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Comparative study of some seed spices for their anti- hyperglycemic and free radical scavenging activity

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Abstract
Diabetes mellitus and free radical toxicity has encountered many lives and safer drugs to manage such etiologies have succeeded only for a short span and have counted more side effects. Spices are consumed with every meal in either form. Therefore, usage of spice/s as a functional food adjunct makes an appropriate choice to treat life style mediated disease. Hence, the present study includes *in vitro* evaluation of some seed spices for their anti- hyperglycemic and free radical scavenging activity. Among studied spices *Myristica fragrans* and *Papaver somniferum* were found to have promising values for both the activities whereas *Trigonella foenum-graecum* had higher inhibition values for alpha carbohydrate hydrolyzing enzymes but contrary it has comparatively low efficacy to quench free radicals.

Keywords: Diabetes mellitus, alpha amylase, alpha glucosidase, free radical scavenging activity, anti-hyperglycemic

1. Introduction

Spices and herbs have been used as functional food [1-5] and also have been reported to have various beneficial effects on human health which include anti-sclerotic, antithrombotic, anti-carcinogenic, anti-inflammatory, antiarrhythmic, anti-rheumatic, gastroprotective, and lipid-lowering action. In addition, spices have radioprotective (protects against radiation), anti-allergic and antimalarial effects. Spices inhibit the oxidation of low-density lipoprotein and protein glycation [6-10].

The therapeutic effects of certain spices are so significant that they have often been included in non-clinical, clinical and therapeutic studies. Studies show that curcumin possesses anti-inflammatory effects and therapeutic effect in gastrointestinal diseases. It is an inhibitor of low density lipoprotein oxidation and also showed effects against neurodegenerative diseases.

India occupies the world with higher number of diabetic subjects earning the dubious distinction. The metformin (biguanide drug), a first-line treatment for diabetes, was developed from *Galega officinalis*. To date, this is the only plant-derived allopathic treatment for diabetes. Early *in vitro* studies of spices such as cinnamon, cloves, bay leaves and turmeric have shown that they display insulin-enhancing activity [11, 12]. Mechanistic studies suggest that extracts of cinnamon increase *in vitro* glucose uptake and glycogen synthesis and increases insulin-receptor phosphorylation [13, 14]. Spices such as fenugreek (*Trigonella foenum-graecum*) seeds, onion (*Allium cepa*), garlic (*Allium sativum*) and black cumin (*Nigella sativa*) are recognized to possess hypoglycemic, hypolipidemic and antioxidant influence in diabetic situation [15, 16]. The control of postprandial hyperglycemia is an important strategy in the management of DM, especially type II diabetes and reducing chronic complications associated with the disease [17, 18]. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolysing enzymes α-glucosidase and α-amylase in the digestive tract [19, 20]. The antioxidant potential of any plant works in multifactorial channels. On one hand they quench free radicals and hence provide a regulated pH zone for metabolic milieu and on the second hand they also maintain the physical properties of biological system and therefore prevent the onset of many diseases. The present study includes some of the seed spices i.e. *Amomum subulatum, Brassica juncea, Elettaria cardamomum, Myristica fragrans, Papaver somniferum, Punica granatum* and *Trigonella foenum-graecum* for the evaluation of their phenolic content, antihyperglycemic potential and free radical scavenging activity.

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2. Material and Methods
2.1 Preparation of extract
Dried products were extracted both with water and methanol: water extraction, 1 g dried material or 10 g fresh material was minced in a small water volume, diluted at 200 ml and boiled until the volume was reduced to 100 ml, then stirred for 24 h in the dark; methanol extraction, 1 g dried material /10 g fresh material was stirred in 100 ml methanol for 24 h at room temperature in the dark as protocol provided by Gugliucci and Stahl, 1995[21]. In all experiments, the activity of each spice was tested using 100 ml aqueous or methanol extract.

2.2 Anti- hyperglycemic Evaluation
For Alpha-amylase inhibition assay, the assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the spice extract in concentration range 20-100 µg/ml were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any spice extract.
The % inhibition was calculated according to the formula [22].

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{540} \text{ (control)} - \text{Abs}_{540} \text{ (extract)} * 100}{\text{Abs}_{540} \text{(control)}}$$

For Alpha-glucosidase inhibition assay Acarbose, P-Nitrophenyl-α-D-glucopyranoside and Baker's Yeast alpha glucosidase were purchased from Sigma. The yeast alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Spice extracts were used in the concentration ranging from 20-100 µg/ml. Different concentrations of spice extracts were mixed with 320 µl of 100 mM phosphate buffer pH 6.8 at 30 °C for 5 minutes. 3 ml of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without any extracts.
The % inhibition was calculated according to the formula [22].

