acid were purchased from Merck (Darmstadt, Germany). Quercetin, quercitrin, kaempferol, apigenin, rabbit lung ACE, captopril, allopurinol, xanthine, L-ascorbic acid, hippuryl-histidyl-leucine, DPPH, ABTS and Trolox were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Samples collection and preparation

A total of 20 ripe cocoa samples were randomly harvested from a local cocoa plantation in Ibadan, Nigeria. The samples were later authenticated at the Plant Breeding Section of Cocoa Research Institute of Nigeria, Ibadan, Nigeria. Thereafter, the pods were cut open, and the beans with the pulps were manually removed. The pod husks were chopped into small irregular sizes and oven-dried to a constant weight at 50°C for 3 d. The beans with the pulps were fermented in a basket lined and covered with banana leaves for 7 d. Thereafter, the fermented beans were manually separated from the pulps and other contaminants, and washed to remove the remnant mucilage. The fermented beans were later oven-roasted at 120°C for 65 min. The roasted beans were cracked and winnowed to separate the shells from the nibs. The nibs were milled using a grinding machine to form cocoa liquor. The cocoa liquor was packed in muslin cloth and pressed into cake using a hydraulic-press in order to extract its fat (cocoa butter). The cocoa cake formed after pressing and the already oven-dried pod husks were ground into powder of 0.5 mm particle size, to obtain the cocoa bean powder (CBP) and the cocoa pod husk powder (CPHP).

Preparation of CBP and CPHP polyphenolic-rich extracts

Polyphenolic-rich extracts of the CBP and the CPHP were prepared by following the method described by Kuo *et al*. (2012). Briefly, 100 g of each sample was extracted with 300 mL absolute methanol at 50°C for 3 h, for three successive times. The samples were filtered with Whatman filter paper (No. 2), and the extracts were collected after each successive extraction. Subsequently, the extracts were partitioned with 200 mL hexane in a separating funnel, and the aqueous phase of each was further extracted with 180 mL ethyl acetate for three successive times. The resulting extracts were later dried in a rotary evaporator at 45°C, under reduced pressure. The residues obtained for each sample after drying were used for subsequent analyses.

Preparation of rats' liver homogenate for XO inhibition assay in vitro

Four adult male Wistar albino rats weighing at 200-220 g were used to prepare liver homogenate that served as the source of XO for the XO inhibition assay. The rats were handled humanely by following the guidelines specified in the public health service policy on humane care and use of laboratory animals of the National Academy of Science, USA (1996). Rats' liver homogenate was prepared based on the

method described by Nakamura *et al*. (2014). Briefly, the rats were mildly anesthetised with ether and then sacrificed. The liver tissue was quickly harvested, washed in ice-cold 0.15 M KCl, and then blotted dry. Next, 1 g liver tissue was homogenised in 9 mL ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA. Subsequently, the homogenate was sonicated twice under ice-cold condition for 30 sec, and centrifuged at 10,000 g for 20 min at 4°C. The cold supernatant was collected and used as the source of the XO for the XO inhibition assay.

XO inhibition assay in vitro

The ability of each extract to inhibit XO was determined based on the method reported by Umamaheswari *et al*. (2007), with little modification. Briefly, 300 µL 50 mM sodium phosphate buffer (pH 7.5), 100 µL of serial concentrations (10, 20, 30 and 40 µg/mL) of each extract in dimethyl sulphoxide (DMSO), 100 µL of the freshly prepared liver tissue homogenate and 100 µL distilled water were mixed in separate test tubes. The mixtures were then pre-incubated at 37°C for 15 min, after which 200 µL of freshly prepared 0.15 mM xanthine solution (substrate) was added. The mixtures were incubated again at 37°C for 30 min, after which 200 µL 0.5 M HCl was added to terminate the reaction. Thereafter, the absorbance was read at 295 nm using a UV spectrophotometer against a reagent blank containing the phosphate buffer in place of the liver tissue homogenate. A reference test containing 100 µL DMSO in place of the extract was included in the assay so as to obtain the maximum uric acid formed; while allopurinol was used as a positive control. The percentage inhibition of XO by each extract was calculated, and the IC_{50} was obtained from the non-linear regression equation of the % inhibition versus extract concentration curve.

$$\% \text{ XO inhibition} = [(Abs_{reference} - Abs_{sample}) \div Abs_{reference}] \times 100;$$

where $Abs_{reference}$ = absorbance of the reference test without the extract, and Abs_{sample} = absorbance of test extract.

