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Physicochemical Characterization and in Vitro Anti-Obesity Potential of *Anethum graveolens* (Dill) Seed Cake

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ABSTRACT

This study investigated the in vitro anti-obesity effects of *Anethum graveolens* L. (Apiaceae) dill seed cake (DSC). DSC is a valuable source of dietary fiber, proteins, lignans, and phenolic compounds, all of which contribute to managing dyslipidemia, metabolic disorders, and obesity. DSC, extracted as a byproduct after oil removal, was subjected to physicochemical evaluation and lignan profiling using HPLC. In addition, its potential was analyzed by determining the hydrolysis rate and enzymatic inhibition of α-amylase, α-glucosidase, and pancreatic lipase. The findings indicated high fiber content, moderate protein levels, and low amounts of moisture, ash, and fat, with minimal foreign particles. HPLC analysis identified lignan-like compounds such as carvone, perillyl alcohol, perillaldehyde, and cineole. The IC50 values for α-amylase, α-glucosidase, and pancreatic lipase inhibition were recorded at 60.18 ± 2.21, 456.42 ± 5.32, and 54.13 ± 2.25 µg/ml, respectively. These results indicate that DSC can help in the management of obesity and its associated metabolic concerns by limiting the intestinal absorption of glucose and lipids. Therefore, dill seed cake holds promise as a functional ingredient for nutraceutical applications in weight management.

Keywords: Obesity, Dill seed cake, *Anethum graveolens*, Lignans, Dietary fiber

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Introduction

Obesity has become a critical global health concern, primarily driven by an imbalance in energy consumption, which leads to various chronic conditions such as cardiovascular diseases and hormonal disturbances [1]. In recent years, complementary and alternative medicine has gained traction, particularly in India, where herbal remedies and dietary interventions are widely used to address obesity. The benefits of dietary antioxidants in preventing oxidative stress related to obesity have been well-documented. *Anethum graveolens*, commonly known as dill, belongs to the Umbelliferae family and is naturally found in the Mediterranean region, India, and Asia. Historically, dill has been employed in traditional medicine in India [2]. Dill seeds are rich in antioxidative phytochemicals like phenolic acids and lignans. The residue left after the extraction of dill seed oil, known as dill seed cake, is packed with dietary fiber, both soluble and insoluble. High-fiber foods play a significant role in managing type 2 diabetes, lowering cholesterol, and preventing obesity. Dill seed cake is typically used as animal feed, but its fiber and antioxidant content suggest it could offer additional health benefits.

Research has already demonstrated that dill juice exhibits significant antioxidant and cholesterol-lowering effects [3]. Studies also show that dill supplementation may improve lipid profiles and insulin sensitivity in those with metabolic syndrome [4]. Clinical trials have highlighted the potential of dill in managing lipid levels in patients with type 2 diabetes [5]. In addition, extracts and tablets containing dill seed have been shown to reduce

cholesterol by inhibiting HMG-CoA reductase activity [6]. Oshaghi *et al*. [7] reported the presence of antioxidants like flavonoids, tannins, and saponins in dill seed tablets, which support their use in promoting metabolic health. Dill seed extract has also demonstrated positive effects in managing antioxidant, lipid, and blood sugar levels in diabetic patients [8].

Further studies, such as those by Haidari *et al*. [9], reveal that the consumption of dill seed powder can positively impact glycemic control, lipid parameters, and inflammatory markers in patients with type 2 diabetes. The treatment group exhibited a noticeable reduction in serum insulin, LDL, cholesterol, and insulin resistance. Among the key compounds in dill seeds, carvone, and limonene are thought to contribute to its ability to prevent obesity by altering inflammatory pathways and lipid metabolism in the adipose and liver tissues [10, 11]. However, despite the promising properties of dill seed, the anti-obesity effects of dill seed cake (DSC) remain underexplored. This study aims to investigate the in vitro anti-obesity potential of DSC, focusing on its phytochemical properties and its ability to inhibit enzymes involved in glucose absorption and fat digestion, such as α-amylase, α-glucosidases, and pancreatic lipase. The findings could support the development of dill seed cake as a potential natural supplement for managing obesity and weight gain.

