IN-VITRO ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE INHIBITION ACTIVITY OF UMBELLIFERONE AND BETA-IONONE ISOLATED FROM CORIANDRUM SATIVUM LINN.

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ABSTRACT

Aims: The objective of the present study was to provide an In-Vitro evidence for the potential inhibitory activity of Ethanolic extract, Umbelliferone and β-Ionone on α-amylase and α-glucosidase enzymes. Materials and Methods: Different concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) of Ethanolic extract, Umbelliferone and Beta-Ionone were subjected to α-amylase and α-glucosidase inhibitory assay. The absorbance was measured at 540 and 405 nm using multiplate reader and the percentage of α-amylase and α-glucosidase inhibitory activity and IC₅₀ values of Ethanolic extract, Umbelliferone and Beta-Ionone were calculated. Result and Discussion: Ethanolic extract of Coriandrum sativum Linn. has shown highest α–amylase and α-glucosidase inhibitory potential with IC₅₀ values of 0.294, 0.211 which was comparable with acarbose (0.125 and 0.93 mg/ml). Whereas, Umbelliferone and β-Ionone have shown lesser activity. Conclusion: The results of the present study indicate that, Ethanolic extract of Coriandrum sativum Linn, rich in triterpenoids and phenolics is effective α–amylase and α–glucosidase inhibitors, which may be helpful to reduce the postprandial glucose levels. Hence, it may useful in the management of T2D.

KEYWORDS: Coriandrum sativum Linn, Umbelliferone, β-Ionone, α-amylase and α-glucosidase.
INTRODUCTION

Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine may contain hundreds of different phytochemicals. It is difficult to isolate the benefits of individual phytochemicals. Research has shown that phytochemicals work together to promote their health benefits.\(^1\) Coriandrum sativum Linn. of family Umbelliferae is an annual herbaceous plant and is cultivated all over the world for its use not only in the indigenous medicines but also as one of the ingredients of all spicy foods especially of Pakistan and India. The plant is a rich source of essential oil and many of the researchers have almost concentrated on extraction, composition, biological activities, and use against various diseases of its crude extracts and essential oils.\(^2\) Coriandrum sativum is an important medicinal plant used against a number of diseases. The plant is a rich source of essential oil and many of the researchers have almost concentrated on extraction, composition, biological activities, and use against various diseases of its crude extracts and essential oils. Various parts of this plant such as seed, leaves, flower and fruit, possess Diuretic, Antioxidant activity, Antidiabetic, Anti-convulsant activity, Sedative Hypnotic Activity, Anti-microbial activity, Antimutagenic and Anthelmintic activity. Many of today’s synthetic drugs originate from the plant kingdom. Herbal drugs are proved as effective as synthetic drugs with lesser side effects Coriander is one of a few savory plants, a potential source of phenolic compounds having biological activities.\(^3\)

Diabetes mellitus is an endocrine disorder characterized by hyperglycemia is associated with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. It is a progressive metabolic disorder of glucose metabolism that eventually leads to microvascular and macrovascular changes causing secondary complications that are difficult to manage. Type 1 diabetes results from inadequate synthesis of insulin by \(\beta\)-cells of the pancreas, while type II diabetes is characterized primarily by insulin resistance or \(\beta\)-cell dysfunction.\(^4\) Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules. On the other hand, mammalian \(\alpha\)-glucosidase in the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human die. Inhibitors \(\alpha\) - amylase and \(\alpha\)-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion. An effective means of lowering the levels of postprandial hyperglycemia have
been offered by α-amylase and α-glucosidase inhibitors. Several inhibitors of α-amylase and α-glucosidase has been isolated from medicinal plants to serve as an alternative drug with increased potency and lesser adverse effects than existing synthetic drugs. Alpha amylase and Alpha-glucosidase inhibitors are drug-design targets in the development of compounds for the treatment of diabetes, obesity and hyperlipaemia. All the existing therapies show ever have limited efficacy, limited tolerability and/or significant mechanism based side effects. Despite the existing pharmacotherapy, it is still difficult to attain adequate glycemic control amongst many diabetic patients due to the progressive decline in β-cell function. Though several studies showed the antidiabetic potential of Umbelliferone and β-Ionone. No previous report has been given on the mechanism by which it exerts this effect. We have also published an article on the In Vitro Cytotoxicity activity of Phytochemicals isolated from Coriandrum sativum Linn in selected cell lines. As a follow up to this, the aim of this study was to evaluate the effect of Umbelliferone and β-Ionone on the activities of α-amylase and α-glucosidase of inhibition of these enzymes.

MATERIALS AND METHODS

Chemicals and Reagents
Alpha-amylase from Aspergillus oryzae, α-glucosidase from Saccharomyces cerevisiae and paranitrophenyl-glucopyranoside were products of Noor Enzymes Pvt Ltd, West Bengal, India while starch soluble (extra pure) was obtained from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. p-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate (Na₂CO₃), sodiumdihydrogen phosphate, di-sodium hydrogen phosphate were purchased from Hi-Media, India. Mumbai Other chemicals and reagents were of analytical grade and water used was glass distilled.

