Progress in Nutrition 2018; Vol. 20, N. 3: 403-414 DOI: 10.23751/pn.v20i3.6196

ORIGINAL ARTICLE

Comparative in vitro analysis of anti-diabetic activity of Indo-Pak black cardamom (*Amomum subulatum Roxb.)* and Chinese black cardamom *(Amomum tsao-ko Crevost et Lemaire)*

Syed Ammar Hussain, Ahsan Hameed*, Jingjing Fu, Haifang Xiao, Qing Liu, Yuanda Song*

Colin RatledgeCenter for Microbial Lipids, School of Agriculture Engineering and Food Science, Shandong University of Technology, Zibo, P.R. China - E-mail: ysong@sdut.edu.cn

Summary. Diabetes mellitus is a metabolic disorder of glucose metabolism. An indispensable strategy for the control of diabetes mellitus, especially diabetes type 2 and its harmful effects, is the efficient management of postprandial hyperglycemia. *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* are considered useful for the treatment of diabetes mellitus in Indo-Pak region and P.R China respectively. In this study, all tested concentrations of aqueous (v/v) extracts of the seeds and rind showed significant inhibitory activity against α-amylase and α-glucosidase. It can be concluded that aqueous extracts of *Amomum subulatum Roxb* and *Amomum tsao-ko Crevost et Lemaire* dry fruit constitutes (seeds and rind) have the significant inhibitory activity against the two carbohydrate hydrolyzing enzymes and the presence of phytochemicals like flavonoids, saponins, and tannins etc may have contributed significantly to the inhibitory potential of plant extracts

Key words: α-amylase inhibitory activity, α-glycosidase inhibitory activity, *Amomum subulatum Roxb.*, *Amomum tsao-ko Crevost et Lemaire*, seed and rind, type 2 diabetes

Introduction

Diabetes mellitus (DM) is the metabolic disorder characterized by increased blood glucose level (hyperglycaemia) with abnormality in carbohydrate, protein and fat metabolism. According to World Health Organization (WHO), there are 346 million people affected worldwide from diabetes and this number will be doubled by the year 2030 (1-2). It is such a progressive endocrine disorder of glucose metabolism that eventually leads to micro- and macro-vascular changes causing secondary complications that are incredibly challenging to manage (3). Type 1 diabetes arisen due to the inadequate synthesis of insulin by β-cells of the pancreas, while type 2 diabetes is regarded as primarily by insulin resistance (a condition in which peripheral cells do not respond normally to insulin) or β-cell dysfunction (4). The drugs which are mostly used to treat the diabetics are: insulin, sulfonylureas, biguanide, glycosidase inhibitors, aldosereductase inhibitor, carbamoylmethyl benzoic acid, thiazolidinediones (5, 6). Now-a-days, there are different kinds of therapeutic strategies for the treatments of diabetes in practice likewise: stimulation of endogenous insulin secretion, increase the activity of insulin at the target tissues and inhibition of α-amylase enzyme activity to reduce the degradation of starch and lower the blood glucose level (6-8). α-amylase is a well-known enzyme found in the pancreatic juice and saliva which break down large insoluble starch molecules into absorbable molecules. While mammalian α-glucosidase in the mucosal brush border of the small intestine catalyzed the last step of digestion of starch *i.e.* the breakdown of disaccharides that are ample in human diet (9, 1). The α-amylase and α-glucosidase involved in the di-

* Both Authors contributed equally to this work

gestion of carbohydrates can considerably decrease the postprandial increase of blood glucose after consumption of a mixed carbohydrate diet and thereby creating a dynamic platform in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients (11). Inhibitors of α-amylase enzyme (EC 3.2.1.1) and α-glucosidase enzyme (EC 3.2.1.20) postpone the breaking down of carbohydrates in the small intestine and reduce the postprandial blood glucose expedition (3, 12). α-amylase inhibitors are one of the anti-diabetic drug families, of which acarbose is the most prominent one. These drugs have a robust advantage and are suitable for healing non-insulin-dependent diabetes mellitus (type-2 diabetes) but also prompt the gastrointestinal side effects that reduce their use in a preventive approach (13, 14). Many researcher and nutritionist are extremely interested to fabricate a novel nutritional approach to perfectly control the postprandial glycaemia without inducing negative circumstances on the digestive system. In the aforesaid context, medicinal plants because of their easy accessibility and less side-effects have gaining special place in pharmaceutics to treat various kind of chronic diseases (15). Plant materials used as traditional medicine for the treatment of diabetes are deliberated as one of the good source for the drugs discovery or a lead to make a new chemical entity (16). Plant extracts or different folk plant preparations are being prescribed by the traditional practitioners and have also been accepted by the users for the treatment of diabetes and any other diseases in many countries all over the world especially in the third world countries (17). Currently more than 400 plants are being used in different forms to lessen the hyperglycaemic effects (11, 15). Several different inhibitors of α-amylase and α-glucosidase has been isolated from the medicinal plants to serve as an alternative drug with better potency and lesser adverse effects than existing synthetic drugs (18). Active compounds derived from the medicinal plants are not only the source of α-amylase and α-glycosidase inhibitors, moreover they also are the rich source of phenolic substances, and consequently they have great antioxidant activity (19). Amomum belongs to family Zingiberaceae is a genus of Rhizomatous terrestrial herb, distributed mainly in tropical Asia and Africa, found in the eastern Himalayas and cultivated in Nepal, northern West Bengal, Assam and Sikkim hills (20). The seeds are reported to possess stimulant, stomachic, alexipharmic and astringent properties, and are used in traditional medicine for the treatment of indigestion, vomiting, abdominal pains and rectal diseases. The seeds are found to promote the elimination of bile and are used to treat congestive jaundice; they are also used in gonorrhoea, while the rind has been reported to be useful in treating headache and stomatitis. The aromatic oil extracted from the seeds is useful to the eyes in cases of inflammation (21). *Amomum subulatum Roxb.* dry fruits locally regarded as black cardamom, have a strong camphor-like flavor, with a smoky character derived from the method of drying. It has many biological activities like: analgesic (22), anti-inflammatory (23), antimicrobial (24-27), antioxidant (28-31), antiulcer (32-36), cardioadaptogen (37), diuretic (38), and hypolipidaemic activity (39-41). While *Amomum tsao-ko Crevost et Lemaire* mostly used in Chinese cuisine and folk medicine preparations for the treatment of throat infection and stomach (42, 43). The methanol extract of *Amomum tsao-ko* had markedly influence on plasma glucose and thiobarbituric acid reactive substances (TBRAS) and antioxidant potential (43). However, the wide use of *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* fruits as a medicinal plant has very less scientific evidences to attest its bioactive components as well as its usage as a medicinal source to treat diabetes. The current study is the first comparative report for in vitro evaluation of their aqueous seeds and rind extracts for bioactive compounds in relation to their anti-diabetic activity.