Materials and Methods

Chemicals

Various analytical-grade chemicals and reagents were utilized in this study. α-Amylase, sodium dodecyl sulfate, N-acetylcysteine, O-phthalaldehyde, and Folin-Ciocalteu phenol reagent were procured from Hi-Media, India. P-nitrophenylα-D-glucopyranoside, sodium phosphate buffer, pancreatic lipase, α-glucosidase, acarbose, and orlistat were sourced from NS Scientific, India. Additionally, sodium dodecyl sulfate, borate buffer, and bovine serum albumin were acquired from Thermo Fisher Scientific, India, while O-phthalaldehyde (OPA) was obtained from Merck, India. All the chemicals used in this research were either HPLC or analytical grade.

Dill seed collection

Fresh *Anethum graveolens* seeds were purchased from a local market in Bhopal, Madhya Pradesh. To confirm their botanical identity, the seeds were authenticated by Dr. S. Naaz, a taxonomist from Saifia College, Bhopal. A reference specimen was then archived in the Departmental Herbarium (147/Bot. Saifia/Sci./College/Bpl).

Preparation of dill seed cake (DSC)

Dill seeds were first shade-dried and then ground into a coarse powder. The powdered seeds were stored under controlled conditions before further processing. To extract the oil, 500 gm of dill seeds were loaded into an Eco Smart ES oil press machine (Surat, India). After the extraction, the dill seed cake (DSC) residue was collected and subsequently finely pulverized.

Phytochemical analysis

A 25 gm sample of dried DSC was extracted using a Soxhlet apparatus with 200 ml of four different solvents—petroleum ether, chloroform, ethyl acetate, and 90% ethanol—for 12 hours. The resulting extracts were filtered and concentrated under a vacuum using a Jyoti Scientific evaporator. The concentrated extracts were weighed and stored at low temperatures for further phytochemical analysis. To identify the presence of alkaloids, flavonoids, phenolics, carbohydrates, saponins, steroids, tannins, and proteins, standard procedures described by Kokate [12] were followed.

Physicochemical analysis

Moisture, ash, and dry matter content: A 2 gm sample of DSC was placed in a pre-weighed silica crucible and heated at 450 °C until all carbon residues were eliminated. The final weight was recorded, and the total ash content was calculated relative to the initial weight. Additionally, water-soluble and acid-insoluble ash were determined using 10% hydrochloric acid instead of deionized water [13]. The moisture content was estimated by drying a weighed DSC sample in an oven at 105 °C until it reached a stable weight, and the percentage of water loss was then calculated [14].

Foreign matter examination: A 100 g sample of DSC was carefully spread out in a thin layer and examined under natural light and a 6x magnification lens to detect any external impurities [12].

Fat content measurement: The AOAC protocol was followed to determine the fat content using Soxhlet extraction. A 40 gm DSC sample was subjected to n-hexane extraction at 70 °C for 8 hours, and the total extracted oil was measured [15].

Protein content estimation: The lowry protein assay was employed to determine total protein content. The absorbance of the DSC extract was compared against a bovine serum albumin (BSA) standard curve and measured at 660 nm using a spectrophotometer [16].

Purification of dill seed cake (DSC) extract

To obtain a purified extract, 20 g of DSC underwent three successive extractions using 500 mL of hexane at room temperature to eliminate fat content. The defatted material was then rinsed thoroughly with distilled water three times (500 mL per wash) to remove soluble proteins and carbohydrates, followed by drying at temperatures below 70 °C. This dried material (20 g) was further subjected to methanol extraction (400 mL) in a soxhlet apparatus for 16 hours. The resulting methanol extract was filtered, vacuum-dried, and weighed. Before analysis, the extract was stored under refrigeration and later dissolved in 100 mL methanol to obtain a solution rich in phenolics and lignans [17].