Extraction and Isolation
The shade dried Aerial part of Coriandrum sativum Linn (2 kg) was extracted exhaustively with methanol (2.5x2 L) at the 50°C for 48 h. After extraction total filtrate was concentrated by distilling off the solvent and evaporated to dryness (304 gm). It was made aqueous with distilled water in a separating funnel and further fractionated with series of organic solvents to obtain the fractions, viz. n- hexane fraction, chloroform fraction and ethyl acetate fraction. The resulting ethyl acetate extract was subjected silica gel column (60–120 mesh) using as a n-hexane/CHCl₃/MeOH as eluent. At uniform interval, the eluents (each of five ml) were collected and the progress of separation was monitored by thin layer chromatography (TLC)
(silica gel G 60 F254 TLC plates of E. Merck, layer thickness 0.2mm) using solvent system chloroform: methanol (90:10) and iodine vapour as detecting agent. Fractions eluted with chloroform: methanol (95:5) and 1-5 fraction of chloroform: methanol (90:10), which showed single spot on TLC (Rf value 0.35) afforded the compound -I .Fractions (12.2 g) were combined and rechromatographed on a silica gel column (CHCl₃/MeOH, 95:5) which yield a single fraction. The fraction was dried by vacuum distillation and crystallization using ethylacetate yielded 2.820 g of yellowish-white crystalline solid, m.p 228- 234°C ,which has a slight solubility in hot water, but high solubility in ethanol\(^6\) Fraction 6-10 were combined and rechromatographed on a silica gel column (n-hexane/ethylacetate 90:10) showed single spot on TLC (Rf value0.92) afforded the compound -II. The fraction was dried by distilling off the solvent and evaporated to dryness and crystallization using ethylacetate yielded 2.142 g of light yellow liquid, b.p 126- 128°C , which has insoluble in water, but high solubility in ethanol. The compounds Umbelliferone and β-Ionone were isolated from Coriandrum sativum Linn and were characterized by UV, IR, \(^1\)H NMR, \(^13\)C NMR and HMBC, as reported the authors earlier.\(^6\)

![Figure-1: Chemical structure of Umbelliferone.](image)

![Figure-2: Chemical structure of β-Ionone](image)

**IN-VITRO ASSAY**

**Alpha-amylase inhibitory activity**

Alpha-amylase inhibitory activity of Ethanolic extract, Umbelliferone and Beta-Ionone were carried out according to the standard method with minor modification.\(^7\) In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl α-amylase (2µ/ml), and 20 µl of varying concentrations of Ethanolic extract, Umbelliferone and Beta-Ionone (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) were preincubated at 37°C for 20 min. Then, the 20 µl of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and
incubated further at 37°C for 30 min; 100 µl of the DNS colour reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate Reader (Multiskta thermo scientific, version 1.00.40). Acarbose at various concentrations (0.1–0.5 mg/ml) was used as a standard. Without test (Ethanolic extract, Umbelliferone and Beta-Ionone) substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

\[
\text{%Inhibition} = \left( \frac{Ac - As}{Ac} \right) \times 100
\]

Where,

\( As \) is the absorbance in the presence of test substance and \( Ac \) is the absorbance of control.

**Alpha-glucosidase inhibitory activity**

Alpha-glucosidase inhibitory activity of Ethanolic extract, Umbelliferone and Beta-Ionone were carried out according to the standard method with minor modification.\(^8\) In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl α-glucosidase (1 U/ml), and 20 µl of varying concentrations of Ethanolic extract, Umbelliferone and Beta-Ionone (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) were preincubated at 37°C for 15 min. Then, 20 µl p-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 µl Na₂CO₃ (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. Without test substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

\[
\text{%Inhibition} = \left( \frac{Ac - As}{Ac} \right) \times 100
\]

Where,

\( As \) is the absorbance in the presence of test substance and \( Ac \) is the absorbance of control.
Statistical Analysis
All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey’s multiple comparisons test for significant differences. Values were considered significant at p<0.05.

Table 1: α-amylase and α-glucosidase inhibitory effects of Ethanolic extract, Umbelliferone and Beta-Ionone, and Acarbose.

<table>
<thead>
<tr>
<th>Substance</th>
<th>IC_{50} values of α-amylase (mg/ml)</th>
<th>IC_{50} values of α-glucosidase (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acarbose</td>
<td>0.125</td>
<td>0.093</td>
</tr>
<tr>
<td>EtOH extract</td>
<td>0.294</td>
<td>0.211</td>
</tr>
<tr>
<td>Umbelliferone</td>
<td>0.577</td>
<td>0.547</td>
</tr>
<tr>
<td>Beta-Ionone</td>
<td>1.372</td>
<td>1.240</td>
</tr>
</tbody>
</table>

Figure 3: α-amylase inhibition of EtOH extract, Umbelliferone and β-Ionone.

