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Hydroethanolic plant extracts from Cameroon positively modulate enzymes relevant to carbohydrate/lipid digestion and cardio-metabolic diseases

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Cardiovascular diseases are the greatest cause of death globally and are frequently associated with type 2 diabetes mellitus and metabolic syndrome, a condition including visceral obesity, hypertension, elevated triglycerides and low HDL cholesterol and hyperglycaemia. Several medicinal plants, including spices, are used in Cameroon as herbal medicines and are traditionally employed for the treatment of several ailments such as diabetes and related diseases. In this study, we chemically characterized eleven Cameroonian spice extracts and evaluated their effects on some enzyme activities relevant to carbohydrate and lipid digestion and cardio-metabolic diseases. Hydroethanolic spice extracts were characterized by GC-MS analysis and screened for their ability to modulate the activity of α-glucosidase, α-amylase, pancreatic lipase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase and angiotensin-converting enzyme (ACE). Among the spice extracts tested, those from *Xylopia parviflora* showed the widest inhibitory spectrum, with a relevant effect on all enzyme activities. *Dichrostachys glomerata* and *Aframomum citratum* extracts were more selective. The selected and strong activity of some plants, such as that of *Aframomum citratum* on pancreatic lipase and that of *Xylopia aethiopica* on ACE, suggests their specific use in obesity and hypertension, respectively. Chemical analysis indicated that for some spice extracts such as *Xylopia parviflora* and *Aframomum citratum* their secondary metabolites (chlorogenic acid, pimaric acid, and catechin and its derivatives) could potentially justify the biological properties observed. Our findings clearly show significant inhibition of cardio-metabolic enzymes by hydroethanolic Cameroonian spice extracts, suggesting the potential usefulness of nutraceuticals derived from these plants to develop novel management strategies for obesity and diabetes complications.

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Introduction

The global prevalence of obesity and type 2 diabetes mellitus (T2DM) has increased significantly over the past decades, in association with the increased prevalence of metabolic syndrome (MetS).[1] Globally, the prevalence of MetS varies between 1.2% and 22.6% among youth and between 9.0% and 35.0% for adults, depending on the chosen definition of MetS, the geographical area, the design of the study, the year of implementation of the study, the age of the cohort and the target population.[2] MetS is a cluster of some pathological features, including visceral adiposity, hypertension, increased glycaemia or T2DM, and atherogenic dyslipidemia, and is associated with a greater risk of cardiovascular morbidity and mortality.[3]

Its main pathophysiological driver is insulin-resistance, which is currently managed by improved nutrition, physical

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activity, and some pharmacological approaches, such as the use of metformin.[4] In addition, a variety of other therapeutic drugs is available for the management of the different components of MetS by targeting specific enzymes involved in the control of cardiovascular and metabolic functions, as well as in the process of nutrient digestion. These agents include hypoglycemic agents such as acarbose, miglitol and voglibose, known to inhibit a wide range of amylases and glycosidases;[5] inhibitors of pancreatic lipase, such as orlistat,[6] able to reduce lipid digestion; statins, which are well-known 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR) inhibitors,[7] leading to reduced circulating cholesterol; and captopril, benazepril and related compounds, working as angiotensin converting enzyme (ACE) inhibitors.[8] In addition to these therapeutic agents, relevant research studies in the cardiometabolic area have recently been focused on the validation of the use of food supplements[9,10] and phytotherapeutical products.[11] In this context, the potential of spices, traditionally used in local contexts for their nutritional and health-promoting properties, for the treatment of MetS is still largely unexplored and could represent an alternative strategy for the validation of novel effective and safe anti-diabetes, anti-obesity, anti-dyslipidemia and anti-hypertension agents.

Since the above-mentioned enzymes, involved in nutrient digestion and cardiovascular and metabolic functions, may be potential targets for phytotherapeutic approaches, we explored the modulatory effects of a series of nutritional spice extracts from Cameroon on the activity of a panel of enzymes, differentially involved in the pathophysiology of MetS. The phytochemical profiles of nutritional products have been preliminarily evaluated to propose potential candidate extract components responsible for the observed activities.

Materials & methods

Chemicals

α-Amylase, α-glucosidase, porcine pancreatic lipase (PPL; triacylglycerol lipase, EC 3.1.1.3), HMG-CoAR, ACE, dinitrosalicylic acid, *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), *p*-nitrophenyl butyrate (*p*-NPB), nicotinamide adenine dinucleotide (NADPH), *N*-[3-(2-furyl) acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG), acarbose, orlistat, pravastatin and captopril were obtained from Sigma-Aldrich (St Louis, MO, USA). All reagents were of the highest grade available.