Estimation of dietary fiber

Dietary fiber content, including both soluble and insoluble fractions, was determined using the approach outlined by Zhao *et al*. [18]. The purified DSC extract was first gelatinized by heating at 100 °C (pH = 6) for 15 minutes in the presence of heat-stable α-amylase. Subsequently, the sample underwent protease digestion at 60 °C (pH = 7.5) for 30 minutes to degrade protein content, followed by enzymatic hydrolysis using amyloglucosidase (60 °C, pH = 4.5) for 30 minutes to remove starch. After digestion, the remaining fraction was washed sequentially with water, 95% ethanol, and acetone, then dried and weighed to determine the insoluble fiber content.

To assess soluble fiber, the filtrate was treated with preheated 95% ethanol (60 °C), causing fiber precipitation. The precipitate was subsequently filtered and washed with 70% ethanol, 95% ethanol, and acetone, then dried and weighed. The total dietary fiber content was computed by summing the soluble and insoluble fiber fractions, with adjustments for ash and protein content.

Quantification of total phenolic compounds

The total phenolic content was determined following the Folin-Ciocalteu method. A 2 mg portion of the purified DSC extract was mixed with 2 mL of 90% ethanol and 10 mL of distilled water. The reaction was initiated by adding 1 mL of 50% Folin-Ciocalteu reagent, which was thoroughly mixed and left to incubate for 5 minutes. Following this, 2 mL of 5% sodium carbonate solution was introduced, vortexed for 15 seconds, and allowed to react at 40 °C for 60 minutes. The resulting solution's absorbance at 725 nm was measured using a Genesys 180 spectrophotometer (Thermo Fisher Scientific Inc., USA). A standard calibration curve was generated using gallic acid, and the total phenolic content was expressed as mg of gallic acid equivalent (GAE) per 100 mg of sample [19].

HPLC-based authentication of lignans

The presence of lignans in DSC was analyzed using a Waters Alliance 2695 HPLC system (Milford, USA), fitted with a photodiode array detector, auto-sampler, and a 20 µL injection loop. The separation process was carried out using a Primesep 200 C18 column (250 × 4.6 mm, 5 µm) in conjunction with a 3 µm guard column (Phenomenex, Macclesfield, UK). A mobile phase consisting of methanol and water (65:35 v/v) was used, filtered through a 0.45 µm PVDF membrane, and degassed before application. The system ran under isocratic conditions at a flow rate of 1.3 mL/min for 20 minutes, with detection performed at 220 nm.

For sample preparation, 1 mg of purified DSC extract was dissolved in 90% methanol, sonicated, and filtered. A diluted stock solution (10 µg/mL in methanol) was then passed through a 0.45 µm microfilter before being injected into the system for analysis [20].

In vitro studies

Hydrolysis analysis

To evaluate the degree of hydrolysis, an OPA reagent was prepared by mixing 50 mmol OPA, 50 mmol NAC, and 20% (w/v) sodium dodecyl sulfate in 0.1 M borate buffer (pH = 9.5). The reaction mixture consisted of a

measured quantity of purified DSC extract combined with the OPA reagent, followed by vortexing for 5 seconds and incubation at room temperature for 2 minutes. The absorbance was recorded at 340 nm using a Genesys 180 spectrophotometer (Thermo Fisher Scientific Inc., USA) to determine the extent of hydrolysis [21, 22].

α-amylase inhibition assay

To assess α-amylase inhibition, a test solution was prepared by mixing equal amounts of purified DSC extract and acarbose with 0.20 mmol α-amylase in phosphate buffer (pH = 6.9), then incubating for 10 minutes at 25 °C. A 1% starch solution in 0.02 M sodium phosphate buffer (pH = 6.9) was subsequently added, followed by another 10-minute incubation at 25 °C. The reaction was stopped by adding a 3,5-dinitrosalicylic acid reagent and heating the mixture in a water bath for 5 minutes. After cooling, absorbance was measured at 540 nm, and α-amylase inhibition (%) was calculated using the formula:

$$A_c - [A_s - A_0]/ A_c \times 100 \quad (1)$$

The ability of DSC extract to inhibit α-glucosidase was assessed using a p-nitrophenyl α-D-glucopyranoside-based assay following the method described in Djoman *et al*. [23]. A 0.006% α-glucosidase solution was prepared in 0.02 M phosphate buffer (pH = 6.9) and incubated with the purified DSC extract (dissolved in phosphate buffer, 0.02 M) for 1 hour at 25 °C. After incubation, 2 M p-nitrophenyl α-D-glucopyranoside was introduced into the reaction mixture, followed by further incubation at 30 °C for 30 minutes.

