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In Vitro and *in Silico* Studies of *Gnaphalium U.* Extract: Inhibition of α-amylase and α-glucosidase as a Potential Strategy for Metabolic Syndrome Regulation

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Abstract

A medicinal plant with significant therapeutic promise, *Gnaphalium uliginosum* was studied for its bioactive components, molecular interactions with α-amylase and α-glucosidase, and inhibitory effects on enzymes that metabolize carbohydrates. High-performance liquid chromatography (HPLC) analysis revealed that the flavonoid tricin and quinic acid derivatives were the main constituents. The anti-inflammatory and antioxidant qualities of these substances are well-known, which adds to their therapeutic value. The enzyme activity decreased in a dose- dependent manner, according to the *in vitro* α-amylase inhibition experiment. The extract reduced the levels of pancreatic and small intestine starch by 27.2 and 44.5 %, respectively, at the highest dose (100 µL). According to α-glucosidase inhibition experiments, the extract significantly reduced the concentration of glucose, outperforming the reference substance acarbose (44.75 %) with a 46.42 % reduction. Key bioactive chemicals' binding interactions with α-amylase and α-glucosidase were revealed by molecular docking studies. With binding energies ranging from −7.3 to −9.5 kcal/mol, tricin, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid had significant binding affinities. These interactions' capacity to block enzymes was supported by hydrogen bonding, pi-pistacking, and other stabilizing factors. These results demonstrate *Gnaphalium uliginosum's* medicinal potential in the treatment of metabolic diseases like diabetes. The bioactive substances showed strong inhibitory effects on the metabolism of carbohydrates, indicating that they may be used as natural antidiabetic medicines. To confirm these benefits and investigate their potential applications in the treatment of metabolic syndrome, more clinical research is necessary.

Keywords: α-amylase, α-glucosidase, *Gnaphalium uliginosum*, Metabolic syndrome, Molecular docking

Introduction

Metabolic syndrome represents a constellation of interconnected metabolic abnormalities, including central obesity, hypertension, dyslipidemia, and insulin resistance, which together significantly elevate the risk of developing type 2 diabetes, cardiovascular diseases, and other chronic conditions. The global prevalence of metabolic syndrome has risen alarmingly, driven by sedentary lifestyles, unhealthy dietary patterns, and an aging population [1]. A key feature of metabolic syndrome is postprandial hyperglycemia, a condition characterized by a sharp rise in blood glucose levels following meals. This phenomenon is primarily mediated by the enzymatic activity of α-amylase and α-glucosidase, which play essential roles in carbohydrate

metabolism by breaking down complex carbohydrates into simple sugars [2].

Targeting these enzymes offers a promising approach for managing postprandial hyperglycemia and, consequently, metabolic syndrome. Synthetic inhibitors of α-amylase and α-glucosidase, such as acarbose, are commonly used but are often associated with undesirable side effects like gastrointestinal discomfort [2]. As a result, there has been growing interest in identifying natural alternatives from medicinal plants. These natural inhibitors (ACE inhibitors, statins, and metformin) are not only effective but also tend to have fewer side effects, making them a safer and more sustainable option for long-term management of metabolic disorders. Among the many medicinal plants being explored, *Gnaphalium.U* (commonly known as Cudweed) has gained attention due to its diverse array of bioactive compounds and traditional use in treating various ailments [3]. Preliminary studies suggest that this plant may possess antidiabetic properties, making it a potential candidate for further exploration. The objective of this study is to investigate the inhibitory potential of *Gnaphalium.U* extracts on α-amylase and α-glucosidase enzymes [4]. Using a combination of *in vitro* assays and *in silico* molecular docking approaches, this research aims to elucidate the mechanisms by which the plant's bioactive compounds exert their effects. The findings will contribute to understanding the therapeutic potential of *Gnaphalium.U* in managing metabolic syndrome and offer insights into its role as a natural source of enzyme inhibitors [5].

Materials and methods

Plant material and extract preparation

Extracts were supplied as a powder by BIOTON LTD, Tashkent, Uzbekistan. To prepare the sample, 5 g of powder is mixed with 60 mL of ethanol (40 %) and extracted using a Soxhlet apparatus (SOX406 Fat Analyser, China) for 2 h at a temperature of 105 °C. The mixture is then filtered using filter paper to obtain the first filtrate. The maceration technique is repeated with the first residue until the second filtrate is obtained. Then, the first and second filtrates are combined and concentrated using a vacuum evaporator (OGAWA, Japanese incubator) at a temperature of 45 °C and a pressure of 80 mmHg [6].

High-performance liquid chromatography (HPLC) analysis (electrospray-ionization mass spectrometry in negative ion mode)

Five grams of *Gnaphalium uliginosum* extract was accurately weighed and dissolved in 300 mL of distilled water. Subsequently, 50 mL of 70 % ethanol was added to the solution [7]. The mixture was stirred for 1 h at a temperature of 40 - 50 °C, followed by 2 h at room temperature, and then centrifuged at 3,500 g for 20 min [8]. The supernatant was collected, and the extraction process was repeated twice more. The supernatants were filtered, combined, and the final volume was adjusted to 100 mL using 70 % ethanol before HPLC analysis. The HPLC analysis utilized approximately 0.1 % trifluoroacetic acid, acetate buffer, and acetonitrile as solvents. Chromatographic conditions for separation were as follows: Agilent HPLC 1260 Infinity autosampler; Eclipse XDB-C18 column (80 Å, 5 µm, 4.6×250 mm); detection at wavelengths of 247, 254, and 276 nm; a flow rate of 1 mL/min; and the eluent gradient program: phosphate buffer at 95:5 from 0 - 5 min, 70:30 from 6 - 12 min, 50:50 from 12 - 13 min, and 95:5 from 13 - 15 min [9]. The column temperature was maintained at 30 °C, and the sample injection volume was 10 µL. This experiment was conducted at the Bioorganic Chemistry Institute, Science Academy of Uzbekistan.

Animal Experiments

All animal procedures adhered to the European Directive 2010/63/EU on the protection of animals used in scientific research (European Union, 2010). The study protocol received approval from the Animal Ethics Committee of the Institute of Bioorganic Chemistry, AS RUz (Protocol Number: 133/1a/h, dated August 4, 2014).

Tissue preparation

Surgical procedures were carried out under sodium pentobarbital anesthesia, with all efforts made to minimize animal distress. The study involved white male rats with a body weight between 180 and 220 g [10].

***In vitro* assays**

Determination of the inhibitory activity of samples against α-amylase in vitro

The inhibitory activity of the *Gnaphalium.U* samples on α-amylase was determined in pancreas homogenates of 5 mature rats [11]. The experimental protocols were approved by the Institute's Animal Ethics Committee based on the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [10].The animals were decapitated by a guillotine, and the pancreas was removed from the abdominal cavity, cleared of adipose tissue, weighed and homogenized in a glass homogenizer with a Teflon pestle at 400 rpm for 1 min. The resulting homogenate was centrifuged at 3,000 rpm for 15 min. For experiments, the supernatant was taken and stored on ice. Starch content in tissues was determined using the method reported by Cavalieri [12].