Preparation of plant extracts

Plant materials consisted of eleven spices (*Xylopia aethiopica* (Dunal) A. Rich., *Xylopia parviflora (A. Rich.) Benth, Scorodophloeus zenkeri* Harms, *Monodora myristica* (Gaertn.) Dunal, *Tetrapleura tetraptera* (Schum. & Thonn.) Taub., *Echinops giganteus* A. Rich., *Dichrostachys glomerata* (Forssk.) Chiov., *Afrostyrax lepidophyllus* Mildbr., *Aframomum melegueta* K.Schum., *Aframomum citratum* (C.Pereira) K. Schum., and *Zanthoxylum leprieurii* Guill. & Perr.) harvested in various localities of the region of West Cameroon in September 2017. Selected samples were made up of different fruits, seeds and roots identified in the National Herbarium of Cameroon (http://irad.cm/national-herbarium-of-cameroun/) in Yaoundé (Cameroon) on the basis of comparison with the preserved specimens. Briefly, air dried and powdered samples (100 g) were subjected to magnetic stirring with 100 mL of a 70% hydroethanolic mixture for 4 and 16 hours, respectively, at room temperature under dark conditions. The mixture was filtered through Whatman cellulose filter paper, and the filtrate obtained was concentrated under reduced pressure, and then frozen in a cold room with dry CO_2 ice and alcohol to give residues which constituted the crude extracts. They were then placed at −20 °C and, after 5 minutes, lyophilized and maintained at −20 °C for subsequent biological studies. Plant solution stocks (100 mg mL^{-1}) dissolved in DMSO were prepared, aliquoted and kept at −80 °C.

Enzyme inhibition assays

The extracts were tested using different enzyme inhibition assays at a concentration of 100 μg mL^{-1}, except for PPL inhibition, where the extract concentration was 5 mg mL^{-1}. The concentration of 100 μg mL^{-1} was selected, since the general guidelines suggest that biological analysis of crude extracts should be performed at concentrations below 100 μg mL^{-1}.[12]

α-Amylase inhibition assay. The α-amylase inhibition assay was conducted according to the protocol described in the Worthington enzyme manual with some modifications.[13] In summary, 20 μL of each plant extract solution (100 μg mL^{-1}) or acarbose (200 μg mL^{-1}), used as the reference inhibitor, and 20 μL α-amylase solution (0.5 mg mL^{-1}) in a 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) were incubated at 25 °C for 10 min. After preincubation, 20 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped with 40 μL of a dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was then diluted by adding 600 μL bidistilled water, and the absorbance was measured at 540 nm using a microplate reader.

α-Glucosidase inhibition assay. The α-glucosidase inhibition assay was performed according to the protocol reported in the Worthington Enzyme Manual with some modifications.[13] The assay involved incubation, in a 96-well plate at 25 °C for 10 min, of 50 μL aqueous plant extracts or acarbose (200 μg mL^{-1}), used as the reference inhibitor, and 100 μL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase (1 unit per mL) solution. After preincubation, 50 μL of 5 mM pNPG solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. Before and after incubation, absorbance readings were recorded at 405 nm using a microplate reader.

Pancreatic lipase inhibition assay. The PPL inhibition assay was conducted according to the study in ref. 14. The assay involved mixing 30 μL PPL (2.5 mg mL^{-1} in 10 mM morpholi-

nepropanesulphonic acid and 1 mM ethylenediamine tetraacetic acid, pH 6.8) with 850 μl Tris buffer (100 mM Tris-HCl and 5 mM $CaCl_2$, pH 7.0). Then, either 100 μL plant extracts or orlistat (100 μg mL^{-1}), used as the reference inhibitor, were added and incubated for 15 min at 37 °C. 10 μL substrate (10 mM *p*-NPB in dimethyl formamide) was then added and incubated for 30 min at 37 °C. Lipase activity was determined by measuring the hydrolysis of *p*-NPB to *p*-nitrophenol at 405 nm using the microplate reader.

HMG-CoAR inhibition assay. The assay was conducted according to the protocol reported in the HMG-CoAR assay kit (Sigma-Aldrich).[15] The assay was conducted by placing 181 μL phosphate buffer with 1 μL plant extract or pravastatin (300 μM), used as the reference inhibitor, into 96-well plates; 4 μL of NADPH (to obtain a final concentration of 400 μM) and 12 μL of HMG-CoAR substrate (to obtain a final concentration of 0.3 mg mL^{-1}) were then added. The analyses were initiated (time 0) by the addition of 2 μL of HMG-CoAR (concentration of the enzyme stock solution was 0.50–0.70 mg protein per mL) and incubated at 37 °C. The rates of NADPH consumed were monitored every 20 s for up to 600 s by reading the decrease in absorbance at 340 nm, using the microplate reader.

ACE inhibition assay. The assay was performed according to a previously described protocol,[16] with some modifications. The assay involved mixing 5 μL ACE (1 U mL^{-1}) with 50 μL of different concentrations of plant extracts, or captopril (100 Mm), used as the reference inhibitor, dissolved in 50 mM Tris-HCl buffer, pH 7.5, containing 0.3 M NaCl, and then 0.25 mL of 5×10^{-4} M FAPGG dissolved in 50 mM Tris-HCl buffer, pH 7.5, containing 0.3 M NaCl, was added. The decrease of absorbance at 345 nm was recorded over 5 min at room temperature using the microplate reader.

