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ORIGINAL PAPER

Making Health Out of Recycling: The Innovative Role of Hawthorn Seed Waste in Diabetes Management

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Abstract
Diabetes mellitus (DM) is a chronic metabolic disorder that affects millions of people worldwide, and its prevalence is increasing due to global population growth and lifestyle changes. One of the key strategies in managing DM is the inhibition of enzymes such as α-glucosidase and Dipeptidyl Peptidase-IV (DPP-IV), as well as reducing protein glycation. In this study, the antidiabetic potential of Crataegus monogyna (hawthorn) seeds, which are generally considered agricultural waste, was investigated. Hawthorn seeds were extracted using water, ethanol, and methanol as solvents, and the obtained extracts were analyzed for their phytochemical composition, total phenolic and flavonoid content, antioxidant activity, enzyme inhibitory effects, and antiglycation potential. The methanol extract exhibited the highest phenolic content (53.21 ± 4.69 mg GAE/g) and antioxidant activity, while the ethanol and methanol extracts showed strong antiglycation effects. The water extract demonstrated the highest inhibitory activity against DPP-IV (IC50: 0.21 mg/mL) and α-glucosidase (IC50: 85.23 µg/mL). In silico molecular docking and dynamics simulations revealed that procyanidin A2 and procyanidin B1 exhibited strong binding affinities for DPP-IV and α-glucosidase, respectively, and that these complexes were stable. These results suggest that hawthorn seeds, traditionally considered waste, possess significant pharmacological potential and could offer a new, natural, and sustainable therapeutic option for diabetes management. This study serves as an important example for the valorization of biomass and the repurposing of waste materials in the pharmaceutical field.

Keywords Hawthorn seeds · Diabetes mellitus · Antidiabetic potential · In silico studies · Waste valorization

Introduction

The increase in agricultural production on an industrial scale has resulted in a concomitant increase in the amount of organic waste from processing processes. Due to their low economic value, these wastes are commonly used in limited ways or directly discarded. However, a significant proportion of these agricultural wastes has the potential to function as bioactive inputs because of their inherent bioactive compounds. Re-evaluation of crop wastes not only reduces the environmental burden but also offers a strategic approach in terms of circular economy and sustainable waste management. Consequently, there has been a significant increase in research in recent years on the conversion of food processing by-products and agricultural wastes into value-added products [1–3]. Agricultural wastes, especially those of plant origin, contain many biologically active compounds in terms of their content, and the potential use of these compounds in the health and pharmaceutical industry is increasing. The highest added value in the recycling of plant wastes is seen in the pharmaceutical industry [4, 5]. For example, different parts of plants, such as fruits, flowers, roots, branches, stems, and seeds, contain many components with therapeutic effects [6]. The global herbal medicine market was valued at approximately US$14 billion in 2009, and it is projected to reach around US$5 trillion by 2050 [7]. This shows the increasing importance of plant-derived compounds in pharmaceutical production. The use

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of bioactive compounds obtained from plant waste in areas such as pharmaceuticals, cosmetics, and perfumes is among the transformations that have reached the top of the value pyramid. This transformation demonstrates how efficiently waste can be utilized in the healthcare industry while simultaneously increasing environmental sustainability.

Hawthorn (*Crataegus spp*.) is a widespread medicinal plant that belongs to the *Rosaceae* and *Maloideae* subfamilies. Based on its traditional use and its status as "generally recognized as safe" (GRAS), the European Medicines Agency's Committee for Herbal Medicinal Products has classified hawthorn as a "traditional herbal medicinal product." [8]. This wild plant has been used for centuries as a traditional remedy, herbal medicine, and dietary supplement [9]. Hawthorn fruit is widely used in processed products such as jam, marmalade, fruit juice [10]. However, the hawthorn seeds produced during these processes are usually not used and are treated as industrial agricultural waste. Although hawthorn seeds are a great source of biomass, their bioactive compounds remain underutilized. Such vegetable wastes generated after food processing not only create environmental waste but also miss opportunities to be transformed into value-added products. The reuse of plant waste, such as hawthorn seeds, an important part of sustainable waste management [11–13]. The use of compounds obtained from these wastes through recycling and biotechnological methods can provide environmental and economic benefits by strengthening our understanding of the circular economy.

Diabetes is a metabolic disease characterized by high blood sugar (hyperglycemia), resulting from low insulin secretion, insulin action, or both. Chronic progression of hyperglycemia is associated with long-term organ damage, mostly in the blood vessels, kidneys, eyes, and heart [14]. Diabetes affects millions of people worldwide, and the global diabetes prevalence in 2019 was estimated to be 9.3% (463 million), rising to 10.2% (578 million) by 2030, and 10.9% (700 million) by 2045 [15]. Type 2 Diabetes Mellitus (T2DM) is among the most prevalent metabolic disorders globally, primarily arising from two key factors: impaired insulin secretion by pancreatic β-cells and the reduced responsiveness of insulin-sensitive tissues to insulin. Disruption in the feedback between insulin action and secretion leads to high blood glucose levels, and β-cell dysfunction reduces insulin secretion and impairs glucose regulation. Insulin resistance (IR) increases glucose production in the liver and decreases glucose uptake in the muscles, liver, and adipose tissue [16].

