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Research Paper

Anti-amylase, anti-lipase and antioxidant effects of aqueous extracts of some Cameroonian spices

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ABSTRACT

Diabetes mellitus and associated co-morbidities including cardiovascular disease (CVD) and obesity are leading causes of mortality. In developing countries, where the per capita income is low, it is necessary to seek affordable alternative therapies. This study investigated 19 different commonly used Cameroonian spices for their polyphenol content, as well as their in vitro antioxidant, anti-amylase and anti-lipase activities. Results indicated that the aqueous extracts of *Aframomum daniellii*, *Hypodapnis zenkeri*, *Echinops giganteus*, *Aframomum citratum*, *Xylopia aethiopica*, had more than 75% inhibitory activity for pancreatic amylase. *Xylopia aethiopica* (92.25%) and *Scorodophloeus zenkeri* (56.39%) were most effective in inhibiting the activity of pancreatic lipase. *Dichrostachys glomerata* (81.58%), *Tetrapleura tetraptera* (83.94%) and *Xylopia parviflora* (90.55%) exhibited the most potent 2, 2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$) radical scavenging activity. These spices therefore exhibited properties that are beneficial to health and could therefore be used as an alternative and/or complementary strategy in managing risk factors and associated co-morbidities of diabetes mellitus.

Keywords: Cameroonian spices; Anti-amylase; Anti-lipase; Anti-oxidants.

INTRODUCTION

Herbs, spices and medicinal plants have been cherished by many ancient cultures for their use in curing common ailments and promoting good health (Lewis and Elvis-Lewis, 2003). Dietary spices are a heterogeneous collection of a wide variety of volatile and non-volatile chemicals obtained from dried aromatic parts of plants-generally the seeds, berries, roots, pods, and sometimes leave. Populations that use spices and/or herbs in their diets have been shown to have lower incidences of chronic disease (Duthie, et al., 2003). Studies have suggested that excessive intake of calories are related to chronic diseases, including type 2 diabetes mellitus, cardiovascular disease (CVD) and obesity. These are all linked to oxidative stress, causing an imbalance of pro oxidants and antioxidants in cellular systems, which impairs normal biological functions (Droge, 2002). One benefit of spices and herbs

is that they contain bioactive components such as polyphenols that can reduce oxidative stress and modulate harmful biological pathways, thereby reducing these chronic diseases. Polyphenols also offer an attractive strategy to control postprandial hyperglycemia, assist in weight management, and in the management of CVD, with minimal side effects. Various polyphenols have been reported to show radical scavenging activity (Sawa, et al., 1999) as well as inhibiting α-amylase and α-glucosidase activities (Kim, et al., 2000).

The present study was aimed at investigating and recording the polyphenol content, anti-oxidative, anti-amylase and anti-lipase activities of nineteen different spices commonly used in cooking in different parts of Cameroon.

MATERIALS AND METHODS

***Plant material*:** Nineteen commonly used spices were collected from local markets in two Cameroonian cities- Yaoundé and Bafoussam- and identified at the National Herbarium. Each of the spices was washed, sun dried, cut into small pieces and ground into a powder with a mechanical grinder. Seed spices containing husks, were processed with and without their husk. The following spices were used: barks of *Hypodaphnis zenkeri* and *Scorodophloeus zenkeri;* roots of *Echinops giganteus, Scleria striatum, Mondia whitei, Dorstenia psilurus,* seeds of *Fagara xanthoxyloide, Fagara leprieurii, Aframomum citratum, Aframomum aulacocarpus, Aframomum melegueta, Monodora myristica, Afrostyrax lepidophyllus, Scorodophloeus zenkeri* fruits of *Xylopia aethiopica, Solanum melongena Aframomum daniellii, Dichrostachys glomerata, Tetrapleura tetraptera,* and *Xylopia parviflora*.
The ground powders were stored separately in air-tight containers and kept in a cool, dry, dark place.

Preparation of aqueous extract: 2.5 gm sample of each spice was suspended in 10 ml of water at 80ºC for 30 minutes with stirring. The suspension was centrifuged at 3000xg for 5 min, and the resulting supernatant filtered with Whatman No. 1 filter paper (England). The filtrate was used for photochemical screening, measurement of the total polyphenolic content, antioxidant and anti-amylase activities.

Determination of phenolic content: The concentration of phenol in the extracts was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965) with catechin used as the standard. Into test tubes containing 1.0ml of Folin- Ciocalteu's reagent (diluted 10 times), was added 20µl of spice filtrate. The tubes were left at room temperature for 30 minutes, and the absorption at 750 nm measured. Results obtained were expressed as catechin equivalents (CAE)/gm of plant material.