In vitro α-glucosidase inhibition study

Rat small intestinal tissue homogenate was employed as an enzyme source. A section of the small intestine was removed, properly cleaned, dried on blotting paper, weighed, and then homogenized in a glass Teflon homogenizer at 1,400 g for 30 min with precooled phosphate buffer saline. The final volume of the supernatant was kept at 20 % (w/v). The spectrophotometric assay method was used in accordance with the method of slight modifications. Here, 40 µL of tissue homogenate was mixed with *Gnaphalium.U* extract in doses 0.01∼5.00 mg/mL and incubated for 15 min at 37 °C. Then, 280 µL of maltose (37 mM) was added, and the mixture was again incubated for 30 min. Finally, the tubes were submerged in hot water for 10 min to halt the process. The tubes were centrifuged, and a Cypress Diagnostics test kit (Germany) was used to determine the glucose concentration in the supernatant. Percent inhibition was calculated, where untreated tissue homogenate was used as an absorbance control [13].

In silico experiments

Datasets and software

This study made use of publicly available academic datasets and software [14]. The Protein Data Bank (PDB) provided the structural information for α-Amylase (PDB ID: 1HNY) and α-Glucosidase (PDB ID: 2ZOX). PubChem was the source of information about the drug, including its pharmacological and chemical characteristics. PyMol (version 1.2) was used for the structural data visualization and analysis. AutoDock 4.2 was used for molecular docking, with assistance from AutoDock Tools (ADT).

Ligand-receptor docking - Ligand and receptor file preparation

To optimize the PDB data for α-amylase and α-glucosidase, polar hydrogens were added, water molecules were eliminated, and Kollman charges were assigned. Ligand files, which were saved in the ".pdbqt" format, were similarly created.

Grid parameter file preparation

To enable ligand flexibility, a 3-dimensional grid box was constructed around the receptor's active site. A grid parameter file (.gpf) contained the parameters.

AutoGrid operation

AutoGrid4 was used to create ligand atom atomic maps, resulting in docking-compatible files.

Docking parameter file preparation

Using the Lamarckian Genetic Algorithm for simulations, the Docking Parameter File (.dpf) specified crucial docking parameters.

Using AutoDock

Ligand-receptor binding energies were calculated using docking simulations. PyMol was used to analyze and show the results in order to comprehend binding energetics and processes.

Statistical analysis

The results are presented as mean ± standard deviation (SD). Each assay was independently performed at least 3 times, and statistical analysis was conducted using the Student's t-test. Data for each treatment represent 3 independent replicates (3 and 6; $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively).

Results

III.I HPLC analysis results

This indicates the similar chemical nature of these compounds. Peaks observed within the retention time range of 1 - 2 min suggest the presence of quinic acid derivatives [15]. Specifically, these peaks are indicative of compounds such as chlorogenic acid, 4-O-caffeoylquinic acid, cynarin, 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, and 3,4,5-tri-O-caffeoylquinic acid [16]. The peak with a retention time of 25.073 min suggests the presence of the flavonoid tricin in the extract, a conclusion supported by the results of mass spectrometry (**Figure 1**).

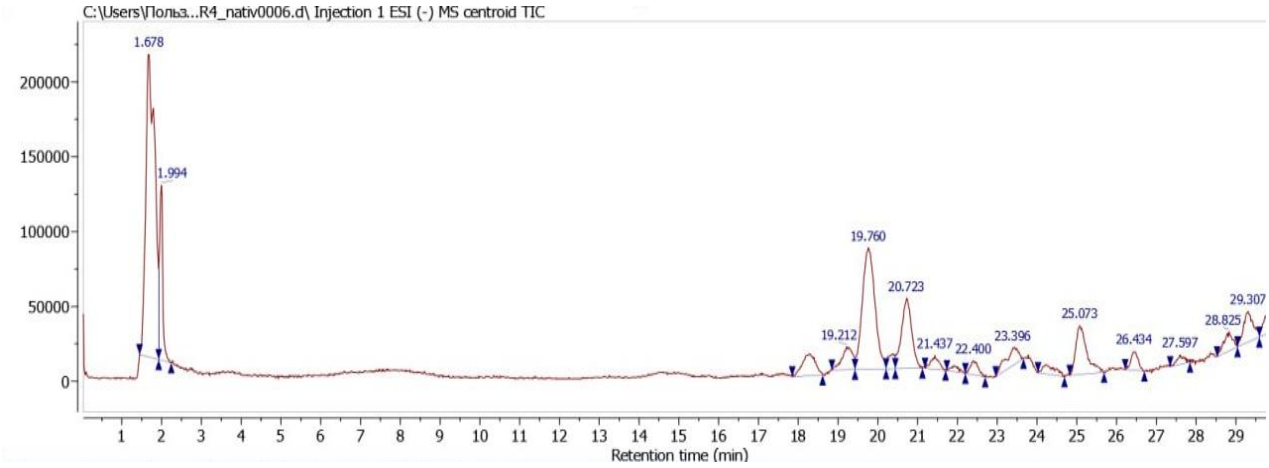


Figure 1 HPLC chromatogram of *gnaphalii uliginosi* sample.

Quinic acid and its derivatives are of particular interest for human health, primarily due to their anti-inflammatory and antioxidant properties. These derivatives contain phenolic hydroxyl groups within a conjugated system with an even number of carbon atoms, enabling them to act as direct antioxidants by neutralizing free radicals. Chlorogenic acid, for instance, has been extensively studied as a nutraceutical for treating metabolic syndrome and associated conditions such as diabetes, obesity, dyslipidemia, and hypertension (**Figure 2**). Studies have demonstrated significant free radical scavenging activity of 3,5-di-O-caffeoylquinic acid, along with its iron-chelating properties. Similarly, cynarin exhibits Fe^{2+}-chelating activity, inhibits DPPH free radicals, and prevents the formation of radicals such as DMPD•$^{+}$, ABTS•$^{+}$, and H_2O_2. The flavonoid tricin and tricin-containing extracts have drawn researchers' attention due to their strong anti-radical activity, lack of toxicity, and absence of side effects. These beneficial biological properties have led to the widespread production and application of tricin and its derivatives in medicine, nutraceuticals, and dietary supplements [17].

