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RESEARCH PAPER

Antidiabetic Screening of Phenolic-rich Extracts of Selected Medicinal Spices

Jubril Olayinka Akolade[2] · Asiat Na'Allah[1] · Abdulhakeem Olarewaju Sulyman[3] · Azeemat Titilola Abdulazeez[1] · Asiata Opeyemi Atoti[1] · Mardiya Bawa Isiaku[1]

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Abstract
Medicinal spices are used by herbal practitioners as condiments in decoctions for management of diabetes and its associated complications. The study assessed and compared the potentials of phenolic-rich extracts from four selected medicinal spices: *Curcuma longa*; *Allium sativum*; *Xylopia aethiopica* and *Syzygium aromaticum* to delay carbohydrate digestion, scavenge free radicals and ameliorate hyperglycemia. Results showed that the seed of *X. aethiopica* has the highest phenolic content (19.25 ± 0.96 mg/g) while *C. longa* extracts was the lowest (10.42 ± 0.86 mg/g). Dose-dependent in vitro bioactivities were exhibited by all the spice extracts, with the best antiradical (EC_{50} = 64.98 ± 5.16 µg/ml) and α-amylase inhibitory (IC_{50} = 11.33 ± 1.10 µg/ml) activities displayed by *A. sativum* and *S. aromaticum*, respectively. Intraperitoneal administration of either of the spice extracts for 5 days caused significant reduction (> 80%) in hyperglycemia. The antidiabetic activities of the spice extracts may be attributed to the presence of notable secondary metabolites such as tumerone and its derivatives in *C. longa* extract or eugenol that was the main constituent of the extract from *S. aromaticum* and also found in minor quantities in *A. sativum* and *X. aethiopica* extracts. These findings showed that extract from these spices could be explored as a functional component of foods or as candidates for development of oral antihyperglycemic agents for the management of diabetes mellitus.

Keywords α-Amylase · Free radicals · Hyperglycaemia · Medicinal spices · Phenolics

1 Introduction

Diabetes mellitus is one of the most common endocrine disorders, characterized by hyperglycemia resulting from deficiencies in secretion and/or action of insulin (ADA 2012). It is a group of metabolic diseases resulting in alterations in metabolism of not only carbohydrate but also lipid and protein. Oxidative stress plays a crucial role in the pathogenesis of diabetes mellitus. Persistence in hyperglycemia causes oxidative stress in tissues prone to secondary complications in diabetic patients (Mahjoub et al. 2012). Production of reactive oxygen and nitrogen species or free radicals has been postulated as the root cause of impaired glucose tolerance, beta-cell dysfunction and development of insulin resistance in type 2 diabetes mellitus (Wright et al. 2006).

Alpha-amylase inhibitors such as acarbose are used in the management of diabetes mellitus to reduce the rate of degradation of complex carbohydrates in the intestine, reducing postprandial hyperglycemia. These synthetic compounds have some side effects. Acarbose, for example, is associated with gastrointestinal side effects such as abdominal pain, flatulence and diarrhea as well as hepatitis (Hoffman and Spengler 1997; WHO 1999; Chiasson et al. 2002), prompting the search for alternative therapies for

✉ Jubril Olayinka Akolade
akoladejubril@yahoo.com; jo.akolade@shestco.gov.ng

✉ Abdulhakeem Olarewaju Sulyman
abdulhakeem.sulyman@kwasu.edu.ng;
aksp4real@yahoo.com

[1] Department of Biological Sciences, Al-Hikmah University, Ilorin, Nigeria

[2] Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, Abuja, Nigeria

[3] Department of Biochemistry, Kwara State University, Malete, Nigeria

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inhibition of carbohydrate digestion and hence, postprandial hyperglycemia in the management of diabetes.

Plant phenolics found abundantly in dietary spices have been shown to exhibit various biological roles in the prevention, management and treatment of chronic diseases such as diabetes mellitus (Scalbert et al. 2005). Phenolics have the potentials to scavenge free radicals, reduce the rate of lipid peroxidation and regulate carbohydrate metabolism by delaying the absorption of glucose in the intestine (preventing hyperglycemia) or by stimulating insulin secretion among other mechanisms (Hanhineva et al. 2010). More so, studies have shown that phenolic compounds play a key role in mediating amylase inhibition (Oboh et al. 2012; Armando et al. 2016).

Spices are one of the most utilized plant species either for their nutritional or medicinal purposes. Nutritionally, spices are dietary adjuncts primarily used as flavoring agents to garnish the taste of foods. Spices are also essential medicines that have been used for millennia (Duke 2002). In North Central Nigeria, spices are used by traditional medicine practitioners (TMPs) as condiments in decoctions for management of diabetes and its associated complications (Okujagu 2006). Epidemiological evidence showed that prevalence of chronic diseases was reduced among populace that consumes diets rich in spice. The habitual consumption of spicy foods was found to be inversely proportional with mortality independent of other risk factors of death (Lv et al. 2015). Bio-actives from dietary spices are a heterogeneous mixture of diverse volatiles such as essential oils and non-volatile compounds such as polyphenols obtained from various parts of plants (Etoundi et al. 2010).