To terminate the reaction, 1 M sodium carbonate (Na_2CO_3) was added, leading to the release of p-nitrophenol, which was quantified spectrophotometrically at 405 nm. The extent of α-glucosidase inhibition was determined by comparing the absorbance values of the control and test samples against acarbose (a standard inhibitor) using the following formula:

$$\% \text{ α-glucosidase inhibition} = A_c - [A_s - A_0]/ A_c \times 100 \quad (2)$$

Porcine pancreatic lipase inhibition assay

The pancreatic lipase inhibitory activity of DSC extract was evaluated using DNPB (2,4-dinitrophenyl butyrate) as a substrate, following the method outlined in Ong *et al*. [24]. Porcine pancreatic lipase (0.1 mmol) was prepared in potassium phosphate buffer (pH = 6.0) and pre-incubated with Tween 80 (0.6%) along with the purified DSC extract (dissolved in 0.1 mmol potassium phosphate buffer, pH = 7.3) for one hour at 30 °C.

To initiate the reaction, 25 mmol DNPB was introduced into the mixture, followed by a five-minute incubation at 30 °C. The reaction outcome was determined by quantifying the release of 2,4-dinitrophenol using spectrophotometric analysis at 400 nm. The percentage of pancreatic lipase inhibition was then calculated using the following formula:

$$I\% = [(A - a) - (B - b)] / (A - a) \times 100 \quad (3)$$

Evaluation of pancreatic lipase suppression

To assess pancreatic lipase inhibition, the following parameters were considered:

A: Enzyme activity measured without the presence of an inhibitor.

a: Negative control where no inhibitor was used, and the reaction was halted immediately upon substrate addition.

B: Enzyme activity assessed in the presence of an inhibitor.

b: Negative control where the enzyme was pre-incubated with the inhibitor before the substrate was introduced.

In condition a, the reaction was stopped right after adding the substrate to the enzyme-containing buffer. Conversely, in b, the inhibitor was allowed to interact with the enzyme mixture before the reaction began.

Data processing and statistical interpretation

All experimental values were expressed as mean ± SEM. The statistical significance of differences among groups was determined through ANOVA (Analysis of Variance), followed by Tukey's post-hoc test to compare multiple datasets. A P-value of less than 0.05 was considered indicative of a statistically meaningful difference.

Results and Discussion

Phytochemical profile and discussion

Analysis of DSC extracts

A detailed phytochemical screening revealed the presence of carbohydrates, steroids, flavonoids, and phenolic compounds in all solvent-based extracts of DSC, including petroleum ether, chloroform, ethyl acetate, and methanol. However, alkaloids, tannins, and saponins were exclusively found in ethyl acetate and methanol extracts, whereas proteins were detected only in petroleum ether and chloroform extracts **(Table 1)**.

Table 1. Phytochemical evaluation of dill seed cake

Chemical class	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Alkaloids	–	–	+	+
Carbohydrate	+	+	+	+
Glycoside	–	–	–	–
Flavonoids and phenolics	+	+	+	+
Saponins	–	+	+	+
Phytosterols and triterpenes	+	+	+	+
Tannin	–	–	+	+
Proteins	+	+	–	–
Fat and oil	+	+	+	+

Where + is Present and – is Absent.

Physicochemical analysis

The total fat concentration in DSC was measured at 4.11 mg per 100 mg. Moisture content constituted 6.39%, while ash content accounted for 8.95% of the sample's composition. The total fiber content, comprising both soluble and insoluble fibers, was determined to be 21.37%. Additionally, the dry matter portion made up 84.36%, whereas the presence of foreign organic matter was minimal at 0.75%. The sample also exhibited phenolic compound levels of 38.23 mg/100 mg and a protein content of 22.21 mg/100 mg **(Table 2)**.

