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Anti- Hyperglycemic Activity and Free Radical Scavenging Activity of Some Apiaceae Spices

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Abstract: The present study encompasses the evaluation of eight fruit spices i.e Anethum graveolens, Apium graveolens, Carum carvi, Coriandrum sativum, Cuminum cyminum, Foeniculum vulgare, Pimpinella anisum and Trachyspermum ammi of Apiaceae for their anti-hyperglycemic potential and antioxidant activity. Anti-hyperglycemic activity was evaluated through inhibition of alpha glucosidase and alpha amylase while antioxidant activity was inferred through their free radical scavenging activities viz. DPPH, SOA and HRA and were correlated to their phenolic content. Pimpinella anisum and Trachyspermum ammi showed significant efficacy to regulate the post prandial hyperglycemia indicating their futuristic role in the management of diabetes mellitus II.
Keywords: α- amylase, α- glucosidase Apiaceae, Diabetes mellitus, DPPH.

I. INTRODUCTION

Apiaceae or older Umbelliferae includes are aromatic plants grown primarily for their flavoring essence of foods, liquors and confectionery. Higher number of its plants are deployed as spices. The characteristic aroma and flavor of members in this family are associated with the specialized essential oil or oils in the edible portions particularly in fruit or seed or other parts. Anatomically nearly all Apiaceae members contains a well-developed secretory system viz. phloem in the stem and leaves, schizogeneus cavities in the root and clearly-delimited tissue i.e vittae in the fruit. These structures deposit essential oils, which in turn provides specific odor and flavor [1].

Nutraceuticals can be designated as food with pharmacological properties and hence add therapeutic values when ingested. They have already been proved as stimulant, carminative, pectoral and with palliative properties. The secondary metabolite constituents of the plants defines it therapeutic values and its application in different maladies [2-4]. Some of the important secondary metabolite includes polyphenolic compounds, polyacetylenes and terpenoids [5].

Diabetes mellitus II has hit many lives. Its persistent life span and associated pathologies makes it a silent killer. In diabetic patients the glycemic trauma is exploded through post prandial hyperglycemia which lasts up to three to four hours. This elevated blood sugar is a root cause of many anomalies [6].

The ability of a drug or diet to delay the production or absorption of glucose by inhibiting carbohydrate hydrolyzing enzymes i.e α-amylase and α-glucosidase is one of the most significant therapeutic approaches to manage elevated postprandial hyperglycemia [7]. At present, the use of insulin secretagogues and sensitizers constitute the predominant line of therapy, however, the use of carbohydrate digesting enzyme inhibitors play a vital role in controlling hyperglycemia by reducing the intestinal absorption of glucose [8]. The diabetic profile gets worst in absentia of endogenous or exogenous antioxidants. Exogenous antioxidant property of plants is generally related to the presence of phenolic compounds. Nutritionally, these compounds are responsible for increasing the shelf life of foods as well as slowing the lipid, protein and enzymatic oxidation, as well as for providing protection against development of cancers, cardiovascular and liver diseases, diabetes, osteoporosis and neurodegenerative diseases in humans [9- 12].

The anti-hyperglycemic and antioxidant activity of apiaceae members are priorly studied in many plants as *Anethum graveolens*[13-16], *Angelica archangelica* [17], *Apium graveolens* [18-20], *Carum carvi* [21-24], *Coriandrum sativum* [25-28], *Cuminum cyminum* [29-32], *Daucus carota* [33,34], *Foeniculum vulgare* [35-38], *Levisticum officinale* [39], *Pimpinella anisum* [40-42] etc.

Phytochemical screening of spices and vegetables which are usually used in diet shows that they possess bioactive constituents of pharmaceutical importance. Therapeutic activity is especially important in prevention and treatment of modern diseases which are directly related to oxidative stress. Present study was designed to evaluate anti-hyperglycemic potential of eight fruit spices from Apiaceae i.e *Anethum graveolens, Apium graveolens, Carum carvi, Coriandrum sativum, Cuminum cyminum, Foeniculum vulgare, Pimpinella anisum* and *Trachyspermum ammi* through α- amylase and α- glucosidase inhibition and free radical scavenging

activity. The study also included the determination of total phenolic content so that the scavenging property and anti-hyperglycemic can be co-related to it.