Inhibition of dietary carbohydrate digestion is an approach adopted for diabetes management. α-Glucosidase (α-G) (EC 3.2.1.20), located on the brush border of small intestines, is a hydrolyze-class enzyme that acts upon α(1→4) glycosidic bonds releasing monosaccharides, such as glucose, which are further absorbed and enter the bloodstream. Inhibition of α-G can delay intestinal glucose absorption and regulate postprandial hyperglycemia [17]. Glucagon-like peptide 1 (GLP1) is an intestinal peptide that enhances insulin secretion and reduces glucagon release after nutrient intake. In addition, GLP1 slows gastric emptying and promotes a sense of fullness. The plasma concentration of GLP1 increases upon ingestion; however, due to its degradation by dipeptidyl peptidase 4 (DPP4), only 10–15% of GLP1 enters the systemic circulation [18]. The discovery that exogenously infused GLP1 can normalize fasting glucose levels in T2DM patients highlights its therapeutic potential. However, its rapid degradation by DPP4, which converts GLP1 to its inactive form, limits its effectiveness. Dipeptidyl peptidase 4 inhibitors (DPP4i), used for type 2 diabetes since 2006, have been widely adopted as second-line treatments because of their low risk of hypoglycemia and complementary action. Their safety profil makes them ideal when other therapies are unsuitable or contraindicated [19]. Preventing diabetic complications is strongly linked to non-enzymatic glycation, commonly referred to as "glycation." This process begins when the free amino group of amino acids reacts with the carbonyl group of glucose, eventually forming advanced glycation end-products (AGEs). Another outcome of hyperglycemic conditions is oxidative stress, which is a state of increased free radical production. Prolonged exposure of cells to free radicals promotes the onset of diabetic complications [20].

In this study, the biological activity of hawthorn seed extract as food processing waste was investigated. The total phenolic and flavonoid contents, antioxidant capacity, antidiabetic effect (α-glucosidase and DPP-4 inhibitory activities), and antiglycation potential of the extract obtained from hawthorn seeds were evaluated using in vitro methods. Furthermore, the interaction of hawthorn seed compounds with these enzymes was analyzed using in silico methods. This study shows that plant waste, such as hawthorn seeds, can be transformed into value-added products by evaluating them in terms of biologically active components and can be an environmentally sustainable resource. Furthermore, this study highlights the potential of recycling hawthorn seed waste for applications in the health and pharmaceutical industries, offering a valuable perspective on sustainable waste management.

Materials and methods

Preparation of Extracts from *Crataegus Monogyna* Seeds

C. monogyna seeds were separated, freeze-dried, and ground. Dried seeds (65 g) were weighed and extracted with water, ethanol (80%), and methanol (80%) for 72 h using an orbital shaker. The crude extract was filtered, and the supernatant was incubated in an oven at 40 °C until all solvents evaporated.

Phytochemical Analysis

Qualitative analysis of quinones, alkaloids, saponins, tannins, anthocyanins, phytosterols, cardiac glycosides, coumarins, and terpenoids was performed according to the following methods [21]. 0.25 mL of the extract was used for all the tests. The reagents used for each method and the indicator reactions of the tests are listed in Table 1.

Total Phenolic and Flavonoid Contents

The total polyphenolic content of *C. monogyna* seed extract was estimated using Folin-Ciocalteu method [22]. 20 µL of the sample was mixed with 100 µL of 1:10 diluted Folin-Ciocalteu reagent and incubated for 5 min. Then, 80 µL 7.5% N2CO3 was added to the mixture and incubated for 1 h at room temperature. The absorbance of the samples was measured at 760 nm. Gallic acid was used as a standard (5–100 µg/mL), and the total phenolic content was calculated in terms of gallic acid equivalents (GAE).

Total flavonoid content of *C. monogyna* seed extract was estimated according to the method described by [23]. Next, 100 µL of 2% $AlCl_3.6H_2O$ was added to 100 µL of the sample and incubated at room temperature for 10 min. Quercetin was used as the standard (5–100 µg/mL). Subsequently, the absorbance of the samples and standards was measured at 405 nm, and the total flavonoid content was calculated in terms of quercetin equivalents (QE).