For all enzyme inhibition assays, the absorbances of sample blanks (buffer instead of enzyme solution) and a control (buffer in place of plant extract) were recorded as well. The final sample absorbance was obtained by subtracting its corresponding sample blank reading.

The inhibition of enzyme activity was calculated according to the equation below:

$$\text{Inhibition } (\%) = \left(\frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{Control}}}\right) \times 100.$$

For all assays, experiments were performed three times (3 replicates) with "%"considered as the percentage of inhibition.

Phytochemical profiling by gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis was conducted according to the study in ref. 17 with minor modifications, using a Bruker Scion SQ instrument (Bruker, Milan, Italy). An aliquot (100 μL) of the hydroalcoholic extract (10 mg mL^{-1}, ethanol/water 7 : 3) was taken to dryness under reduced pressure and subsequently derivatized by the addition of 30 μL of pyridine and 70 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). After 1.5 h of incubation at 62 °C, 100 μL of ethylacetate was added and 1.0 μL of the solution was injected into the GC-MS system. Identification was done by comparison with the retention times and fragmentation patterns of authentic standards when available, or by comparison with those present in the National Institute of Standards and Technology (NIST) spectral library (NIST, 2011 vers. 2.0), or by comparison with those present in the literature. Kovats retention indexes (KI) were calculated using chromatographic data of a homologous series of *n*-alkanes C7–C29 (data not shown). The percentage composition of the extracts was computed by the normalization method from the GC peak areas, calculated as the mean of three injections for each extract, without correction factors.

Statistical analysis

Results from at least three independent experiments carried out in triplicate were expressed as mean(± SD) values or as a mean percentage (%) compared to a control. Mean differences were determined by one-way ANOVA. The Waller–Duncan test was used to test for differences of means. Pearson's linear test was used to test for correlation and the values of less than 0.05 were considered to be statistically significant. All graphs were generated using SPSS (Version 25).

Results

Description of plant extracts

The extraction yield was 7.1–32.7% for seeds, 6.4–49.2% for fruits and 13.1% for the root sample (Table 1). In general, most of the extracts were in solid form (crystalline powders). Only in the case of *Monodora myristica* seeds, an oily extract was found.

Effects of plant extracts on enzyme activities

α-Amylase and α-glucosidase. We found that the activity of α-amylase was significantly ($p < 0.05$) inhibited by extracts of *Xylopia parviflora* (40.9%) *and Dichrostachys glomerata* (25.0%), whereas α-glucosidase activity was significantly ($p < 0.05$) inhibited by *Xylopia parviflora* (34.0%) and *Xylopia aethiopica* (27.3%) (Table 2). However, this inhibitory activity was significantly lower ($p < 0.05$) compared to that of acarbose, which reduced the activity of α-amylase and α-glucosidase by 86.7% and 52.5%, respectively.

Pancreatic lipase. *Xylopia parviflora* (92.8%) and *Aframomum citratum* (89.6%) extracts significantly inhibited PPL activity, with a greater effect compared to orlistat (58.8%), used as the positive control (Table 2). *Tetrapleura tetraptera* (35.7%) and *Dichrostachys glomerata* (43.4%) extracts showed a non-significant inhibitory activity compared to orlistat. It is noteworthy that the plant extracts were used here at a concentration of 5 mg mL^{-1}, since no activity was observed using 100 μg mL^{-1} and 1 mg mL^{-1} concentrations (data not shown).

HMG-CoAR. All plant extracts showed a low, non-significant inhibitory activity on HMG-CoAR, compared to pravastatin (81.7%). However, compared to other extracts, *Xylopia parvi-*

Table 1 Identification and yield of extracts of plants

Plant name	Part used	Family	Herbarium voucher number	Extract color	Extract aspect	Extraction yield (%)
Xylopia aethiopica (Dunal) A. Rich	Fruits	Annonaceae	16419/SRF-Cam	Brown-strand	Powder	23.9
Xylopia parviflora (A. Rich.) Benth	Seeds	Annonaceae	6431/SRF-Cam	Brown-beige	Powder	20.5
Scorodophloeus zenkeri Harms	Seeds	Fabaceae	44803/HNC	Brown-auburn	Crystal	16
Monodora myristica (Graertm.) Dunal	Seeds	Annonaceae	2949/SRF-Cam	Yellow-saffron	Oil	27.9
Tetrapleura tetraptera (Schum. & Thonn.)Taub	Fruits	Fabaceae	12117/SRF-Cam	Brown-bistra	Powder	49.2
Echinops giganteus Var Lellyi C. D. Adams	Roots	Asteraceae	23647/SRF-Cam	Yellow-topaz	Powder	13.1
Afrostyrax lepidophyllus Mildbr	Seeds	Huaceae	44853/HNC	Yellow-amber	Crystal	7.1
Dichrostachys glomerata (Forssk.) Hutch	Seeds	Fabaceae	15220/SRF-Cam	Brown-coffee	Crystal	27.7
Aframomum melegueta (Roscoe) K.Schum	Fruits	Zingiberaceae	39065/HNC	Brown-acajou	Powder	11.5
Aframomum citratum (Pereira ex Oliv. and Hanb) K. Shum.	Fruits	Zingiberaceae	37736/HNC	Beige	Powder	6.4
Zanthoxylum leprieurii Guill. Et Perr.	Seeds	Rutaceae	37632/HNC	Brown-bistra	Powder	32.7

HNC: Cameroon national herbarium; SRF-Cam: société de réserves forestière du Cameroun.

