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Original Article

Evaluation of Antidiabetic, Neuroprotective and Antihyperpigmentation Effects of *Medicago lupulina* and *Trifolium arvense* by Determining Enzyme Inhibition Activity

Serap Koksal Bellikli,[1] Ismail Sen,[1] Gokhan Zengin,[2] Yavuz Selim Cakmak[1]

[1]Department of Molecular Biology and Genetics, Aksaray University, Aksaray, Türkiye
[2]Department of Biology, Selcuk University, Konya, Türkiye

ABSTRACT

Objective: In today's world where chronic diseases are increasing, research on effective treatment methods continues. Different methods and drug development studies for treatment continue in a multidisciplinary manner. In order to reduce side effects, natural products are the primary choice and are in the first place in research.

Materials and Methods: The present study was undertaken to study enzyme-inhibiting activity of ethyl acetate and methanol extracts from *Trifolium arvense* and *Medicago lupulina* in order to explore these plants for their therapeutic use. Inhibition effects for cholinesterases (acetylcholinesterase and butyrylcholinesterase), tyrosinase, α-amylase and α-glucosidace tests were performed.

Results: The results showed that the methanol extracts of *Medicago lupulina* and *Trifolium arvense* had strong α-glucosidase inhibitory activity with values 568±0.01 mg ACAEs/g extract and 525±4.24 mg ACAEs/g extract. Although both plants showed comparable activity for other enzymes, the ethyl acetate extracts exhibited a slightly higher enzyme inhibition profile than that of methanol extract, especially against α-glucosidase.

Conclusion: The extracts of the plants studied may possess significant therapeutic potential and could be particularly advantageous in the pharmaceutical industry, especially in terms of their inhibition activity on the glucosidase enzyme.

Keywords: Antidiabetic, enzyme inhibitions, *Trifolium arvense*, *Medicago lupulina*, neuroprotective

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Address for correspondence:
Serap Koksal Bellikli.
Department of Molecular Biology and Genetics, Aksaray University, Aksaray, Türkiye
E-mail: serapkoksalctf@gmail.com

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INTRODUCTION

While many biochemical processes in our bodies keep us metabolically active and healthy, enzymes also play an important role. Therefore, although diseases occur as a result of insufficient enzyme activity and therefore involve an enzyme deficiency in the disease process, it can also result in exuberant genesis where there is too much enzyme activity leading to abnormalities. When there is too much enzyme activity, enzyme activity is reduced or eliminated by inhibitory mechanisms. Enzyme inhibitors: These are small chemical compounds that have the ability to inhibit enzymes and reduce their catalytic activity [1]. They act by inhibiting the activity of enzymes, by degrading an enzyme or its cofactors, by causing allosteric changes in enzyme conformation, or by interrupting activation in such a way as to destroy the tissues involved. Because of these effects, enzyme inhibitors can be used as drugs under physiological conditions [2].

The prevalence of disorders of enzyme activity caused by diseases such as diabetes and Alzheimer's has highlighted the search for treatments through enzyme inhibition [3, 4]. Acetylcholine (ACh) is a neurotransmitter present in many pathways of the central and peripheral nervous systems, and it is hydrolyzed by acetylcholinesterase (AChE) to terminate stimulation. Alzheimer's disease affects most of the cells that produce and use ACh, leading to lower production in these neurons and abnormal communication between them. AChE breaks down ACH into choline and acetic acid, so inhibitors of this enzyme (arginine deiminase) prevent the breakdown of ACh, allowing it to remain at higher levels. These are supported by what I have stated above, suggesting that AChE inhibitors reverse the deficit of cholinergic neurotransmission and consequently may reduce cell loss within accepted limitations in some areas [5].