Table 1 Qualitative phytochemical tests

Analyzed species	Reagent	Indicator Reaction
Quinones	0.25 mL H_2SO_4	Red color development
Alkaloids	0.25 mL 3:1 KI/I	Brown precipitate
Saponins	0.25 mL dH_2O	Foam formation
Tannins	0.5 mL %52 $FeCI_3$	Green color development
Anthocyanins	0.5 mL 2 M HCl + 0.25 mL 4 M NH_3	Pinkish red color development
Phytosterols	0.25 mL chloroform + 0.25 mL H_2SO_4	Yellow color development
Cardiac glycosides	1 mL Acetic acid + a few drops of %5 $FeCI_3$ + 0.5 mL H_2SO_4	Brown color development
Coumarins	0.25 mL %10 NaOH	Yellow color development
Terpenoids	1 mL chloroform + a few drops of H_2SO_4	Red color development

Antioxidant Activity

2.2-Diphenyl-1-picrylhydrazyl (DPPH) [24] and 2.29-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [25] assays were conducted with slight modifications for the free radical scavenging activity of *C. monogyna* seed extract. DPPH solution was prepared in methanol at a concentration of 0.004%, and 50 µL of the solution was mixed with 50 µL of the sample (2 mg/mL). Four replicates of each sample were studied. The reaction mixture was incubated at room temperature in the dark for 30 min before measuring the absorbance of the samples and standards at 517 nm. Trolox was used as the standard, and free radical activity was calculated in terms of Trolox equivalent (QE) using the following formula: DPPH radical scavenging activity (%) = $[(A_0 - A_1)/ (A_0)] \times 100$. Four replicates of each sample were studied.

ABTS radical cation was prepared by mixing 7.4 mM ABTS solution with 2.45 mM $K_2S_2O_8$ and left at room temperature for 16 h for radical development. The $ABTS^{\bullet+}$ solution was diluted until it gave an absorbance of 0.700 ± 0.02 absorbance at 732 nm prior to the experiment. The sample (25 µL) was added to 200 µL of $ABTS^{\bullet+}$ solution and incubated at room temperature for 30 min before reading the absorbance of the samples and the standards at 517 nm. Trolox was used as the standard and absorbance values were interpreted as Trolox equivalents.

Enzyme Inhibition and Antiglycation Activity Assays

α-glucosidase inhibitory activity of *C. monogyna* seed extract was analyzed according to [26] with slight modifications. Sodium phosphate buffer (125 µL, pH 6.8) and 75 µL of 2 mM p-nitrophenyl α-D-glucopyranoside were mixed in a 96-well plate. Then, 10 µL of seed extract was added to the wells and the reaction mixture was pre-incubated at 37 °C for 15 min. Finally, 50 µL of 0.1 U α-glucosidase from Saccharomyces cerevisiae was pipetted into the wells and the plate was incubated for another 15 min at 37 °C. The absorbance was measured at 405 nm. Acarbose was used as the standard inhibitor. The activity assay for dipeptidyl peptidase-IV (DPP-IV) was performed according to [27] using the chromogenic substrate Gly-Pro-pNA.

The antiglycation activity of the extracts was determined using an in vitro glycation model [28]. In this context, each

seed extract was dissolved in 100 mM potassium phosphate (pH 7.4) buffer and the fluorescence intensity was measured at 370 nm excitation and 440 nm emission using a fluorescence spectrometer after incubation for 24 h at 50 °C in a glucose-BSA in vitro glycation system [10 mg/mL BSA and 500 mM D-glucose containing 100 mM potassium phosphate (pH 7.4; 0.02% sodium azide)].

The IC_{50} value was defined as the concentration of glycation, α-glucosidase inhibition, and inhibition of 50% of glycation, α-glucosidase, and DPP-IV activity, which were calculated using a graphically.

Molecular Docking

The phenolic compounds of hawthorne seeds identified by the method of UPLC-ESI-TQD-MS/MS were used as ligands for the molecular docking with both α-Glucosidase and DPP-IV ([29]. The stuructures of the ligands were retrieved from Pubchem [30]. The crystal structures of α-Glucosidase (3A4A) and DPP-IV (1WCY) were downloaded from Protein Data Bank [31]. Molecular docking simulations were conducted using AutoDock4, a widely utilized tool for studying protein-ligand interactions [32, 33]. The target protein structures were prepared using AutoDockTools (ADT) by removing water molecules, adding polar hydrogen atoms, and assigning Gasteiger-Marsili partial charges. Ligand structures were also processed in ADT, where torsional degrees of freedom were defined, and Gasteiger charges were calculated. Both the protein and ligand structures were converted into PDBQT format, the input format required by AutoDock4. The grid box for each docking simulation was defined based on the binding sites of the original inhibitors that co-crystallized with the respective proteins, ensuring that the grid encompassed all critical residues involved in ligand binding. The docking simulations employed the Lamarckian Genetic Algorithm (LGA), with the number of genetic algorithm runs set to 20, ensuring thorough exploration of the ligand-binding conformational space. Docking poses were ranked according to their binding free energies (ΔG), allowing the identification of the most favorable binding modes and interactions. Additionally, acarbose and diprotin A were used as the control ligands for α-glucosidase and DPP-IV, respectively, to validate the docking protocol. The best poses for each protein were visualized and analyzed using BIOVIA Dassault Systèmes (BIOVIA Discovery Studio, 2024 Client, San Diego, CA, USA).