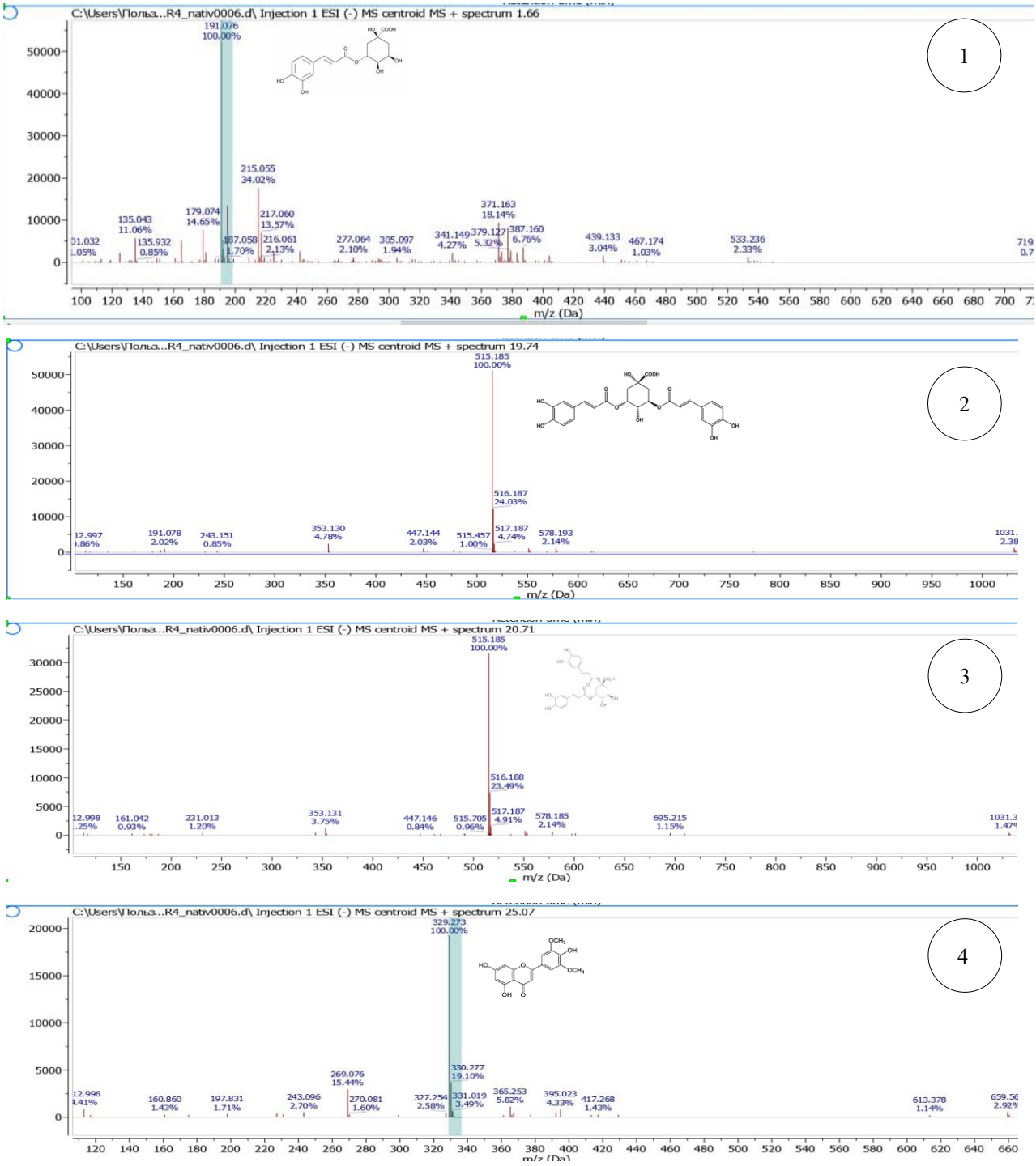


Figure 2 Mass chromatograms of fractions 1, 2, 3, and 4 of *gnaphalii uliginosi* extract.

Determination of the inhibitory activity of samples against α-amylase in *vitro*

The study investigated the effect of *Gnaphalium* plant extract on α-amylase activity. Homogenates of rat pancreas and small intestine were used for this purpose. Based on the results obtained using the spectrophotometric method, the amount of starch (mg/min/mL) was calculated, and the dose-dependent effect of the plant extract on α-amylase activity was observed (**Table 1**). In the healthy rat pancreas, the starch amount was 3934.25 ± 2.43 mg/min/mL. Upon the addition of the plant extract, a dose-dependent decrease in starch levels was observed. With the addition of 10 µL of the extract, the starch amount

decreased to 3604 ± 181.2 mg/min/mL, corresponding to an 8.4 % reduction. At 25 µL, the reduction was 9.6 %, at 50 µL, it was 14.3 %, and at the highest dose of 100 µL, the starch amount dropped to 2863 ± 102.6 mg/min/mL, indicating a 27.2 % reduction. A similar effect was observed in the small intestine [18]. In healthy rat small intestine, the initial starch level was 35.43 ± 0.63 mg/min/mL, which decreased under the influence of the extract. With 10 µL of the extract, the starch amount was 32.04 ± 0.74 mg/min/mL, showing a 9.7 % reduction. At 25 µL, the starch amount was 30.43 ± 1.16 mg/min/mL; at 50 µL, it decreased to 27.27 ± 0.61 mg/min/mL. Finally, at 100 µL of the extract, the starch amount was 19.7 ± 1.65 mg/min/mL, indicating a 44.5 % reduction (**Table 2**). Based on the results, the dose-dependent inhibitory effect of the *Gnaphalium* plant extract on α-amylase activity was confirmed [19]. The reduction in starch levels indicates that the plant extract decreases enzyme activity. The findings suggest that the extract's effect is dose-dependent, with higher dose 100 µL showing the strongest inhibitory effect. These results highlight the potential of this plant extract for antidiabetic applications and its practical use in treating metabolic disorders [20].

Table 1 α-amylase activity in pancreatic and intestinal homogenates *in vivo*.

Samples	**"Activity of pancreatic α-amylase, mg/min/mL of starch"**			
Control	**3934,25 ± 2,43**			
	"Concentration of extracts, mg/mL"			
	10	**25**	**50**	**100**
Gnaphalium	3604 ± 181,2	3558 ± 64,2	3370 ± 164	2863 ±102,6

Table 2 Changes in α-amylase activity in intestinal homogenates *in vivo.*

Samples	**"Activity of intestinal α-amylase, mg/min/mL of starch"**			
Control	**35,43 ± 0,63**			
	"Concentration of extracts, mg/mL"			
	10	**25**	**50**	**100**
Gnaphalium	32,04 ± 0,74	30,43 ± 1,16	27,27 ± 0,61	19,7 ± 1,65

***In vitro* α-glucosidase inhibition study**

In the next experiment, the effect of *Gnaphalium* plant extract on the activity of the α-glucosidase enzyme was studied, with acarbose used as a reference compound [21]. This enzyme plays a crucial role in carbohydrate metabolism, as it breaks down carbohydrates into monosaccharides in the small intestine [22]. The results indicate that both acarbose and the *Gnaphalium* plant extract exhibit a dose-dependent inhibitory effect on α-glucosidase, leading to a reduction in glucose levels. The plant extract showed the most significant effect at a high dose, where at a concentration of 0.385 mg/mL, the glucose level was 12.58 ± 0.11 mmol/L, representing a 46.42 % reduction in glucose concentration. In comparison, Acarbose achieved only a 44.75 % reduction. Thus, *Gnaphalium uliginosum* extract, at a high dose, may serve as an effective agent for reducing glucose levels [23]. Future clinical trials of this plant extract could help confirm its efficacy and potential therapeutic applications (**Figure 3**).

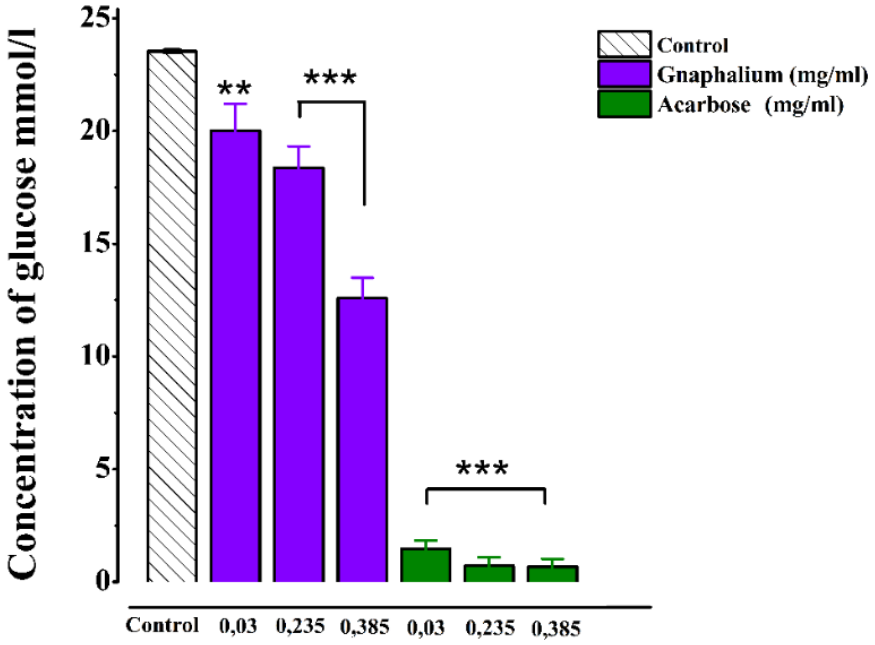


Figure 3 Dose-Dependent Inhibitory Effects of *Gnaphalium* Extract and Acarbose on Glucose Concentration n = 6; $p < 0.05$; $p < 0.001$.