Many dietary spices are used worldwide in the management of diabetes. Among the most commonly used in North Central Nigeria is: turmeric (*Curcuma longa*), garlic (*Allium sativum*), clove (*Syzygium aromaticum*) and selim pepper (*Xylopia aethiopica*). *C. longa* is a rhizomatous herbaceous perennial plant that belongs to the Zingiberaceae family. The active component of turmeric, curcumin, is a potential candidate for the management of diabetes and its associated complications (Goel et al. 2008). *A. sativum* is a member of the Liliaceae family and most commonly used worldwide as dietary spice. It is recognized as one of the herbal drug ingredients in China for treating diabetes mellitus (Jia et al. 2003). Studies have shown that *S*-allyl cysteine sulphoxide (allicin), a sulfur containing amino acid in garlic had the potential to reduce the hyperglycemia in rats (Shakya et al. 2010). *S. aromaticum* is an angiosperm of the Myrtaceae family popularly referred to as clove. Cloves are the pink flowering bud of the plant which are dried and used for culinary and medicinal purposes (Bhowmik et al. 2012). The major constituent of clove extract; eugenol and its derivatives, has been reported to modulate physiological responses in hyperglycemic rats and ameliorate hyperglycemia-induced secondary complications (Nangle et al. 2006; Shukri et al. 2010). *X. aethiopica* also known as Ethiopian or Selim pepper belongs to the Annonaceae family. Decoction from *X. aethiopica* fruits is commonly used locally in West Africa for the treatment of diabetes (Soladoye et al. 2012).

The multifactorial pathogenicity of diabetes mellitus has necessitated management with multimodal therapies repository in medicinal spices. The inhibition of postprandial hyperglycaemia and amelioration of oxidative stress are very significant in the management of diabetes mellitus. Thus, this study is designed to assess and compare the potentials of phenolic-rich extracts from four selected medicinal spices: *C. longa*; *A. sativum*; *X. aethiopica*; *S. aromaticum* on inhibition of carbohydrate metabolizing enzyme and scavenging of free radicals as well as amelioration of hyperglycemia in alloxan-induced rats.

2 Materials and Methods

2.1 Materials

2.1.1 Spices

Rhizomes of *Curcuma longa* (UILH/005/1208) and *Allium sativum* (UILH/002/1209) were collected from Oja-Oba, Ilorin, Kwara State, while seeds of *Syzygium aromaticum* (UILH/003/1204) and *Xylopia aethiopica* (UILH/004/1089) were obtained from Paiko, Niger State, North Central Nigeria. The spices were authenticated at the herbarium unit of the Department of Plant Biology, University of Ilorin, where voucher specimens were deposited.

2.1.2 Chemicals and Apparatus

Alloxan monohydrate, α-amylase, 2,2-diphenyl-1-picrylhydrazyl, ethanol, acarbose, Folin–Ciocalteu reagent and sucrose (SIGMA, St Louis, MO, USA), trichloroacetic acid, gallic acid, thiobarbituric acid (JHD, China), metformin (Qianjin Pharm. Co. Ltd., China) and other chemicals used were of analytical grades. Accu-chek active glucometer and strips from Roche diagnostic (Mannheim, Germany), orbital shaker (Bio Gene Shaker, India), rotary evaporator (Toption, China), laboratory oven (Thermo Fischer scientific, MA, USA), UV–Visible Spectrophotometer (Simtronics, Haryana) and analytical balance (Ohaus Corporation, NJ, USA) were used.

2.1.3 Experimental Animals

Albino rats (*Rattus norvegicus*) of average weight of 110.88 ± 5.33 were obtained from the animal house of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State. They were acclimatized for 2 weeks to standard laboratory housing conditions. The research adheres strictly and conforms to the Principles of Laboratory Animal Care (NIH Publication No. 85-23, 1996). The research protocols were requested to and approved by Department of Biological Sciences, Al-Hikmah University, Ilorin, Nigeria.

2.2 Methods

2.2.1 Preparation of Extracts

Extracts were prepared based on simulation of local method as described by traditional medicine practitioners in Ilorin, Nigeria. The raw spices (seeds of *C. longa* and *A. sativum* as well as the rhizomes of *S. aromaticum and X. aethiopica*) were washed separately, oven-dried at 40 °C, pulverized and sieved. The pulverized spices (100 g) were weighed separately into a 1-L flask labeled CL, AS, SA and XA, respectively, and soaked with 500 ml of absolute methanol. The flasks were capped and placed on a shaker for 30 min and then allowed to stand for 24 h. The various extracts were filtered, concentrated using vacuum-assisted rotary evaporator at 40 °C to about 5 ml and further oven-dried at 40 °C. The dried extract was labeled appropriately and stored in air-tight container at 4 °C.

2.2.2 Determination of Total Phenolic Content

Total phenolic content (TPC) of methanolic extracts from the various spices were determined by Folin–Ciocalteu reagent using gallic acid as standard as described by McDonald et al. (2001) with slight modifications. Stock solutions of the extracts (0.1 g in 10 ml of methanol; 10 mg/ml) were prepared and further diluted to give 1 mg/ml. The diluted solutions (0.1 ml) were mixed with 1 ml of 0.2 N Folin–Ciocalteu reagent (1 ml) and allowed to stand for 5 min before 0.9 ml of 1 M Na_2CO_3 solution was added. The mixtures were allowed to stand for 30 min and the absorbance was determined at 765 nm. Standard curves of gallic acid (0.005–0.1 mg/ml) were also prepared. TPC of extracts were derived by regressing absorbance readings of test samples onto the standard curve and expressed as mg gallic acid equivalent per gram of sample.