Table 2. Physicochemical evaluation of dill seed cake

Physicochemical parameters	Values (% w/w)
Total ash	8.95 ± 0.24
Acid insoluble ash	1.89 ± 0.03
Water soluble ash	4.86 ± 0.11
Dry matter	84.36 ± 3.23
Moisture content	6.39 ± 0.21
Foreign organic matter	0.75 ± 0.006
Total fat	4.11 ± 0.21
Total fiber	21.37 ± 2.21
Soluble fiber	4.32 ± 0.17
Insoluble fiber	17.31 ± 1.02
Total phenolic compound	38.23 ± 7.36
Total protein	22.21 ± 0.05

All the data are presented as mean ± SEM of three determinations.

HPLC analysis

The HPLC profile of the DSC revealed the presence of several lignan-like substances, such as carvone (Rt: 9.985), perillyl alcohol (Rt: 10.255), perillaldehyde (Rt: 11.605), and cineole. To analyze the lignins from dill seeds, Tao

and Pereira's method was slightly modified, using a mobile phase composed of methanol and water (65:35) **(Figures 1 and 2)** [20].

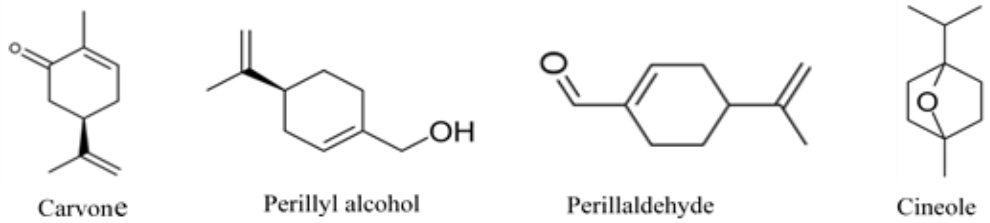

Figure 1. Chemical structure of dill seed lignan

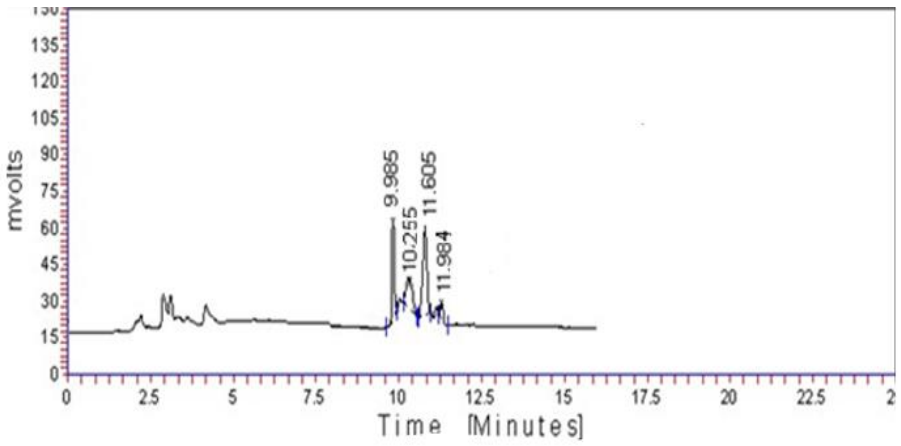

Figure 2. HPLC chromatogram of dill seed cake purified extract displaying peak of carvone (Rt: 9.985), perillyl alcohol (Rt: 10.255), perillaldehyde (Rt: 11.605) and cineole (Rt: 11.984).