2. Materials and Methods

2.1. Plant material

Dry fruits of *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* were purchased from Punjab province, Pakistan and Shandong province, P.R China respectively. Their identification was authenticated by M. Jafar Jaskani from UAF. Pakistan and Haifang Xiao from SDUT. China.

2.2. Chemicals and reagents

α-amylase from porcine pancreas, α-glucosidase from Saccharomyces cerevisiae, and para-nitrophenyl-

glucopyranoside were products of Sigma-Adrich Co., St Louis, USA, while soluble starch (extra pure) was obtained from J. T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents were of analytical grade and water used was glass distilled.

2.3. Preparation of aqueous extract of fruit's constituent

Dried fruits (seeds with rind) of *Amomum subula-tum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* were washed with water to remove all contaminants; these were dried under room temperature, the seeds were separated from rind, and then both fruits parts grounded separately to powder using disintegrator (Ultra Centrifugal Mill, MRK CO., Ltd., Tokyo Japan). 10 grams of the seeds and rind powder from both fruits were extracted by maceration in 100 mL of distilled water (v/v) for 3 days with frequent agitation speed of 280 rpm at 28°C in the dark. The supernatants were collected, filtered through Whatman No. 1 filter paper and the filtrate was then concentrated at 60°C using a rotary evaporator (BuchiLabortechnik, Flawil, Switzerland). Finally the concentrates were freeze dried (Labconco Corporation, Kansas City, MO, USA) to yield a dry powder. Dried extracts were weighed and dissolved in 10% dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared.

2.4. Phytochemical screening

Phytochemical compositions of the seeds and rind of the said plants were determined using the methods previously described by Trease and Evans (44) and Sofowora (45).

2.4.1. Test for anthraquinones:

5 mL of chloroform was added to 0.5 g of the plant extracts of each specimen. The resulting mixture was shaken for 5 min after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink color in the aqueous layer indicated the presence of anthraquinones.

2.4.2. Test for flavonoids:

A portion of the plant extract was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. Development of yellow coloration was an indication of the presence of flavonoids.

2.4.3. Test for reducing sugar:

To about 1 g of each plant extract in the test tube, 10 mL distilled water was added and the mixture boiled for 5 min. The mixture was filtered while hot and the cooled filtrate made alkaline to litmus paper with 20% sodium hydroxide solution. The resulting solution was boiled with an equal volume of Benedict qualitative solution on a water bath. The formation of a brick-red precipitate depicted the presence of reducing compound.

2.4.4. Test for saponin:

Approximately 2 g of plant extract was boiled in 20 mL of distilled water in a water bath and filtered. Next, 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again and then observed for the formation of emulsion as an indication of saponin.

2.4.5. Test for steroids:

In this test, 2 mL of acetic anhydride was added to 0.5 g of plant extract with 2 mL concentrated H_2SO_4. The color change from violet to blue or green is an indication of steroids.

2.4.6. Test for Tannins:

In the test for tannins, 0.5 g of plant extract was boiled in 20 mL of water in a test tube and filtered. Few drops of 0.1 % ferric chloride were added and observed for a brownish green or blue black coloration as an indication of tannins.

2.4.7. Test for terpenoids:

In brief, 0.5 g of plant extract was mixed with 2 mL chloroform and 3 mL H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was an indication of terpenoids.

2.5 Determination of total phenolic compounds

Total soluble phenolic compounds in the extracts

were determined with Folin-Ciocalteu reagent according to the method described by Hameed et al. (46). Briefly, 1 mL of extract (1000 μg/mL) in a volumetric flask was diluted with distilled water (46 mL). One mL of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min, Na_2CO_3 (3 mL, 2 % w/v) was added and then allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The total concentration of phenolic compounds in the extract determined as microgram of pyrocatechol equivalent by using an equation that was obtained from the standard pyrocatechol graph:

Absorbance = 0.0054 × total phenols (pyrocatechol equivalent) (μg) 0.0058.

2.6 Assay for total flavonoid content

Total flavonoid content was determined using the method given elsewhere (47, 48). Briefly, aluminium trichloride (1 mL, 2 % w/v) in methanol was mixed with the same volume of the extract (1 mL, 2000 μg/mL). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of an extract (1 mL, 2000 μg/mL) with methanol (1 mL) and without $AlCl_3$. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

Absorbance = 0.0338 quercetin (μg) - 0.0002; R2= 0.9998.

2.7. A-amylase inhibition assay

The inhibition of α-amylase was determined using an assay modified from the Worthington Enzyme Manual (48). Aliquot 0-4 mg/mL in DMSO (v/v 1:1) of aqueous extract of fruit's constitutes was prepared and 500 μL of each concentration extract was mixed with 500 μL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) and incubated at 25°C for 10 min. After pre-incubation, 500 μL of a 1.0 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) 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 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 15 mL of distilled water, and the absorbance was measured at 540 nm using a micro-plate reader (Thermomax, Molecular device Co., Virginia, USA). The experiments were performed in triplicate and the absorbance of sample blanks (buffer instead of enzyme solution) and a control (buffer in place of sample extract) were also recorded. The absorbance of the final extract was obtained by subtracting its corresponding sample blank reading. Acarbose was prepared in distilled water and used as positive control.

The percentage inhibition was calculated using the formula:

% Inhibition = {(Ac – Ae)/Ac} 100

where Ac and Ae are the absorbance of the control and extract, respectively.

IC_{50} values (inhibitor concentration at which 50 % inhibition of the enzyme activity occurs) of seed and rind extracts were determined by plotting graph with varying concentrations of the said extracts against the percent inhibition.

2.8. α-glucosidase inhibition assay

The α-glucosidase was assayed using a method modified by Apostolidis et al. (49). Aliquot 0-4 mg/mL in DMSO (v/v 1:1) of aqueous extract of fruit's constitutes was prepared. 50 μL of each concentration extract was mixed well with 100 μL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/mL) and the mixtures were then incubated in 96-well plates at 25°C for 10 min. After pre-incubation, 50 μL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were in-cubated at 25°C for 5 min. Before and after incubation absorbance readings were recorded at 405 nm using a micro-plate reader (Thermomax, Molecular device Co., Virginia, USA) and compared to a control which contained 50 μL of the buffer solution instead of the extracts. The experiments were performed in triplicate and the α-glucosidase inhibitory activity was expressed as percentage inhibition. Acarbose was prepared in distilled water and used

as positive control. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \{(Ac - Ae)/Ac\}\ 100$$

where Ac and Ae are the absorbance of the control and extract, respectively.