Table 2 Phytochemical screening of *C. monogyna* seed methanol, ethanol and water extract

Phytochemical	Ethanol	Methanol	Water
Saponins	++	+	-
Alkoloids	-	++	+
Phytosterol	+	++	-
Flavanoids	++	++	+
Coumarins	+	+	+
Kinon	+	+	+
Cardiac	+	+	-
Phenol	+++	+++	+
Terpenoids	+	+	+
Carbohydrates	++	+	+
Anthocyanins	+	+	-
Tannins	++	++	+

Molecular Dynamics

Molecular dynamics (MD) simulations were used to examine the stability, binding interactions, and dynamic behavior of the ligand-enzyme complex, thereby providing a comprehensive understanding of its structural and energetic properties under physiological conditions. Desmond module of Schrödinger was used to utilize for the analysis of molecular interactions between the target proteins and the ligands with the highest docking score [34]. The system was solvated in an orthorhombic cubic simulation box with a size of X = 10 Å, Y = 10 Å, and Z = 10 Å. using the Simple Point Charge (SPC) water model. An OPLS3e force field was employed to minimize the energy of the system. The net charge of the system was neutralized by adding counterions Na+, Cl-. The ionic strength was adjusted to the physiological conditions by adding 0.15 M NaCl. Isothermal-isobaric (NPT) ensemble was employed for the simulations with a time interval of 100 ns.

Results and Discussion

Percentage Extraction Yields and Phytochemical Screening

The extraction yields of *C. monogyna* seeds were 77.4%, 71.1%, and 35.5% for ethanol, methanol, and water, respectively. The best extraction yield was achieved with ethanol, which is classified as a green solvent. The green solvents are nature-friendly due to their non-toxic, recyclable biodegradable, and renewable properties [35].

Phytochemicals are a variety of chemical compounds produced by plants and possess significant biological activities, such as antioxidant, antimicrobial, anti-inflammatory, anti-hyperglycemic, antifungal, and immunomodulatory activities [36]. In the evaluation of the results (Table 2), grading was conducted based on color intensity, with +++ denoting the darkest color, + indicating the lightest color, and - signifying no change (graded according to the foaming intensity in the saponin test). Qualitative phytochemical screening of *C. monogyna* seeds under different extraction

conditions revealed significant amounts of phenols, flavonoids, tannins, and terpenoids. The negative or very low results of tests for potential toxic components in hawthorn seeds, such as cardiac glycosides, terpenoids, and anthraquinones, suggest that the extracts are non-toxic and may be suitable for incorporation into functional foods, nutraceuticals, or functional beverages with potential health benefits. The chlorogenic and caffeic acid contents of *Crataegus pinnatifida* seeds have been reported previously ([37], and the extraction was carried out using ethyl acetate. It should be noted that the chemical content of fruits varies among species, and different types of extraction methods may lead to the screening of different sample compositions. Phytochemical tests generally serve as a fundamental preliminary approach for determining the predominant components in the prepared plant extracts. In this context, these tests require confirmation using more sensitive and quantitative methods in future investigations.

Total Phenolic and Flavonoid Contents

Extraction methods involve the separation and recovery of biologically active components from plant tissues using selective solvents. The secondary metabolite composition of an extract depends on extraction type, duration, temperature, solvent quality, solvent concentration, and polarity. Owing to the dynamic nature of biological samples, it is not feasible to obtain the desired compounds in a single step, and an optimized extraction and analysis method is necessary for the effective separation and characterization of these components. Total phenol and flavonoid concentrations were calculated as gallic acid equivalent (mg GAE/g) and quercetin equivalent (mg QE/g), respectively. The methanol extraction of *C. monogyna* seeds resulted in the highest total phenolic content (53.21±4.69 µg GAE/mL) (Table 3).Żurek et al., (2024) investigated the phenolic properties of six species of hawthorn using 70% methanol extraction. According to the results, the phenolic content of *C. monogyna* was found to be 47.1±0.9 mg GAE/g plant, and other species gave the results ranging between 46 and 71 mg GAE/g. As for the total flavonoid content, ethanol and methanol extractions showed similar results.

Antioxidant Activity

Free radicals and reactive oxygen species (ROS) are formed during vital activities under the influence of endogenous and environmental factors. When free radicals are not neutralized, they can cause several serious pathophysiological events in the body through various mechanisms [38] The free radical scavenging activities of *the C. monogyna* seed extracts are presented in Table 4. The EC_{50} values for the DPPH and ABTS methods varied from 338 to 73.83 µg/mL and from 16.34 to 47.66 µg/mL, respectively. In previous studies, the antioxidant activities of *Crataegus elburensis* seed methanolic extract, a different hawthorn species, were determined by DPPH radical removal method and the EC_{50} value was found as 92.88 µg/mL [39]. It is clear that the antioxidant capacity of *C. monogyna* seed extracts prepared within the scope of this study was better than the reported results. In both ABTS and DPPH methods, the highest antioxidant activity result was obtained in the methanol extract (4.06±0.03 mmol trolox equivalent 100 g-1 dry weight) and the lowest value was obtained in the water extract (0.66±0.06 mmol trolox equivalent 100 g-1 dry weight). These values are consistent with previously determined phenolic and flavonoid contents.