2.2.3 Gas Chromatography and Mass Spectroscopy (GC–MS) Analysis

Gas chromatography–mass spectrometry (MS) analysis was carried out at the Shimadzu Training Center (STC) for Analytical Instruments, Lagos, Nigeria, using a Shimadzu-QP2010SE 15A operating at 70 eV ionization energy, equipped with Optima-5MS capillary column (phenyl methyl siloxane 30 m × 0.25 mm, 0.25 µm film thicknesses). Helium was the carrier gas, at flow rate of 1 ml/min and a split ratio of 1:20. Acquisition mass range was 45–600 and scan time was 0.5 s/scan. The injector temperature was 250 °C and the detector (ion source) temperature was 200 °C. The conditions for analysis were set as follows; column oven temperature was programmed from 60 to 280 °C (temperature at 60 °C was raised to 180 °C at 10 °C/min and held for 2 min, and then finally to 280 °C at 15 °C/min and held for 4 min). The percentage composition of the plant extracts was computed in each case from GC peak areas. The identification of the components was based on the comparison of retention indices (determined relative to the retention time of series of n-alkanes) and mass spectral with those of authentic samples with NIST 11 mass spectral library collection and with data from literature (Jennings and Shibamito 1980; Adams 2007; Joulain and Koenig 1998).

2.3 In Vitro Studies

2.3.1 Free Radical (DDPH) Scavenging Assay

The extracts were evaluated using DPPH radical scavenging activity according to the method described by Terpinc et al. (2009). DPPH radical solution (0.1 mM) was prepared in 90% v/v methanol. The DPPH solution (2.9 ml) was added to 0.1 ml solution of the extracts or gallic acid at concentrations ranging from 10 to 160 µg/ml. The resulting solution was mixed and incubated in the dark for 30 min. The absorbance was read at 517 nm against a blank. The control solution consisted of 0.1 ml of 90% methanol in lieu of the extracts. Methanol (90% v/v) was used as a blank. Analyses were carried out in triplicates. Percentage inhibition of DPPH radical was calculated as:

$$\% \text{ Radical scavenging activity} = \left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\left(\text{Abs}_{\text{control}}\right) \times 100$$

Mean effective concentration (EC_{50}) was also computed using regression analysis.

2.3.2 Alpha-Amylase Inhibitory Assay

Alpha-amylase inhibitory activity assay was carried out according to the method reported by Oboh et al. (2012).

Varying concentrations (1–16 µg/ml) of the extracts or acarbose (50 µl) were incubated with 500 µl of 20 mM phosphate buffer (pH, 6.9, with 6 mM NaCl) containing porcine pancreatic α-amylase (0.5 mg/ml) at 25 °C for 15 min, respectively. Then, 500 µl of 1% starch solution prepared in phosphate buffer was added to each tube to initiate the reaction and the reaction mixtures were further incubated at 37 °C for 15 min. The reaction was terminated by the addition of 0.5 ml of 3, 5-dinitrosalicylic acid (DNS). Thereafter, the mixture was heated in a boiling water bath for 5 min and allowed to cool to room temperature (≤ 27 °C). The resulting mixture was diluted by adding 5 ml of distilled water and absorbance was read at 540 nm against the blank (distilled water). The control experiment was performed without the test samples. Analyses were performed in triplicates and the amylase inhibitory activity was expressed as percentage inhibition using the following formula:

$$\% \text{ Inhibition of } \alpha\text{-amylase} = \left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right) / \left(\text{Abs}_{\text{control}}\right) \times 100$$

Mean inhibitory concentration (IC_{50}) was also computed using regression analysis.

2.4 In Vivo Study

2.4.1 Induction of Experimental Hyperglycaemia

Hyperglycaemia was induced in rats as previously described by Akolade et al. (2014) with little modification. Rats were fasted for 12 h and then intraperitoneally administered a single dose of 150 mg/kg b. wt. (body weight) of alloxan monohydrate freshly dissolved in normal saline solution. Also, six of the rats were given normal saline to serve as normoglycaemic control. Blood samples were taken from the caudal vein and fasting blood glucose (FBG) was determined prior to induction and after 48 h, using a glucose oxidase-based Accu-chek glucometer and compatible strips. Rats with FBS ≥ 250 mg/dl after 48 h and sustained hyperglycemia for the next 72 h (5 days after alloxan induction) were considered hyperglycemic and were used in the study.

2.4.2 Animal Grouping and Extract Administration

The rats were randomly selected into seven groups of six rats each. The extracts were reconstituted in 5% DMSO and administered intraperitoneally once a day, for 5 days to the experimental rats as follows:

1. Hyperglycaemic rats treated with the vehicle; 5% DMSO to serve as non-treated control.
2. Hyperglycaemic rats treated with 50 mg/kg b. wt. Metformin; the standard drug.
3. Hyperglycaemic rats treated with 50 mg/kg b. wt. *C. longa.*
4. Hyperglycaemic rats treated with 50 mg/kg b. wt. *A. sativum.*
5. Hyperglycaemic rats treated with 50 mg/kg b. wt. *S. aromaticum.*
6. Hyperglycaemic rats treated with 50 mg/kg b. wt *X. aethiopica.*
7. Normoglycaemic rats treated with 5% DMSO to serve as non-induced control.