II. MATERIAL AND METHODS

2.1 Preparation of Extract

Dried fruit powder of targeted Apiaceae spices were extracted with both water and methanol. For water extraction, 1 g of dried //10 g fresh material fruit material was minced in a small water volume and was subsequently diluted at 200 ml and boiled until the volume was condensed to 100 ml, followed by stirring for 24 h in the dark whereas for 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[43]. In all experiments, the activity of each spice was tested using 100 ml aqueous or methanol extract.

2.2 Anti- hyperglycemic Evaluation

Alpha-amylase inhibition assay was carried out spectro-photometrically by evaluating absorbance of the studied mixture at 540 nm**.** The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the spice fruit 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 experimental tests. The reaction was terminated with the addition of 400 µl DNS reagent and was further placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was read at 540 nm. The control samples were prepared without any spice extract.

The % inhibition was calculated as formulated by Jung *et al*., 2006[44].

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

For Alpha-glucosidase inhibition assay yeast alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and was used as the enzyme extract while P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Spice extracts were used in the concentration ranging from 20-100 µg/ml and 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. All the chemicals were purchased from Sigma. As similar to alpha-amylase inhibition assay the % inhibition of alpha-glucosidase was also calculated according to the formula provided by Jung *et al*., 2006[44] .

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

The IC 50 values for both inhibitor enzymes was determined through 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. For statistical analysis, all tests were performed in triplicate.

2.3 Determination of Total Phenol

Total soluble phenolics in the extracts were determined with Slinkard and Singleton, 1977[45] method using Folin-Ciocalteu reagent and gallic acid as a standard phenolic compound. 1 ml of extract solution was mixed with 45 ml distilled water and one ml of Folin-Ciocalteu reagent and were mixed thoroughly. After 3 min, 3 ml of Na_2CO_3 (2%) was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm and the concentration was determined as microgram of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph.

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

2.4 Free Radical Scavenging Activity

Free radical scavenging activity was estimated through three parameters i.e. 2,2-diphenyl-1-picrylhydrazyl(DPPH), superoxide and hydroxyl radicals. Reduction of DPPH radical was evaluated by Cervato et al., 2000[46] method. 3 ml of 60mM DPPH in ethanol was added to 100ml of each spice extract, and absorbance was registered at 517 nm (Abs 517 extract) and the same was also recorded for control tests which were devoid of spice extract. The inhibition percentage was calculated as:

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

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 observed by nitroblue tetrazolium (3 mM) and read spectro-photometrically at 560 nm. Superoxide radical scavenger activity of 100ml each spice extract was measured as their ability to inhibit this reaction as described by Cervato et al., 2000[46] with respect to control samples (distilled water or methanol) and was calculated as:

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

Hydroxyl radical was generated by incubating reaction mixture containing 100mM $FeCl_3$, 100mM ascorbate, 1 mM hydrogen peroxide, 2.8 mM deoxyribose in phosphate buffer 20 mM, pH 7.4 at 37˚C for 60 mins and their concentration was estimated using the thiobarbituric acid (TBA) method [46] for both test and control samples. Percent inhibition was calculated as:

$$\text{Percentage inhibition} = \frac{(\text{Abs}_{532}\ \text{control} - \text{Abs}_{532}\ \text{extract}) * 100}{\text{Abs}_{532}\ \text{control}}$$

III. RESULT AND DISCUSSION

Highest inhibition for both α-glucosidase and α-amylase in aqueous extract was projected by *Carum carvi* i.e 21.33 % and 21 % respectively followed by *Apium graveolens* (14.6 %) and *Pimpinella anisum* (14.44 %) for α-glucosidase and *Anethum graveolens* (13.87 %) and *Pimpinella anisum* (13.5 %) for α-amylase. *Anethum graveolens* had better values for α-amylase inhibition (13.87%) in aqueous extract while the values for α-glucosidase (9.08%) was comparatively low than other spices. Contrary *Apium graveolens* has high inhibition efficacy for α-glucosidase (14.6%) but low values for α-amylase (12.67%) in aqueous extract. Low values for α-glucosidase and α-amylase inhibition in both aqueous and methanolic extract was marked for *Coriandrum sativum* and *Trachyspermum ammi* (Table 1).