IC_{50} values (inhibitor concentration at which 50 % inhibition of the enzyme activity occurs) of seed and rind extracts were determined by plotting graph with varying concentrations of the said extracts against the percent inhibition

2.9. Statistical Result

All the measurements were done in triplicate and results are expressed in terms of mean ± standard deviation and IC_{50} values were calculated using Graph Pad Prism 5 version 5.01 (Graph pad software, Inc., La Jolla, CA, USA.) statistical software.

3. Results and discussion

To control the diabetes complications, the management of the blood glycemic (sugar) level is considerable and a novel approach. Inhibitors of carbohydrate hydrolyzing enzymes (*i.e.* α-amylase and α-glycosidase) have been practically valuable as oral hypoglycemic drugs for the control of diabetes especially in patients with type-2 diabetes mellitus (50-52). Inhibitors of α-glucosidase postpone the breaking down of carbohydrate in the small intestine and reduce the postprandial blood glucose expedition in a person suffering from diabetes (53). Several α-amylase inhibitors including acarbose, miglitol and voglibose are clinically useful to treat diabetes but these are expensive and have considerable clinical side effects. Medicinal plants have great potential to retard the absorption of glucose by inhibiting the saccharides hydrolyzing enzymes (54, 55). There is an attempt to explore the alternative drugs from medicinal plants with increased potency and less adverse effects than existing drugs (56-58).

Therefore, screening and isolation of inhibitors from plants for these enzymes are escalating.

The phytochemical composition of aqueous extracts of *Ammomum Subulatum Roxb.* fruit's constitutes (seeds and rind) indicated the presence of anthraquinones, flavonoids and tannins, in both the seed and rind part, while saponin, steroids and terpenoids were only present in rind extract and reducing sugar was only detected in its seed extract (Table 1). Whilst the phytochemical composition of aqueous extracts of *Ammomum tsao-ko Crevost et Lemaire* fruit's constitutes (seeds and rind) illustrated the presence of flavonoids and tannins, in its both seed and rind extract, while anthraquinones, steroids and reducing sugar were only present in seed extract and saponin and terpenoids were only detected in its rind extract (Table 2).

In the present study, anti-diabetic activity of the aqueous extracts from seeds and rind were evaluated with reference to α-amylase and α-glycosidase inhibi-

Table 1. Phytochemical composition of aqueous extract of *Ammomum subulatum Roxb.*

Phytochemicals	Seed	Rind
Anthraquinones	+	+
Flavonoids	+	+
Reducing Sugar	+	-
Saponin	-	+
Steroids	-	+
Tannins	+	+
Terpenoids	-	+

(+): Present, (-) Not detected

Table 2. Phytochemicals composition of aqueous extract of *Ammomum tsao-ko Crevost et Lemaire*

Phytochemicals	Seed	Rind
Anthraquinones	+	-
Flavonoids	+	+
Reducing Sugar	+	-
Saponin	-	+
Steroids	+	-
Tannins	+	+
Terpenoids	-	+

(+): Present, (-) Not detected

tion. It was found that the plants used in this study, *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* fruit's constitutes showed potential anti-diabetic activities. The in vitro α-amylase and α-glucosidase inhibition study illustrated that the aqueous extract of *Amomum subulatum Roxb.* fruit's constitutes at concentrations of 4.0, 3.2, 2.4, 1.6 and 0.8 mg/mL inhibited α-amylase and α-glucosidase enzyme activities in a dose dependent manner. At the highest concentration of 4.0 mg/mL, the seed extract of *Amomum subulatum Roxb*. exhibited maximum α-amylase and α-glucosidase inhibitory activity of 87.1 % and 59.1 % respectively, while at the lowest concentration of 0.8 mg/mL showed a minimum inhibition of 67.3 % and 10.7 % respectively as well. The IC_{50} values of aqueous seed extract of *Amomum subulatum Roxb*. were 1.70 mg/mL and 1.98 mg/mL respectively, while its rind extract at 4.0 mg/mL have highest α-amylase and α-glucosidase inhibitory activity of 86.9 % and 61.8 % respectively and at the lowest concentration of 0.8 mg/mL showed a minimum inhibition of 53.2 % and 22.9 % respectively as well (Figure 1, 2). The IC_{50} values of rind extract of *Amomum subulatum Roxb*. were 1.10 mg/mL and 1.18 mg/mL respectively. Whilst acarbose at the concentration of 4.0 mg/mL, showed a maximum percentage inhibition of 74.3 % for α-amylase with an IC_{50} of 2.1 mg/mL and 84.01 % for α- glycosidase with IC_{50} 1.90 mg/mL value respectively. Our results suggested that the seed and rind extract of *Amomum subulatum Roxb*. have almost same percentage inhibition for α-amylase and α-glucosidase but higher than that of acarbose activity.

Likewise, the aqueous extract of *Amomum tsao-ko Crevost et Lemaire* fruit's constitutes (seeds and rind) also showed α-amylase and α-glucosidase inhibition in a dose dependent manner for concentrations of 4.0,

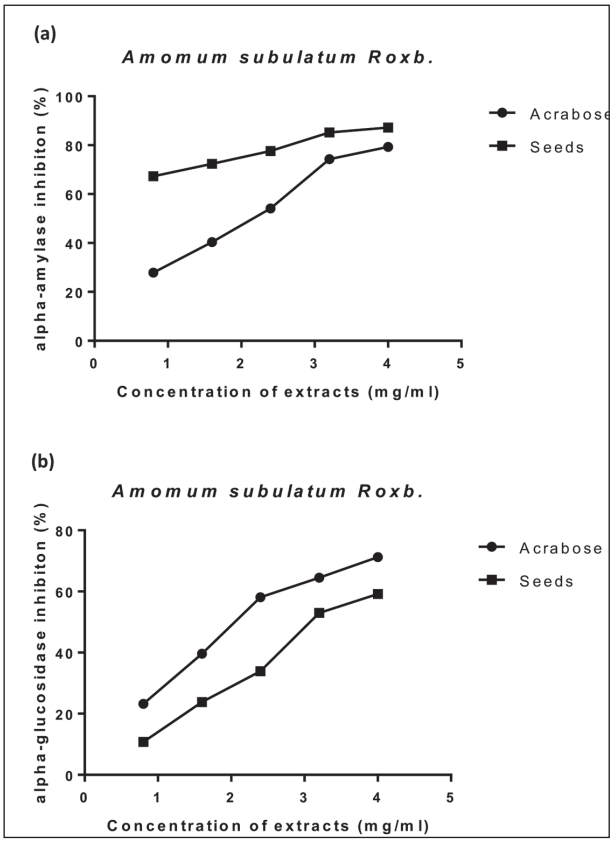

Figure 1. α-amylase inhibitory activities (a), α-glucosidase inhibitory activities (b), of seeds extracts of *Amomum subulatum Roxb.*