ACE inhibition assay in vitro

The ability of the extracts to inhibit ACE was tested following the method reported by Cushman and Cheung (1971). Briefly, 50 µL of serial concentrations (10, 20, 30 and 40 µg/mL) of the extract was mixed with 50 µL ACE solution (4 mU/mL), and the mixture was incubated at 37°C for 15 min. Thereafter, 150 µL 8.33 mM of the substrate (hippuryl-histidyl-leucine) in 125 mM Tris–HCl buffer (pH 8.3) was added to the reaction mixture, and incubated for 30 min at 37°C. Next, the reaction was terminated by adding 250 µL 1 M HCl to the mixture, and the hippuric acid (HA) formed was extracted with 1.5 mL ethyl acetate. The ethyl acetate layer of the mixture was separated by centrifugation, and 1.0 mL aliquot of it was dispensed into a clean test tube and evaporated to dryness in a hot-air oven. The HA residue was dissolved in 1.0 mL deionised water, and its absorbance was measured at 228 nm using a UV spectrophotometer. A positive control test (containing 64 nmol/L captopril) and a reference test (without the extract) were included in the assay. The percentage inhibition of ACE by the extracts was calculated, and the IC_{50} was obtained from the non-linear regression equation of the % inhibition versus extract concentration curve.

$$\% \text{ ACE inhibition} = [(Abs_{reference} - Abs_{sample}) \div Abs_{reference}] \times 100;$$

where $Abs_{reference}$ = absorbance of the reference test without the extract, and Abs_{sample} = absorbance of test extract.

DPPH scavenging assay*

The method reported by Cervato *et al*. (2000) was followed to test the ability of the extracts to scavenge DPPH*. Briefly, 1.0 mL of serial concentrations (4, 8, 12 and 16 µg/mL) of the extracts was mixed with 3.0 mL DPPH* solution (60 µM) in test tube. The test mixture was then kept in the dark for 30 min, after which the absorbance was measured at 517 nm using a UV spectrophotometer. A reference test (containing methanol in place of the extract), and a positive control test (containing ascorbic acid solution in place of the extract) were included in the assay. The ability of the extracts to scavenge DPPH* was calculated as % scavenging ability and expressed as half-maximal scavenging concentration (SC_{50}), which was obtained from the non-linear regression equation of the % scavenging ability versus extract concentration curve.

$$\% \text{ DPPH scavenging ability} = [(Abs_{reference} - Abs_{sample}) \div Abs_{reference}] \times 100;$$

where $Abs_{reference}$ = absorbance of the reference test without the extract, and Abs_{sample} = absorbance of test extract.

ABTS^{+} scavenging assay*

The method reported by Re *et al*. (1999) was followed to test for the ability of the extracts to scavenge ABTS*$^{+}$. ABTS*$^{+}$ reagent was prepared by mixing equal volume of 7 mM ABTS*$^{+}$ and 2.45 mM $K_2S_2O_8$ aqueous solutions, and incubating at room temperature in the dark for 16 h. Subsequently, its absorbance was adjusted to 0.7 ± 0.02 with 95% ethanol at 734 nm. Next, 2.0 mL ABTS*$^{+}$ reagent was mixed with 0.2 mL extract, and the mixture was kept in the dark for 15 min. Thereafter, its absorbance was read at 734 nm using a UV spectrophotometer. The ability of the extract to scavenge ABTS*$^{+}$ was later calculated from a standard curve prepared using Trolox, and expressed as Trolox equivalent antioxidant capacity (TEAC).