Highest total phenolic content (TPC) was observed in aqueous extract of *Pimpinella anisum* (263 mg GAE/g) followed by its own methanolic extract (251 mg GAE/g). TPC of *Trachyspermum ammi, Apium graveolens* and *Anethum graveolens* was higher in methanolic phase as compared to that of aqueous extract. *Coriandrum sativum, Foeniculum vulgare* and *Anethum graveolens* had comparatively very low values of TPC in both extracts (Figure 1).

DPPH or 1,1-diphenyl-2-picrylhydrazine radical scavenging activity refers to trapping of free radicals in any system and therefore marks the antioxidant property . Generally, it is variably displayed in different solvents. In our present study the DPPHA was higher than DPPHM for *Anethum graveolens, Foeniculum vulgare* and *Pimpinella anisum* while nearly equal DPPHA and DPPHM was seen in *Carum carvi* and *Coriandrum sativum.* Highest DPPH was shown by *Pimpinella anisum*(73.91%) followed by *Trachyspermum ammi* (48.46%) , *Apium graveolens* (25.2%) and *Cuminum cyminum* (25.02%) with similar lineage in methanolic extract. Slightly opposite lineage was observed for both aqueous and methanolic extracts for superoxide quenching efficacy where *Pimpinella anisum* values were higher than *Trachyspermum ammi* while the values of *Apium graveolens* were at par to DPPH scavenging efficacy. Hydroxyl quenching was higher for *Pimpinella anisum* (63.06%), *Trachyspermum ammi* (41.8%) and *Carum carvi* (26.15%) in aqueous extract while *Trachyspermum ammi* (67.31%), *Pimpinella anisum* (61.57%) and *Apium graveolens* (39.73%) in methanolic extract (Table 2).

In present study, spice extracts also exhibited appreciable anti-glycant activities in addition to their anti-oxidant properties which were nearly akin to prior studies [40, 41]. *Pimpinella anisum* had higher phenolic content. Its higher anti-hyperglycemic activity as well as higher free radical scavenging activity for all the three parameters studied i.e DPPH, SO and HRO can be correlated to its higher phenolic content and therefore further can be investigated for other parameters to be therapeutically used for the management of diabetes mellitus II. *Trachyspermum ammi* also had promising values for radical scavenging activities and possess significant phenolic content but as compared to other studied spices its value for the inhibition of both the enzymes were not at par. Methanol extraction has been shown to increases the recovery of hydrolysable tannins; however, this class of phenolic compounds did not contribute to anti-oxidant action measured by the DPPH method [47]. This could be one of the causes of the lack of correlation between DPPH radical inhibition and the phenol contents of methanol extracts. *Carum carvi* revealed significant anti-hyperglycemic activity but had poor values for phenolic content and free radical scavenging activities.

IV. CONCLUSION

Studies of antihyperglycemic activity and free radical scavenging activity reveals *Pimpinella anisum* and *Trachyspermum ammi* can be deployed to manage post- prandial hyperglycemia in diabetic patients. These spices also possess high antioxidant potential therefore will assist in reducing the associated pathologies of DMII.

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Table 1 : α-glucosidase and α -amylase inhibitory activity of Apiaceae spices

Spice	AGI(A) (%)	AGI(M) (%)	AAI(A) (%)	AAI(M) (%)
Anethum graveolens	9.08 ± 0.66	6.32 ± 1.33	13.87 ± 0.86**	9.0 ± 1.33***
Apium graveolens	14.6 ± 1.00	7.77 ± 0.38**	12.67 ± 0.86	8.75 ± 0.86

Carum carvi	21.33 ± 0.50	10.5 ± 2.15*	21.0 ± 0.12	16.87 ± 1.15
Coriandrum sativum	1.06 ± 0.50	0.84 ± 0.12	2.34 ± 0.10	1.0 ± 0.50
Cuminum cyminum	14.2 ± 1.75	9.56 ± 1.33	12.33 ± 0.66***	9.5 ± 0.66
Foeniculum vulgare	4.34 ± 1.33**	1.32 ± 0.66	5.6 ± 0.12	4.5 ± 0.33
Pimpinella anisum	14.44 ± 0.12***	16.95 ± 0.86	13.5 ± 2.15	15.0 ± 1.00**
Trachyspermum ammi	4.0 ± 0.50	2.85 ± 0.66**	2.82 ± 0.10	1.0 ± 0.33**