Table 3 Total phenolic and flavonoid contents of *C. monogyna* seed extracts

Extraction solvent	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
Ethanol	37.7±3.46	10.46±0.06
Methanol	53.21±4.69	10.35±0.25
Water	16.08±5.3	9.88 2.35

Table 4 Antioxidant activity of *C. monogyna* seed extracts

Extraction solvent	DPPH (EC_{50})	ABTS(EC_{50})
Methanol	73.83±2.1 µg/mL	16.34 µg/mL±0.728
Ethanol	85.8±6.04 µg/mL	22.52 µg/mL±0.846
Water	337.99±5.083 µg/mL	47.66 µg/mL±1.33

Antiglycation and Glucosidase Inhibitory Activities

Antiglycation activity was measured by increasing the concentration of seed extracts to inhibit glycation. The percentage inhibition of the extracts was plotted against the extract concentrations and IC_{50} values were calculated using the linear regression equation of the plot. This study is the first to demonstrate the anti-glycation activity of hawthorn seeds, which are high in polyphenols. Upon comparison of all extracts with the reference aminoguanidine for the prevention of AGE (advanced glycation end products) formation, it was observed that ethanol (69.99 µg/mL) and methanol (66.25 µg/mL) extracts exhibited higher efficiency in preventing glycation than aminoguanidine (70.03 µg/mL). The antiglycation efficiency of the distilled water extract of *C. monogyna* seed (453.74 µg/mL) was substantially lower (Fig. 1). Although aminoguanidine serves as a prototype anti-AGE agent approved for the treatment of certain diabetic complications, it also exhibits significant adverse effects. Consequently, alternative glycation inhibitors are required [40]. Therefore, *C. monogyna* seeds should be considered for the discovery of new antiglycation agents.