Acetylcholinesterase, an enzyme overexpressed in the brains of people with Alzheimer's disease, metabolizes acetylcholine (ACh) released from presynaptic terminals that remain at synapses. Acetylcholinesterase inhibitors: Research shows that these drugs improve cognition and reduce behavioral symptoms by increasing acetylcholine levels at the synapse [6]. BChE, a serine hydrolase, belongs to the family of esterases that hydrolyze various types of esters in the human body. While BChE is not nearly as potent in the hydrolysis of acetylcholine, it has a broader spectrum of substrates. And the consequences... but more on that later! Some studies have shown that blocking the BChE enzyme will raise acetylcholine in the brain. In the framework of Alzheimer's disease, BChE is an important stabilizing enzyme [7], and causes abnormal accumulation of β-amyloid (Aβ). The researchers believe that the findings indicate that inhibiting BChE could be a treatment for advanced Alzheimer's disease. Furthermore, amylase and glucosidase are essential enzymes for the digestion and metabolism of carbohydrates in the human body. It is crucial to understand the functions of both enzymes to explore their therapeutic properties in diabetes. Amylase is produced and secreted by the salivary glands in the mouth and the pancreas in the small intestine. In the mouth, salivary alpha-amylase starts the carbohydrate digestion process by breaking down starch into smaller polysaccharides and maltose. This process continues in the small intestine under the action of pancreatic alpha-amylase. Amylase is crucial for regulating the rapid rise in blood glucose levels that occurs during food digestion. Glucosidase enzymes facilitate the hydrolysis of complex carbohydrates such as disaccharides and oligosaccharides into simpler sugars such as glucose. Glucosidase inhibitors reduce the activity of glucosidase enzymes, primarily in the small intestine. This slowdown in enzyme activity delays the digestion and absorption of complex carbohydrates, leading to a more gradual increase in blood glucose levels after a meal. Because of these important functions, inhibition of alpha-amylase and alpha-glucosidase enzymes is vital for diabetes management and treatment [8-14].

The enzyme tyrosinase plays a crucial role in the production of melanin in melanocytes through three main reactions in the body. The first pathway involves the conversion of tyrosine to L-DOPA through hydroxylation. In the second pathway, L-DOPA is oxidized to form dopachrome. The third pathway involves the conversion of dopachrome to melanin, which occurs through a series of complex reactions including cyclization and oxidative polymerization [15]. Abnormal melanin production can lead to significant aesthetic problems in humans, particularly in middle-aged and elderly individuals [16]. Common external factors, especially exposure to ultraviolet (UV) light, contribute to pigmentary disorders such as melasma, solar lentigines and freckles [17]. In addition, exposure to certain drugs and chemicals and certain medical conditions can also cause hyperpigmentation. Depigmenting agents, including inhibitors of the enzyme tyrosinase, are frequently used to treat hyperpigmentation disorders [18]. Therefore, the investigation of tyrosinase inhibitors is essential to develop effective treatments for hyperpigmentation disorders.

Recently, the side effects of chemical drugs have led to a detailed study of the enzyme inhibition activities and therapeutic properties of plants used in traditional medicine. Phytotherapy research on medicinal plants and their active ingredients has increased significantly in recent years [19-23]. Many trees, shrubs, and herbaceous plants in the Fabaceae (Leguminosae) family, which are commonly found in temperate, subtropical, and tropical regions, hold considerable economic value [24]. *Medicago lupulina* L. is a perennial herbaceous plant belonging to this family. While it is primarily utilized in the fodder industry like other species of Medicago, it is also valued for its medicinal and ornamental properties. It has low soil requirements and can thrive in cold-resistant, arid, and stony soils [25]. In a study conducted in the Isparta region, it was found that this plant adapts very well and can spread rapidly. It was suggested that it could serve as an alternative to grasses due to this characteristic [26]. Medicago species are highly valued as animal feed because of their beneficial components and high protein content [27]. In some countries, the leaves are used in soups, salads, and sandwiches due to their nutritional value [28]. These plants also enrich the soil around them with nitrogen. Their dense fiber structure, similar to that of other clover species, together with their nitrogen-fixing ability make them notable. They are also known for their antibacterial properties and calming effects on the human body [29].