2.4.3 Glycaemic Study

The animals were subjected to a 12-h overnight fast with free access to water (feeds were withdrawn from 7 to 7 am). The rats were treated as shown above. FBG of all the experimental rats were determined prior to treatment and during the 5 days of administration of the extracts. Percentage reduction in blood glucose was also computed (Sulyman et al. 2016).

2.5 Statistical Analysis

All data are expressed as the mean of three or six replicates ± standard error of mean. One way analysis of variance with Dunnett's post hoc test was used for multiple comparisons of treatment groups using GraphPad prism version 5.02. Values were considered statistically significant at the 95% confidence level.

3 Results

3.1 Phenolic Contents of Methanolic Extracts from the Selected Spices

Figure 1 shows the total phenol contents (TPC) determined in methanolic extracts of *C. longa*, *A. sativum*, *X. aethiopica* and *S. aromaticum.* The analysis revealed that extracts from the seed of *X. aethiopica* contains the highest TPC (19.25 ± 0.96 mg GAE/g) while *C. longa* extracts was the lowest (10.42 ± 0.86 mg GAE/g). Appreciable amount of TPC was quantified in *A. sativum* (17.63 ± 1.81 mg GAE/g) which was not significantly different from those of *S. aromaticum* (14.04 ± 1.28 mg GAE/g).

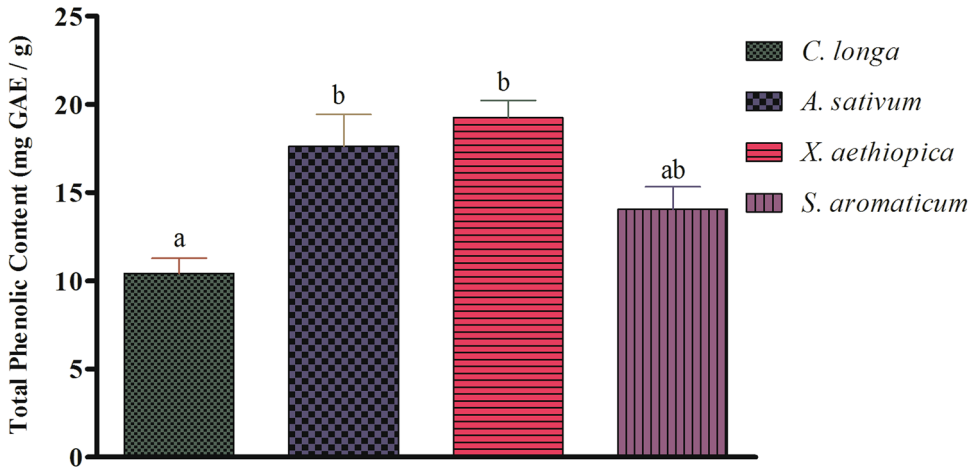


Fig. 1 Total phenol content of methanolic extracts from selected spices *mg GAE/g = milligram gallic acid equivalent per gram of sample. Bar values are expressed as mean of three replicates ± SEM and those with different superscripts are significantly different ($p < 0.05$) from each other

3.2 Chemical Constituents of Methanolic Extracts from the Selected Spices

The chemical constituents quantified in methanolic extracts of *C. longa*, *A. sativum*, *X. aethiopica* and *S. aromaticum* using GC–MS technique are shown in Table 1. Ar-tumerone (5.87%), Curlone (2.83%) and tumerone (1.57%) were the main secondary metabolites of *C. longa* extract, while eugenol (61.18%) predominate the extract of *S. aromaticum.* Eugenol was also found in minor quantities in *A. sativum* (0.62%) and *X. aethiopica* (0.60%).

3.3 Free Radical Scavenging Activities of Methanolic Extracts from the Selected Spices

Figure 2 shows the DPPH radical scavenging activities of methanolic extracts of *C. longa*, *A. sativum*, *X. aethiopica* and *S. aromaticum* at various concentration (10–160 µg/ml). The free radical scavenging activity of the extracts or the standard (gallic acid) was dose dependent and increases as concentration increases. At the low concentration of 10 µg/ml, radical scavenging activities of the extracts (4.78–50.80%) were significantly ($p < 0.05$) lower than gallic acid (88.84 ± 1.23%), whereas, at the high concentration of 160 µg/ml, there was no significant difference between the radical activity of the standard and that of the *A. sativum* extract. Comparatively, among the spice extracts, *A. sativum* extract showed the best free radical scavenging activity (48.24 ± 1.48% at 10 µg/ml to 93.16 ± 0.1% at 160 µg/ml) and this reflected in low IC_{50} values 64.98 ± 5.16 µg/ml computed for the extract which was not significantly different from that computed for gallic acid (58.70 ± 6.89 µg/ml; Table 2).

3.4 Alpha-Amylase Inhibitory of Methanolic Extracts from the Selected Spices

Alpha-amylase inhibitory activities of methanolic extracts of *C. longa*, *A. sativum*, *X. aethiopica* and *S. aromaticum* at various concentrations (1–16 µg/ml) is shown in Fig. 3. The capacity of the extracts or the standard (acarbose) to inhibit the activities of the enzyme increases as the concentration of inhibitors increase. At all concentration evaluated the scavenging activities of acarbose (4.78–50.80%) was significantly ($p < 0.05$) higher than any of the spice extract (88.84 ± 1.23%). Among the spice extracts, *X. aethiopica* showed the least inhibitory effect (33.33 ± 0.83%) on the enzyme, while *S. aromaticum* extract showed the best effect (56.12 ± 0.53%). The mean alpha-amylase inhibitory concentration (IC_{50}) computed for *S. aromaticum* extract (11.33 ± 1.10 µg/ml) was not significantly different from that of the standard (8.50 ± 0.74 µg/ml; Table 3).

3.5 Glucose Lowering Effects of Methanolic Extracts from the Selected Spices

Figure 4 shows the glucose lowering effect of methanolic extract of *C. longa*, *A. sativum*, *X. aethiopica* and *S. aromaticum* administered intraperitoneally for 5 days in alloxan-induced hyperglycaemic rats. Administration of alloxan monohydrate to rats significantly ($p < 0.05$) increased the blood glucose level within 48 h, when compared to the non-induced group administered normal saline to serve as control. Hyperglycaemia was sustained for 5 days prior to treatment with the extracts or standard drug. Administration of extracts from the spices or metformin caused reduction in hyperglycaemia over the course of treatment. Significant ($p < 0.05$) reductions in hyperglycemia were observed 24 h after single daily dose administration of the extracts from *X. aethiopica* but after 48 h in rats treated with either *S. aromaticum or C. longa* extracts and 72 h in the group treated with *A. sativum*

Table 1 Chemical constituents of methanolic extracts from selected spices

S/n	RT (min)	Compounds	% Composition			
			C. longa	*A. sativum*	*X. aethiopica*	*S. aroamticum*
1.	8.04	5-Hydroxymethylfurfural	–	1.60	–	–
2.	6.00	*cis*-4-methoxy thujane	–	–	0.61	–
3.	6.04	Terpinen-4-ol	–	–	0.53	–
4.	6.64	Dimethyl malate	–	–	–	0.55
5.	8.93	Eugenol	–	0.62	0.60	61.18
6.	9.09	1-Benzylidene-2,2,3-trimethylcyclopentylidene	1.03	–	–	–
7.	10.89	11.alpha.-Hydroxyprogesterone	0.69	–	–	–
8.	10.91	1,3-Dimethyladamantane-5,7-diol	–	–	–	0.62
9.	11.10	beta.H,5.alpha.-Eremophil-1(10)-ene, 11-(trimethylsiloxy)-	0.22	–	–	–
10.	11.24	Aromadendrene oxide-(2)	–	–	0.41	–
11.	11.40	Spathulenol	–	–	0.69	–
12.	11.76	Tumerone	1.57	–	–	–
13.	11.79	Methyl 7-(dimethoxymethyl) bicyclo [2.2.1]heptane-1-carboxylate	–	–	0.50	–
14.	11.83	Ar-tumerone	5.87	–	–	–
15.	12.06	Eudesma-5,11(13)-dien-8,12-olide	1.31	–	0.39	–
16.	12.18	Curlone	2.83	–	–	–
17.	12.47	4-(4-Methylphenyl)pentanal	0.74	–	–	–
18.	12.62	3-(1-Methyl-2-phenylethoxy)-2-butanol	0.43	–	–	–
19.	13.10	12,12-Dichlorobicyclo [8.2.0]dodecan-11-one	0.55	–	–	–
20.	13.17	Methyl 14-(2-octylcyclopropyl)tetradecanoate	0.43	–	–	–
21.	13.46	Methyl hexadecanoate	1.07	0.91	5.70	1.13
22.	13.72	Palmitic acid	1.85	2.22	0.82	0.63
23.	13.90	4a,7,7,10a-Tetramethyl-2,3,4a,5,7,8,9,10,10a, 10b-decahydro-1H-benzo[f]chromene	1.00	–	–	–
24.	14.09	1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3R-(3.alpha.,4a.beta.,6a.alpha.,10a.beta.,10b.alpha.)]	–	–	1.41	–
25.	14.55	Methyl cis-6-octadecenoate	2.27	–	–	–
26.	14.55	Methyl linoleate	–	–	–	1.84
27.	14.56	Methyl elaidate	–	0.99	15.57	–
28.	14.70	Methyl stearate	–	–	1.26	0.33
29.	14.84	Oleic acid	1.84	2.51	1.05	0.69
30.	14.96	Stearic acid	–	0.76	–	–
31.	16.24	Pimara-7,15-dien-3-one	–	–	1.00	–

RT retention time (min)

extract. The antihyperglycemic effect was maintained until the end of the treatment. Normoglycemic ($\leq$ 120 mg/dl) level was observed in rats treated with either *S. aromaticum* or *X. aethiopica* extracts after 3 days of treatment and in those treated with *C. longa* extracts after 5 days of treatment. Above 98% reduction in hyperglycemia were recorded after 5 days of intraperitoneal administration of metformin or the extracts except in the group administered with *A. sativum* extract with 80% percentage reduction (Table 4).

4 Discussion

Phenolic compounds are essential to the physiology of plants and are involve in diverse functions such as pathogen resistance and allelopathy (Haslam 1998). Phenolics are found abundantly in dietary spices and vary in quantity as well as composition. In this study, total phenolic content (TPC) varies in methanolic extracts from various spices. TPC in

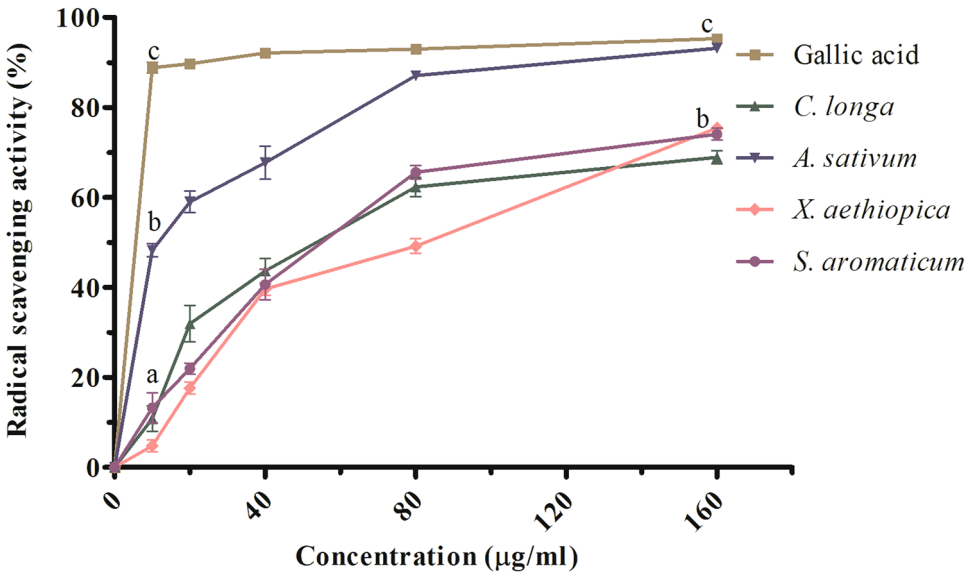


Fig. 2 Free (DPPH) radical scavenging activities of methanolic extracts of selected spices. Point values are expressed as mean of three replicates ± SEM and those with different superscripts are significantly different ($p < 0.05$) from each other

Table 2 Mean DPPH radical scavenging concentration (EC_{50}) of methanolic extracts of selected spices

	EC_{50} (µg/ml)
Gallic acid (Standard)	58.70 ± 6.89^{a}
C. longa	92.15 ± 5.32^{b}
A. sativum	64.98 ± 5.16^{a}
X. aethiopica	94.82 ± 3.17^{b}
S. aromaticum	88.42 ± 4.16^{b}

Values are expressed as mean of three replicates ± SEM and those with different superscripts are significantly different ($p < 0.05$) from each other

Table 3 Mean alpha-amylase inhibitory concentration (IC_{50}) of methanolic extracts of selected spices

	IC_{50} (µg/ml)
Acarbose (Standard)	8.50 ± 0.74^{a}
C. longa	13.97 ± 1.16^{b}
A. sativum	14.57 ± 0.62^{bc}
X. aethiopica	18.63 ± 2.18^{c}
S. aromaticum	11.33 ± 1.10^{ab}

Values are expressed as mean of three replicates ± SEM and those with different superscripts are significantly different ($p < 0.05$) from each other

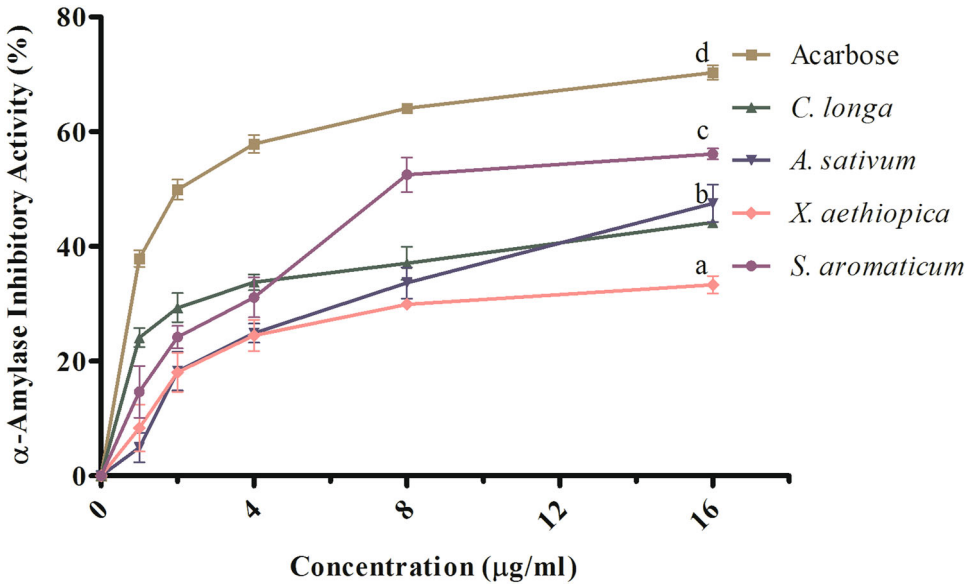


Fig. 3 Alpha-amylase inhibitory activities (IC_{50}) of methanolic extracts of selected spices. Point values are expressed as mean of three replicates ± SEM and those with different superscripts are significantly different ($p < 0.05$) from each other