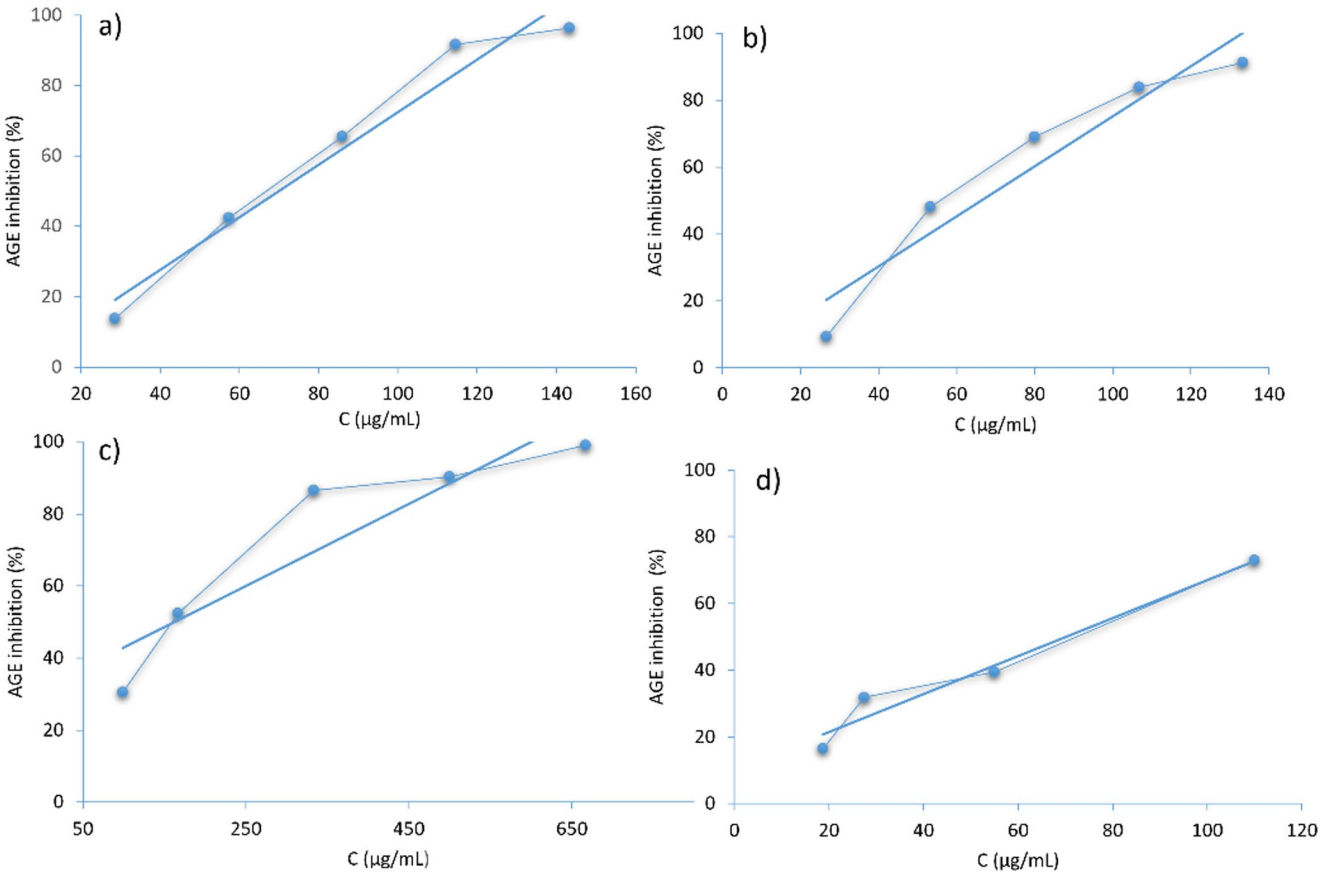

Fig. 1 AGE inhibition activities of *C. monogyna* seed extracts (**a**) Ethanol, (**b**) Methanol, (**c**) Water, (**d**) Aminoguanidine (control)

Targeting enzymes is one way to treat diabetes. Each enzyme related to pathology acts via a different mechanism for the progression of the disease. In this study, we analyzed the potential of *C. monogyna* seed extracts to inhibit dipeptidyl peptidase IV (DPP-IV) and α-glucosidase (Fig. 2). DPP- IV inhibitors are an important class of drugs that inhibit DPP- IV enzymes keeping glucagon-like peptide-1 (GLP-1) intact to maintain blood glucose levels [41]. To the best of our knowledge, there are no published studies on DPP-IV inhibition by hawthorn species. According to our findings, water extraction resulted in the highest inhibitory activity (IC_{50}: 0.21 mg/mL), but it still fell far away from the reference drug diprotin A (IC_{50}: 9.54 µg/mL) [42]. Similarly, hawthorn seed water extraction gave the highest inhibition (IC_{50}: 85.23 µg/mL) against α-glucosidase compared to ethanol and methanol extraction. The IC_{50} value of the reference drug for α-glucosidase inhibition, acarbose was measured as 1.39 ± 0.23 mg/mL in a previous work we published before [43].

Molecular Docking and Molecular Dynamics Simulations

The binding affinities of the selected ligands with their respective target proteins were calculated using AutoDock4, and the results are presented in Table 5. The binding energies (kcal/mol) reflect the strength of interaction between the ligands and the active site residues of the proteins. Lower binding energy values indicate stronger interactions. For DPP-IV, the control ligand diprotin A demonstrated a binding energy of -6.95 kcal/mol and among the tested ligands, Procyanidin A2 displayed a comparable binding energy of -7.9 kcal/mol, indicating a potential for strong inhibitory activity. As for α-glucosidase, the control ligand acarbose exhibited a binding energy of -5.91 kcal/mol, serving as a benchmark for comparison with the test ligands. Among the test compounds, Procyanidin B1 showed a remarkable binding with a ΔG of -10.92 kcal/mol, suggesting a high affinity for the active site. This finding is further supported by prior experimental evidence reported in the literature, which demonstrates that procyanidins act as an inhibitor of DPP-IV, confirming its potential as a therapeutic candidate [44]. The docking results align with these experimental