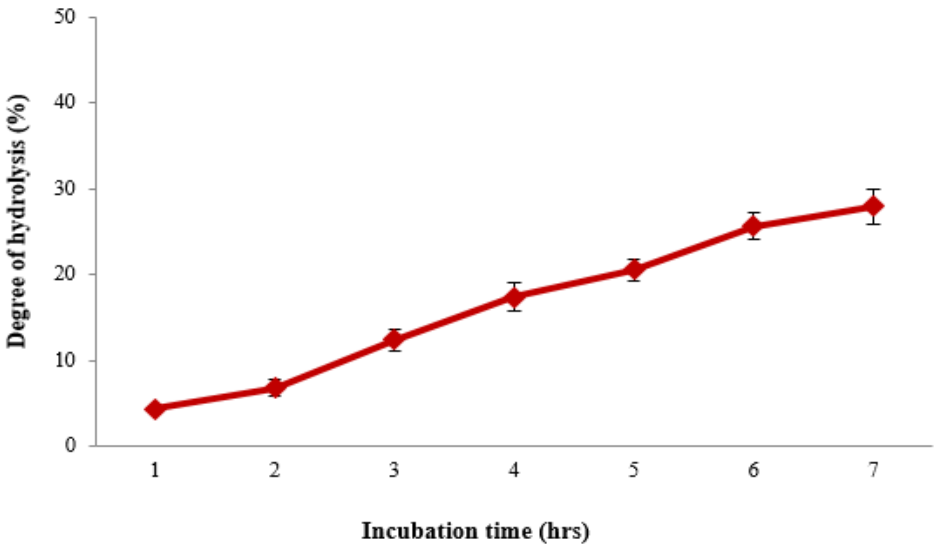

Figure 3. Percent degree of hydrolysis of dill seed cake at different time intervals.

In-vitro studies

The hydrolysis rate of DSC varied between 4.32% and 27.92%, as illustrated in **Figure 3**, indicating that protein breakdown in the dill seed cake occurred at a slow pace. The hydrolysis process began gradually over the first 2 hours, with only 6.75% hydrolysis, and continued at a steady pace afterward ($P < 0.01$).

DSC demonstrated a dose-dependent inhibition of α-amylase, with an IC50 value of 60.18 ± 2.21 µg/ml. The inhibition effect began at 10 µg/ml, showing a gradual increase, reaching 60.33% at a 100 µg/ml concentration.

Similarly, DSC inhibited α-glucosidase enzyme activity across all tested concentrations (ranging from 100 to 1000 µg/ml) in a dose-dependent manner, achieving an IC50 of 456.42 ± 5.32 µg/ml. At the highest concentration (1000 µg/ml), 56.16% inhibition was observed for α-glucosidase. The inhibition of pancreatic lipase by DSC was evaluated against orlistat, with DSC exhibiting a concentration-dependent increase in inhibitory activity. At 100 µg/ml, DSC achieved a 58.34% inhibition, with an IC50 of 54.13 ± 2.25 µg/ml **(Figure 4)**.

