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INHIBITORY EFFECTS OF GARDEN CRESS (*LEPIDIUM SATIVUM L.*) SEED COAT PHENOLICS ON α-AMYLASE, α-GLUCOSIDASE AND TRYPSIN

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Abstract: Garden cress (*Lepidium sativum*) seeds are well known traditionally for their medicinal and health beneficial properties such as hypoglycemic, hepatoprotective, nephroprotective and anti-carcinogenic etc. Diabetes mellitus, a metabolic disorder, is increasing in prevalence drastically worldwide. Dietary supplements from natural sources are a well-accepted phenomenon for prevention or management of diabetes mellitus. Hence, inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase by plant phenolics is an effective technique to lower postprandial glucose level. The present study was investigated to evaluate the inhibitory effect of garden cress seed coat phenolics on pancreatic α-amylase and intestinal α-glucosidase. It was observed that seed coat phenolics inhibited pancreatic α-amylase and intestinal α-glucosidase with IC_{50} values of 5.5 and 33.2 µg/ml respectively, suggesting strong inhibition towards α-amylase. Protease inhibitors are known to have anti-carcinogenic activities and hence, garden cress seed coat phenolics were also evaluated for inhibition of trypsin. Garden cress seed coat phenolics inhibited trypsin with IC_{50} value of 14.6 µg/ml. These results indicate the potential of Garden cress seed coat as a functional ingredient for development of specialty health food formulations.

Keywords: Garden cress seed coat, Phenolics, Pancreatic α-amylase, Intestinal α-glucosidase, Trypsin, inhibition, IC_{50}

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INTRODUCTION

Garden cress (*Lepidium sativum L.*) is native to Egypt and South west Asia. It is cultivated in India [1], North America and parts of Europe [2]. Garden cress (Gc) seed is a pseudo cereal and seeds are rich in proteins, fat, dietary fiber, vitamins and minerals [3]. It is also a rich source of omega-3-fatty acids [4]. Gc seeds possess varied medicinal properties like abortifacient, diuretic, expectorant, antibacterial, gastrointestinal, stimulant, hypoglycemic, gastroprotective, laxative and stomadic. Gc seed is reported to posses 28% of bran fraction (seed coat) and it contains about 13% protein, 6% fat, and is a rich source of dietary fiber (75%) [3]. Gc seeds are also a rich source of polyphenolic compounds [5, 6].

Diabetes mellitus (DM) is the most common endocrine disorder known and characterized by abnormally high blood glucose concentration due to relative or absolute deficiency of insulin or the resistance of the β-cells to the action of insulin. The dynamics of the diabetes epidemic are changing rapidly. According to the International Diabetes Federation, diabetes affects at least 285 million people worldwide, and that number is expected to reach 438 million by the year 2030[7]. The fast uptake of glucose in the intestine by the action of α-amylase and α-glucosidase helps in the breakdown of complex carbohydrates (starch and oligosaccharides) into simple sugars such as maltose and glucose is an important factor which results in postprandial hyperglycemia [8].

Many synthetic drugs are commonly used for treating DM either alone or in combination by inhibiting α-amylase and α-glucosidase activity and thus, reduce glucose absorption in the body. They prevent the rise in blood glucose efficiently, but the continuous use of these drugs is often associated with undesirable side effects, such as liver toxicity, renal tumors and adverse gastrointestinal symptoms [9, 10].

Therefore, there is an increasing need to have a safe natural source of α- amylase and α-glucosidase inhibitors. The food and pharmaceutical industries are continuously looking for newer natural sources of these carbohydrate hydrolyzing enzymes inhibitors. Protease inhibitors have long been considered to be anti-nutritional compounds, but recent studies suggested that certain plant protease inhibitors were effective at preventing or suppressing carcinogenic processes in a wide variety of *in vitro* and *in vivo* animal model systems [11].

Several findings have been reported on the inhibitors of carbohydrate-hydrolysing enzymes from plant source [8, 12, 13]. Although Gc seed possesses a good amount of seed coat with polyphenols content, but there are no reports on the enzyme inhibitory activities of Gc seed coat phenolics. Hence, the objective of the present study was to evaluate the inhibitory activity

of Gc seed coat phenolic extract against pancreatic α- amylase, intestinal α- glucosidase and also to investigate trypsin inhibitory activity for a potential anti-carcinogenic property.