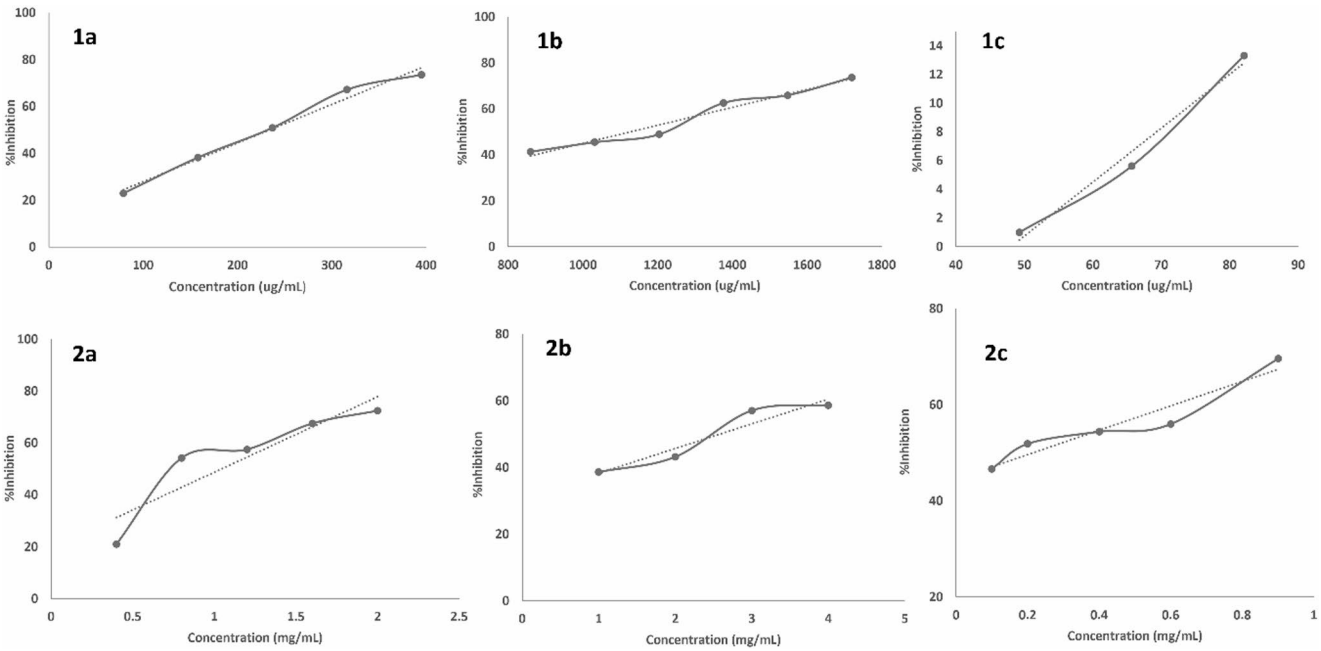

Fig. 2 Enzyme inhibition acitivities of *C. monogyna* seed extracts (1:Inhibition of α-glucosidase; 2:Inhibition of DPP-IV; **a**:Methanol; **b**:Ethanol; **c**:Water)

Table 5 Molecular Docking scores (kcal/mol) of ligands for DPP-IV and α-Glucosidase

Compounds	DPP-IV	α-Glucosidase
(+)-Catechin	-7.35	-7.43
Procyanidin C1	-5.94	-0.78
Procyanidin A3	-5.22	-5.87
Procyanidin A2	-7.9	-8.61
3-O-Caffeoylquinic acid	-5.46	-7
5-Caffeylquinic acid	-5.08	-5.53
Procyanidin B1	-6.59	-10.92
Coumarylquinic acid	-5.16	-3.95
Diprotin A	-6.95	-
Acarbose	-	-5.91

observations, highlighting the molecule's high affinity and ability to interact effectively with key residues involved in DPP-IV inhibition.

Molecular docking analysis showed a high affinity of procyanidin A2 and procyanidin B1 for DPP-IV and α-glucosidase, respectively. The binding interactions and stability of the enzyme-ligand complexes were further analyzed by molecular dynamics simulations (Fig. 3). The root mean square deviation (RMSD) of DPP-IV and procyanidin A2 demonstrated a stable complex due to low fluctuations between 1,2 − 1,6 Å. The RMSD of the ligand was close to 1 Å, which supported the stability of the complex RMSF (Root Mean Square Fluctuation) of the protein gave maximum fluctations around 3.6 Å with a medium level flexibility (Supplementary File). The interaction timeline demonstrates significantly stable contacts throughout the 100 ns simulation, with key residues maintaining consistent interactions. The total number of contacts fluctuated between 8 and 16 during the simulation, centered around 12, indicating a stable binding mode. The key residues TRP629, GLN553, and HIS740, which exhibit persistent contact patterns. Notably, TRP629 established a strong π–π stacking interaction with the aromatic ring system of the ligand, which occurred in approximately 69% of the simulation time. The 2D interaction diagram revealed several critical binding features: ASP545 formed water-mediated hydrogen bonds with a 52% occupancy rate; TRP629 exhibited both hydrophobic and π-π stacking interactions; and HIS740 established polar contacts with 92% persistence. Additionally, water bridges play a crucial role in stabilizing the complex. The RMSD values for α-glucosidase increased over time, reaching approximately 3.2 Å for the protein and 5.4 Å for the ligand. This indicates that some conformational changes occurred, potentially leading to a less stable binding interaction. Higher flexibility was observed in the RMSF graph, with peaks reaching 4.0–4.5 Å, particularly in specific loop regions. The average total number of contacts (15–20) indicated a more diverse interaction network involving residues LYS156, SER241, and ASP242. Water-mediated interactions, which play a crucial role in the binding stability, were also observed. According to the 2D ligand interaction diagram, ASP242 formed strong water-mediated hydrogen bonds with a 60–63% occupancy rate, and GLN279 established consistent polar contacts with 57% persistence. TYR158 exhibited π–π stacking interactions