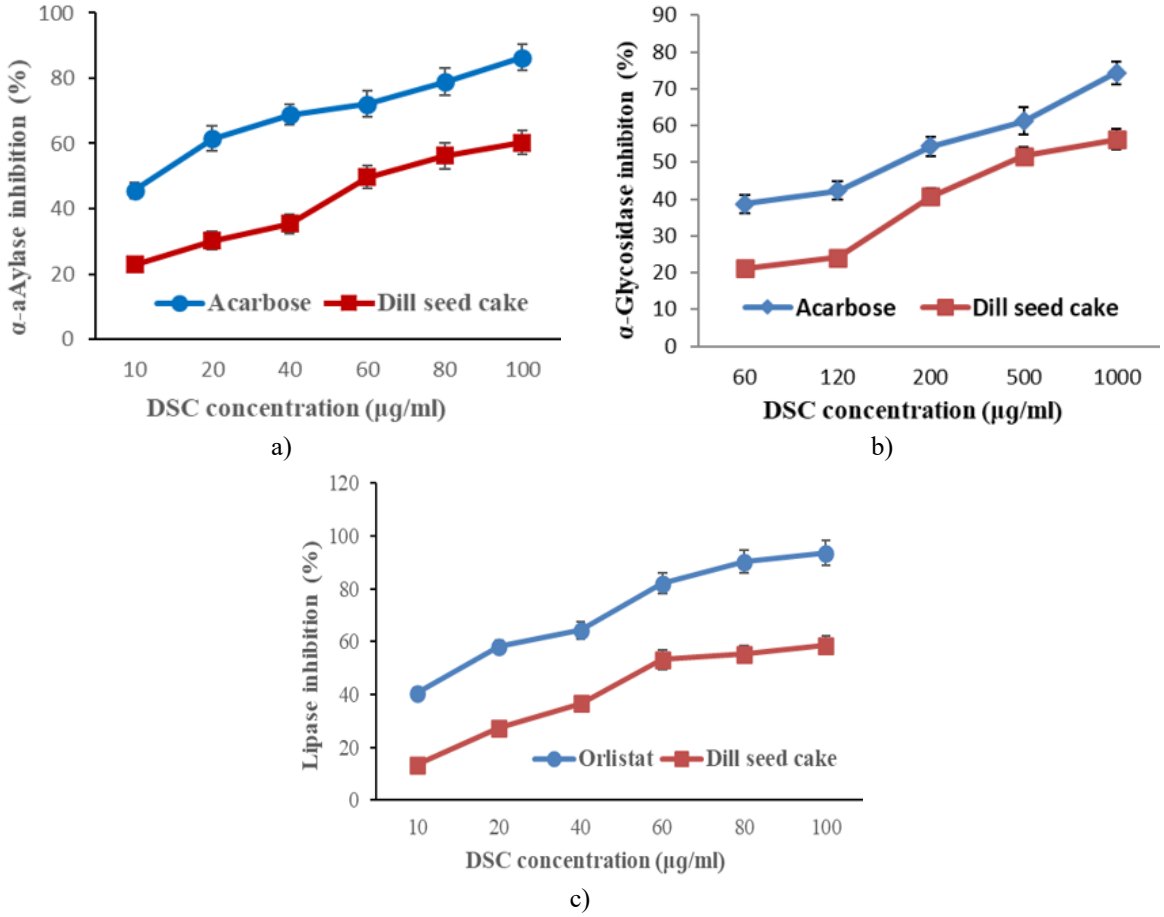

Figure 4. α-Amylase, α-glycosidase, and lipase inhibitory activity of dill seed cake at different concentration ranges.

The physicochemical attributes and potential anti-obesity effects of the leftover dill seed cake, after the oil extraction process, were evaluated in this study. The results suggest that dill seed cake has promising anti-obesity properties, making it a potential candidate for developing a safe and effective nutraceutical product aimed at managing obesity. Edible seeds are globally recognized for their nutritional benefits, particularly for their high fiber, protein, lignan, and polyphenol content. Due to their growing popularity as supplements to counter obesity and associated health risks, the market for these seeds is expanding. Dill seed cake, produced economically after oil extraction, serves as an accessible and affordable dietary supplement for obesity control [25-29].

Dill is known for its antioxidative properties, and it has demonstrated potential in reducing cholesterol levels. It has also shown effectiveness in improving insulin sensitivity, which is a key aspect of managing metabolic syndrome in type 2 diabetes. Research indicates that dill powder supplementation can lower blood glucose, insulin resistance, cholesterol levels, and malondialdehyde in type 2 diabetes patients. Other seeds, including quinoa, chia, pumpkin, fenugreek, and chandrashoor, are also recognized for their anti-obesity potential. The ability of dill seeds to reduce obesity is likely due to their impact on oxidative stress, inflammation, and metabolic factors like blood sugar and lipids [28-30].

Before this study, the anti-obesity effects of dill seed cake had not been explored experimentally. Our analysis found that dill seed cake contains a variety of bioactive compounds such as carbohydrates, steroids, proteins, alkaloids, flavonoids, and phenolics. The moisture and ash content of the cake were both under 10%, and the fat content was notably higher than expected. The fiber content—both soluble and insoluble—was substantial,

correlating with the high dry matter percentage. Moderate amounts of phenolic compounds and proteins were also present. The predominant lignans in the seed cake were carvone, perillyl alcohol, perillaldehyde, and cineole. Hydrolysis of dill seed cake occurred slowly, with a rate below 30%. The length of hydrolysis had an inverse relationship with the size of protein particles in the resulting hydrolysate. Dill seed cake's slow and low hydrolysis rate suggests that it produces longer peptide chains, which might offer unique health benefits [30-33].