Trifolium arvense, commonly known as rabbit's foot clover, is a herbaceous clover species that typically grows upright and rises to a height of about 5 to 30 cm over its one-year life cycle. It has purple or pink flowers and leaves divided into three sessile leaflets [30]. This plant is beneficial for poor soils, as it fixes nitrogen, thereby enhancing soil fertility. Native to many parts of Europe and western Asia, excluding polar regions, *Trifolium arvense* is also grazed by goats and sheep. The aerial parts of the plant are harvested during flowering and then air-dried for use. It thrives best in sunny conditions and sandy soils [31]. *Trifolium arvense* has various applications in herbal medicine. It is commonly used to treat gastrointestinal disorders, gout, and rheumatism, as well as for wound care and addressing foot sweating in young children [32].

Our study aimed to investigate the known properties of some plant species as well as their potential medicinal uses in our country and province. We investigated the ability of various extracts to inhibit cholinesterase enzymes associated with Alzheimer's disease, α-amylase and α-glucosidase enzymes linked to diabetes mellitus, and tyrosinase enzymes linked to hyperpigmentation. The results may improve our understanding of the biological effects of chemical compounds found in these plant species. This research can also contribute to basic scientific knowledge in fields such as biochemistry, biotechnology and pharmacology.

MATERIALS AND METHODS

Plant Material

Plant specimens were collected from their natural habitats around Hasandağı, Aksaray, during the 2020 vegetation period. They were dried in the shade and stored in a dark, suitable environment. The plant samples were identified by Dr. Bülent ESKİN and Mustafa KESKİN.

Preparation of Plant Extracts

Extracts are obtained from plant samples using organic solvents. The solvent portion of the extracts is completely evaporated and the extracts are dried before stock solutions are prepared for enzyme inhibition activity analysis at a concentration of 2 mg/ml. Preliminary tests are performed on the prepared stock solutions to determine the relevant concentration ranges for further research. For each plant sample, 15 g of powdered material is transferred into a 200 ml round bottom flask using two different solvents: methanol and ethyl acetate. The samples are then mixed and placed in an ultrasonic bath for extraction, following a protocol of four repetitions lasting 15 minutes each, with a total break of one hour and five minutes between repetitions. After the ultrasonication process is completed, filtration is performed. Evaporation is used to obtain pure plant extracts by removing solvents. To eliminate the remaining water, which is also a solvent, the lyophilization method is used. Upon completion of these methods, extracts from both plants are obtained using two different solvents.

Enzyme Inhibition Tests

Cholinesterase inhibition

Cholinesterase (ChE) inhibitory activity was measured using the Ellman method [33]. A sample solution (50 µL) was combined with DTNB (125 µL) and either acetylcholinesterase or butyrylcholinesterase solution (25 µL) in a Tris-HCl buffer at pH 8.0. The mixture was incubated for 15 minutes at 25°C in a 96-well microplate. The reaction was initiated by adding acetylcholine iodide or butyrylcholine chloride. Additionally, a control tube without the enzyme was prepared for each extract. After further incubation at 25°C for 10 minutes, the absorbances of the sample and control were measured at 405 nm. The cholinesterase inhibitory activity is expressed as galantamine equivalent (GALAE).

α-amylase inhibition

Alpha-amylase inhibitory activity was assessed using the Caraway-Somogyi iodine/potassium iodide (IKI) method [34]. In a 96-well microplate, 25 µL of sample solutions were mixed with 50 µL of α-amylase solution in phosphate buffer (pH 6.9, 6 mM sodium chloride). The mixture was incubated at 37°C for 10 minutes. Following the pre-incubation, the reaction was initiated by adding 50 µL of starch solution (0.05%). An enzyme-free blank solution was prepared in the same way. The reaction mixture was incubated at 37°C for an additional 10 minutes, and the reaction was halted by adding 25 µL of 1 M HCl. Afterward, 100 µL of iodine-potassium iodide solution was added. The absorbance of both the sample and the blank was measured at 630 nm. The results of α-amylase inhibitory activity were expressed as acarbose equivalent (ACAE).