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{410} \text{ (control)} - \text{Abs}_{410} \text{ (extract)} * 100}{\text{Abs}_{410} \text{(control)}}$$

The IC 50 values for both α-amylase and α-glucosidase were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference α-amylase and α-glucosidase inhibitor. All tests were performed in triplicate.

2.3 Total Phenol Determination
Total soluble phenolics in the extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton, 1977 [23] using gallic acid as a standard phenolic compound. 1 ml of extract solution contains 1000 mg extract was mixed with 45 ml distilled water. One millilitre of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min, 3 ml of Na$_2$CO$_3$ (2%) was added then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the both extracts was determined as microgram of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph. The equation is given below-

$$\text{Absorbance} = 0.0008 * \text{Gallic acid}$$

2.4 Free Radical Scavenging Activity
Reduction of DPPH radical was performed as described by Cervato et al., 2000 [24].
3ml of 60mM DPPH in ethanol were added in 100ml each spice extract, and absorbance was monitored at 517 nm (Abs 517 extract). The absorbance of a control (distilled water or methanol instead of spice extract) was also recorded at the same wavelength (Abs 517 control). Therefore, the percentage of inhibition was calculated by the formula:

$$\text{Percentage inhibition} = \frac{(\text{Abs}_{517} \text{ control} - \text{Abs}_{517} \text{ extract}) * 100}{\text{Abs}_{517} \text{ control}}$$

Superoxide was generated by oxidation of xanthine (30 mM) with xanthine oxidase (5 U) in 60 mM phosphate buffer, pH 7.4, 30 mM ethylenediamine tetra acetic acid, and was detected by nitroblue tetrazolium (3 mM) followed spectrophotometrically at 560 nm. Superoxide radical scavenger activity of 100ml each spice extract were measured by their ability to inhibit this reaction [24] with respect to control samples (distilled water or methanol) and determined by the formula:

$$\text{Percentage inhibition} = \frac{(\text{Abs}_{560} \text{ control} - \text{Abs}_{560} \text{ extract}) * 100}{\text{Abs}_{560} \text{ control}}$$

Hydroxyl radical was generated by incubation for 60 min at 37^{0}C of a reaction mixture containing 100mM FeCl$_3$, 100mM ascorbate, 1 mM hydrogen peroxide, 2.8 mM deoxyribose in phosphate buffer 20 mM, pH 7.4. Deoxyribose degradation by hydroxyl radical occurring in the presence of 100ml each spice extract or control (distilled water or methanol) was estimated using the thiobarbituric acid (TBA) method [24]. Therefore, the percentage of inhibition was calculated by the formula:

3. Result and Discussion
α-amylase begins the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides and α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia [25, 26]. Hence, inhibitors of α-amylase and α-glucosidase are useful in the control of hyperglycemia as they delay carbohydrate digestion, which consequently reduce the postprandial plasma glucose level.
Highest α-glucosidase inhibitory activity in aqueous extract was observed in *Trigonella foenum-graecum* (46.72%) followed by *Myristica fragrans* (23.46%) and *Papaver somniferum* (21.9%). The same lineage was also observed for α -amylase inhibitory activity in aqueous phase with inhibition of 45.92% in was maximum in *Trigonella foenum-graecum,* 24 % in *Myristica fragrans* and 23% in *Papaver somniferum* (Table 1).

Table 1: α-glucosidase and α -amylase inhibitory activity of seeds used as spices

Spice	AGI(A) (%)	AGI(M) (%)	AAI(A) (%)	AAI(M) (%)
Amomum subulatum	17.8 ± 1.75*	14.3 ± 0.86**	15.32 ± 0.50**	9.34 ± 0.12**
Brassica juncea	11.86 ± 0.12*	8.2 ± 0.86	9.63 ± 0.10***	9.1 ± 1.33
Elettaria cardamomum	3.4 ± 0.50	2.23 ± 0.66	2.0 ± 0.38**	1.0 ± 0.50*
Myristica fragrans	23.46 ± 1.00***	16.78 ± 1.00	24.0 ± 0.12	15.2 ± 1.15
Papaver somniferum	21.9 ± 0.33	26.43 ± 1.00	23.0 ± 0.33	27.82 ± 2.15
Punica granatum	4.46 ± 0.66	1.0 ± 0.12**	5.23 ± 1.33	4.85 ± 1.00
Trigonella foenum-graecum	46.72 ± 0.12	22.45 ± 1.20***	45.92 ± 0.50**	27.45 ± 0.12
AGI(A): α-glucosidase inhibition (Aqueous Extract); AGI(M): α-glucosidase inhibition (Methanolic Extract); AAI(A): α-amylase inhibition (Aqueous Extract); AAI(M): α- amylase inhibition (Methanolic Extract).The activity of each spice was tested using 100 ml aqueous or methanol extract, corresponding to 1 mg dry spice. Values are mean ± SEM and P *<0.05; **<0.01; ***<0.001				