Obesity is often caused by disruptions in how the body uses energy, leading to imbalances in carbohydrate and lipid metabolism. The management of blood sugar and weight is closely tied to the digestion and absorption of carbohydrates. The enzyme α-amylase breaks down starch into maltose and isomaltose, which are then converted into monosaccharides by α-glucosidases for absorption. Slowing or inhibiting the digestion of carbohydrates plays a crucial role in weight management by reducing post-meal blood sugar spikes. Dill seed cake exhibited moderate inhibitory effects on both α-amylase and α-glucosidases enzymes, showing its potential for managing blood sugar and obesity [30, 32, 34].

Regarding fat metabolism, pancreatic lipase is essential for breaking down dietary fats into triglycerides. These triglycerides are further processed into free fatty acids and monoglycerides, which are absorbed into the body and can lead to elevated blood lipid levels. Inhibiting pancreatic lipase activity—similar to the action of the weight-loss drug orlistat—can help prevent obesity. Dill seed cake demonstrated a strong inhibitory effect on pancreatic lipase, supporting its potential role as an anti-obesity agent. Overall, the ability of dill seed cake to inhibit pancreatic lipase, α-amylase, and α-glucosidases reinforces its anti-obesity potential [32-34].

Adipose tissue plays a crucial role in energy storage and providing fuel for various body functions. When excess energy-dense foods are consumed, surplus fats, often referred to as body fat, are deposited in adipose tissue, leading to obesity. The high content of soluble and insoluble fibers, along with proteins in dill seed cake, may contribute to weight reduction and fat burning. The effectiveness of these benefits depends on factors such as the fiber type, its function, and the dosage or frequency of consumption. Dill seed is rich in dietary fiber, proteins, and phenolic compounds, with moderate fat content. A diet low in carbohydrates and glycemic index, combined with high fiber and protein intake and moderate healthy fats, positively influences weight control. The presence of lignans and fibers may also help lower blood cholesterol levels. Lignans, which have anti-obesity effects, can reduce the risk of developing obesity-related chronic diseases. Dill seeds are abundant in carvone, a compound that is known to have antihyperglycemic effects by improving glycoprotein abnormalities in organs such as the liver, kidney, and plasma [33-35].

Dietary fibers, especially soluble ones, are crucial for improving insulin sensitivity and blood sugar regulation in people with type 2 diabetes. Dill seed cake, rich in fiber left behind after oil extraction, has been shown to enhance leptin release, thereby suppressing appetite. Additionally, dill seed has demonstrated a hypolipidemic effect in animal models, partially by inhibiting the enzyme HMG-CoA reductase. The variety of phytochemicals found in dill seeds might assist in controlling body fat and weight by activating pathways that promote fat breakdown and lipolysis. The inhibitory effects of dill seed cake on various intestinal enzymes in vitro have been reported for the first time. Ongoing studies are examining its in vivo anti-obesity potential when applied to different high-calorie diets. Thus, dill seed cake could become a valuable nutraceutical product in combating obesity and promoting fat loss in overweight individuals. These findings suggest that dill seed cake could be developed into a safe, effective anti-obesity supplement, and its by-products may also offer commercial opportunities as functional, value-added food products with enhanced health benefits [32-36].

Conclusion

Dill seeds offer a rich source of essential nutrients, including fiber, protein, and phenolic compounds, with lignans playing a significant role in their potential as nutraceutical ingredients. The inhibitory effect of dill seed cake on key digestive enzymes, such as α-amylase, α-glucosidase, and pancreatic lipase, can help limit the breakdown of carbohydrates and fats, contributing to better weight management. This research highlights the promising use of dill seed cake as a dietary supplement for controlling obesity and improving metabolic health.

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