Table 2 Enzyme inhibition assays: effect of plant extracts

	Inhibition (%, relative to control = 100)				
	α-Amylase	α-Glucosidase	Pancreatic lipase	HMG-CoAR	ACE
Acarbose (200 μg mL^{-1})	86.7 ± 0.4[a]	52.5 ± 2.9[a]			
Orlistat (100 μg mL^{-1})			58.8 ± 2.7[a]		
Pravastatin (300 μM)				81.7 ± 3.2[a]	
Captopril (100 mM)					71.1 ± 12.5[a]
Xylopia aethiopica	nd	27.3 ± 1.0[c]	nd	4.3 ± 3.1[d]	100[b]
Xylopia parviflora	40.9 ± 2.7[b]	34.0 ± 2.6[b]	92.8 ± 8.5[b]	12.9 ± 1.1[bc]	96.9 ± 3.6[ab]
Scorodophloeus zenkeri	nd	3.3 ± 0.4[f]	22.1 ± 8.4[e]	1.9 ± 2.3[b]	4.2 ± 16.8[d]
Monodora myristica	16.6 ± 3.5 [de]	4.7 ± 3.1[f]	nd	12.8 ± 3.0[bc]	50.7 ± 11.6[c]
Tetrapleura tetraptera	18.9 ± 3.5[d]	6.0 ± 2.5[e]	35.7 ± 2.2	8.0 ± 2.3[c]	96.1 ± 8.8[ab]
Echinops giganteus	nd	4.7 ± 3.2[f]	27.8 ± 7.8[d]	0.9 ± 3.3[c]	18.8 ± 17.6 [de]
Afrostyrax lepidophyllus	nd	nd	nd	1.8 ± 2.1[d]	26.2 ± 11.6[d]
Dichrostachys glomerata	25.0 ± 4.1[c]	11.5 ± 0.84[d]	43.4 ± 2.9[c]	1.4 ± 1.2[e]	69.6 ± 7.5[a]
Aframomum melegueta	12.4 ± 1.6[e]	11.2 ± 2.3[d]	nd	nd	37.2 ± 16.6[d]
Aframomum citratum	11.1 ± 0.7[e]	10.0 ± 3.7[d]	89.6 ± 5.8[b]	10.0 ± 2.5[d]	54.6 ± 12.9[c]
Zanthoxylum leprieurii	nd	4.5 ± 3.2[f]	nd	nd	100[b]

Data are mean ± SD; $n = 3$. The concentration of all plant extracts was 100 μg mL^{-1} for all assays, except for pancreatic lipase (concentration: 5 mg mL^{-1}). In a row, the assigned values of the different letters are significantly different at the 5% probability threshold (Waller–Duncan test). HMG-CoAR: 3-hydroxy-3-methylglutaryl coenzyme a reductase; ACE: angiotensin converting enzyme; nd: no inhibition detected; 100: complete inhibition.

flora (12.9%), *Monodora myristica* (12.8%) and *Aframomum citratum* (10.0%) were found to be more active (Table 2).

ACE. Some inhibitory activity on ACE was detected for all plant extracts, although to a different extent (Table 2). The active control captopril reduced ACE activity by 71.1%. A significant inhibitory effect was observed for extracts of *Monodora myristica* (50.7%), *Aframomum citratum* (54.6%), *Dichrostachys glomerata* (69.6%), *Tetrapleura tetraptera* (96.1%), and *Xylopia parviflora* (96.9%). In addition, *Zanthoxylum leprieurii* and *Xylopia aethiopica* extracts completely abolished ACE activity (100%). Extracts of *Scorodophloeus zenkeri* (4.2%), *Echinops giganteus* (18.8%), *Afrostyrax lepidophyllus* (26.2%) and *Aframomum melegueta* (37.2%) showed a lower, non-significant inhibitory activity.

The combined analysis of the previous assays (Table 2) showed a significant correlation ($p < 0.01$) between the results obtained from the α-amylase and α-glucosidase assays ($R = 0.957$) (Fig. 1). Moreover, a negative correlation was observed between the results obtained for the α-amylase and the HMG CoA reductase inhibition assays ($R = -0.303$) (Fig. 2). No correlation was observed between the results obtained from other different assays.

Plant extract metabolomic fingerprinting

The phytochemical compositional profile of the tested hydroalcoholic extracts was investigated by GC-MS analysis. Before analysis, the samples were derivatized by silanization to allow the simultaneous detection of analytes, from the most highly polar to the lipophilic components. In general, all extracts showed the presence of primary metabolites such as oligosaccharides, dicarboxylic acids and long chain fatty acids (Table 3). Among the extracts showing the most significant enzymatic inhibitory activity, beside glycerol and oligosaccharides (fructofuranose and isomeric forms of glucopyranose), *Xylopia parviflora* seeds showed inhibitory activity on PPL and ACE and partially on HMG-CoAR. In this extract, significant pro-

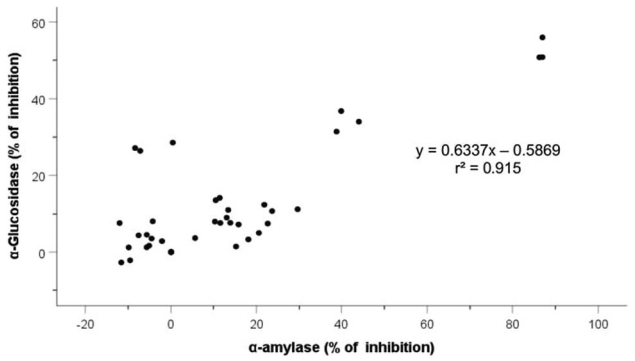

Fig. 1 Correlation analysis between the inhibition profiles of α-amylase and α-glucosidase produced by different spice extracts. The inhibitory activities on both enzymes are significantly correlated ($P < 0.01$, $R = 0.957$). Data are expressed as % inhibition compared to the control set at 100%.

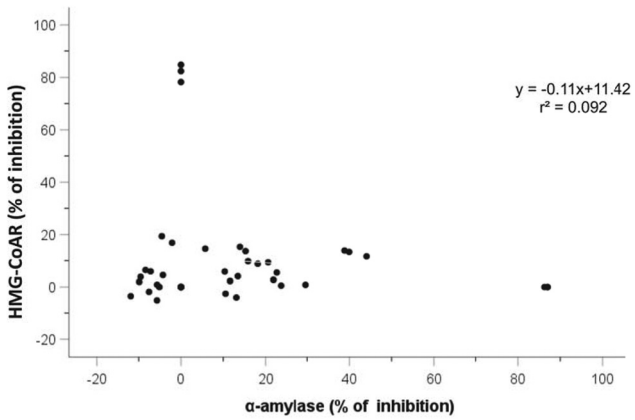

Fig. 2 Correlation analysis between the inhibition profiles of α-glucosidase and HMG-CoAR produced by different spice extracts. The two inhibitory activities on both enzymes are significantly inversely correlated ($P < 0.01$, $R = -0.303$). Data are expressed as % inhibition compared to the control set at 100%.

portions of pimaric acid (8.73%), catechin (total 3.81%) and chlorogenic acid derivatives (total 3.34%) were detected (Fig. 3A). The presence of these bioactive secondary metabolites can explain the observed inhibition of PPL activity.[18,19]

The GC-MS profile of *Aframomum citratum* fruits was dominated by the characteristic aliphatic acids malic acid and citric acid, as well as by a series of major monosaccharide and disaccharide sugars (Fig. 3B). 3,4-Dihydroxy-benzoic acid (0.49%), cinnamic acid (0.13%) and pimaric acid (0.05) were the main secondary metabolites identified at lower concentration levels.

In contrast, the partially HMG-CoAR inhibiting oily extract of *Monodora myristica* seeds (Fig. 3C) was constituted by a mixture of long-chain fatty acids with minor proportions of constituents typical of essential oils (*e.g.* 2,3-pinanediol, taucadinol, and alpha-cadinol) (Table 3). *Tetrapleura tetraptera* and *Zanthoxylum leprieurii*, notwithstanding their significant inhibitory activity against ACE, did not show the presence of bioactive substances in their GC-MS profiles (not shown).

Discussion

The modulation of enzyme activities relevant to the pathophysiology of metabolic disorders is an important target for the treatment of these diseases. In this regard, in addition to a wide range of specific drugs, a significant contribution can also be offered by the study of the potential activities of phytochemicals present in nutritional/medicinal plants.

In the present study, we explored the ability of hydroethanolic extracts, obtained from a series of nutritional plants traditionally used in Cameroon as both spices and medicaments,[20,21] to inhibit some enzyme activities with relevance to cardio-metabolic disorders. Interestingly, we found that some spice extracts showed potent inhibitory activity (in some cases up to 70–100%).