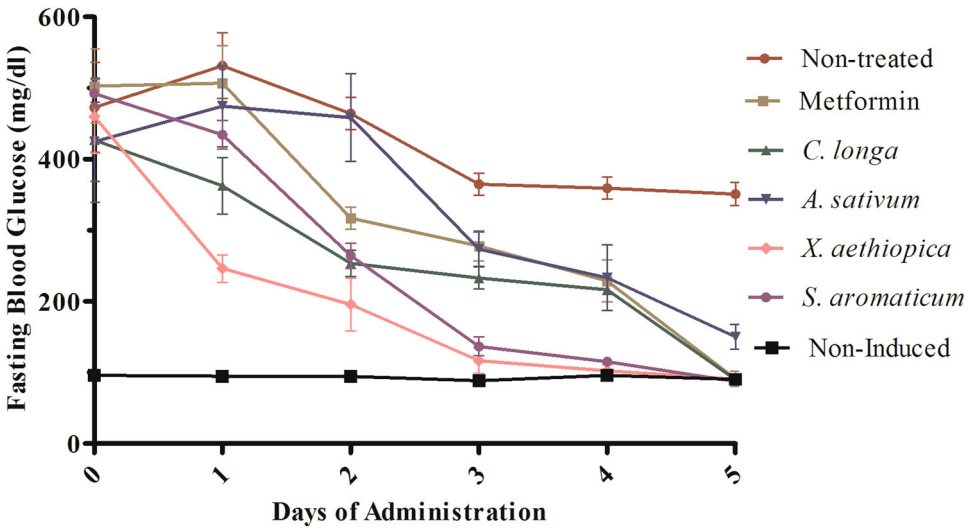


Fig. 4 Antihyperglycaemic effect of methanolic extracts from selected spices administered intraperitoneally for 5 days in alloxan-induced rats. Point values are expressed as mean of three replicates ± SEM and those with different superscripts are significantly different ($p < 0.05$) from each other

Table 4 Glucose (mg/dL) lowering effect of intraperitoneal administration of methanolic extracts from selected spices

	Day-5 Prior to induction	Day 0 Before treatment	Day 5 After treatment	% Glucose reduction
Non-treated	87.0 ± 2.89[a]	472.7 ± 63.17[b]	351.0 ± 15.95[d]	31.55[A]
Metformin	85.7 ± 1.20[a]	503.0 ± 52.08[b]	91.0 ± 10.69[a]	98.73
C. longa	89.3 ± 1.86[a]	427.0 ± 87.20[b]	90.0 ± 3.64[a]	99.79
A. sativum	85.0 ± 2.52[a]	424.7 ± 55.64[b]	150.0 ± 17.32[c]	80.86
X. aethiopica	83.3 ± 1.86[a]	460.0 ± 51.73[b]	90.67 ± 1.76[a]	98.04
S. aromaticum	86.0 ± 1.15[a]	492.3 ± 19.88[b]	87.33 ± 4.37[a]	99.67
Non-induced	91.0 ± 2.65[a]	96.0 ± 4.73[a]	90.33 ± 1.76[a]	ND

Values are expressed as mean of six replicates ± SEM and those with different superscripts along a row or column are significantly different ($p < 0.05$) from each other

% Glucose reduction = [(Day 0–Day 5)/(Day 0–Day-5)] × 100

ND not determined

[A]Significantly lower than treatment group

X. aethiopica ≥ *A. sativum* ≥ *S. aromaticum* ≥ *C. longa* (Fig. 1); eugenol was the most abundant compound in *S. aromaticum* but only found in trace amount in *X. aethiopica* and *A. sativum* but not detected in *C. longa* (Table 1).

Dietary intake of phenolic compounds are beneficial and affect diverse processes in vivo. Many phenolic compounds can act as antioxidants. DPPH radical assay is widely used for in vitro evaluation of antioxidant capacity of plant-derived chemicals. Figure 2 shows that DPPH radical scavenging activity of extracts from dietary spices were concentration dependent. Mild to moderate mean effective concentration was exhibited by the spice extracts. The mild antiradical activity of *C. longa* extracts may be attributed to the absence of curcuminoids, the extract was a turmerone-chemotype. Although, antioxidant capacity of curcumin-free *C. longa* extract has been demonstrated (Yu et al. 2008), turmerone-rich extract of *C. longa* has been reported to exhibit only a mild scavenging effect on DPPH radical (Liju et al. 2011).

In correlation with the study of Adefegha and Oboh (2012b) on aqueous extracts from spices, despite the lower TPC content of *S. aromaticum*, the DPPH radical scavenging activity of its methanolic extracts was higher than those of *X. aethiopica*. Variation in composition of the extracts and quantity of the predominant compound may be responsible for the relatively better antiradical activity of *S. aromaticum.* The antioxidant properties of eugenol, a phenylpropanoid detected in high concentration in *S. aromaticum* extract have been reported by various authors (Bondet et al. 1997; Bortolomeazzi et al. 2010; Gulcin 2011). In general, free radical activities of phenolic compounds are attributed to the ability of the OH functional group to donate H atom (Duthie et al. 2003). Formation of dehydroeugenol following interaction between DPPH and eugenol may also contribute to the free radical scavenging activity of eugenol (Bortolomeazzi et al. 2010). Presence

and synergism of terpinen-4-ol and cis-4-methoxy thujane with eugenol despite in low amount may account for moderate activity of *X. aethiopica.* Proposed mechanisms of synergistic antiradical action may involve dimerization between two phenoxyl radicals (Bondet et al. 1997).