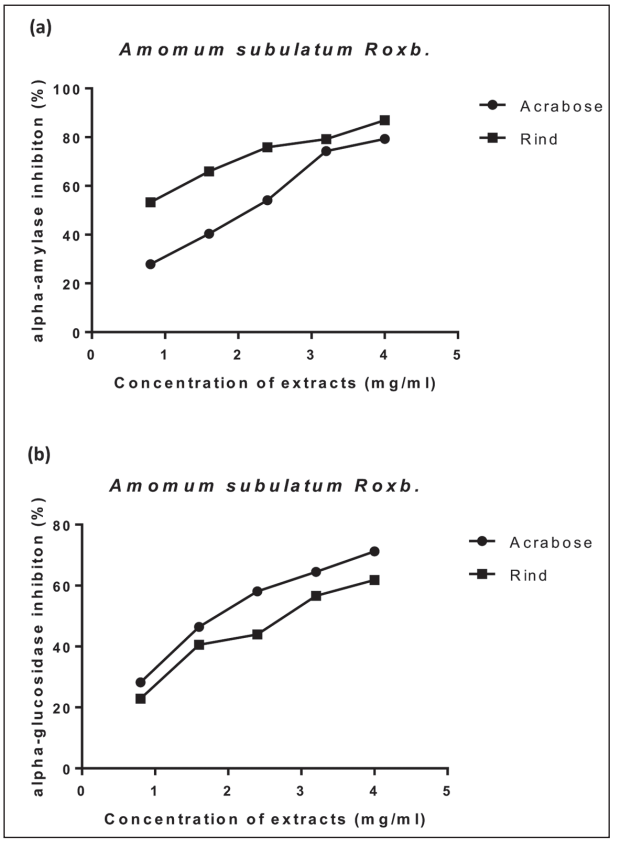

Figure 2. α-amylase inhibitory activities (a), α-glucosidase inhibitory activities (b), of rind extracts of *Amomum subulatum Roxb.*

3.2, 2.4, 1.6 and 0.8 mg/mL. At the highest concentration of 4.0 mg/mL, the seed extract of *Amomum tsao-ko Crevost et Lemaire* exhibited maximum α-amylase and α-glucosidase inhibitory activity of 83.9 % and 54.7 % respectively, while at the lowest concentration of 0.8 mg/mL showed a minimum inhibition of 52.5 % and 18.2 % respectively as well, with IC_{50} values of 1.04 mg/mL and 1.4 mg/mL respectively. While its rind extract at 4.0 mg/mL have maximum α-amylase and α-glucosidase inhibitory activity of 69.3 % and 29.1 % respectively and at the lowest concentration of 0.8 mg/mL, rind extract showed minimum inhibition for α-amylase and α-glucosidase of 26.1 % and 4.02 % respectively as well (Figure 1, 2). The IC_{50} values of aqueous rind extract of *Amomum tsao-ko Crevost et Lemaire* were 1.24 mg/mL and 2.4 mg/mL respectively. Among the two extracts of said plant, it is suggested that the seed extract of *Amomum tsao-ko Crevost et Lemaire* was more effective than the rind extract, and both extracts were relatively analogous to acarbose activity.

In the conclusive manner, it is suggested that seeds and rind extracts of *Amomum subulatum Roxb.* and seed extract of *Amomum tsao-ko Crevost et Lemaire* are much potent than acarbose on equal weight basis.

In this era of inquisitiveness, inventions and discoveries, it has now become indispensible to compare our findings with previous studies on different medicinal plants for inhibition against α-amylase and α-glucosidase activities. Kargbo et al. (59) suggested that at a concentration of 4.0 mg/mL, the ethanolic leaves extract of *Anisophyllea laurina* R. Br. ex Sabine exhibited α-amylase and α-glucosidase inhibitory activity of 78.5 % and 58.2 % respectively, with IC_{50} values of 2.40 mg/mL and 3.11mg/mL respectively. The stem extracts showed α-amylase and α-glucosidase inhibitory activities of 69.5 % and 63.6 % respectively, with IC_{50} values of 2.6 mg/mL and 3.5 mg/mL respectively. Dastjerdi et al. (60) found that α-amylase activity was inhibited by different *Teucrium* species. The IC_{50} value of hydro-alcoholic extract of *T. polium* against α-amylase activity was 3.63 mg/mL. The IC_{50} value of *T. oliverianum* and *T. Orientale* against α-amylase activities were 3.86 and 13.93 mg/mL, respectively. Kazeem et al. (61) worked on *Morinda lucida* Benth leaves, they revealed that the aqueous extract of *Morinda lucida* Benth leaves possesses IC_{50} value of 2.30 mg/mL against α-amylase and IC_{50}value of 2.00 mg/mL against α-glucosidase activities. Mohamed et al. (62) investigated the α-amylase and α-glucosidase inhibitory activities of 50 % ethanolic extract of *Orthosiphon stamineus*, they found that IC_{50} value of 36.70 mg/mL for α-amylase and IC_{50} value 4.63 mg/mL for α-glucosidase. Balasubramaniam V. et al. (63) discovered that ethanol extract of *E. denticulatum* (red edible seaweed) at 10 mg/mL, significantly inhibited the α-amylase activity by 67 %. From this discussion, it is concluded that *Amomum subulatum Roxb.* and *Amo-mum tsao-ko Crevost et Lemaire* seed and rind extracts have best inhibitory activities against α-amylase and α-glucosidase in comparison with different medicinal plants stated above (Table 3).