MATERIALS:

Garden cress seeds were procured from local market, Dharawad, Karnataka, India. Gc seeds were cleaned, stored in closed container and refrigerated (6ºC±0.5) until further use.

Chemicals:

Pancreatic α-amylase from porcine pancreas (EC 3.2. 1.1), intestinal α-glucosidase (EC 3.2.1.20) from rat intestinal acetone powder, trypsin from bovine pancreas (EC 3.4.21.4), Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA) and *p*-nitrophenyl-α-D-glucopyranoside (PNPG),were purchased from Sigma Chemical Co. (St. Louis, MO).All other chemicals were of analytical grade.

METHODS:

Preparation of Gc Seed coat: Garden cress seeds were mixed thoroughly with 10% water (V/W), tempered for 15 minute at room temperature and were decorticated using plate mill (Model: Chowdhary & Co. Pvt. Lmtd., India). The decorticated seeds were subjected to air separation at the volumetric air flow rate of 25m^3/hr in a laboratory air classifier (Petkus, Germany) to obtain seed coat. Seed coat was collected and powdered in laboratory mill (IKA M 20 universal mill) to pass through 500 µm sieve. Gc seed coat powder was defatted using petroleum ether (60-80ºC) in Soxhlet apparatus for eight cycles. Defatted seed coat powder was again pulverized finely using laboratory mill (IKA M 20 universal mill) to pass through 250µm sieve and was used for polyphenol extraction.

Extraction of Gc seed coat polyphenols:

Polyphenols from Gc seed coat were extracted using 1% HCl-Methanol (100 ml/g) at 60^{0}C for 70 minutes (repeated three times) by refluxing [6]. Extract was pooled and concentrated using rotary flash evaporator and was used for analysis.

Determination of total polyphenol content (TPC) of Gc seed coat:

Spectrophotometric method[14] was used to determine the TPC of the Gc seed coat polyphenol extracts. Extract (50 µl) was treated with Folin–Ciocalteu's reagent (0.5ml, 1:10 diluted with water) and 7.5% sodium carbonate solution (1.5 ml), mixed thoroughly, and volume was made

up to 6 ml with water. The blue colour developed was read at 760 nm after 30 min. A standard curve of gallic acid was used to calculate the TPC of Gc seed coat extract. Results were expressed as mg of gallic acid equivalents (GAE)/ g of defatted Gc seed coat.

Enzyme Inhibitory activities:

Inhibition of pancreatic α- amylase: The procedure described by Bernfeld (1955) [15] was used with minor modifications to evaluate the inhibitory effect of Gc seed coat phenolics extract against pancreatic α-amylase. The amylase activity was determined using 1% soluble starch as a substrate which was prepared in 0.1 M sodium citrate buffer containing 1 mM $CaCl_2$, pH 6.9. Suitable aliquots of Gc seed coat phenolic extract and the enzyme solution (0.33 units; one unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per minute under assay conditions) were pre-incubated for 10 min at 37ºC. The residual amylase activity was measured by adding starch solution and incubating at 37ºC for 30 min. Dinitrosalicylic acid solution (1.0 ml) was added to stop the reaction and boiled for 5 min in a boiling water bath, and the total volume was made up to 10 ml with distilled water. The absorbance was measured at 540 nm and enzyme activity was quantified. Appropriate solvent controls were maintained.

Enzyme inhibition (%) = 100 X (Absorbance for enzyme standard – Absorbance for sample)/ Absorbance for enzyme standard

Inhibition of intestinal α-glucosidase: The inhibitory effect of Gc seed coat phenolics extract against intestinal α-glucosidase was assayed according to the method of Matsui *et al.* 2001[16]. The intestinal α-glucosidase activity was determined, using 2mM PNPG (p-nitrophenyl-α-D-glucopyranoside) as a substrate in 0.05 M sodium phosphate buffer, pH 6.8. The enzyme solution (0.02 units; one unit of enzyme activity is defined as the amount of enzyme required to release one micromole of para nitro phenol from PNPG per minute under assay conditions) and suitable aliquots of Gc seed coat phenolic extract were pre-incubated for 10 min at 37ºC. The residual enzyme activity was measured by adding 3mM PNPG solution and incubating at 37ºC for 30 min. The reaction was terminated by addition of 0.67M Na_2CO_3 solution. Enzyme activity was quantified by measuring the absorbance at 405 nm. Appropriate solvent controls were maintained.