Alpha-amylase inhibitory activity: The activity of α-amylase was measured using the starch-iodine method (Komaki, et al., 2003). Briefly, 20 µl of α-amylase solution (0.030 mg/ml) was mixed with 1.3 ml of Tris-HCl buffer (0.01 M containing 0.006 M NaCl, pH 6.8) and 80 µl of the aqueous of extract. After incubation at 37ºC for 20 min, 100 µl of the starch solution (0.1%) was added, and the mixture re-incubated for 20 min, after which 2 ml of 0.01% acidic iodine solution was added, and the absorbance measured at 565 nm. The percentage inhibition was calculated by comparing to the control which did not have the extract.

Inhibition of enzyme activity was calculated as (%) = (A-C) × 100/ (B-C)

- where, A = absorbance of the sample, B = absorbance of blank (no extract), and C = absorbance of control (no starch).

Lipase inhibitory activity: Inhibition of lipase by the aqueous extract of selected spices was determined using a modified assay described by Smeltzer, et al., (1992). Briefly, a suspension containing 1% (v/v) of triolein, and 1% (v/v) Tween 40 in

0.1 M phosphate buffer (pH 8) was prepared and emulsified. Assays were then initiated by adding 800 µl of the triolein emulsion to 200 µl of porcine pancreatic lipase (0.5 gm pancreatin in 15 ml 0.1 M phosphate buffer at pH 8.0) and 200 µl of extract (or 0.1 M Phosphate buffer, pH 8). The contents were mixed and the absorbance measured immediately at 450 nm and designated as T_0. The test tubes were incubated at 37°C for 30 min and at the end of the incubation; the absorbance at 450 nm was recorded and designated as T_{30}.
The variation in absorbance = $[A_{450}\ (T_0)\ -\ A_{450}(T_{30})]$ was calculated for both control and the treatment and the % inhibition was calculated using the formula:

$$\% \text{ inhibition} = ([\Delta A_{450Control} - \Delta A_{450Extract}] / \Delta A_{450Control}) \times 100$$

Antioxidant assay: 2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)-(ABTS) radical scavenging activity was used to screen for antioxidant activity. The ABTS assay was conducted by modifying a previously described method (Re, et al., 1999). Briefly, to 1 ml of 7 mM ABTS (in water, activated overnight with 140 mM potassium persulfate) was added 50 µL of filtrate. The resulting mixture was incubated for 30 min at room temperature, and the absorbance measured at 734 nm against a control containing ethanol in place of the extract.

The percentage inhibition in ABTS radical due to the extract was calculated by the formula;

$$I = (A_B - A_A)/A_B * 100$$

- Where I = percentage $ABTS^+$ inhibition; A_B = absorbance of a blank sample (t = 0 min); A_A = absorbance of the test sample at the end of the reaction (t = 30 min).

Statistical analysis: The results of phenol content and $ABTS^+$ activity were expressed as the Mean ± SD of at least three determinations (n = 3). Analyses were done using the SPSS 10.5 for Windows and the Kolmogorov test. The effect of the extracts on pancreatic amylase and lipase were expressed as percentage of inhibition.

RESULTS

***Phenolic content*:** The phenolic content of the different spices ranged from 2.06 to 60.75 mg CAE/gm of extract (Table-1). *Dichrostachys glomerata, Xylopia parviflora* and *Tetrapleura tetraptera*, had the highest content of phenolic compounds.
***$ABTS^+$ radical scavenging activity*:** The $ABTS^+$ radical scavenging activities of the different extracts are also shown in Table 1. The free radical ($ABTS^+$) scavenging activity was highest in the extract of *Dichrostachys glomerata* (87.58%), *Tetrapleura tetraptera* (83.94%), *Xylopia parviflora* (90.55%), *Xylopia aethiopica* (54.72%) and *Fagara xanthoxyloide* (48.82%). The other spices had free radical scavenging activities of less than 35%.
***Anti-amylase activity*:** Spices were classified to be of high potency (75-100% inhibitory effect on pancreatic alpha amylase activity), moderate potency (50-75% inhibitory effect on pancreatic alpha amylase), low potency (25-50% inhibitory effect on pancreatic alpha amylase) or with no potency (less than 25% inhibitory effect on pancreatic alpha amylase). Spices of high potency included *Aframomum daniellii, Hypodapnis zenkeri, Echinops giganteus, Aframomum citratum,* and *Xylopia aethiopica*, while those of moderate potency were *Monodora myristica* (with husk and without husk), *Fagara leprieurii, Fagara xanthoxyloide , Scorodophloeus zenkeri (barks and seeds without husk), Aframomum aulacocarpus* (with husk), *Solanum melongena*. On the other hand spices of low potency were *Aframomum melegueta, Afrostyrax lepidophyllus* (with husk), *Dorstenia psilurus, Aframomum citratum*

(without husk), *Scorodophloeus zenkeri* (with husk) *Dichrostachys glomerata.* Aqueous extracts of *Scorodophloeus zenkeri* (without husk) and *Tetrapleura tetraptera* were considered as not potent (Table 2).