α-glucosidase inhibition

α-glucosidase inhibitory activity was determined using the methodology proposed by Palanisamy et al. [35]. A solution of the sample, glutathione, α-glucosidase solution, phosphate buffer, and PNPG solution was prepared and transferred to a 96-well microplate, which was then incubated at 37°C for 15 minutes. Similarly, an enzyme-free blind was prepared. The reaction was terminated by the addition of sodium carbonate (50 µL, 0.2 M). The absorbances of the sample and blind were then measured at 400 nm. The α-glucosidase inhibitory activity was expressed as acarbose equivalent (ACAE).

Tyrosinase inhibition

The inhibitory activity of tyrosinase was determined using the dopachrome method with L-DOPA as the substrate [36]. A solution of the sample (25 µL) was combined with a solution of the tyrosinase (40 µL) and phosphate buffer (100 µL, pH 6.8) in a 96-well microplate and incubated for 15 minutes at 25°C. L-DOPA (40 µL) initiated the reaction. An enzyme-free blind solution was prepared. After 10 minutes at 25°C, the sample and blind absorbances were read at 492 nm. Tyrosinase inhibitory activity is expressed as kojic acid equivalents (CAE).

RESULTS

Enzyme Inhibition Activity

Thanks to the discovery of new enzyme inhibitors, global health problems such as Alzheimer's disease, diabetes mellitus and hyperpigmentation can be treated with the enzyme inhibition approach. Enzyme inhibition is being used to treat global health problems such as Alzheimer's disease, diabetes mellitus and neurodegenerative diseases that are difficult to treat. It is possible to treat Alzheimer's disease with acetylcholinesterase and butyrylcholinesterase inhibitors, skin hyperpigmentation with tyrosinase inhibitors, diabetes with alpha-amylase and alpha-glycosidase inhibitors [37]. In our study, enzyme inhibition activities of ethyl acetate and methanol extracts of *Trifolium arvense* and *Medicago lupulina* were evaluated (Table 1, Fig. 1 and Fig. 2).

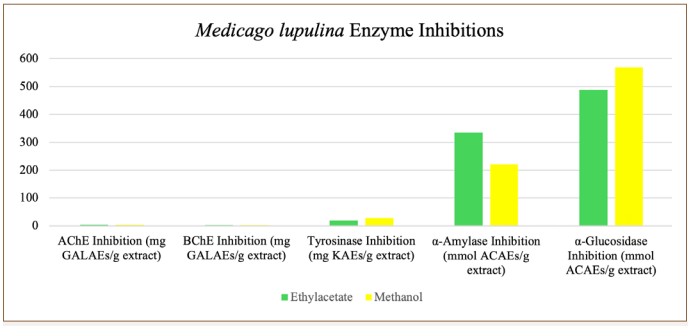

Figure 1. Enzyme inhibition activities of Medicago lupulina extracts.

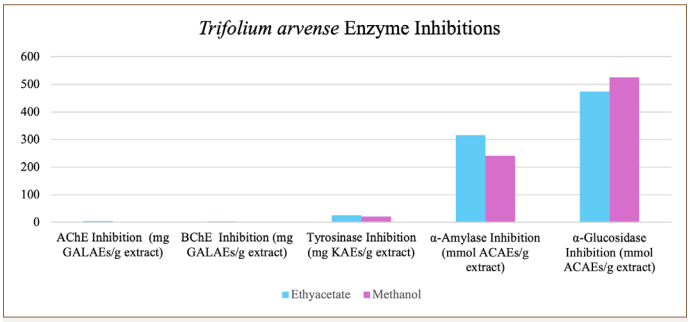

Figure 2. Enzyme inhibition activities of Trifolium arvense extracts.

Cholinesterase inhibitions (acetylcholinesterase and butyrilcholinesterase) of extracts

Most current treatments for Alzheimer's disease are based on the cholinergic hypothesis, which involves the breakdown of cholinergic neurons and the rapid hydrolysis of the neurotransmitter acetylcholine (ACh) by cholinesterases, which are the major causes of various AD symptoms. The human brain contains two types of cholinesterases: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Inhibiting these enzymes plays an important role in ameliorating cognitive deficits by blocking ACh hydrolysis. Therefore, it is very important to search for natural sources that can inhibit these enzymes [38].