Molecular docking analysis

Interactions of protein (α-amylase) and ligands

Molecular docking studies were conducted to investigate the binding interactions of bioactive compounds from *Gnaphalium uliginosum* with α-amylase, aiming to validate the *in vitro* experimental findings. The docking results revealed key insights into the binding affinities, interactions, and binding energies of the compounds with α-amylase, providing a molecular basis for their inhibitory activity [24].

Tricin

Tricin demonstrated significant binding interactions with α-amylase, primarily forming a conventional hydrogen bond with HIS A:299. Additional interactions included unfavorable bumping with HIS A:101, Pi-Pi stacked interactions with HIS A:305, TYR A:62, and TYR A:59, as well as alky and pi-alkyl interactions with LEU A:165. Furthermore, a potential carbon hydrogen bond with THR A:163 was identified. The binding energy for tricin was calculated as −9.0 kcal/mol, indicating a stable and favorable binding conformation (**Figure 4**).

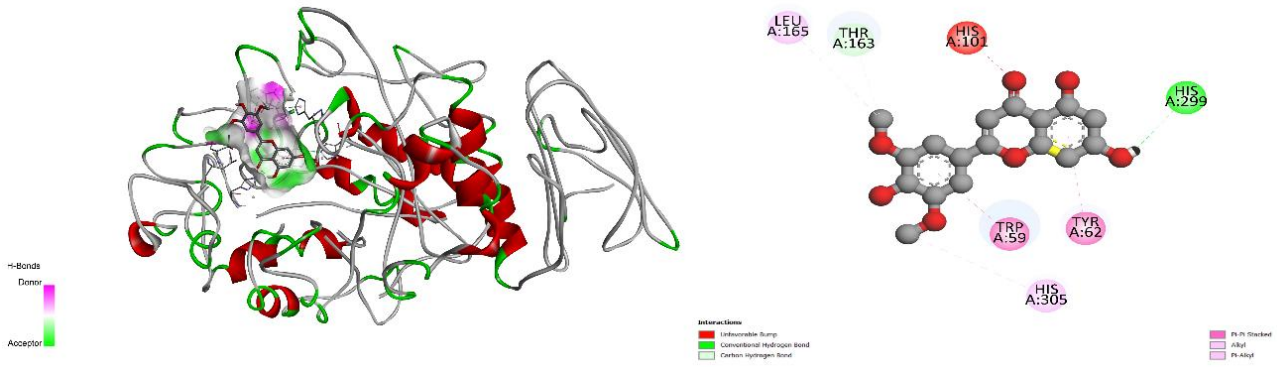


Figure 4 Molecular interactions of Tricin with α-amylase.

3,5-di-o-caffeoylquinic acid

This compound exhibited strong binding affinity to α-amylase with a binding energy of −7.9 kcal/mol. Key interactions included conventional hydrogen bonding with ASN A:105, pi-alkyl interactions with VAL A:107, pi-Sigma interactions with ILE A:51, and pi-pi stacked interactions with TRP A:59. These results suggest robust binding and the potential for effective α-amylase inhibition (**Figure 5**).

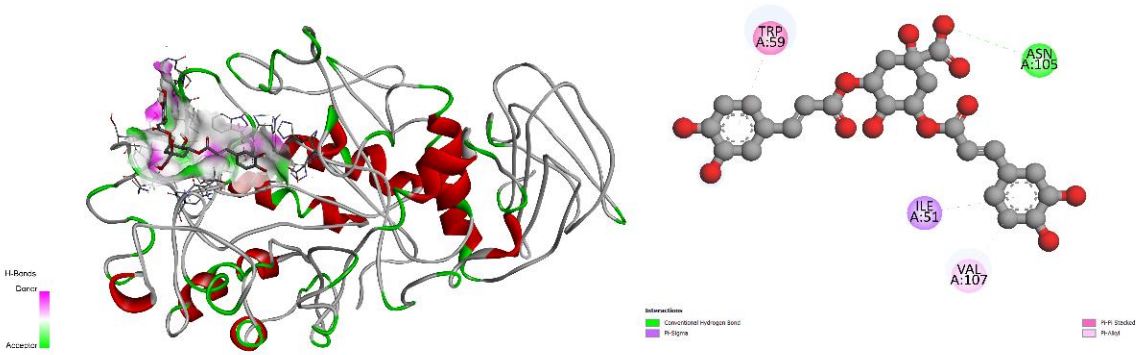


Figure 5 Molecular interactions of 3,5-Di-O-Caffeoylquinic Acid with α-amylase.

4,5-di-o-caffeoylquinic acid

The docking analysis revealed multiple conventional hydrogen bonds with ASP A:197, ARG A:195, GLU A:233, TRP A:59, and GLN A:63. Additionally, a pi-pi stacked interaction was observed with TYR A:62. The binding energy was −8.7 kcal/mol, indicating a strong and stable interaction with the α-amylase active site (**Figure 6**).

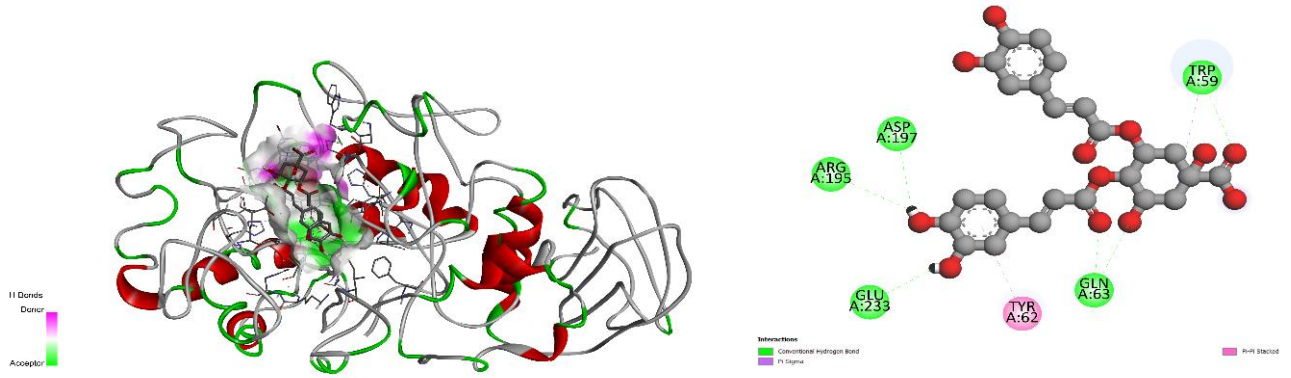


Figure 6 Molecular interactions of *4,5-Di-O-Caffeoylquinic Acid* with α-amylase.

4-o-caffeoylquinic acid

This compound showed conventional hydrogen bonding with GLU A:233, a carbon hydrogen bond with HIS A:305, and a pi-pi stacked interaction with TRP A:59. The binding energy of −7.6 kcal/mol reflects moderate binding stability (**Figure 7**).