Inhibition of trypsin: The inhibitory effect of Gc seed coat phenolics extract against trypsin was assayed according to the procedure described previously by Kakade *et al.* (1969)[17].The trypsin inhibitory activity of Gc seed coat phenolic extract was determined using chromogenic substrate BAPNA in 0.05 M Tris-HCl buffer containing 0.02 M $CaCl_2$. The enzyme solution and suitable aliquots of Gc seed coat phenolic compounds were pre-incubated for 10 min at 37ºC. The

residual enzyme activity was measured by adding BAPNA solution and incubating at 37ºC for 10 min. The reaction was terminated by addition of 30% acetic acid solution. Enzyme activity was quantified by measuring the absorbance at 405 nm. Appropriate solvent controls were maintained.

Determination of IC_{50}

IC_{50} is defined as the concentration of phenolics required to inhibit 50% of the enzyme activity.

To determine the IC_{50} values, the enzyme activities were determined in the presence of the Gc seed coat extract at various concentrations. IC_{50} values were obtained graphically by plotting least square regression curve.

Data analysis: All the data were analyzed using the Graphad Instat software. Each experiment was performed in triplicate, and the results were expressed as the mean values ± standard deviation. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by multiple comparison test (Tukey's test) at 5% level of significance. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION:

Hydrolysis of dietary starch is the major source of glucose in blood, with pancreatic α-amylase and intestinal α-glucosidase being the key enzymes involved in starch breakdown and intestinal absorption, respectively. A calcium metalloenzyme, pancreatic α-amylase catalyses the cleavage of α-D-(1-4) glycosidic linkages of starch, amylose, amylopectin, glycogen and various maltodextrins into shorter oligosaccharides. Other amylolytic enzymes also participate in the process of starch breakdown, but the contribution of α -amylase is a prerequisite for the initiation of this process. A second enzyme, intestinal α-glucosidase, which is also located in the brush-border surface membrane of intestinal cells, activates the final step of the digestive process. This exo type carbohydrase enzyme catalyses the hydrolysis of complex carbohydrates and disaccharides to release monosaccharides. Consequently, inhibitors of these hydrolytic enzymes suppress the influx of glucose from the intestinal tract to blood vessels resulting in a decrease in postprandial hyperglycemia [13].

Total polyphenol content of Gc seed coat was evaluated. It contained 9.2 mg GAE/g of defatted Gc seed coat. Gc seed coat phenolic extract was evaluated for inhibition of pancreatic α-amylase and intestinal α- glucosidase at different concentration of phenolics (4-17 µg/ml and 21-75 µg/ml respectively), to obtain the inhibition in the range of 20-80%. It was observed that after certain concentration of phenolics, inhibition of pancreatic α-amylase and α- glucosidase

showed a level of saturation i.e. inhibition above 80% (Figure 1 and 2). IC_{50} values for inhibition of these enzymes were determined. IC_{50} values of 5.5 µg/ml and 33.2 µg/ml for inhibition of pancreatic α-amylase and intestinal α-glucosidase (Table 1), respectively were obtained from the linear regression curves. These results revealed that Gc seed coat is more effective inhibitor of pancreatic α-amylase than that of intestinal α-glucosidase with significantly lower IC_{50} value. Shobhana *et al.* (2009) [18] also reported higher inhibition of pancreatic α- amylases than α-glucosidase by finger millet seed coat phenolics. Hence, it may be concluded that Gc seed coat phenolics are a potential source of pancreatic α-amylase inhibitor.

Garden cress seed coat phenolics were also evaluated for inhibition of trypsin at different concentration levels of 15-22 µg/ml. There are reports suggesting the anti-carcinogenic properties of protease inhibitors. Armstrong *et al.* (2003) [19] reported the chemoprotective activity of Bowman-Birk inhibitors against oral cancer. Gc seed coat phenolics inhibited trypsin in dose-dependent manner (Figure 3) and showed IC_{50} value of 14.6 µg/ml (Table 1). Many of the theories on mechanism of action of the anti-carcinogenic protease inhibitors are related to the fact that these agents prevent the release of the superoxide anion radical and hydrogen peroxide from polymorphonuclear leukocytes and other cell types stimulated with tumor-promoting agents [20] and other possible mechanism of their action is inhibition of matrix metalloproteinases and thus, prevent tumor progression [21].