Phytochemicals especially polyphenols has established increasing attention due to fascinating discoveries considering their biological activities (64). Previous findings proved that the methanolic extract of *Amomum subulatum Roxb.* fruit's constitutes showed high level of total phenolic content and total flavonoid content (65), it's essential oil demonstrated high level of total phenolic content due to presence of components like; 1,8-cineole, α-terpineol, terpinen-4-ol, spathulenol, α-pinene (66), Oleoresin of *Amomum subulatum Roxb.* seeds had moderate level of total phenoilc content, total flavonoid contant and total tannin content (67), its fruit's constitutes also had good antioxidant potential (68, 69). Many active compounds have already been explored in fruit's constitutes such as protocatechuic acid (70), petunidin-3,5-diglucoside, leucocyanidin-3-O-β-D-glucopyranoside, subulin, 1,8-cineole, α-terpinylacetate (71), protocatechualdehyde, 1,7-bis(3,4-dihydroxyphenyl) hepta-4E, 6E-dien-3-one, 2, 3, 7trihydroxy-5-(3,4-di-hydroxy-E-styryl)-6, 7, 8, 9-tetrahydro-5H benzocyclo-heptene (72), essential oil mainly consist of 1,8-cineole, lamonene, sabeinene, pinenes and terpinols (73), While earlier studies on *Amomum tsao-ko Crevost et Lemaire* fruit's constitutes demonstrated that it had moderate level of total phenolic content (TPC) (74,75) and total flavonoid content (TFC) (75). In the past, many phenolic compounds have been investigated in *Amomum tsao-ko Crevost et Lemaire* fruit's constitutes like; hannokinol, mesohannokinol, catechin, epicatechin, β-sitosterol, β-sitosterol 3-O-glucoside, 2,6 dimethoxyphenol, protocatechualdehyde, protocatechuic acid vanillic aci *p*-hydroxybenzoic acid

Table 3. IC_{50} values for α-amylase and α-glucosidase inhibitory potential of different plants extracts

Source Plants	IC_{50} (mg/mL)	
	α-amylase	α-glucosidase
Amomum subulatum Roxb. (seed)	1.7	1.9
Amomum subulatum Roxb. (rind)	1.1	1.1
Amomum tsao-ko Crevost et Lemaire (seed)	1.07	1.4
Amomum tsao-ko Crevost et Lemaire (rind)	1.24	2.4
Anisophyllea laurina R. Br. ex Sabine (leaf)	2.4	3.1
Anisophyllea laurina R. Br. ex Sabine (stem)	2.6	3.5
Teucrium polium	3.63	–
Teucrium oliverianum	3.86	–
Teucrium orientale	13.93	–
Morinda lucida Benth (leaves)	2.3	2
Orthosiphon stamineus	36.7	4.6

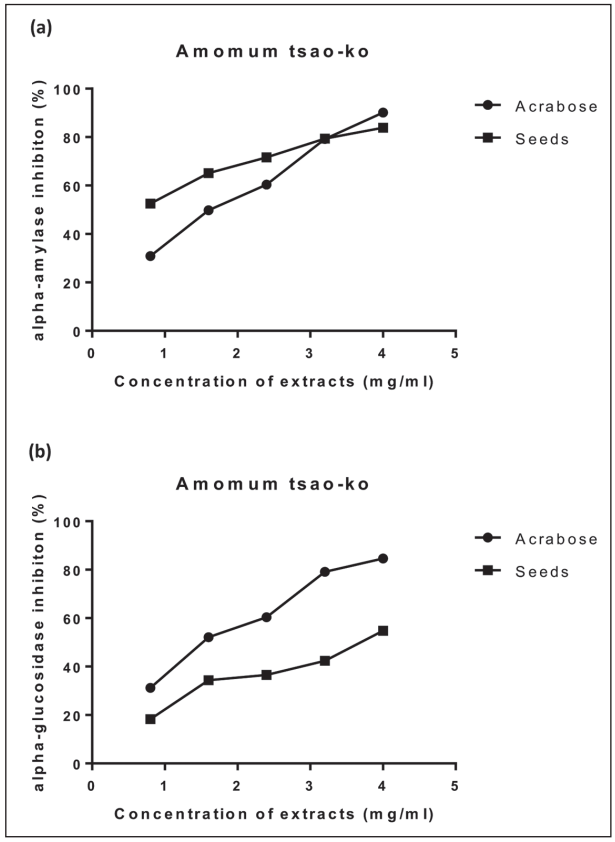

Figure 3. α-amylase inhibitory activities (a), α-glucosidase inhibitory activities (b), of seed extracts of *Amomum tsao-ko Crevost et Lemaire*

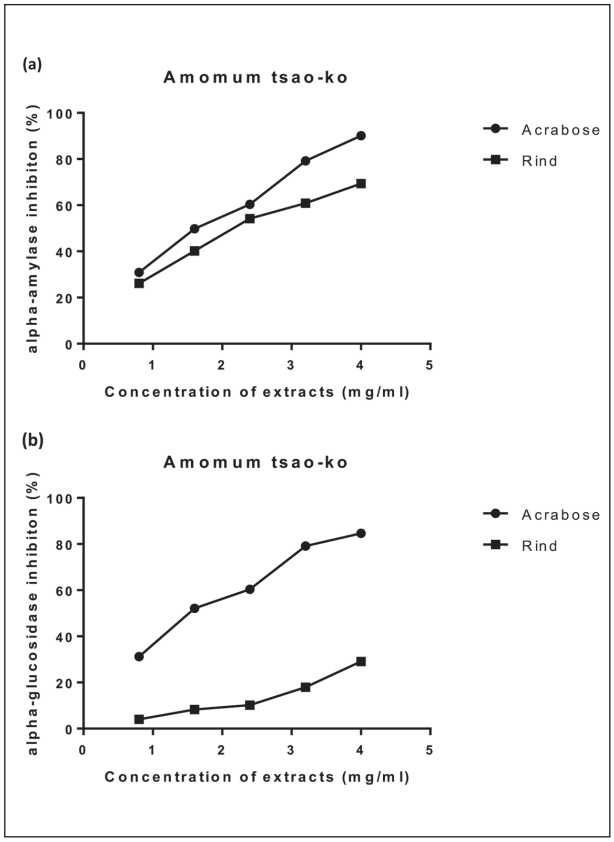

Figure 4. α-amylase inhibitory activities (a), α-glucosidase inhibitory activities (b), of rind extracts of *Amomum tsao-ko Crevost et Lemaire*

(76, 77), daucosterol, quercetin, quercetin-7-O-β-glucoside, quercetin-3-O-β-glucoside, catechol (78), 2-methoxy-1,4-biphenol-1-O-[6-O-(3-methoxy-4-hydroxybenzoyl)]-β-D-glucopyranoside, 3′, 5′-di-C-β-D-glucopyranosylphloretin, rutin, pyrogallic acid (79), and also fat soluble polar active components that might be responsible for decreased blood glucose and TBARS concentrations (80). The α-amylase and α-glucosidase inhibitory activities might be due to the individual or synergistic outcome of these bioactive compounds, the results of current work are very interesting, still sufficient in vivo studies (in rats or rabbits) are required to extrapolate its usage in humans.