In our study, when the ethyl acetate and methanol extracts prepared from *Trifolium arvense* species were investigated in terms of cholinesterase enzyme inhibition, 3.48±0.06 mg GALAEs/g extract activity in the ethyl acetate extract of

Table 1. Enzyme inhibitions activity Medicago lupulina and Trifolium arvense of extracts

Extracts/Assays	AChE Inhibition (mg GALAEs/g extract)	BChE Inhibition (mg GALAEs/g extract)	Tyrosinase Inhibition (mg KAEs/g extract)	α-amylase Inhibition (mmol ACAEs/g extract)	α-glucosidase Inhibition (mmol ACAEs/g extract)
Medicago lupulina-EA	3.48±0.01*	5.76±0.09*	18.66±1.23*	334.99±2.40*	488±3.54*
Medicago lupulina-MeOH	3.31±0.11	1.30±0.01	27.84±0.07	221.21±0.97	568±0.01
Trifolium arvense-EA	3.48±0.06*	3.21±0.08*	24.95±0.46*	316.02±4.87*	474±7.07*
Trifolium arvense-MeOH	Na	0.62±0.02	20.97±0.40	241.35±0.28	525±4.24

*Average of three paralel analysis±standard deviation; AChE: Acetylcholinesterase; BChE: Butyrilcholinesterase; GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalen; EA: Ethyl acetate; MeOH: Methanol.

Trifolium arvense for acetylcholinesterase and no activity was detected in the methanol extract. When butyrylcholinesterase activities were analyzed, 3.21±0.08 mg GALAEs/g extract activity was detected in the ethyl acetate extract of *Trifolium arvense* and 0.62±0.02 mg GALAEs/g extract activity was detected in the methanol extract.

The investigation into the inhibition of *Medicago lupulina* against cholinesterases yielded the following results: the inhibition against acetylcholinesterase was determined to be 3.48±0.01 mg GALAEs/g extract in ethyl acetate extract and 3.31±0.11 mg GALAEs/g extract in methanol extract. The butyrylcholinesterase activities were analyzed, and the results indicated that 5.76±0.09 mg GALAEs/g extract of the ethyl acetate extract and 1.30±0.01 mg GALAEs/g extract of the methanol extract exhibited activity. The ethyl acetate extracts of both samples demonstrated comparable activity concerning acetylcholinesterase. The ethyl acetate extract of *Medicago lupulina* exhibited the highest level of activity

α-amylase and α-glucosidase inhibitions of extracts

The basic premise of enzyme inhibition, a contemporary strategy for the effective management of diabetes mellitus, is to reduce diet-associated hyperglycemia by inhibiting the digestion of ingested carbohydrates through inhibition of carbohydrate hydrolysis enzymes. Pancreatic α-amylase represents an important therapeutic target within the digestive tract and facilitates the initial hydrolysis of starch to maltose. This is then converted to glucose by α-glucosidases. Accordingly, the delay of starch hydrolysis achieved by reducing the action of α-amylase plays a crucial role in regulating the glucose rise in postprandial hyperglycemia [47].

From this point of view, the identification of inhibitors that can inhibit α-amylase and α-glucosidase enzymes is a very important discovery. In this context, the objective of our study was to determine the ability of different extracts prepared from *Trifolium arvense* and *Medicago lupulina* species to inhibit α-amylase and α-glucosidase enzymes, which are enzymes involved in glucose metabolism. The data obtained from the study indicated that the α-amylase enzyme inhibition of the extracts prepared from *Trifolium arvense* species was 316.02±4.87 mmol ACAE/g extract for the ethyl acetate extract and 241.35±0.28 mmol ACAE/g extract for the methanol extract, respectively. Furthermore, the α-glucosidase inhibition was found to be 474±7.07 mmol ACAEs/g extract for the ethyl acetate extracts of T. arvense and 525±4.24 mmol ACAEs/g extract for the methanol extracts. The α-amylase inhibition of the species *Medicago lupulina* was determined to be 334.99±2.40 mmol ACAEs/g extract for the ethyl acetate extracts and 221.21±0.97 mmol ACAEs/g extract for the methanol extracts. Upon analysis of the α-glucosidase inhibition results for the M. lupulina species, the following values were determined: 488±3.54 mmol ACAEs/g extract activity for ethyl acetate extracts and 568±0.01 mmol ACAEs/g extract activity for methanol extracts.