CONCLUSION:

Garden cress seed coat phenolics were evaluated for inhibition of pancreatic α-amylase, intestinal α-glucosidase and trypsin. Gc seed coat phenolics showed inhibition against all these three enzymes and results indicated that it was most effective towards pancreatic α-amylase. The present study reveals that Gc seed coat phenolics with α-amylase and α-glucosidase inhibitory activity can be used as a potential hypoglycemic ingredient for the development of specialty functional foods. In addition trypsin inhibitory activity of Garden cress seed coat suggests a possibility of its anti-carcinogenic activity. Future studies are required to establish these efficacies of Garden cress seed coat phenolics by *in-vivo* animal model experiments.

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Table 1. IC_{50} values (µg/ml) for inhibition of enzymes by Garden cress seed coat phenolic extract

Enzyme	IC_{50} value (µg/ml)
Pancreatic α-amylase	5.50±0.42[a]
Intestinal α-glucosidase	33.25±1.50[c]
Trypsin	14.62±0.93[b]

Results are mean of three determinations ± SD. Values with different letters are significantly different at p≤0.05.

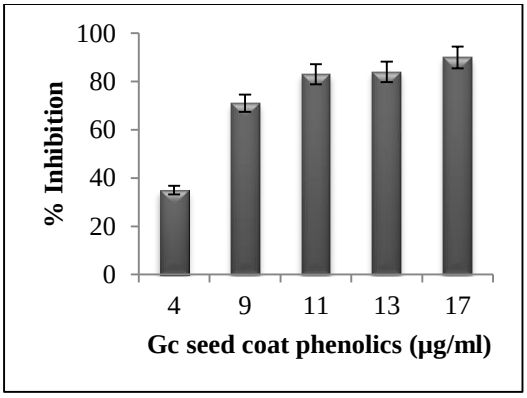

Figure 1. Inhibitory effect of Garden cress seed coat phenolic extract on the activity of pancreatic α-amylase

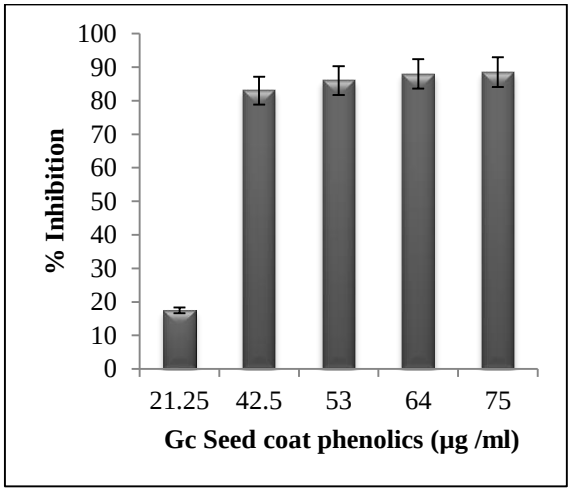

Figure 2.Inhibitory effect of Garden cress seed coat phenolic extract on the activity of intestinal α-glucosidase

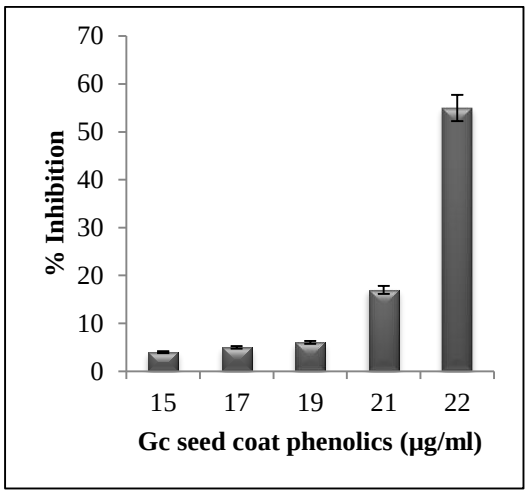

Figure 3. Inhibitory effect of Garden cress seed coat phenolic extract on the activity of trypsin

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