4. Conclusion

In the present study, *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* dry fruit's constitutes (seeds and rind) were evaluated to find out the possible mechanism for their anti-diabetic mode of action. *Amomum subulatum Roxb.* seeds and rind extract could inhibit starch digestion enzymes more efficiently than acarbose. Among the four aqueous extracts, seed extracts of both *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* showed best inhibitory effect on α-amylase and better on α-glycosidase activities, whilst the rind extract of both *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* showed good inhibitory potential for α-amylase and sufficient inhibitory potential for α-glycosidase activity. Based on the results presented in this study, it can be concluded that aqueous extracts of *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* dry fruit's constitutes (seeds and rind) have high inhibitory effect on α-amylase and α-glycosidase activities. These activities might be attributed to presence of phenolic compounds earlier identified in these plants, which may have individual or synergistic consequence for such activities. Therefore our results suggested the potential use of these plants as a dietary supplement or in the manufacture of drugs for the control of increased blood glucose (sugar) level in the body. However, further studies are also needed to elucidate the active principle(s) constitutes in these plants which are responsible for such activities.

References

1. Kumar PS, Sudha S. Evaluation of α amylase and α glucosidase inhibitory properties of selected seaweeds from Gulf of Mannar. International research journal of pharmacy (2012) 3(8) 128-130.
2. Lavelli V. Grape skin phenolics as inhibitors of mammalian α-glucosidase and α-amylase effect of food matrix and processing on efficacy. Food Function (2016) 7, 1655, DOI:10.1039/c6fo00073h
3. KazeemMI, Adamson JO, Ogunwande IA. Modes of Inhibition of α-Amylase and α-Glucosidase by Aqueous Extract of *Morinda lucida* Benth Leaf BioMed Research International Volume 2013, Article ID 527570, http://dx.doi.org/10.1155/2013/527570
4. Heise T, Nosek L, Rønn BB, et al. Lower within-subject variability of insulin detemir in comparison to NPH insulin and insulin glargine in people with type 1 diabetes. Diabetes, vol. 53, no. 6, pp. 1614–1620, 2004.
5. Funke I, M.F. Melzing, 2006. Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of *a*-amylase activity. Brazilian journal of pharmacognosy., 16: 1-5.
6. Dastjerdi ZM, Namjoyan F, Azemi ME. Alpha Amylase Inhibition Activity of Some Plants Extract of *Teucrium* Species. European Journal of Biological Sciences 7 (1): 26-31, 2015, DOI: 10.5829/idosi.ejbs.2015.7.01.91127
7. Tarling A, K Woods, R Zhang, HC Brastianos GD Brayer, RJ Andersen, S.G. Withers, 2008. The Search for Novel Human Pancreatic alpha-Amylase Inhibitors: High-Through put Screening of Terrestrial and Marine Natural Product Extracts. ChemBioChem, 9: 433-438
8. Bailey CJ. 2003. New approaches to the pharmacotherapy of diabetes. In: Pickup, J.C., William, G. (Eds.), Textbook of Diabetes, Vol. 2, 3rd. Blackwell Science Ltd, pp: 73.1-73.
9. AF. Afifi, EM. Kamel, AA. Khalil, MA. Foaad, E. M. Fawziand, M. Houseny. Purification and characterization of a-amylase from penicilliumolsonii under the effect of some antioxidant vitamins. Global Journal of Biotechnology and Biochemistry, vol. 3, no. 1, pp. 14-12, 2008.
10. V. Manohar, NA. Talpur, BW. Echard, S. Lieberman, HG. Preuss. Effects of a water-soluble extract of maitakemushroomon circulating glucose/insulin concentrations in KK mice. Diabetes, Obesity and Metabolism, vol. 4, no. 1, pp. 43-48, 2002.
11. Kargbo MBR, Onivogui G, Song Y. In vitro anti-diabetic activity and phenolic compound profile of ethanol extracts of *Anisophyllealaurina R*. Br. ex Sabine leaves and stem bark. European academic Research, Vol. II, Issue 12/ 2015
12. Y-I Kwon, E Apostolidis, K Shetty. Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension. Journal of Food Biochemistry, vol. 31, no. 3, pp. 370-385, 2007.
13. Cheng AYY, IG Fantus, 2005. Oral anti-hyperglycemic therapy for type 2 diabetes Mellitus. Can Med Assoc J, 172: 213-226.
14. De Sales PM, PM de Souza, LA Simeoni, PO Magalhães