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

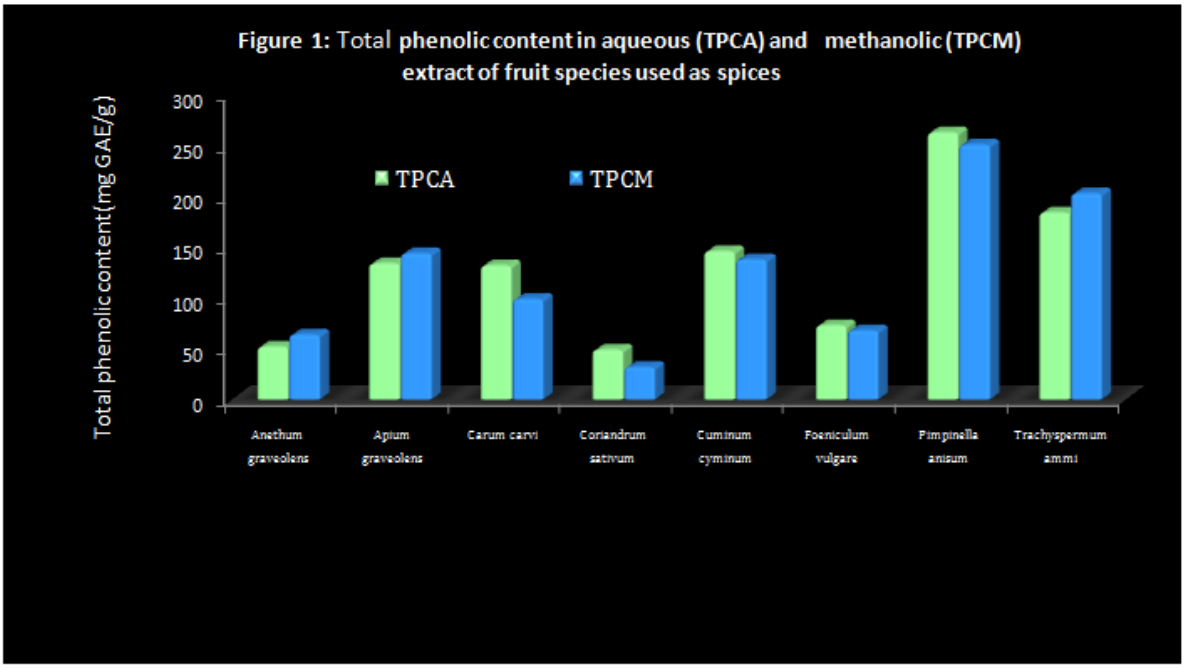

Table 2 : Free Radical scavenging activity of apiaceae spices in aqueous and methanolic extracts

Spices	**% Inhibition**					
	DPPHA	**DPPHM**	**SOAA**	**SOAM**	**HRA**	**HRM**
Anethum graveolens	19.89 ± 1.00	12.8 ± 0.12***	10.71 ±1.04	11.04 ± 0.38	13.77 ± 0.38	15.73 ± 0.38
Apium graveolens	25.2 ± 0.38**	39.56 ± 0.66	35.10 ± 0.66	37.58 ± 0.12*	25.93 ± 0.66	39.73 ± 1.50*
Carum carvi	24.14 ± 0.33**	23.52 ± 0.66	15.48 ± 1.00**	21.32 ±1.04	26.15 ± 1.00	23.54 ±1.04*
Coriandrum sativum	7.2 ± 0.58***	7.28 ±1.04	5.96 ± 1.60*	7.08 ± 0.38**	6.19 ± 0.33	6.68 ± 0.86***
Cuminum cyminum	25.02 ± 0.38	30.55 ± 0.86	25.20 ± 0.38	30.29 ± 0.50	19.33± 0.66	31.64 ± 0.12
Foeniculum vulgare	14.14 ±1.04***	17.43 ± 0.33**	13.75 ± 1.50	14.37 ± 1.60*	14.64 ± 0.58	16.22 ± 1.60
Pimpinella anisum	73.91 ± 1.50***	63.23 ± 0.38	53.74 ±1.04**	47.70 ± 0.33	63.06 ± 0.38	61.57 ± 0.50
Trachyspermum ammi	48.46 ± 0.58	60.53 ± 0.86	60.11 ± 1.50**	51.65 ±1.04*	41.8 ± 1.00	67.31 ± 0.38

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