Analysis of phenolic acids and flavonoids by HPLC-DAD

The phenolic acid and flavonoid constituents of the extracts were quantified using a reverse-phase high performance liquid chromatography (HPLC-DAD) system (Shimadzu, Kyoto, Japan), equipped with SPD-M20A diode array detector and LC solution 1.22 SP1 software. Extracts were injected at a concentration of 12 mg/mL, and chromatographic separations were carried out using Phenomenex C_{18} column (4.6 mm × 250 mm × 5 µm particle size). The mobile phase was HPLC grade water with 1% formic acid (v/v) (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 0.6 mL/min and injection volume of 40 µL. The gradient programme used followed the method described by Fernandes *et al*. (2016) with slight modification. The extracts and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath before use. Stock solutions of standard references were prepared in methanol:water (1:1, v/v) at a concentration range of 0.030 – 0.500 mg/mL. Phenolic acids and flavonoids in the extracts were measured at the following wavelengths: 257 nm (gallic acid); 326 nm (caffeic and chlorogenic acids) and 365 nm (quercetin, quercitrin, apigenin and kaempferol). The chromatography peaks were confirmed by comparing their retention time with those of reference standards and by DAD spectra matching (200 to 600 nm). All chromatography analyses were carried out in triplicate at ambient temperature. The limit of detection (LOD) and limit of quantification (LOQ) of the HPLC-DAD were calculated as earlier defined by Boligon *et al*. (2015).

Statistical analysis

Results of triplicate experiments were presented as mean ± standard deviation (SD). Independent samples *t*-test was performed on the data at 95% confidence level, using version 17 of SPSS statistical software package.

Result

The abilities of the cocoa bean powder (CBP) and cocoa pod husk powder (CPHP) extracts to inhibit XO and ACE, expressed in terms of their half-maximal inhibitory concentrations (IC_{50}), are presented in Table 1. CBP had significantly ($p < 0.05$) lower IC_{50} (41.08 ± 2.09 µg/mL) than CPHP (IC_{50}: 42.97 ± 2.21 µg/mL) on XO. However, the IC_{50} (5.60 ± 0.23 µg/mL) of allopurinol, a reference XO inhibitor, was much lower than those of the CBP and CPHP. Similarly, CBP had significantly ($p < 0.05$) lower IC_{50} (26.34 ± 1.63 µg/mL) than CPHP (IC_{50}: 31.03 ± 1.75 µg/mL) on ACE; but captopril, a reference ACE inhibitor, had a much lower IC_{50} (4.35 ± 0.18 µg/mL) than both.

Table 1. IC_{50} of cocoa bean powder (CBP) and cocoa pod husk powder (CPHP) extracts on xanthine oxidase (XO) and angiotensin I-converting enzyme (ACE) activities.

Extract/ Standard	XO IC_{50} (µg/mL)	ACE IC_{50} (µg/mL)
CBP	41.08 ± 2.09^{b}	26.34 ± 1.63^{b}
CPHP	42.97 ± 2.21^{c}	31.03 ± 1.75^{c}
Allopurinol	5.60 ± 0.23^{a}	ND
Captopril	ND	4.35 ± 0.18^{a}

Results are presented as mean ± standard deviation of triplicate (n = 3) determinations. Values with different lowercase superscript letter along the same row vary significantly at $p < 0.05$; ND: not detected.

The free radicals-scavenging abilities of CBP and CPHP extracts, tested using DPPH* and ABTS*$^{+}$ assays, are presented in Table 2. The half-maximal scavenging concentration (SC_{50}) of CBP (23.16 ± 1.85 µg/mL) on DPPH* was significantly ($p < 0.05$) lower than that of CPHP (SC_{50}: 28.24 ± 2.06 µg/mL); indicating that CBP had a stronger DPPH*-scavenging ability than CPHP. In comparison, ascorbic acid, a reference antioxidant with SC_{50} of 7.13 ± 0.54 µg/mL, exhibited a much stronger DPPH*-scavenging potential than CBP and CPHP. However, the abilities of CBP and CPHP to scavenge ABTS*$^{+}$ (1.83 ± 0.09 and 1.61 ± 0.04 mmol TEAC/g, respectively) were comparable ($p > 0.05$).