Inhibitory effect of both *Amomum subulatum* and *Elettaria cardamomum* for both the enzymes i.e α-glucosidase and α –amylase in aqueous phase was higher for seed extracts as compared to fruit extract. Except *Papaver somniferum* all seeds showed prominent inhibition in aqueous phase as compared to methanolic form. Seeds of *Brassica juncea* were equally inhibitory for α -amylase in both aqueous and methanolic phases while *Myristica fragrans* and *Trigonella foenum-graecum* were nearly equal for both α-glucosidase and α –amylase inhibition in aqueous forms. α-glucosidase inhibition of methanolic extract of *Trigonella foenum-graecum* was nearly half as that compared to aqueous extract.

Total phenolic content was higher in aqueous extract of all studied seed spices and it exceeded from methanolic extract by 2.5 to 17.02%. The highest difference in content was observed in *Brassica juncea* (17.02%) and *Punica granatum* (11.45%) while the phenolic content only differed by 2.5% in *Trigonella foenum-graecum*.

Highest total phenolic content in aqueous extract was obtained in *Myristica fragrans* (324mg GAE/g) and nearly equal in *Punica granatum* (323mg GAE/g) followed by *Amomum subulatum* (313mg GAE/g) while in methanolic extract the phenolic content was 301mg GAE/g, 295mg GAE/g and 286mg GAE/g in *Myristica fragrans, Amomum subulatum* and *Punica granatum* respectively (Figure 1).
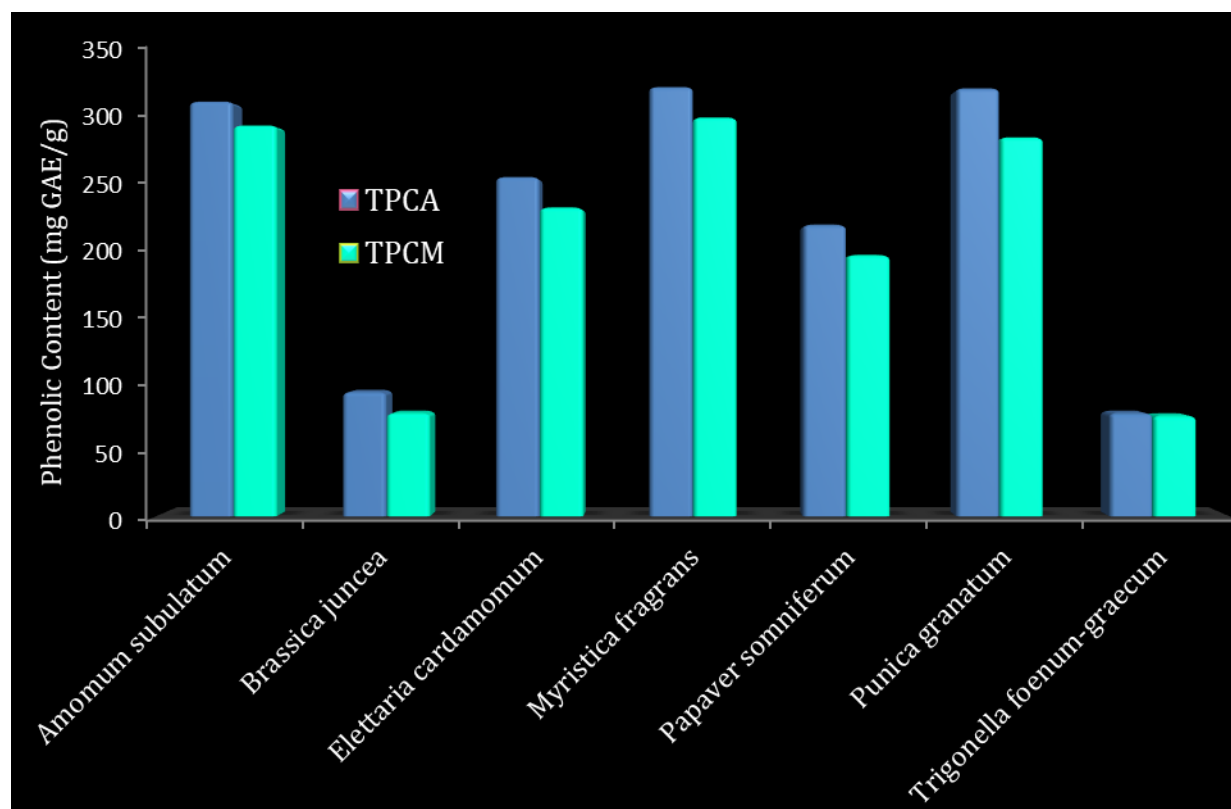
Fig 1: Total phenolic content in aqueous (TPCA) and methanolic (TPCM) extract of seed species used as spices