The inhibition of α-amylase and α-glucosidase is important in order to modulate/reduce the digestion/absorption of carbohydrates, with relevant advantages under pathological conditions such as insulin resistance, T2DM and metabolic syndrome.[21] In particular, inhibition of pancreatic α-amylase and α-glucosidase by acarbose is utilized in T2DM to slow down carbohydrate absorption and reduce post-prandial insulin secretion, in addition to promoting incretin (glucagon-like peptide (GLP)-1) release.[22] In this study, the evaluation of the ability of extracts to inhibit α-amylase activity revealed that *Xylopia parviflora* and *Dichrostachys glomerata* showed the highest inhibition (in the 25–40% range). Moreover, a relevant α-glucosidase inhibitory (27–34% of inhibition) activity was displayed by *Xylopia parviflora* and *Xylopia aethiopica*. For all plant extracts tested, we also found a significant correlation between the ability to inhibit α-amylase and that to inhibit α-glucosidase. Aqueous extracts from some tropical plants, including some studied here (*Xylopia aethiopica, Monodora myristica*, and *Aframomum Melegueta*)[23] and *Gynura divaricata* (L.)[24] were also found to inhibit α-amylase and α-glucosidase activities.

Taken collectively, these data indicate that inhibition of enzymes involved in the digestion of polysaccharides by dietary phytochemicals has promising potential.[25]

In the context of obesity treatment, inhibition of pancreatic lipase, thereby reducing the hydrolysis of dietary fats and thus their absorption, is a clinically validated approach, mainly through the drug orlistat.[6] Here we report that *Xylopia parviflora* and *Aframomum citratum* extracts showed a strong inhibitory effect (about 90%) on PPL, while a lower inhibition (about 40%) was observed with *Dichrostachys glomerata* and *Tetrapleura tetraptera.* These results suggest that polyphenolic species such as 3,4-dihydroxy benzoic acid, catechin and chlorogenic derivatives can concur, at least in part, significantly in the inhibition of this important enzyme.

Plants appear to be a good source of anti-lipase compounds, since inhibition of PPL by medicinal herbs has been reported for some Asian plants, including *Prunella vulgaris* L. and *Rheum palmatum* L. methanolic extracts.[26] Similar to our study, other plant extracts (*Oxalis cordata*,[27] *Afromomum melegueta* and *Spilanthes acmella*)[28] in a concentration range of

Table 3 Identification of the main representative secondary metabolites in the hydroalcoholic extracts of fruits or seeds from different plant species. RI: retention index and RT: retention time. For reasons of clarity, the silanized portions in the species name (where applicable) have been omitted

Species	RI	RT	Identification	(%)
Xylopia aethiopica	1432	29.988	Chlorogenic acid	2.36
	1863	39.238	Methylcopalate	0.31
	1948	40.361	Pimaric acid TMS	0.45
	2061	41.690	9-Methyl-prost-13-en-1-oic acid	0.34
Xylopia parviflora	1952	40.408	Pimaric acid	8.73
	3151	50.242	Catechin	2.81
	3224	50.667	Catechin	0.50
	3314	51.176	Chlorogenic acid	0.24
	4034	54.753	Chlorogenic acid	0.58
	4205	55.501	Chlorogenic acid	1.15
	4264	55.754	Chlorogenic acid	1.61
Scorodophloeus zenkeri	—	—	—	—
Monodora myristica	927	8.135	*o*-Cymene	0.05
	960	8.817	Benzene, butyl-	0.03
	1044	10.801	Benzene, pentyl-	0.06
	1096	12.315	*Trans*-1-phenyl-1-pentene	0.01
	1143	13.927	Thymol	0.02
	1149	14.177	Phenol-2-ethyl-4,5-dimethyl	0.03
	1160	14.609	2,3-Pinanediol	0.12
	1181	15.522	α-Cubebene	0.06
	1196	16.258	Ylangene	0.02
	1203	16.619	Isoleptospermone	0.06
	1206	16.773	α-Cubebene	0.01
	1219	17.499	2,3-Pinanediol	0.05
	1225	17.858	β-Copaene	0.01
	1253	19.609	α-Muurolene	0.05
	1259	20.009	γ-Muurolene	0.28
	1262	20.232	δ-Cadinene	0.24
	1266	20.492	Naphthalene-1,2,3,4,4α,7-hexahydro-1,6-	0.06
	1268	20.637	α-Copaene	0.05
	1281	21.605	Cubedol	0.07
	1283	21.735	Butyl-6,9,12,15-octadecatetraenoate	0.04
	1286	21.943	Cubedol	0.12
	1307	23.441	τ-Cadinol	0.44
	1312	23.813	α-Cadinol	0.16
	1500	32.224	Hexadecanoic acid, ethyl-ester-	2.97
	1521	32.816	Manoyl oxide	0.07
	1548	33.514	Hexadecanoic acid, trimethylsilyl-ester	1.78
	1648	35.721	*cis*-Vaccenic acid	19.09
	1666	36.070	Linoleic acid, methyl ester	30.97
	1673	36.213	(*E*)-9-Octadecenoic acid ethyl ester	31.67
	1679	36.316	Oleic acid, methyl ester	2.70
	1703	36.749	1-Ethyl-13-methyl-tetradecanoate	4.42
Tetrapleura tetraptera	—	—	—	—
Echinops giganteus	1287	21.980	6-*epi*-Shyobunol	1.1
	4370	56.197	Stigmasterol	0.26
	4457	56.552	α-Amyrin	0.98
	4516	56.792	β-Sitosterol	0.25
	4546	56.910	β-Amyrin	0.47
	4577	57.034	1,1,6-Trimethyl-3-methylene	1.42
	4654	57.333	Geranylgeraniol-*tert*-butyl	0.81
	4769	57.778	β-Amyrin	0.92
	4876	58.178	Lup-20(29)-en-3-ol, acetate	1.66
	5074	58.901	Lup-20(29)-en-3-ol, acetate	0.62
Dichrostachys glomerata	3151	50.246	Catechin	1.44
	3223	50.662	Catechin	0.32
	4945	58.432	4′,5,7-Trihydroxyflavanone	1.02
Afrostyrax lepidophyllus	—	—	—	—
Aframomum melegueta	1872	39.359	Paradol	3.71
	1946	40.336	Pimaric acid TMS	1.85
	1963	40.549	Shogaol	2.05
	2028	41.312	Gingerol derivative	1.86
	2169	42.833	Gingerol derivative	16.06
	2202	43.162	Gingerol	0.7
	4624	57.218	Catechin fragment	2.08
	4816	57.953	Catechin fragment	0.18