and D Silveira, 2012.Alpha Amylase inhibitors: A review of raw material and isolated compounds from plant source. J Pharm Pharm Sci, 15: 141-183.
15. Suleria HAR, Butt MS, Khalid N, Sultan S, Raza A, Aleem M, Abbas M. (2015). Garlic (Allium sativum): diet based therapy of 21st century–a review. Asian Pacific Journal of Tropical Disease, 5(4), 271-278.
16. Suleria HAR, Butt MS, Anjum FM, Saeed F, Khalid N (2015). Onion: nature protection against physiological threats. Critical Reviews in Food Science and Nutrition, 55(1), 50-66.
17. Suleria H, Butt M, Anjum F, Ashraf M, Qayyum M, Khalid, N, Younis M. (2013). Aqueous garlic extract attenuates hypercholesterolemic and hyperglycemic perspectives; rabbit experimental modeling. Journal of Medicinal Plants Research, 7(23), 1709-1717.
18. H. Matsuda, T. Morikawa, M. Yoshikawa. Antidiabetogenic constituents from several natural medicines. Pure and Applied Chemistry, vol. 74, no. 7, pp. 1301-1308, 2002.
19. Cicero LT, L Yenshou, PB Arlene, Ch Yi-Chin, Ch Shao-Chih Y Wen-Chin, 2013. Herbal Therapies for Type 2 Diabetes Mellitus: Chemistry, Biology and Potential Application of Selected Plants and Compounds, 43: 1-33.
20. Anonymous, Wealth of India. A dictionary of Indian Raw material& Industrial Products, NISCAIR 2004; 1: A-C55
21. Agnihotri S, Wakode S. Antimicrobial Activity of Essential Oil and various Extracts of Fruits of Greater Cardamom. Indian J Pharm Sci. 2010 Sep-Oct; 72(5): 657-659. doi: 10.4103/0250-474X.78542
22. Shukla SH, Mistry HA, Patel VG, Jogi BV. Pharmacognostical, preliminary phytochemical studies and analgesic activity of *Amomum subulatum Roxb.* Pharm Sci Monit 2010, 1(1), 90-102.
23. Alam K, Pathak D, Ansari SH. Evaluation of Anti-Inflammatory activity of Amomium Subulatum fruit extract. Int. J. Pharmaceu. Sci.Drug. Res. 2011, 3(1), 35-37.
24. Kumar U, Kumar B, Bhandari A, Kumar Y. 2010. Phytochemical investigation and comparison of antimicrobial screening of Clove and Cardamom. Inter. J. Pharmaceu. Sci. Res. 2010, 1(12), 138-147.
25. Jain PC, Agarwal SC. Activity of some plants extracts against some keratinophilic species of Nannizzia. Ind. Drugs. 1976, 23(12), 25-26.
26. Hussain T, Arshad M, Khan S, Hamid S, Qureshi MS. In vitro screening of methanol plant extracts for their antibacterial activity. Pak.J. Bot. 2011,43(1). 531-538.
27. Aneja KR, Joshi R. 2011. Antimicrobial activity of Amomum subulatum and Elettaria cardamomum against dental carries causing microorganisms. Ethnobot Leaflets 2011,13, 840-849.
28. Willet W. Diet and health-what should we eat? Science. 1994, 264, 532-537.
29. Kikuzaki H, Kawai Y, Nakatani N. Diphenyl-2-picrylhydrazylradical-scavenging active compounds from greater cardamom (*Amomum subulatum Roxb.*). J. Nutr. Sci. Vitaminol. 2001,47(2), 167-171.
30. Jessie SW, Krishnakantha TP. Inhibition of human platelet aggregation and membrane lipid peroxidation by food spice saffron. Mol. Cell Biochem. 2005, 278, 59-63.
31. Verma SK, Rajeevan V, Bordia A, Jain V. Greater cardamom (*Amomum subulatum Roxb.*)– A cardio-adaptogen against physical stress. J. Herb. Med. Toxicol. 2001, 4(2), 55-58.
32. Chan FKL, Leung WK. Peptic ulcer disease. The Lancet. 2002,360,933-941.
33. Jafri MA, Farah KJ, Singh S. Evaluation of the gastricantiulcerogenic effect of large cardamom (fruits of *Amomum subulatum Roxb.*). J. Ethnopharmocol. 2001,75, 89-94.
34. Sen S, Chakraborty R., De B, Mazumder J. Plants and phytochemicals for peptic ulcer: an overview. Pharmagonosy Rev. 2009, 3(6), 270-279.
35. Farah AJ, Siddiqui A, Aslam M, Javed K, Jafri MA. Antiulcerogenic activity of Elettaria cardamomum Maton. *Amomum subulatum Roxb.* seeds Ind. J. Trad. Know. 2005, 4(3), 298-302.
36. Parmar MY, Shah P,Thakkar V, Gandhi, TR, 2009. Hepatoprotectiveactivity of *Amomum subulatum Roxb.* against ethanol-induced liverdamage. Intern. J. Green Pharm. 3(3), 250-254.
37. Verma SK, Rajeevan V, Bordia A, Jain V. Greater cardamom (*Amomum subulatum Roxb.*)– A cardio-adaptogen against physical stress. J. Herb. Med. Toxicol. 2010, 4(2), 55-58.
38. Nakatani N. and Kikuzaki H. Antioxidants in Ginger Family. Quality Management of Nutraceuticals. Chapter 16, pp 230–240, DOI: 10.1021/bk-2002-0803.ch016
39. Hafidh RR, Abdulamir AS, Bakar FA, Abas F, Jahanshiri F, Sekawi Z. Antioxidant research in Asia in the period from 2000-2008. Am. J. Pharmacol. Toxicol. 2009, 4(3), 48-66. 11.
40. Dhuley JN. Anti-oxidant effects of cinnamon (Cinnamomumverum) bark and greater cardamom (Amomum subulatum) seeds in rats fed high fat diet. Indian J. Exp. Biol. 1999, 37(3), 238-242.
41. Yadav AS, Bhatnagar D. "Modulatory effect of spice extracts on iron-induced lipid peroxidation in rat liver". Biofactors. 2007, 29(2-3), 147-157.
42. Ebihara T, Ebihara S, Maruyama M, et al. A randomized trial of olfactory stimulation using black pepper oil in older people with swallowing dysfunction. J Am Geriatr Soc 54: 1401-1406.
43. Longguan YU et al. 2008. Effect of Lipid Extracted from Tsao-ko (*Amomum tsao-ko Crevost et Lemaire*) on Digestive Enzyme Activity, Antioxidant Activity, Plasma and Liver Lipids, and Blood Glucose Levels of Mice. Journal of Nutritional Science and Vitaminology, Vol. 54 (2008) No. 5 P 378-383.doi.org/10.3177/jnsv.54.378
44. GE. Trease, WC. Evans,Pharmacognosy, WB Saunders, Philadelphia, Pa, USA, 1996.
45. A. Sofowora, Medical Plants and Traditional Medicine in Africa, Spectrum Books, Ibadan, Nigeria, 2006.
46. Hameed A, Hussain SA. Yang J, Ijaz MU, Liu Q, Suleria HAR, Song Y. (2017). Antioxidants Potential of the Filamentous Fungi (Mucor circinelloides). Nutrients, 9(10),