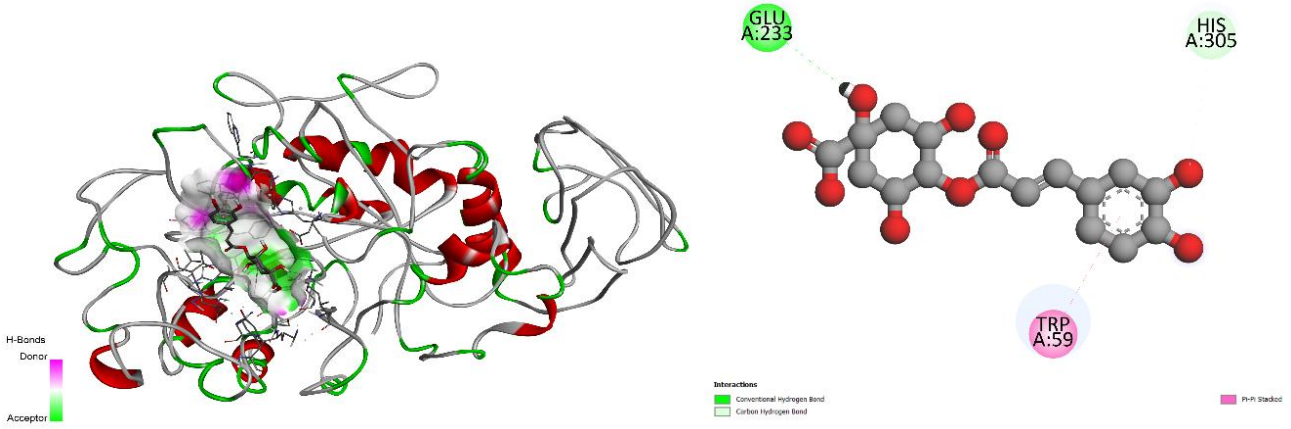


Figure 7 Molecular interactions of *4-O-Caffeoylquinic Acid* with α-amylase.

Chlorogenic Acid

Chlorogenic acid formed a conventional hydrogen bond with GLN A:63, pi-alkyl interactions with ALA A:198 and LEU A:162, pi-sigma interactions with ILE A:235, pi-pi stacked interactions with HIS A:201, and a carbon hydrogen bond with HIS A:101. The binding energy for chlorogenic acid was calculated as −7.3 kcal/mol, suggesting moderate inhibitory potential (**Figure 8**).

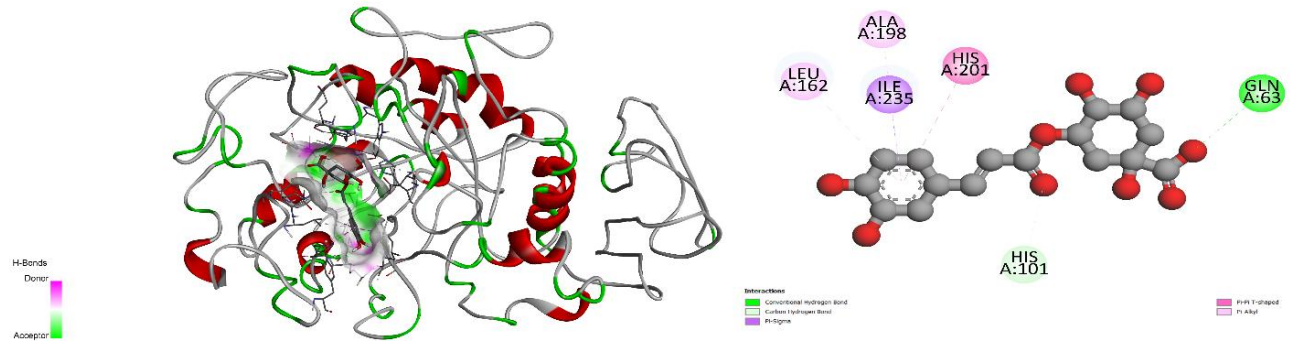


Figure 8 Molecular interactions of Chlorogenic Acid with α-amylase.

Cynarin

Cynarin exhibited robust interactions, forming conventional hydrogen bonds with ASP A:300, GLN A:63, GLU A:233, and THR A:163. Additional interactions included pi-alkyl bonding with LEU A:162, pi-pi stacked bonding with TYR A:162, and an unfavorable donor-donor interaction with HIS A:305. The calculated binding energy was −8.3 kcal/mol, reflecting strong and stable binding to α-amylase (**Figure 9**).

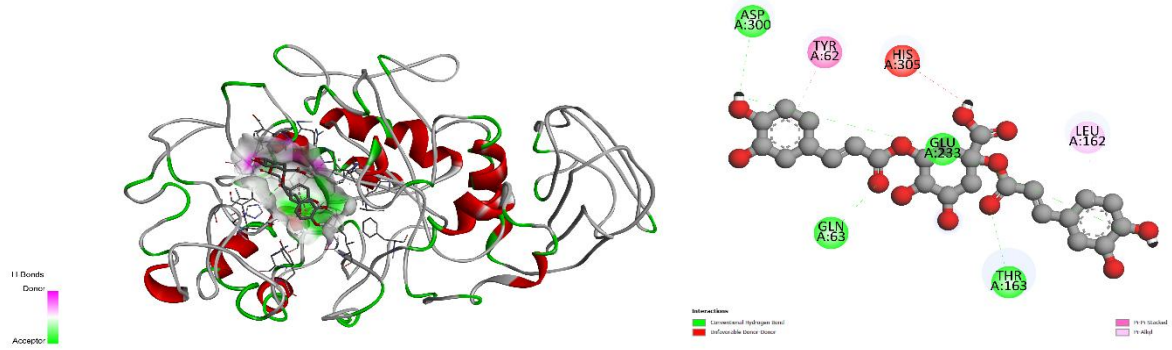


Figure 9 Molecular interactions of Cynarin with α-amylase.

The molecular docking analysis revealed that all tested compounds extracted from *Gnaphalium uliginosum* exhibited favorable interactions with α-amylase, supporting their potential as α-amylase inhibitors. Among the compounds, 3,5-di-O-caffeoylquinic acid demonstrated the strongest binding affinity with a binding energy of −9.0 kcal/mol, followed by 4,5-di-O-caffeoylquinic acid and cynarin. Tricin showed notable interactions and moderate binding stability, highlighting its therapeutic potential. These findings provide molecular-level evidence for the enzyme inhibitory effects observed *in vitro* and support the potential application of these compounds in metabolic syndrome management.

Interactions of protein (α-amylase) and ligands

Molecular docking studies were performed to evaluate the binding interactions of bioactive compounds from *Gnaphalium uliginosum* with α-glucosidase, aiming to corroborate the findings from *in vitro* experiments (**Table 2**). The results provide a detailed understanding of the binding mechanisms and binding energies, highlighting the potential inhibitory effects of these compounds [25] (**Table 3**).

Tricin

Tricin exhibited significant interactions with α-glucosidase, forming conventional hydrogen bonds with GLU A:424, TRP A:345, and GLN A:165. It also formed a carbon hydrogen bond with GLU A:373, pi-alkyl interactions with TYR A:309 and VAL A:168, a Pi-Sigma interaction with VAL A:227, and a pi-pi stacked interaction with PHE A:225. The binding energy was calculated as −7.9 kcal/mol, indicating moderate binding stability and inhibitory potential (**Figure 10**).