Highest DPPHA was obtained for *Myristica fragrans* (89.11%), *Punica granatum* (72.66%) and *Papaver somniferum* (69.52%) and DPPHM for *Myristica fragrans* (91.68%), *Papaver somniferum* (77.92%) and *Amomum subulatum*(67.74%). Marked efficacy to quench DPPH in both the extracts was observed in all seeds except *Brassica juncea* and *Trigonella foenum-graecum*. *Amomum subulatum* showed nearly equal DPPH scavenging efficacy in both the extracts. All the seeds exhibited lower SOA scavenging activity as compared to DPPH except in *Punica granatum* and

Trigonella foenum-graecum where the SOAA and SOAM significantly raised upto 91.63% and 69.64% in *Punica granatum* and 59.4% and 42.77% in *Trigonella foenum-graecum* respectively. SOAA and SOAM values were nearly equal for *Papaver somniferum*. Hydroxyl radical scavenging efficacy showed intermediate lineage between DPPH and SOA. Highest values of HRA was observed in *Papaver somniferum* (79.01%) followed by *Punica granatum* (69.64%) and *Elettaria cardamomum* (67.41%) while *Myristica fragrans* (79.93%), *Punica granatum* (65.66%) and *Amomum*

subulatum (52.19%) for HRM (Table 2). *Amomum subulatum* and *Myristica fragrans* had better radical scavenging efficacy in methanolic extracts as compared to aqueous phase while *Punica granatum* had comparatively higher scavenging efficacy for all the three studied free radicals in both the extracts.

Table 2: Radical scavenging activity of Seed spices in aqueous and methanolic extracts

Spices	% Inhibition					
	DPPHA	DPPHM	SOAA	SOAM	HRA	HRM
Amomum subulatum	67.13 ± 1.00	67.74 ± 0.33*	48.33 ± 0.86**	53.16 ± 0.66	48.49 ± 1.00***	52.19 ± 0.33
Brassica juncea	36.99 ± 0.33	42.03 ± 0.50*	40.6 ± 0.33	36.13 ± 1.00	34.91 ± 1.00	33.93 ± 0.50
Elettaria cardamomum	64.86 ± 0.86**	60.05 ± 0.66*	57.72 ± 0.50*	38.1 ± 0.33*	67.41 ± 0.50	43.2 ± 1.20**
Myristica fragrans	89.11 ± 0.33*	91.68 ± 0.50	29.4 ± 1.20	39.1 ± 0.50*	41.47 ± 1.20	79.93 ± 0.33
Papaver somniferum	69.52 ± 0.50	77.92 ± 0.33	45.88 ± 0.33	45.42 ± 0.86**	75.01 ± 0.66	47.5 ± 0.50
Punica granatum	72.66 ± 0.66**	66.43 ± 1.00*	91.63 ± 1.00**	69.64 ± 0.86	69.0 ± 0.33**	65.66 ± 0.33*
Trigonella foenum-graecum	48.0 ± 0.33	35.65 ± 0.86	59.4 ± 0.86	42.77 ± 1.00	26.26 ± 0.50	17.52 ± 0.66
Radical scavenger activity of spice extracts towards DPPH, superoxide anion (SOA) and hydroxyl radicals (HRA) in aqueous (A) and methanolic (M) extract. Values are mean ± SEM and *P* *<0.05; **<0.01; ***<0.001						

Comparative study of aqueous seed extract for their scavenging efficacy reveals that *Brassica juncea* showed poor quenching for either free radicals while *Elettaria cardamomum* and *Punica granatum* had ample efficacy for DPPH, SOA and HR. *Punica granatum* quenched SOA up to 91.63%. *Amomum subulatum* and *Myristica fragrans* had significant quenching efficacy for DPPH but relatively poor values for both SOA and HR. The antioxidant capacity of studied spices may be due to phenolic diterpenes [27].

4. Conclusion
Myristica fragrans, Punica granatum and *Elettaria cardamomum* had high phenolic content. Among these three spices *Myristica fragrans* and *Punica granatum* along with *Papaver somniferum* showed a significant free radical scavenging activity. *Myristica fragrans, Papaver somniferum* and *Trigonella foenum-graecum* had maximum inhibition for both alpha amylase and alpha glucosidase. Therefore, it can be inferred that *Myristica fragrans* and *Papaver somniferum* can be used for both in regulating diabetes mellitus as well as its associated pathologies whereas *Trigonella foenum-graecum* had higher inhibition values for alpha carbohydrate hydrolyzing enzymes but contrary it has comparatively low efficacy to quench free radicals due to which it can suppress the post prandial glycemic hikes but will not be able to manage the associated pathologies. Therefore, if *Trigonella foenum-graecum* is formulated with *Punica granatum* or *Papaver somniferum* with higher antioxidant spices then it will lead to better management of DM II.

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