Tyrosinase inhibition of extracts

Tyrosinase is a crucial enzyme involved in the biosynthesis of melanin in a diverse range of organisms, including bacteria, mammals, plants, and fungi. It is well documented that the melanin enzyme protects the skin from UV damage. However, its overexpression can result in the formation of freckles, mottled pigmentation, skin cancer, and age spots. The use of tyrosinase inhibitors is of great importance in the pharmaceutical and cosmetic industries, as they have preventive effects on skin aging and pigmentation disorders. Flavonoids, a class of plant secondary metabolites, possess metal-chelating capabilities due to their polyhydroxy phenolic structure, which enables interaction with copper ions within the active site of tyrosinase. Accordingly, they represent a promising class of compounds for the discovery of tyrosinase inhibition [53]. In light of the aforementioned evidence, this study was designed to investigate the inhibitory activity of the tyrosinase enzyme.

The present study investigated the tyrosinase inhibition activities of different extracts prepared from the *Medicago lupulina* and *Trifolium arvense* species. The results of the study indicated that the tyrosinase enzyme inhibition activity of the *Medicago lupulina* species was 18.66±1.23 mg KAEs/g extract in the ethyl acetate extract and 27.84±0.07 mg KAEs/g extract in the methanol extract, respectively. The inhibitory effect of *Trifolium arvense* tyrosinase was found to be 24.95±0.46 mg KAEs/g extract in the ethyl acetate extract and 20.97±0.40 mg KAEs/g extract in the methanol extract, respectively.

DISCUSSION

Cakmak et al. [39], the enzyme inhibition activity for acetylcholinesterase and butyrylcholinesterase for the methanol extract of *Medicago rigidula* was found to be 2.05±0.08 and 0.94±0.11 mg GALAEs/g extract, respectively. In a separate study, Pamukçu et al. [40] quantified the acetylcholinesterase inhibition activity of methanol, ethyl acetate, and water extracts derived from the *Medicago murex* species. The respective inhibition activities were determined to be 1.84±0.06 mg GALAEs/g extract, 1.17±0.14 mg GALAEs/g extract, and 2.02±0.16 mg GALAEs/g extract. The anti-acetylcholinesterase activity of the extract prepared from *Sophora mollis* leaf parts by Fatima et al. [41] demonstrated the highest inhibition percentage (114.60%±19.95%) at the highest concentration of 1000 µg/mL. Furthermore, the activity of the Sophora mollis, *Mucuna*