Table 2. DPPH* SC_{50} and ABTS*$^{+}$ scavenging ability of cocoa bean powder (CBP) and cocoa pod husk powder (CPHP) extracts.

Antioxidant activity	CBP	CPHP	Ascorbic acid
DPPH* SC_{50} (µg/mL)	23.16 ± 1.85^{b}	28.24 ± 2.06^{c}	7.13 ± 0.54^{a}
ABTS*$^{+}$ scavenging ability (mmol TEAC/g)	1.83 ± 0.09^{a}	1.61 ± 0.04^{a}	ND

Results are presented as mean ± standard deviation of triplicate (n = 3) determinations. Values with different lowercase superscript letter along the same row vary significantly at $p < 0.05$; ND: not detected.

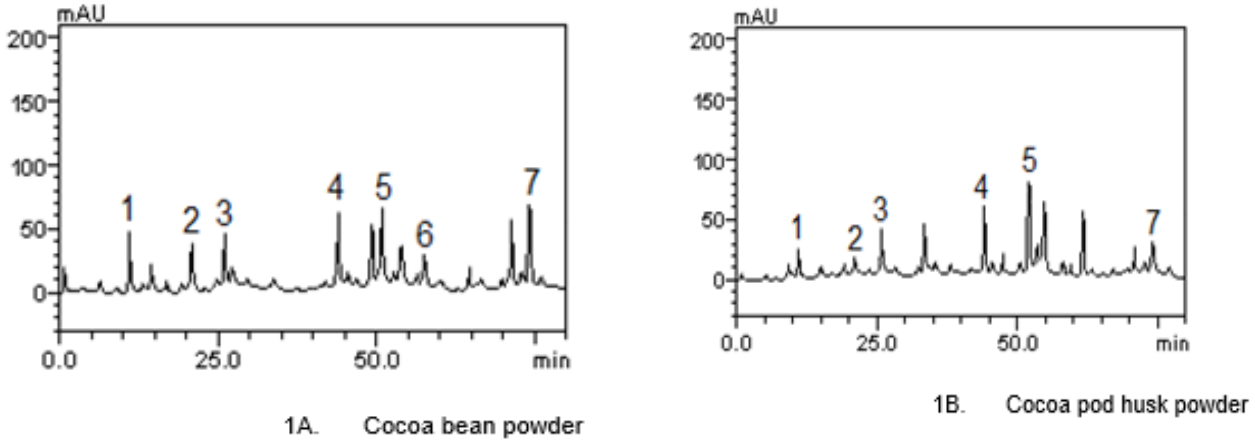

Figure 1. Representative HPLC-DAD profiles of cocoa bean powder (1A) and cocoa pod husk powder (1B) extracts. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), quercitrin (peak 4), quercetin (peak 5), kaempferol (peak 6) and apigenin (peak 7).

Table 3. Phenolic acids and flavonoid constituents of cocoa bean powder (CBP) and cocoa pod husk powder (CPHP) extracts.

Phenolic constituents (mg/g)	CBP	CPHP	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	1.73 ± 0.02^{a}	0.83 ± 0.03^{b}	0.016	0.053
Chlorogenic acid	1.54 ± 0.01^{a}	0.17 ± 0.04^{b}	0.027	0.089
Caffeic acid	1.69 ± 0.04^{a}	1.52 ± 0.01^{a}	0.008	0.026
Quercitrin	2.37 ± 0.01^{a}	2.24 ± 0.02^{a}	0.011	0.035
Quercetin	2.41 ± 0.01^{a}	2.63 ± 0.01^{a}	0.023	0.079
Kaempferol	0.90 ± 0.03	ND	0.009	0.030
Apigenin	2.45 ± 0.02^{a}	0.89 ± 0.05^{b}	0.025	0.081

Results are presented as mean ± standard deviation of triplicate (n = 3) determinations. Values with different lowercase superscript letters along the same row differ significantly at p < 0.05. LOD: Limit of detection; LOQ: Limit of quantification; ND: not detected".