Anti-lipase activity: Spices were classified to be of high potency (75-100% inhibitory effect on pancreatic lipase activity); moderate potency (50-75% inhibitory effect on pancreatic lipase), low potency (25-50% inhibitory effect on pancreatic lipase) or with no potency (less than 25% inhibitory effect on pancreatic lipase). Only *Xylopia aethiopica,* was considered to be of high potency, while the extract of the seeds of *Scorodophloeus zenkeri* (with husk) was moderately potent. *Afrostyrax lepidophyllus* (with husk), *Tetrapleura tetraptera, Dichrostachys glomerata* and the roots of *Scleria striatum* were of low potency, while the rest were considered as not potent (Table 2). None of the extracts enhanced pancreatic lipase activity.

DISCUSSION

It has been suggested that, the management of diabetes and its accompanying oxidative stress should include therapeutic strategies to reduce postprandial hyperglycemia, hypertriglyceridemia and decrease or control body weight. Natural alpha-amylase inhibitors from food-grade herbal sources offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia by decreasing glucose release from starch, which may be potentially useful in the treatment of diabetes mellitus and obesity (McCue and Shetty, 2004). To support the quest for alternative therapies to treat diabetes and prevent weight gain as well as oxidative stress, we investigated the in vitro potential of some Cameroonian spices to reduce oxidative stress as well as carbohydrate and lipid digestion via the enzymes alpha amylase and lipase respectively. Aqueous extracts of five spices showed high anti amylase potencies. Thus, aqueous extracts of these spices can potentially be offered in teas, foods and/or dietary supplements. This anti-amylase activity may be due to the presence of phenolic compounds, which have been shown to interact with and/ or inhibit enzymes (Rhon, et al., 2002; Arts, et al., 2002). The reaction mechanism involved in the inhibition of alpha amylase may be due to the presence of flavonoids such as flavonols which have been reported to inhibit alpha amylase (Kim, et al., 2000). The difference in structure of flavonoids contained in tested extracts may therefore explain the differences in inhibitory activity obtained, which could also be a result of the presence of different concentrations and types of phenolic compounds. We observed an increase in the inhibitory activity of certain spices in the presence of their husk, suggesting that phenolic components present in the husk may modify the inhibitory activity, perhaps through synergistic mechanisms. On the other hand, for some other spices, the inhibitory activity was reduced or completely absent in the presence of their husk, suggesting the inhibition of this effect by other compounds present in the husk.

Five of the selected spices showed high to moderate $ABTS^{+}$ scavenging potency, which could be related to their polyphenolic compounds as proposed by Hossain, et al., 2009. This potential is variable because, a number of factors influence the effectiveness of antioxidants in complex heterogeneous foods and biological systems. These include the lipid/aqueous phase partitioning properties of the antioxidant, the oxidation conditions, and the physical state of the oxidizable substrate or of the antioxidant (Frankel and Mayer, 2000).

CONCLUSION

Aqueous extracts of some Cameroonians spices have anti-amylase and anti-lipase activities as well as antioxidant potential. These spices could be useful in the management of some chronics diseases.

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Table-1: Phenolic content and %ABTS inhibition.