pruriens and *Indigofera atropurpurea* species was recorded as follows: *Sophora mollis* (IC50 value 75.96 µg/mL) > *Mucuna pruriens* (IC50 value 508.20 µg/mL) > *Indigofera atropurpurea* (IC50 value 560 µg/mL). Ahmad et al. [42] investigated the acetylcholinesterase inhibition activity of n-hexane (Tr.Hex), chloroform (Tr.Chf), ethanol (Tr.Et), methanol (Tr.Cme), and water (Tr.Aq) extracts of *Trifolium repens*. The enzyme inhibition values for Chf were found to be 82.4±0.57%, 77.9±2.24%, 72.1±0.47%, 67.9±0.96% and 62.3±0.57% at concentrations of 1000, 500, 250, 125 and 62.5 µg/ml, respectively. The IC50 value was established at 21 µg/mL, indicating that Tr.Chf exhibited the most pronounced inhibitory effect among the tested samples. In the same study, the butyrylcholinesterase inhibitor was also examined. The Tr.Chf and Tr.Et extracts of the plant demonstrated 86.9±1.30% and 77.5±0.49% inhibition, respectively, at the highest concentration of 1 mg/mL. The IC50 values were calculated as 15 and 125 µg/mL for Tr.Chf and Tr.Et, respectively. Eruygur et al. [43], the highest value of acetylcholinesterase activity was observed at 561.29 IC50 (µg/mL) in the pre-flowering leaves of the plant, which were collected at different growth stages of *Medicago sativa*. The butyrylcholinesterase activity of the extracts prepared from the flower part was found to be the highest, with an IC50 value of 1794.77 (µg/mL). In the study conducted by Edrizi et al. [44] with methanol extracts of *Marrubium alysson and Peganum harmala*, the most active butyrylcholinesterase activity was observed in the methanol extract of *Marrubium alysson* at a concentration of 1 mg/ml and at 37°C, with inhibitory activity of 72.5±2.07%. The methanol extracts from *Peganum harmala* demonstrated an inhibitory effect of 50.53±1.98%. At a concentration of 10 µg/ml and a temperature of 37°C, the methanol extracts of *Marrubium alysson* and *Peganum harmala* demonstrated an inhibitory effect on butyrylcholinesterase activity, with an inhibition percentage of 38.23±2.45% and 27.35±1.54%, respectively. In their study, Alhawari et al. [45] employed the use of *Cassia timoriensis* and *Cassia grandis* plants. The flower parts of *C. timoriensis* were subjected to a series of extractions, namely with water, ethyl acetate, methanol, and n-hexane. Extracts were prepared from the bark of *C. grandis* using four different solvents: water, ethyl acetate, methanol and n-hexane. The inhibitory effects of the extracts on acetylcholinesterase were examined, and the findings revealed that the n-hexane extract exhibited an inhibition rate of 47.70±0.35%, the water extract demonstrated an inhibition rate of 51.23±0.07%, the methanol extract displayed an inhibition rate of 83.90±0.75%, and the ethyl acetate extract exhibited an inhibition rate of 86.19±1.75%. Al-Hmadi et al. [46], the inhibitory effects of chloroform, ethyl acetate, and methanol extracts derived from the aerial and subterranean parts of the *Glycyrrhiza foetida* species on the activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were investigated. The findings of the study revealed that the chloroform extract exhibited the highest AChE activity with a value of 2.23±0.11 mg GALAE/g, while the ethyl acetate extract showed the highest BChE activity with a value of 3.39±0.79 mg GALAE/g. When the root parts were analyzed, the ethyl acetate extract exhibited the highest AChE inhibition activity with a value of 2.29±0.20 mg GALAE/g. Similarly, the chloroform extract showed the most pronounced BChE inhibition activity with a value of 3.75±0.30 mg GALAE/g.

Cakmak et al. [39], the α-amylase enzyme inhibition activity was determined to be 0.73±0.04 mmol ACAEs/g extract, while the α-glucosidase enzyme inhibition activity was found to be 1.30±0.09 mmol ACAEs/g extract for a methanol extract prepared from the above-ground parts of the *Medicago rigidula* species. Pamukcu et al. [40] examined the α-amylase and α-glucosidase inhibitory effects of methanol, ethyl acetate, and water extracts derived from the above-ground parts of *Medicago murex* species. The study revealed that the ethyl acetate extract exhibited the highest inhibitory activity against both α-amylase and α-glucosidase, with IC_{50} values of 1.01±0.02 mmol ACAE/g extract and 6.34±0.17, respectively. In a separate study, Ceylan et al. [48] examined the α-amylase and α-glucosidase inhibitory properties of ethyl acetate and water extracts derived from *Lathyrus czeczottianus* species. They determined that the ethyl acetate extract exhibited α-amylase inhibitory activity with an IC_{50} value of 0.34±0.01 mmol ACAEs/g extract, while the water extract demonstrated α-amylase inhibitory activity with an IC_{50} value of 0.14±0.01 mmol ACAEs/g extract. Concerning α-glucosidase inhibition, the findings were presented as 1.56±0.13 mmol ACAEs/g extract for the ethyl acetate extract and 6.49±0.09 mmol ACAEs/g extract for the water extract. Llorent-Martinez et al. [49], investigated the α-amylase and α-glucosidase inhibitory properties of methanol extracts derived from the above-ground components of the *Lathyrus nissolia* species. The study yielded the following determinations: α-amylase inhibition was found to be 3.66±0.06 µg/ml, while α-glucosidase inhibition was determined to be 1.01±0.01 µg/ml. Ashafa et al. [50] investigated the α-amylase and α-glucosidase inhibition properties of a range of extracts prepared from the shell and seed parts of the *Lessertia montana* species. The ethanol extract exhibited the highest α-amylase inhibition activity, with a concentration of 1815±247.80 µg/ml in the bark part and 1191±10.46 µg/ml in the aqueous extract in the seed part. About the α-glucosidase inhibition of the extracts, the aqueous extract from the shell part exhibited the highest activity, with a value of 2609±47.54 µg/ml, while the aqueous extract from the seed part demonstrated the highest activity, with a value of 2947±223.20 µg/ml. Tundis et al. [51] investigated the α-amylase and α-glucosidase inhibition of ethanol extracts