The HPLC profiles of the phenolic constituents of CBP and CPHP extracts are shown in Figures 1A and 1B. Both extracts contained gallic acid (retention time-tR = 11.37 min, peak 1), chlorogenic acid (tR = 21.06 min, peak 2), caffeic acid (tR = 25.18 min, peak 3), quercitrin (tR = 43.11 min, peak 4), quercetin (tR = 50.13 min, peak 5), and apigenin (tR = 74.03 min, peak 7). In addition to these, CBP also contained kaempferol (tR = 56.94 min, peak 6) (Figure 1A). These phenolic compounds fall into the categories of phenolic acids (gallic, chlorogenic and caffeic acids) and flavonoids (quercitrin, quercetin and apigenin). As presented in Table 3, the levels of gallic and chlorogenic acids, and apigenin were significantly ($p < 0.05$) higher in CBP than in CPHP; whereas their levels of caffeic acids, quercitrin and quercetin were comparable ($p > 0.05$).

Discussion

In the present work, both CBP and CPHP extracts inhibited XO. A comparison of their IC_{50} values (41.08 ± 2.09 and 42.97 ± 2.21 µg/mL for CBP and CPHP, respectively) shows that CBP, with a significantly ($p < 0.05$) lower IC_{50}, displayed a stronger XO inhibition than CPHP. However, the IC_{50} values of both extracts (CBP and CPHP) were almost similar to the IC_{50} of some other plant extracts that have been recently reported to inhibit XO, including *Tetrapleura tetraptera* fruit (IC_{50}: 45.71 ± 1.44 µg/mL) (Irondi *et al*., 2015) and Olea europaea leaf (IC_{50}: 42 µg/mL) (Flemmig *et al*., 2011). The abilities of CBP and CPHP to inhibit XO might be attributed to the phenolic acids and flavonoids present in each of them. Possibly, these polyphenolics (phenolic

acids and flavonoids), like allopurinol, inhibit XO by binding at its purine binding site (Hawkes *et al*., 1984), and consequently, prevent the final formation of uric acid.

Similarly, both CBP and CPHP extracts inhibited ACE. However, the IC_{50} of CBP (26.34 ± 1.63 µg/mL) on ACE was significantly ($p < 0.05$) lower than that of CPHP (31.03 ± 1.75 µg/mL), indicating that CBP had stronger ACE inhibitory effect than CPHP. Relative to some other plant extracts that have been reported to inhibit ACE, the CBP and CPHP extracts in the present work had stronger inhibitory potency on ACE than *Ocimum gratissimum* (IC_{50}: 56.63 ± 3.12 µg/mL), as reported by Shaw *et al*. (2017); but had a weaker potency than guava (*Psidium guajava*) leaves (IC_{50}: 21.06 ± 2.04 µg/mL) (Irondi *et al*., 2016). The ACE inhibitory potency of CBP and CPHP observed in the present work might be due to their constituent phenolic acids and flavonoids. Some salient features of phenolic acids, including the ease of formation of charge-charge interactions between the oxygen atom in their carboxylate moiety with the Zn^{2+} in the active site of ACE; formation of a stable complex with the ACE through their interaction with the amino acids residues at the active site of ACE; and the overall contribution of their functional groups (COO^- and OH^-), all help to enhance their ability to inhibit ACE (Shukor *et al*., 2013). Similarly, some structural features in flavonoids, including the catechol group they have in their B-ring, the double bond existing between their C2 and C3 at the C-ring, and the cetone group present in the C4 of their C-ring all contribute to boost their ability to inhibit ACE (Guerrero *et al*., 2012). Interestingly, caffeic acid (Bhullar *et al*., 2014) and quercetin (Shukor *et al*., 2013), one of the most predominant phenolic acids and flavonoids, respectively, in both CBP and CPHP had been reported to be potent ACE inhibitors by earlier studies.