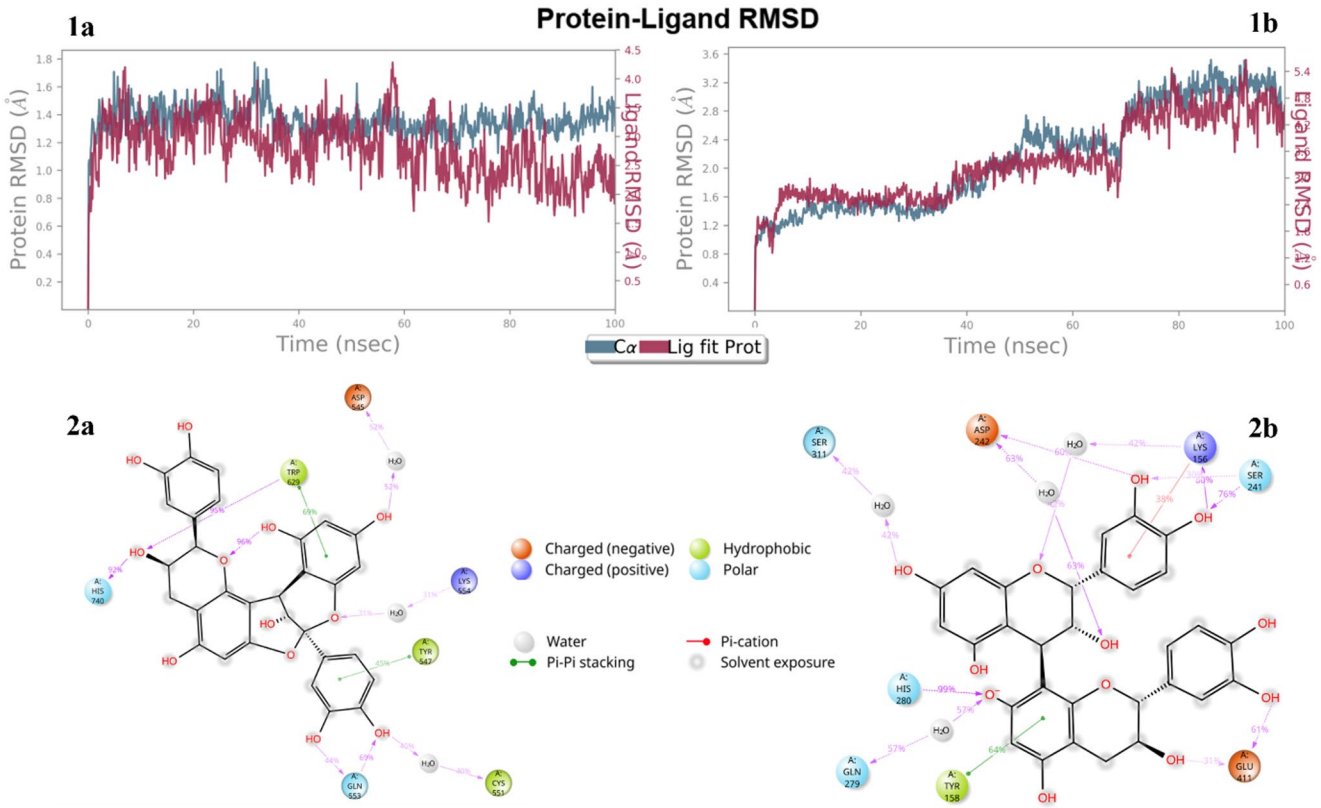

Fig. 3 RMSD graph (1) and 2D interaction diagram (2) for DPP-IV (**a**) and α-glucosidase (**b**) ligand-enzyme-complex

with a 64% occupancy rate. The overall results of the simulations highlighted that the DPP-IV- procyanidin A2 simulation gave a more robust interaction pattern, whereas the α-glucosidase-procyanidin B1 simulation exhibited a more dynamic behavior. Despite the movement of both the protein backbone and ligand molecule, the interaction pattern remained conserved, particularly for three amino acid residues: LYS156, SER241, and ASP242. These residues appeared to play a crucial role in ligand binding by engaging in specific interactions that contribute to the overall stability of the complex.

Conclusion

This study highlights the potential of *Crataegus monogyna* (hawthorn) seed extracts as a valuable source of bioactive compounds with antidiabetic properties, while also addressing the increasing need for sustainable waste recovery in the face of a growing global population. As food production and consumption continue to rise, the generation of agricultural and food processing wastes has become a significant environmental and economic challenge. The use of inedible fruit components such as hawthorn seeds offers an innovative approach for converting waste into valuable bioactive resources. Our findings demonstrate that hawthorn seed extracts exhibit significant antioxidant, anti-glycation, and enzyme inhibitory activities, suggesting their potential role in diabetes management. Phytochemical analysis confirmed that the seeds were rich in phenolic compounds and flavonoids. With the increasing burden of diabetes worldwide and the rising need for eco-friendly solutions, the conversion of food waste into health-promoting compounds presents an opportunity for both environmental sustainability and public health improvements. Future studies should focus on in vivo validation and clinical trials to further explore the therapeutic potential of hawthorn seed extract.

Author Contributions Conceptualization, S.S. and E.K.; Experiments, Validation, Formal analysis, Resources, Data curation, S.S.; E.K.; E.M.; and E.G.; C. Manuscript writing—original draft preparation, S.S.;E.K.; E.M.; Writing—editing, E.K.,E.M.; and E.G.; Supervision, Project administration, Funding acquisition; E.K and S: S All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Data Availability All relevant data are within the paper.

Declarations

Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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