prepared from the flower parts of *Trifolium pratense* and *Trifolium repens* species. The results demonstrated that all extracts exhibited hypoglycaemic activity in a concentration-dependent manner. The α-amylase inhibition of *T. repens* extracts was determined to be 25.0±2.9 µg/mL, while the α-glucosidase inhibition was found to be 69.5±3.1 µg/mL. For *T. pratense* extracts, the α-amylase inhibition was observed to be 78.7±2.1 µg. Thilak et al. [52], the inhibitory potential of α-amylase and α-glucosidase enzymes was evaluated in acetone, methanol, and water extracts prepared from the leaf parts of the *Senna alata* species at varying concentrations (20, 40, 60, and 80 µg/ml). The study yielded the following results for α-amylase inhibition: 59.88±1.40% for acetone, 27.74±0.30% for methanol, and 13.53±1.23% for water at an 80 µg/ml concentration. The highest results for α-glucosidase inhibition were 21.23±0.60% for the acetone extract, 82.940±0.157% for the methanol extract and 81.326±0.738% for the water extract at an 80 µg/ml concentration, respectively.

Cakmak et al. [39], the tyrosinase enzyme inhibition activity was determined to be 16.36±0.85 mg KAEs/g extract for the methanol extract prepared from the above-ground parts of the *Medicago rigidula* species. Pamukcu et al. [40] investigated the tyrosinase enzyme inhibition ability of ethyl acetate, methanol, and water extracts prepared from the above-ground parts of *Medicago murex* species. The findings indicated that the tyrosinase inhibition ability ranged from 4.23±0.56 mg KAE/g extract to 28.29±1.02 mg KAE/g extract. Al-Hmadi et al. [46] detected the tyrosinase inhibition ability of chloroform, ethyl acetate and methanol extracts prepared from the aboveground and root parts of the *Glycyrrhiza foetida* species. The study concluded that all extracts demonstrated the potential to inhibit the tyrosinase enzyme, with a range of 52.84±3.10 to 80.34±1.98 mg KAE/g. Maneeachi and Rinthong [54] investigated the tyrosinase inhibitory activities of methanol, dichloromethane, and water extracts derived from the root, stem and seed parts of the *Cajanus cajan* plant. The findings demonstrated that the extracts exhibited tyrosinase inhibitory activity, with IC_{50} values spanning a range of 3.55 to 12.43 mg/ml. Thummajitsakul et al. [55] examined the tyrosinase inhibitory activity of extracts prepared from *Vigna mungo* (L.) species. The study yielded the following results: the ethanol extract exhibited tyrosinase inhibition at a concentration of 22.02±6.74 mg/ml, while the water extract demonstrated this activity at a concentration of 398.86±243.29 mg/ml.

CONCLUSION

Both plants are widely cultivated in our country and are preferred as animal feed in regions where animal husbandry is widespread. They are also preferred as protein sources in human nutrition today. In addition, our findings indicate that the high level of inhibition activity observed in both enzymes associated with diabetes mellitus is worthy of further investigation. It is suggested that these plant extracts can be used in the pharmacological treatment of diabetes mellitus by utilizing their secondary metabolites.

DECLARATIONS

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