1101–1121.doi.org/10.3390/nu9101101.
47. Hansawasdi C, Kawabata J, Kasai T. Alpha-amylase inhibitors from Roselle (Hibiscus sabdariffa Linn.) tea. BiosciBiotechnolBiochem. 2000;64:1041-3.
48. Young-In K, Emmanouil A, Kalids S: Inhibitory potential of wine and tea against α-amylase and α-glucosidase for management of hyperglycemia linked to type 2 diabetes. J Food Biochem 2008, 32:15-31.
49. Apostolidis E, Kwon YI, Shetty K. Inhibitory potential of herb, fruit, and funga-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Inn Food Sci-Emerg Technol. 2007;8:46–54.
50. Hussain SA, Hammed A, Nazir Y, Naz T, Wu Y, Suleria HAR, Song Y, (2018). Microencapsulation and the Characterization of Polyherbal Formulation (PHF) Rich in Natural Polyphenolic Compounds. Nutrients, 10 (7), 843-868. https://doi.org/10.3390/nu10070843.
51. Gin H, Rigalleau V. Post-prandial hyperglycemia and Diabetes. DiabMetabol 2000; 26: 265-272
52. Sima AA, Chakrabarti S. Long-term suppression of postprandial hyperglycaemia with acarbose retards the development of neuropathies in the BB/W-rat. Diabetologia.1992; 35:325-30.
53. Y-I Kwon, E Apostolidis, K Shetty. Evaluation of pepper (Capsicum annuum)for management of diabetes and hypertension. Journal of Food Biochemistry, vol. 31, no. 3, pp. 370-385, 2007.
54. Sultan MT, Buttxs MS, Qayyum MMN, Suleria HAR. (2014). Immunity: plants as effective mediators. Critical Reviews in Food Science and Nutrition, 54(10), 1298-1308.
55. Kim JS, Kwon CS, Son KH.Inhibition of α-glucosidase and amylase by Luteolin, a flavonoid.Biosci Biotech Biochem 2000; 64: 2458-2461.
56. T Matsui, IA Ogunwande, KJM Abesundara, K Matsumoto. Anti-hyperglycemicpotential of natural products. Mini-Reviews in Medicinal Chemistry, vol.6,no.3, pp.349-356, 2006.
57. H. Matsuda,T. Morikawa, M. Yoshikawa. Antidiabetogenic constituents from several natural medicines. Pure and Applied Chemistry, vol. 74, no. 7, pp. 1301–1308, 2002.
58. IA Ogunwande, T Matsui, T Fujise, K Matsumoto. α-Glucosidase inhibitory profile of Nigerian medicinal plants in immobilized assay system. Food Science and Technology Research, vol. 13, no. 2, pp. 169-172, 2007.
59. Kargbo MR, et al. In vitro anti-diabetic activity and phenolic compound profile of ethanol extracts of Anisophyllealaurina R. Br. ex Sabine leaves and stem bark. European Academic Research Vol. II, Issue 12, (2015)
60. Dastjerdi Z.M, Namjoyan F, Azemi E. Alpha Amylase Inhibition Activity of Some Plants Extract of Teucrium Species. European Journal of Biological Sciences 7 (1): 26-31, (2015). DOI: 10.5829/idosi.ejbs.2015.7.01.91127
61. Kazeem MI, Adamson JO, Ogunwande IA. Modes of Inhibition of α-Amylase and α-Glucosidase by Aqueous Extract of Morinda lucida Benth Leaf. BioMed Research International Volume (2013), Article ID 527570, 6 pages, doi. org/10.1155/2013/527570
62. Mohamed EAH, Yam MF, Siddiqol MJA, Chan SH. Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% extracts and sinensetin from Orthosiphonstamineus Benth as anti-diabetic mechanism. BMC Complementary and Alternative Medicine (2012)12:176. DOI: 10.1186/1472-6882-12-176
63. Balasubramaniam V, Lee JC, Noh MFM, et al. J Appl Phycol (2016) 28: 1965. doi:10.1007/s10811-015-0690-6
64. Cho EJ, Yokozawa T, Rhyu DY, Kim SC, Shibahara N, Park JC. (2003). Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1, 1- diphenyl-2-picryl-hydrazyl radical. Phytome- dcine 10: 544-551.
65. Ghimire BK, Seong ES, Kim EH, et al. A comparative evaluation of the antioxidant activity of some medicinal plants popularly used in Nepal. Journal of Medicinal Plants Research (2011) Vol. 5(10), pp. 1884-1891.
66. Kapoor IPS, Singh B, Singh G, Szczepaniak L, et al. Chemistry, antifungal and antioxidant activities of cardamom (Amomum subulatum) essential oil and oleoresins. Int J Essent Oil Therapeut (2008) 2, 29-40.
67. Pant S, Adhikari B.M, Baral M.P. Antioxidant Assay of Oleoresin of Large Cardamom (Amomum ubulatum) Seeds. conference paper: (2012), 6th National Conference on science and Technology entitled "Economic Growth through Science, Technology and Innovation" organized by National Academy of Science and Technology (NAST), Khumaltar, Nepal, At Kathmandu, Nepal.
68. Rajani R.S, Nathwani S.V, Manek R.A, Patel NM. Evaluation of free radical scavenging activity of fruit constituents of *Amomum subulatum Roxb.* IJPRBS, (2012); Volume 1(6):103-111
69. Kikuzaki H, Kawai Y, Nakatani N. 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging active compounds from greater cardamom (*Amomum subulatum Roxb.*). J Nutr Sci Vitaminol (Tokyo) 2001;47(2):167-171. 11508709
70. Shivanand P, Mahalaxmi R. Identification and determination of Protocatechuic acid present in Greater Cardamom fruit extracts by HPTLC technique. International Journal of Pharmaceutical Sciences Review and Research (2010) volume 1, issue 1, article 006
71. Ritender, Bhatt M, Juyal V, Singh A. *Amomum subulatum Roxb.*: A Critical Review of Pharmacological and Phytochemical Data.International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR), (2014) Volume-3, Issue-5
72. Kikuzaki H. Kawai Y, Nakatani N. 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging active compounds from greater cardamom (*Amomum subulatum Roxb.*). J Nutr Sci Vitaminol (Tokyo). (2001; 47 (2):167-71.
73. Bisht V.K, Negi J.S, Bhandari A.K, Sundriyal R.C. *Amomum subulatum Roxb.*: Traditional, phytochemical and biological activities-An overview. African Journal of Agricultural Research Vol. 6(24), pp. 5386-5390, 26 (2011), DOI: 10.5897/AJAR11.745.
74. Lu M, Yuan B, Zeng M, Chen J. Antioxidant capacity and

major phenolic compounds of spices commonly consumed in China Food Research International 44(2):530-536 (2011)
75. Jeong SC, Tulasi R, Koyyalamudi S.R. Antioxidant Capacities of Hot Water Extracts and Endo-polysaccharides of Selected Chinese Medicinal Fruits. MDPI, Cancers (2016), 8(3), 33; doi:10.3390/cancers8030033
76. Martin TS, Kikuzaki H, Hisamoto M, Nakatani N. Constituents of Amomumtsao-ko and their radical scavenging and antioxidant activities. Journal of the American Oil Chemists' Society (2000), 77: 667
77. Nakatani N, Kikuzaki H. Antioxidants in Ginger Family. Quality Management of Nutraceuticals. Chapter 16, pp 230–240, DOI: 10.1021/bk-2002-0803.ch016
78. Zhang TT, Lu CL, Jiang JG. Bioactivity evaluation of ingredients identified from the fruits of *Amomum tsao-ko Crevost et Lemaire*, a Chinese spice. See comment in PubMed Commons below Food Function. (2014);5(8) :1747-54. doi: 10.1039/c4fo00169a.
79. Wang W, Yang CR, Zhang YJ. Phenolic Constituents from the Fruits of *momum tsao-ko Crevost et Lemaire* (Zingiberaceae). Acta Botanica Yunnanica 31(3):284-288 · (2009), DOI: 10.3724/SP.J.1143.2009.09031
80. Yu L, Shirai N, Suzuki H. et al. Effect of lipid extracted from tsao-ko (*momum tsao-ko Crevost et Lemaire*) on digestive enzyme activity, antioxidant activity, plasma and liver lipids, and blood glucose levels of mice. J Nutr Sci Vitaminol (Tokyo), (2008); 54(5):378-83.

Correspondence:
Yuanda Song, PhD.
Colin Ratledge Center for Microbial Lipids,
School of Agriculture Engineering and Food Science,
Shandong University of Technology, Zibo, P.R. China,
Tel: +86-139-06174047
E-mail: ysong@sdut.edu.cn