Figure 4: α-amylase inhibition of EtOH extract, Umbelliferone and β-Ionone.

RESULT AND DISCUSSION
In the present study, Ethanolic extract, Umbelliferone and Beta-Ionone were evaluated for their inhibitory effect on α-amylase and α-glucosidase enzymes by In-Vitro method. The Ethanolic extract, Umbelliferone and Beta-Ionone (at a concentration of 0.5 mg/ml) exhibited...
60.24, 45.05 and 329.22. α-amylase inhibitory activity [Figure 3] and 62.22, 47.25 and 26.34. α-glucosidase inhibitory activity [Figure 4], respectively. Acarbose was used as a standard reference drug, which showed α-amylase inhibitory activity with an IC$_{50}$ value of 0.108 mg/ml and α-glucosidase inhibitory activity with an IC$_{50}$ value of 0.083 mg/ml. Among all, Ethanolic extract has shown best enzyme inhibitory activity with an IC$_{50}$ value 0.294 and 0.211 (α-amylase and α-glucosidase) [Table 1] which was comparable with that of acarbose.

The use of herbal drugs as complementary approaches in existing medications for the treatment of diabetes and its complications is growing worldwide and many plants in different countries are known to have antidiabetic effects.\textsuperscript{[9]} The ancient Indian literature reports more than 800 plants with antidiabetic properties while ethnopharmacological surveys indicate that more than 1200 plants can be used for hypoglycemic activity.\textsuperscript{[10]} Mainly two carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) are responsible for postprandial hyperglycemia. 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.\textsuperscript{[11,12]} 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. There was no information available in the literature about the In-Vitro (α-amylase and α-glucosidase inhibitory activity) antidiabetic studyof Coriandrum sativum Linn. Hence, the present study aimed to evaluate α-amylase and α-glucosidase inhibitory activitie of Ethanolic, extract, Umbelliferone and Beta-Ionone.

Many bioactive compounds from different plants have been reported to have hypoglycemic effect, in that mostly phenolics and triterpenoids such as oleanane, ursane, lupane, and flavonoids have a positive correlation as antidiabetic agents.\textsuperscript{[13,15]} The presence of triterpenoids and phenolics in Ethanolic extract might have attributed to the highest enzyme inhibition activity compared to Umbelliferone and Beta-Ionone.\textsuperscript{[16]} Hence, the triterpenoids of this plant may be responsible for enzyme inhibitory activity. Apart from that polyphenolic compounds were found in Ethanolic extract, may interact or inhibit specific positions in enzymes thereby reducing the potency of α-amylase and α-glucosidase.\textsuperscript{[17]} The presence of flavonoid compounds in Ethanolic extract may act against diabetes mellitus either through
their capacity to avoid glucose absorption or to improve glucose tolerance by competitive inhibition of sodium-dependent glucose transporter-1.\textsuperscript{18} Another possible mechanism followed by flavonoid compounds (luteolin, kaempferol, chrysin, and galangin) to control blood glucose levels is the inhibition of α-amylase and α-glucosidase activity in the intestine.\textsuperscript{19-20} Due to above reasons, Ethanolic extract showed comparable results with that of acarbose. With the help of result in correlation with previous reports it can be hypothesized that the significant enzyme inhibitory activity of Ethanolic extract may interfere or delay the absorption of dietary carbohydrates as well as disaccharides in the small intestine, leading to the suppression of meal-induced increase of plasma glucose. Hence, it may useful in the management of T2D. The Ethanolic extract showed significant inhibition activity, so further the compound isolation and characterization which is responsible for inhibiting activity, has to be done for the usage of anti diabetic agent. However the results of this study provide the information about the significant anti-diabetic properties of Ethanolic extract, Umbelliferone and Beta-Ionone by in vitro methods, these effects need to be confirmed by employing different In Vivo models and clinical trials for their effective utilization as therapeutic agents.

CONCLUSION
The results of the present study prove that the Ethanolic extract of Coriandrum sativum Linn is effective α-amylase and α-glucosidase inhibitors, which may helpful to reduce the postprandial glucose levels. However, the principle compounds responsible for the inhibitory action of α-amylase and α-glucosidase need to be further comprehensive chemical and pharmacological investigation should be carried out to isolate, purify and characterize the active compound and appropriate elucidation of its mechanism of action. This may be useful for the development of new antidiabetic agents from native plant resources. However the results of this study provide the information about the significant anti-diabetic properties of Ethanolic extract, Umbelliferone and Beta-Ionone by in vitro methods, these effects need to be confirmed by employing different in vitro methods and clinical trials for their effective utilization as therapeutic agents.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES