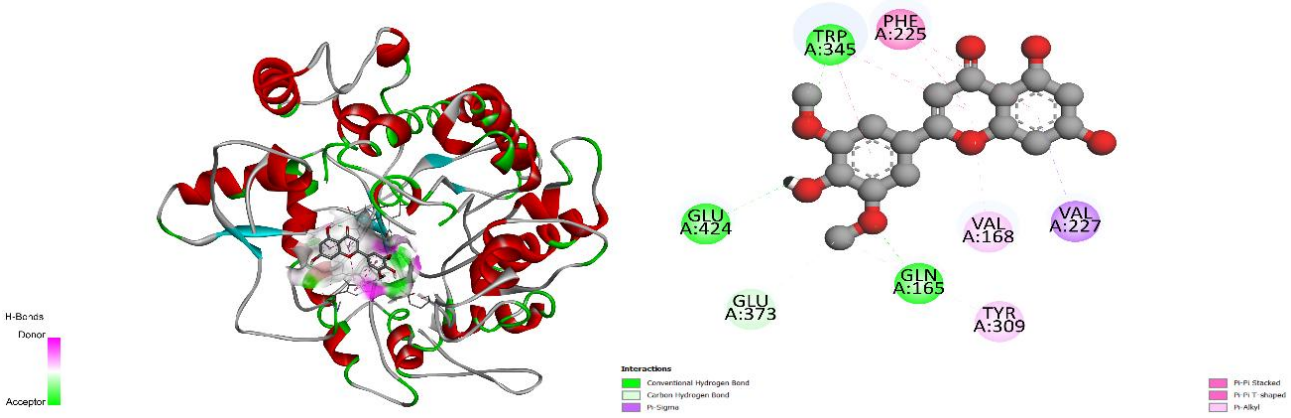


Figure 10 Molecular interactions of Tricin with α-glucosidase.

3,5-di-o-caffeoylquinic acid

This compound displayed the strongest binding affinity among the analyzed molecules, with a binding energy of −9.5 kcal/mol. Key interactions included conventional hydrogen bonds with GLN A:424, ASN A:167, GLN A:165, ILE A:326, and ASP A:175. Additional interactions were observed as pi-sigma bonds with LEU A:327 and LEU A:176 and pi-pi stacked interactions with TRP A:345. These results suggest a robust and stable binding, aligning with its potential as a strong α-glucosidase inhibitor (**Figure 11**).

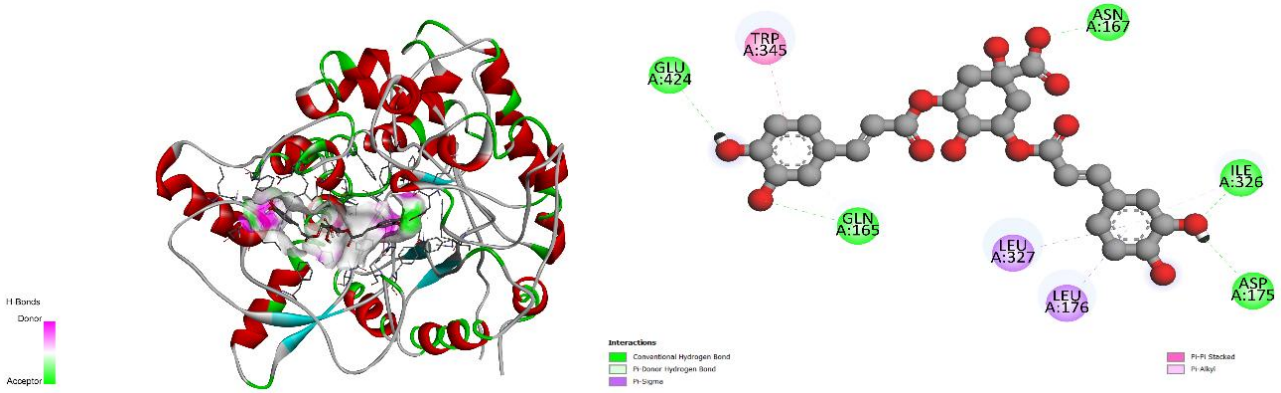


Figure 11 Molecular interactions of 3,5-Di-O-Caffeoylquinic Acid with α-glucosidase.

4,5-di-o-caffeoylquinic acid

4,5-Di-O-caffeoylquinic acid exhibited a binding energy of −9.3 kcal/mol, indicating high binding stability. The molecule formed pi-alkyl interactions with ILE A:326 and VAL A:168, a pi-sigma interaction with VAL A:171, a pi donor hydrogen bond with PHE A:334, and a pi-pi stacked interaction with TRP A:345. Additionally, a conventional hydrogen bond was observed with GLU A:424 (**Figure 12**).

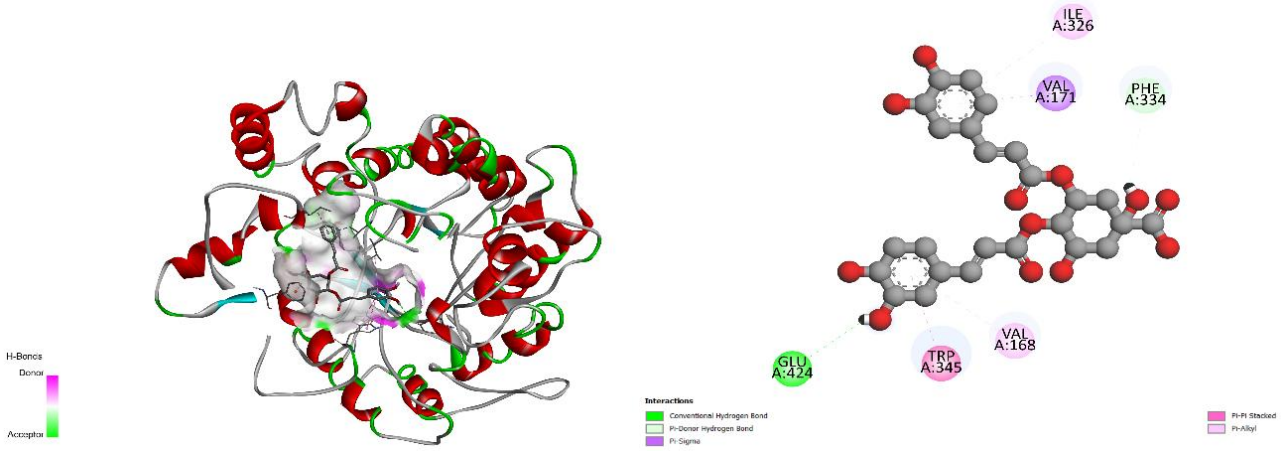


Figure 12 Molecular interactions of 4,5-Di-O-Caffeoylquinic Acid with α-glucosidase.

4-o-caffeoylquinic acid

This compound demonstrated a binding energy of −8.4 kcal/mol. It formed conventional hydrogen bonds with ASN A:164 and TRP A:425 and a pi-pi stacked interaction with TRP A:345 and PHE A:225. Additional pi-alkyl interactions were noted with VAL A:168, while an unfavorable donor-donor interaction was observed with GLN A:165 (**Figure 13**).