Table 3 *(Contd.)*

Species	RI	RT	Identification	(%)
Aframomum citratum	1393	28.393	3,4-Dihydroxy-benzoic acid	0.49
	1464	31.123	Cinnamic acid	0.13
	1946	40.335	Pimaric acid	0.05
	3219	50.636	(*E*)-3,7-Dimethylocta-2,6-dienyl isobutyl carbonate	5.02
Zanthoxylum leprieurii	—	—	—	—

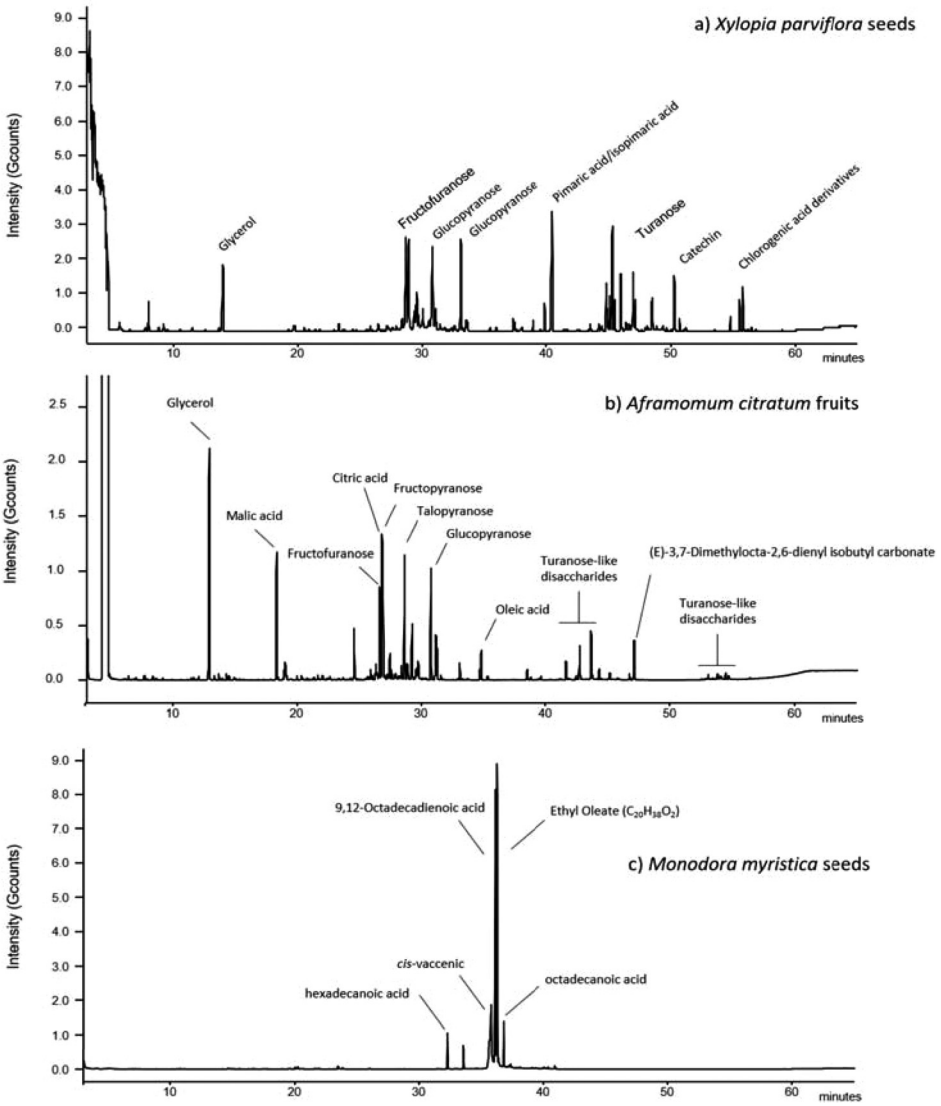

Fig. 3 GC-MS analysis of the phytochemical composition profile of selected hydroalcoholic spice extracts. (a) *Xylopia parviflora*; (b) *Aframomum citratum*; and (c) *Monodora myristica*.