XO- and ACE-catalysed reactions generate free radicals and ROS in the body cells; making oxidative stress a denominator of both hyperuricemia and hypertension. Therefore, enhancing the body's antioxidant defence system is important for the management and/or treatment of hyperuricemia and hypertension (Irondi *et al*., 2017). In the present work, CBP scavenged DPPH* more than CPHP; whereas their $ABTS^{*+}$ scavenging potencies were comparable. When compared to the findings of previous studies, the CBP in the present work exhibited stronger DPPH* scavenging effect (SC_{50}: 23.16 ± 1.85 µg/mL) than what was observed (IC_{50}: 7.4 mg/mL) by Ali *et al*. (2015). However, the DPPH* scavenging effect of the CPHP (SC_{50}: 28.24 ± 2.06 µg/mL) was comparable with the value (EC50: 26.10 µg/mL) previously reported for cocoa pod extract by Abdul Karim *et al*. (2014). The $ABTS^{*+}$ scavenging capacities of CBP and CPHP in the present work (1.83 ± 0.09 and 1.61 ± 0.04 mmol TEAC/g, respectively) were both higher than the $ABTS^{*+}$ scavenging activities reported for two pure cocoa powder samples (0.280 and 0.238 mmol/g) by Oboh and Omoregie (2011). The free radicals scavenging activities of CBP and CPHP might be attributed to their constituent phenolic acids and flavonoids. These phenolic compounds possess redox properties that enable them to act as hydrogen donors, singlet oxygen quenchers and reducing agents (Chang *et al*., 2001).

It is well-known that the various health benefits of food and medicinal plants are due to their phytochemical components, including phenolic acids and flavonoids (Ajila *et al*., 2011). Quercetin in particular has been reported to possess anti-hyperuricemic effect through the inhibition of XO (Zhu *et al*., 2004), and anti-hypertensive activity through the inhibition of ACE (Guerrero *et al*., 2012). Three phenolic acids (gallic, chlorogenic and caffeic acids) and three flavonoids (quercitrin, quercetin and apigenin) were detected in both CBP and CPHP extracts. In addition to these, kaempferol, another flavonoid, was also detected but only in CBP. The levels of gallic and chlorogenic acids, and apigenin were higher in CBP than in CPHP; while their caffeic acid, quercitrin and quercetin levels were comparable. The levels of gallic and chlorogenic acids (1.73 ± 0.02 and 1.54 ± 0.01 mg/g, respectively) detected in CBP in the present work were higher than the quantities of gallic acid (0.84 ± 0.45 mg/g) and chlorogenic acid (1.18±0.33 mg/g) in polyphenol fraction of cocoa powder extract reported earlier by Ali *et al*. (2015). Furthermore, the gallic acid, caffeic acid and quercetin we detected in CPHP were reported to be absent in cocoa pod extract (Abdul Karim *et al*., 2014). The variations in the polyphenolic constituents and free radicals scavenging abilities of CBP and CPHP observed in the present work and those reported previously by other researchers might be due to differences in genetic and environmental factors, as well as methods of extraction (Mpofu *et al*., 2006), which are known to affect the phytochemical constituents and antioxidant activities of plants.

Conclusion

Cocoa bean powder and cocoa pod husk powder extracts inhibited xanthine oxidase and angiotensin 1-converting enzyme *in vitro*. The extracts also scavenged DPPH* and $ABTS^{*+}$. These bioactivities

could be attributed to the phenolic acids and flavonoids constituents of the extracts. Therefore, both cocoa bean powder and cocoa pod husk powder might be useful for the prevention and/or management of hyperuricemia and hypertension, and mitigation of oxidative stress in both diseases. However, cocoa bean powder might be more effective than the cocoa pod husk powder due to its stronger xanthine oxidase and angiotensin 1-converting enzyme inhibitory effects, and higher levels of some phenolic compounds.

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