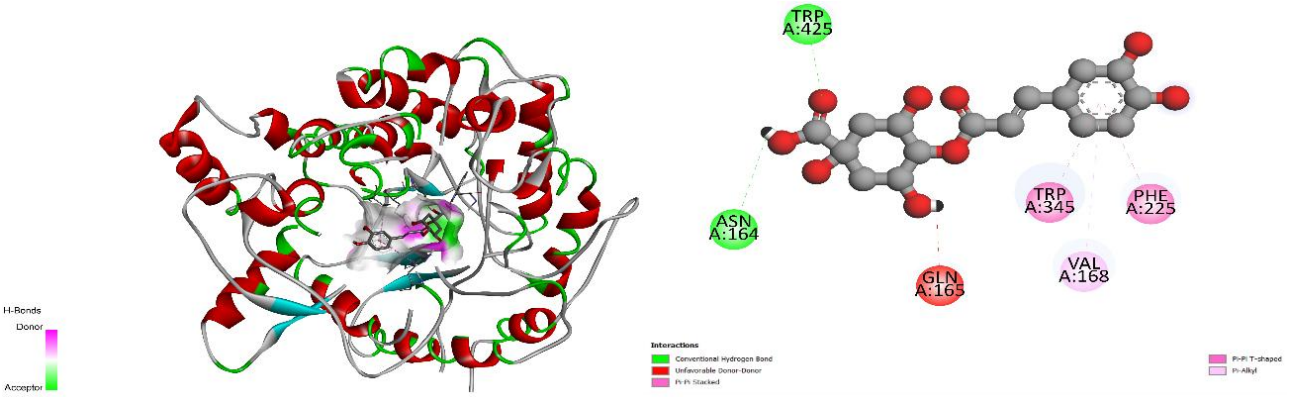


Figure 13 Molecular interactions of 4 -O-Caffeoylquinic Acid with α-glucosidase.

Chlorogenic Acid

Chlorogenic acid showed a binding energy of −8.4 kcal/mol. It formed conventional hydrogen bonds with GLU A:424, GLU A:373, and GLN A:165. Pi-pi stacked interactions were observed with TRP A:345. However, unfavorable bumps were identified with ARG A:312, TRY A:309, and TRP A:417, which may influence the overall binding stability (**Figure 14**).

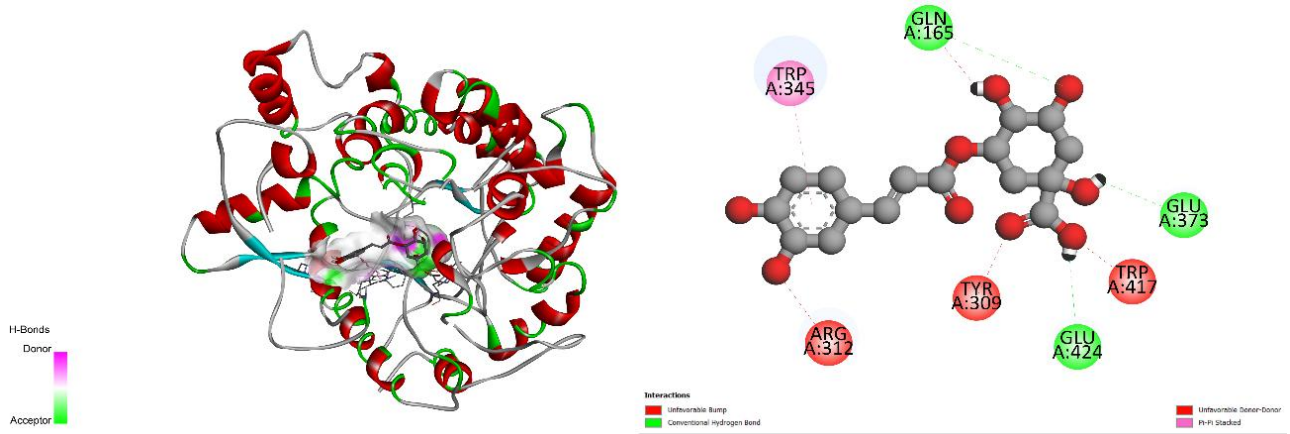


Figure 14 Molecular interactions of Chlorogenic Acid with α-glucosidase.

Cynarin

Cynarin exhibited a binding energy of −8.6 kcal/mol, demonstrating significant interactions. It formed pi-pi stacked interactions with PHE A:334, conventional hydrogen bonds with ALA A:246, and pi-sigma interactions with VAL A:171. A carbon hydrogen bond was observed with HIS A:250, supporting its potential as an α-glucosidase inhibitor (**Figure 15**).

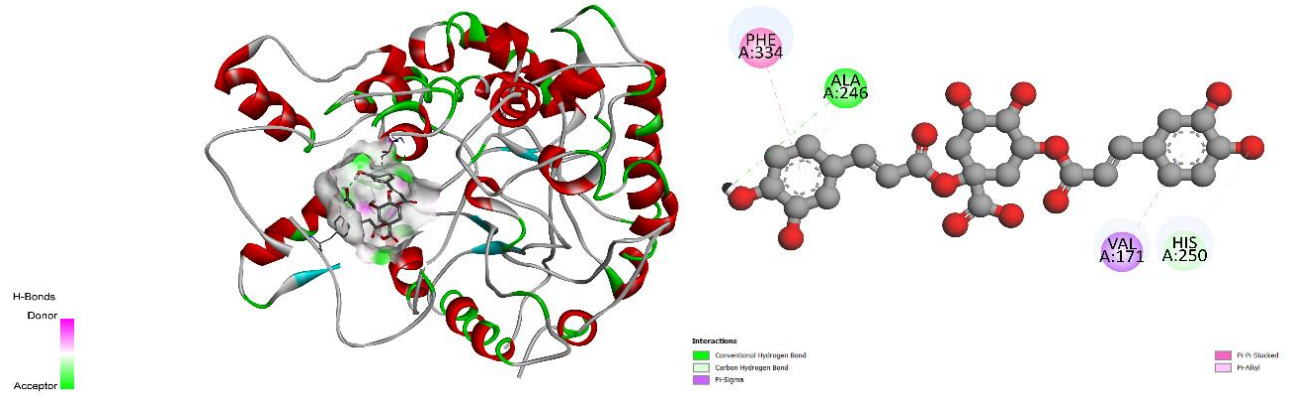
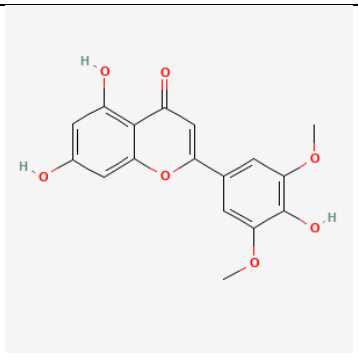
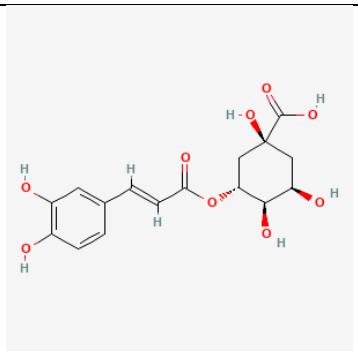
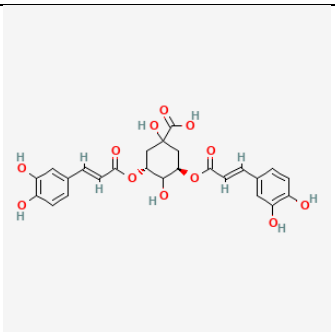
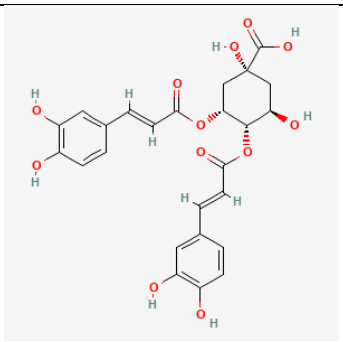
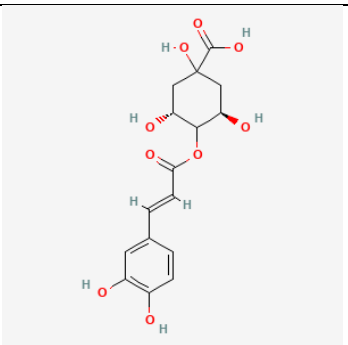
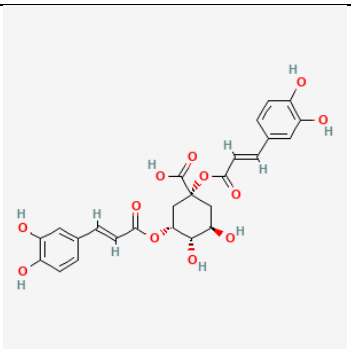


Figure 15 Molecular interactions of Cynarin with α-glucosidase.