1–5 mg mL^{-1} (but not lower) have also been shown to inhibit PPL. Another set of plant extracts, including *Salicis Radicis Cortex* as the most active, were found to inhibit PPL at lower concentrations (100 μg mL^{-1}), but to a lower extent (maximum 42%) than in our study.[29]

HMG-CoAR, the rate-limiting enzyme of cholesterol synthesis, is a well-known target of statins, a class of widely used drugs for the prevention and treatment of atherosclerotic cardiovascular diseases.[30] In this study, this enzyme, however, does not appear to be a target for all spice extracts tested, since they showed a very low inhibition (<13%) ability against it. On the other hand, HMG-CoAR activity was found to be inhibited up to 74% by some Malaysian medicinal herb extracts, including *Basella alba*.[7]

ACE, whose enzymatic activity leads to the generation of angiotensin II, the most active hypertensive effector of the

renin–angiotensin–aldosterone system,[31] is an important drug target for the management of arterial hypertension, also in the context of T2DM and obesity.[32]

We found that several spice extracts showed a potent inhibition of ACE activity. Specifically, *Xylopia aethiopica*, *Zanthoxylum leprieurii*, *Xylopia parviflora* and *Tetrapleura tetraptera* inhibited ACE by 96–100%, which is even greater than that observed for the positive control captopril. *Dichrostachys glomerata*, *Aframomum citratum* and *Monodora myristica* produced a lower, but relevant 50–70% inhibition. The lack of a clear indication of potential candidate substances responsible for these activities suggests that the observed activity arises from extract components different from secondary metabolites detected in the phytochemical profiling experiments, and/or from species with molecular weight and molecular characteristics not suitable to be monitored by the methodology employed (GC-MS after silanization). Further studies are warranted to clarify this apparent discrepancy.

Among the plants tested here, ACE activity inhibition has been previously reported for *Aframomum melegueta.*[33] ACE inhibition activity has also been reported for some plants different from those tested in this study, including *Salvia elegans Vahl.* (Lamiaceae)[34] and *Gynura divaricata* (L.).[24]

In addition to specific inhibition of one or two enzyme activities, some plant extracts, such as those from *Xylopia parviflora* and *Dichrostachys glomerata*, showed a wider inhibitory spectrum, with a relevant effect on all or almost all the enzyme activities tested. These effects may make these plant extracts even more interesting in the context of a complex cardiometabolic condition such as MetS.[11]

On the other hand, the selected and strong activity of the plant products selected for this study, such as that of *Aframomum citratum* on PPL and that of *Xylopia aethiopica* on ACE, makes them specifically useful in obesity and hypertension, respectively.

Similarities and discrepancies between the findings of this study compared to those from previous investigations on the same plant products may also arise from different experimental protocols (*i.e.* aqueous extraction *vs.* organic solvent extraction done with solvent systems different from the classic hydroethanolic mixture used in the present study).

Conclusions

Over the decades, the use of medicinal plants represented an important interaction between humans and the environment. According to the World Health Organization, about 80% of the human population depends on traditional/alternative medicine for the primary treatment of various diseases (https://apps.who.int/iris/bitstream/handle/10665/312342/9789241515436-eng.pdf?ua=1).

The present study provides data indicating that selected Cameroonian spice extracts are capable of inhibiting a set of enzyme activities relevant for nutrient digestion and cardiometabolic diseases, thereby suggesting that they may be used as potential complementary therapeutic agents for the prevention and management of MetS. Studies in cells or/and *in vivo* models could give further insights into the effects and roles of these spice extracts. Moreover, the mechanisms of these extracts in inhibiting the tested enzymes are unknown and the contribution of the multiple potentially active components detected requires further studies.

Abbreviations

ACE	Angiotensin converting enzyme
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
DMSO	Dimethylsulfoxide
FAPGG	*N*-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine
GC-MS	Gas chromatography-mass spectrometry
GLP-1	Glucagon-like peptide
HMG-CoAR	3-Hydroxy-3-methylglutaryl-coenzyme A reductase
KI	Kovats retention indexes
MetS	Metabolic syndrome
NADPH	Nicotinamide adenine dinucleotide
NIST	National Institute of Standards and Technology
T2DM	Type 2 diabetes mellitus
p-NPB	*p*-Nitrophenyl butyrate
*p*NPG	*p*-Nitrophenyl-α-D-glucopyranoside
PPL	Porcine pancreatic lipase
R	Correlation coefficient
SD	Standard deviation

Conflicts of interest

This study is an original research carried out by the cited authors and, therefore, the authors declare that there is no conflict of interests regarding the publication of this paper.

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