Local name	Scientific name	Family	Part used	Phenolic content (mg Equivalent catechin/gm of plant	% ABTS inhibition
Tchouoko	*Aframomum daniellii*	*Zingiberaceae*	Fruit	3.14 ± 0.09	8.09± 0.00
Ndong	*Aframomum melegueta*	*Zingiberaceae*	Seeds	9.37 ± 0.58	22.99 ± 7.94
			Seeds + husk	11.04 ± 2.25	30.05 ± 4.93
Bongo baliem	*Aframomum aulacocarpus*	*Zingiberaceae*	Seeds	2.73 ± 0.42	8.55 ± 0.80
			Seeds + husk	3.69 ± 0.64	9.94± 3.22
Bongo nguel	*Aframomum citratum*	*Zingiberaceae*	Seeds	3.25 ± 0.63	18.31 ± 1.74
			Seeds + husk	3.37 ± 1.58	10.60 ± 2.39
Lumtom (with husk)	*Afrostyrax lepidophyllus*	*Huaceae*	Seeds	4.94 ± 0.49	23.22 ± 4.44
			Seeds + husk	7.27 ± 0.61	32.40 ± 4.36
Tenchouakoua	*Dichrostachys glomerata*	*Mimosaceae*	fruit	60.75 ± 5.47	87.58 ± 2.17
Ngaana	*Dorstenia psilurus*	*Moraceae*	Root	5.37 ± 0.66	22.85± 4.71
Tchanmgne	*Echinops giganteus*	*Asteraceae*	Root	4.22 ± 0.49	18.09 ± 1.20
Melam	*Fagara leprieurii*	*Rutaceae*	Seeds	5.05 ± 1.09	27.28 ± 4.50
Natchou	*Fagara xanthoxyloide*	*Rutaceae*	Seeds	6.61 ± 0.46	48.82 ± 1.02
Nkouppo	*Hypodphnis zenkeri*	*Lauraceae*	Bark	5.22 ± 0.27	34.20 ± 2.19
Limtse	*Mondia whitei*	*Asclepiadaceae*	Root	4.32 ± 0.69	9.46 ± 2.37
Pèpè (with husk)	*Monodora myristica*	*Annonaceae*	Seeds	2.06 ± 1.24	10.33 ± 3.88
			Seeds + husk	2.85 ± 0.31	8.09 ± 0.00
Ngunnock	*Scleria striatum*	*Cyperaceae*	Root	3.65 ± 0.46	8.09 ± 0.00
Omi plat (with husk)	*Scorodophloeus* zenkeri.	*Caesalpiniaceae*	Seeds	8.77 ± 0.41	15.16 ± 2.51
			Seeds + husk	6.93 ± 0.71	12.51 ± 1.21
Nguedjou	*Solanum melongena*	*Solanaceae*	Fruit	8.98 ± 0.25	12.38 ± 0.79
Sèsè	*Tetrapleura tetraptera*	*Mimosaceae*	Fruit	16.63 ± 1.25	83.94 ± 3.58
Bikwi	*Xylopia aethiopica*	*Annonaceae*	Fruit	5.86 ± 0.34	54.72 ± 4.31
Nkey (with husk)	*Xylopia parviflora*	*Annonaceae*	Seeds	51.83 ± 3.55	90.55 ± 2.13
			Seeds + husk	35.09 ± 3.03	88.54 ± 2.02

Table-2: Inhibition (%) of amylase and lipase activities.

Scientific name	Family	Part used	Alpha amylase inhibition (%)	Lipase inhibition (%)
Aframomum aulacocarpus (with husk)	*Zingiberaceae*	Fruits	66.54	0.00
Aframomum citratum (with husk)	*Zingiberaceae*	Seeds	83.58	0.00
Aframomum citratum (without husk)	*Zingiberaceae*	Seeds	47.37	0.00
Aframomum daniellii	*Zingiberaceae*	Fruits	87.69	0.00
Aframomum melegueta	*Zingiberaceae*	Fruits	30.61	8.91
Afrostyrax lepidophyllus (with husk)	*Huaceae*	Seeds	49.45	35.08
Dichrostachys glomerata	*Mimosaceae*	Fruits	49.73	48.64
Dorstenia psilurus	*Moraceae*	Roots	46.16	5.04
Echinops giganteus	*Asteraceae*	Roots	84.42	0.00
Fagara leprieurii	*Rutaceae*	Seeds	55.17	0.00
Fagara xanthoxyloide	*Rutaceae*	Seeds	55.76	0.00
Hypodapnis zenkeri	*Lauraceae*	Bark	86.03	0.00
Mondia whitei	*Asclepiadaceae*	Roots	0.00	0.00
Monodora myristica (with husk)	*Annonaceae*	Seeds	53.60	0.00
Monodora myristica (without husk)	*Annonaceae*	Seeds	54.73	17.64
Scleria striatum	*Cyperaceae*	Roots	0.00	49.61
Scorodophloeus zenkeri	*Caesalpiniaceae*	Bark	58.12	-
Scorodophloeus zenkeri (with husk)	*Caesalpiniaceae*	Seeds	34.71	56.39
Scorodophloeus zenkeri (without husk)	*Caesalpiniaceae*	Seeds	15.36	5.04
Solanum melongena	*Solanaceae*	Fruits	73.23	0.00
Tetrapleura tetraptera	*Mimosaceae*	Fruits	3.84	42.83
Xylopia aethiopica	*Annonaceae*	Fruits	81.36	92.25
Xylopia parviflora	*Annonaceae*	Fruits	77.14	23.45