Comparatively, among all the spices studied, *A. sativum* had the highest antiradical activity and computed mean effective concentration determined via linear regression of the dose response curve competes favorably with gallic acid used as standard (Table 2). Presence of 5-hydroxymethylfurfural in *A. sativum* may contribute immensely to its free radical activity as established by other studies (Zhao et al. 2013). 5- Hydroxymethylfurfural is found in dietary spice and produced through the catabolism of hexoses. The antiradical activity may be attributed to its structure which contains a hydroxyl group that can donate hydrogen and also its electron-withdrawing oxygenated furan ring (Li et al. 2009).

Oxidative stress has been linked with the pathogenesis and pathophysiology of diabetes mellitus. Studies have shown that reactive oxygen can trigger hyperglycemia-induced secondary complications such as macrovascular dysfunction (Adefegha and Oboh 2012a). Thus, management of diabetes and its oxidative stress-associated complications should include strategies to reduce not only postprandial hyperglycemia but also scavenge reactive oxygen and nitrogen species. The inhibition of enzymes involve in the liberation of glucose from dietary carbohydrates such as starch may be crucial to the reduction in postprandial hyperglycemia. Phenolics have been shown to have modulatory effect on postprandial glucose by inhibition of alpha-amylase activity. In correlation with other studies, methanolic extracts of dietary spices inhibited activities of alpha-amylase in a concentration-dependent manner (Adefegha and Oboh 2012a; Lekshmi et al. 2012; Salehi et al. 2013). Acarbose was a more effective inhibitor of alpha-amylase than any of the spice extracts, although mean inhibitory concentration computed for *S. aromaticum* were not significantly different from those of acarbose (Table 3). Similar to its antioxidant properties, alpha-amylase inhibitory activity of *S. aromaticum* has been attributed to the presence of eugenol (Thoroski et al. 1989; Mnafgui et al. 2013; Jelenkovic et al. 2014). Using laboratory media, Thoroski et al. (1989) reported that eugenol at sub-lethal concentration inhibited the production of alpha-amylase in *Bacillus subtilis*. Activity of the enzyme has been shown to be significantly inhibited by eugenol both in in vitro and in vivo models (Mnafgui et al. 2013). Substantial inhibition of the carbohydrate metabolizing enzymes was also exhibited by the turmerone-rich *C. longa* extract. Previous studies revealed that ar-turmerone, the major volatile component of *C. longa*, inhibited activity of alpha-amylase (Lekshmi 2012), however other components of the *C. longa* extracts such as curlone might also contribute to its inhibitory effect (Ponnusamy et al. 2011).

The high in vitro antidiabetic potentials of extracts from *S. aromaticum* and *C. longa* was replicated in in vivo hyperglycemic models. Hyperglycemia was induced in rats using alloxan monohydrate. Degranulation and loss of beta cells integrity occurs following administration of alloxan resulting in impaired insulin production and persistence in hyperglycemia (Lenzen 2008; Akolade et al. 2014). Intraperitoneal administration of *S. aromaticum* and *X. aethiopica* extracts to alloxan-induced hyperglycemic rats caused significant reduction in the elevated blood glucose to normoglycemic level within 3 days, whereas baseline level was achieved after 5 days of administration of *C. longa* phenolic-rich extract. Absolute glucose reduction to baseline level was achieved at the end of the experiment except in rats administered *A. sativum* extract. Generally, antihyperglycemic effects of dietary phenolics derived from the highly branched phenylpropanoid pathway have been documented in literature with detailed review on their mechanism of action (Bahadoran et al. 2013; Xiao 2014; Xiao and Hogger 2015). Antihyperglycemic effect of *C. longa* has been attributed to its curcuminoid fraction (Babu and Srinivisan 1995; Sharma et al. 2006). Other findings have suggested that synergistic interactions between curcuminoids and sesquiterpenes contribute to the antihyperglycemic properties (Nishiyama et al. 2005; Honda et al. 2006). However, this study showed that curcumin-free (turmerone-rich) extract also has the capacity to reduce hyperglycemia in rats.

Similarly, administration of eugenol as low as 10 mg/kg b. wt. significantly reduced elevated glucose levels as well as glycosylated hemoglobin in hyperglycaemic rats (Mnafgui et al. 2013; Srinivasan et al. 2014). In addition, level of plasma insulin was increased and alterations in carbohydrate metabolizing, regulatory and rate-limiting enzymes were normalized on administration of eugenol (Srinivasan et al. 2014). Thus, the beneficial effects of *S. aromaticum* rich in eugenol on the management of diabetes may be attributed to its ability to reduce intestinal absorption of dietary carbohydrate as evident in this study from its strong inhibitory effect on alpha-amylase. Modulation of other enzymes involve in glucose metabolism or stimulation of insulin secretion may also contributes to its glucose lowering property.

5 Conclusion

The study revealed that the selected medicinal spices both in vitro and in vivo have effective antioxidant, antiradical and antihyperglycemic activities, implying their potential use in formulation of diabetic diets and nutritional

supplements. Further studies are required to isolate their active components to validate their pharmacological effect which will be of great medicinal importance for future develoment of antidiabetics.

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