The molecular docking analysis revealed that all tested compounds from *Gnaphalium uliginosum* showed favorable binding interactions with α-glucosidase, supporting their potential as enzyme inhibitors. 3,5-Di-O-caffeoylquinic acid exhibited the strongest binding affinity (−9.5 kcal/mol), followed by 4,5-di-O-caffeoylquinic acid (−9.3 kcal/mol) and cynarin (−8.6 kcal/mol). These findings validate the *in vitro* results and suggest that these compounds could serve as effective α-glucosidase inhibitors, with applications in managing metabolic disorders. Further research, including structural optimization and delivery strategies, could enhance their therapeutic potential (**Table 3**).

Table 3 Structural illustrations of the α-amylase and α-glucosidase - ligand complex.

Compounds of *Gnaphalium.U*	**Chemical structures**	**α-Amylase Affinity (kcal/mol)**	**α-Glucosidase Affinity (kcal/mol)**
Tricin		−7.9 kcal/mol	−7.9 kcal/mol
Chlorogenic acid		−7.3 kcal/mol	−8.4 kcal/mol

Compounds of *Gnaphalium.U*	Chemical structures	α-Amylase Affinity (kcal/mol)	α-Glucosidase Affinity (kcal/mol)
3,5-Di-O-Caffeoylquinic acid		−9.0 kcal/mol	−9.5 kcal/mol
4,5-Di-O-Caffeoylquinic acid		−8.7 kcal/mol	−9.3 kcal/mol
4-O-Caffeoylquinic acid		−7.6 kcal/mol	−8.4 kcal/mol
Cynarin		−8.3 kcal/mol	−8.6 kcal/mol

Discussion

HPLC analysis results

The chromatographic analysis of the *Gnaphalium uliginosum* extract revealed multiple peaks indicative of its diverse chemical composition. Peaks with retention times between 1 and 2 min suggest the presence of quinic acid derivatives, including chlorogenic acid, cynarin, and various di- and tri-caffeoylquinic acids. These compounds are well-documented for their potent antioxidant and anti-inflammatory properties. The peak at 25.073 min, identified as the flavonoid tricin, aligns with mass spectrometry results and underscores the

extract's bioactive potential. Quinic acid derivatives, particularly chlorogenic acid, have been extensively studied for their role in mitigating metabolic syndrome. Their ability to neutralize free radicals, chelate iron, and inhibit oxidative stress positions them as valuable nutraceutical candidates. Similarly, tricin has garnered attention for its anti-radical activity and minimal toxicity, making it a promising compound for therapeutic applications. The identification of these bioactive compounds highlights the pharmacological relevance of *Gnaphalium uliginosum* and its potential in developing antioxidant-rich dietary supplements [27].

Inhibitory activity against α-amylase

The dose-dependent inhibition of α-amylase activity by *Gnaphalium uliginosum* extract demonstrates its potential for managing hyperglycemia. The extract significantly reduced starch hydrolysis in both pancreatic and intestinal homogenates, with higher doses yielding greater inhibitory effects. At the highest dose (100 µL), starch hydrolysis was reduced by 27.2 % in the pancreas and 44.5 % in the small intestine. These findings suggest that the extract effectively suppresses α-amylase activity, thereby limiting glucose release from starch. The observed inhibitory activity can be attributed to the bioactive compounds identified in the extract. For instance, chlorogenic acid and its derivatives are known to interact with digestive enzymes, reducing carbohydrate metabolism. The significant reduction in starch levels underscores the extract's potential as an antidiabetic agent, warranting further exploration through clinical studies [28].

Inhibitory activity against α-glucosidas.

Similar to its effects on α-amylase, the *Gnaphalium* extract exhibited a dose-dependent inhibition of α-glucosidase activity. At a concentration of 0.385 mg/mL, the extract reduced glucose levels by 46.42 %, outperforming the reference compound acarbose, which achieved a 44.75 % reduction. This highlights the extract's efficacy in delaying carbohydrate digestion and glucose absorption in the small intestine. The potent inhibitory activity of the extract against α-glucosidase can be linked to its flavonoid and phenolic acid content. Tricin, for instance, has been reported to interact with α-glucosidase, disrupting its catalytic function. The extract's superior performance compared to acarbose suggests its potential as a natural alternative for managing postprandial hyperglycemia [29].

Molecular docking analysis

Molecular docking studies provided insights into the interactions between bioactive compounds from *Gnaphalium uliginosum* and digestive enzymes. Among the tested compounds, 3,5-di-O-caffeoylquinic acid exhibited the strongest binding affinity to both α-amylase and α-glucosidase, with binding energies of −9.0 kcal/mol and −9.5 kcal/mol, respectively. These interactions involved conventional hydrogen bonds and pi-pi stacked interactions, stabilizing the ligand-enzyme complex and inhibiting enzymatic activity. Tricin also demonstrated notable interactions with both enzymes, forming hydrogen bonds and pi-alkyl interactions. While its binding energy was slightly lower compared to caffeoylquinic acids, tricin's stability and favorable binding conformation highlight its therapeutic potential. Other compounds, such as cynarin and chlorogenic acid, also showed strong and stable interactions, further validating the *in vitro* findings. The molecular docking results corroborate the experimental data, establishing a molecular basis for the inhibitory effects of *Gnaphalium uliginosum* extract on α-amylase and α-glucosidase. These findings emphasize the extract's potential for managing metabolic disorders, particularly diabetes and obesity.

Conclusions

The comprehensive analysis of *Gnaphalium uliginosum* extract highlights its pharmacological potential, particularly in managing metabolic syndrome. The identification of bioactive compounds such as chlorogenic acid, tricin, and caffeoylquinic acids underscores the extract's antioxidant and enzyme inhibitory properties. The dose-dependent inhibition of α-amylase and α-glucosidase activity, supported by molecular docking studies, positions this extract as a promising natural therapeutic agent. Future studies should focus on clinical validation and exploring the extract's efficacy *in vivo*, paving the way for its application in functional foods and nutraceuticals.

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Declaration of Generative AI in Scientific Writing

Only minimal assistance was used from QuillBot for paraphrasing selected sentences. All scientific content, interpretation, and conclusions were developed independently by the authors.

CRediT author statement

Mekhrangiz Zaripova performed in vivo and in vitro experiments. Izzatullo Abdullaev was responsible for the *in silico* analysis and wrote the initial draft. Anvarbek Bogbekov contributed to the *in silico* modeling. Ulugbek Gayibov supervised the *in silico* methodology. Sirojiddin Omonturdiev conducted statistical analysis. Rustam Makhmudov performed the HPLC-MS experiments. Nurali Ergashev supervised the in vivo experimental work. Gulchekhra Jabbarova provided general supervision. Sabina Gayibova supervised the project implementation. Takhir Aripov served as the main supervisor and provided final approval of the manuscript.

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