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α-amylase, α-glucosidase, tyrosinase, acetylcholine esterase enzyme inhibition properties and essential oil composition of *Thermopsis turcica* Kit Tan, Vural&Küçüködük

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ABSTRACT

Thermopsis turcica Kit Tan, Vural & Küçüködük is a member of the legume family (Fabaceae), which has many plant species with economic value. It is one of the most narrowly distributed endemic plant species in Turkey. The most important feature that distinguishes *Thermopsis turcica* from other legume species is that it forms 3 fruits in one flower. α-amylase, α-glucosidase, acetylcholine esterase and tyrosinase inhibitory properties of different extracts and essential oil content of *Thermopsis turcica* were determined. Inhibition activities of enzymes were determined α-amylase using Caraway-Somoygi iodide/potassium iodide method, α-glucosidase with Palanisamy method, acetylcholine esterase enzyme with Ellman method and tyrosinase using dopachrome method. Hydrodestilation process (HD) was applied by using Clevenger device to determine the amount and components of essential oils and analyzed in GC-MS. The highest content of plant fatty acid was palmitic acid (51.8 %), myristic acid (17.5 %) and hexahydrofarnesyl acetone (5.3 %). It was determined that the plant did not show inhibitory activity against acetyl choline esterase and tyrosinase enzymes. Acetone and diethyl ether extracts have been shown to inhibit α-amylase. In particular, the diethyl ether extract has been found to exhibit an inhibitory activity close to the standard substance acarbose. Methanol and acetone extracts of the plant have a high inhibitory effect on the α-glucosidase enzyme. It is important with studies on the antidiabetic effects of this valuable species, which is in danger of extinction.

Keywords:
Thermopsis turcica,
Enzyme inhibition,
α-amylase,
α-glucosidase,
Tyrosinase,
Acetylcholine esterase

Thermopsis turcica Kit Tan, Vural&Küçüködük'ün α-amilaz, α-glukozidaz, tirozinaz, asetilkolin esteraz enzim inhibisyon özellikleri ve uçucu yağ bileşimi

ÖZET

Thermopsis turcica Kit Tan, Vural & Küçüködük, ekonomik değeri olan birçok bitki türüne sahip baklagil ailesinin (Fabaceae) bir üyesidir. Türkiye'de en dar yayılış gösteren endemik bitki türlerinden biridir. *Thermopsis turcica*'yı diğer baklagil türlerinden ayıran en önemli özelliği bir çiçekte 3 meyve oluşturmasıdır. *Thermopsis turcica*'nın farklı ekstraktlarının α-amilaz, α-glukozidaz, asetilkolin esteraz ve tirozinaz inhibe edici özellikleri ve uçucu yağ içeriği belirlendi. Caraway-Somoygi iyodür/potasyum iyodür yöntemi ile α-amilaz, Palanisamy yöntemi ile α-glukozidaz, Ellman yöntemi ile asetilkolin esteraz enzimi ve dopakrom yöntemi ile tirozinaz ile enzimlerin inhibisyon aktiviteleri belirlendi. Uçucu yağların miktarı ve bileşenlerinin belirlenmesi için Clevenger cihazı kullanılarak hidrodestilasyon işlemi (HD) uygulanmış ve GC-MS'de analiz edilmiştir. En yüksek bitki yağ asidi içeriği palmitik asit (% 51,8), miristik asit (% 17,5) ve hekzahidrofarnesil aseton (% 5,3) dir. Bitkinin, asetil kolin esteraz ve tirozinaz enzimlerine karşı inhibitör aktivite göstermediği belirlendi. Aseton ve dietil eter özütlerinin α-amilazı inhibe ettiği gösterilmiştir. Özellikle, dietil eter özütünün, standart madde akarbozuna yakın bir önleyici aktivite sergilediği bulunmuştur. Bitkinin metanol ve aseton ekstraktları, α-glukozidaz enzimi üzerinde yüksek inhibitör etkiye sahiptir. Yokolma tehlikesiyle karşı karşıya olan bu değerli türün antidiyabetik etkileri üzerine yapılacak çalışmalar önemlidir.

Anahtar kelimeler:
Thermopsis turcica,
Enzim inhibisyonu,
α-amilaz,
α-glukozidaz,
Tirozinaz,
Asetilkolin esteraz

1. Introduction

Legumes play an important role in meeting people's food needs by growing in harsh conditions, while bacteria that bind the nitrogen of the air to the soil are found in their roots. *Thermopsis turcica* is a perennial, herbaceous, long rhizome, yellow flower, upright plant that can grow up to 30-89 cm. Its leaves are trifoliate, flowers are at the tip, clustered and gives fruits with 3-10 seeds. It is located only on the southern coasts of Akşehir and Eber lakes in the world (Aksoy and Suyundikov, 2020). *Thermopsis turcica* is the only species of Thermopsis genus in the Thermopsideae tribus in terms of taxanomics of the Fabaceae (Legumes) family. *Thermopsis turcica* is in the CR (Critically Endangered) hazard category (Ekim et al., 2000). The most important feature that distinguishes *Thermopsis turcica* from other legume species is that it forms 3 fruits in one flower. This feature has not been found in any of the legumes with around 18,000 species (Cenkci et al., 2012).

Nowadays, the trend towards plant treatment continues increasingly. Herbal medicines are preferred because they are inexpensive and easily available, are the precursors of synthetic drugs, and are models for synthetic drugs. In addition, herbal medicines have a greater and synergistic effect. The therapeutic properties of plants are due to the chemicals in their natural structure and called secondary compounds and the derivatives of these chemicals (Karaşin 2011).

Diabetes mellitus is a metabolic disease caused by the inability of the pancreas to produce sufficient insulin or the inability of the body to use the insulin produced effectively. The use of various inhibitors that reduce the digestion of carbohydrates are used in the treatment of diabetes. Recent studies have been on hypoglycemic foods rich in α-amylase and α-glucosidase inhibitors. It is evaluated in bioactive compounds that make food less digestible and provide less energy by inhibiting the activity of digestive enzymes by carbohydrates. Bioactive phytochemicals found in plants affect the digestibility of starch and glycemic index in the gastrointestinal system as natural enzyme inhibitors. It is known that especially polyphenolic compounds modulate nutrient absorption by inhibiting α-amylase and α-glucosidase enzymes involved in starch digestion (Gonçalves et al., 2011; Tucci et al., 2010). Neurodegenerative diseases are irreversible, progressive and neurological disorders that occur due to high rates of brain damage. Although the trend in the treatment of neurodegenerative diseases is to prevent amyloid β-peptide-induced plaque formation, cholinesterase inhibitors have also been frequently used in treatment in recent years. It is known that the use of cholinesterase inhibitors is palliative in the treatment of the disease. Tyrosinase is an enzyme whose main function is the synthesis of melanin. Tyrosinase inhibitors are used as whitening or antihyperpigment agents because they suppress dermal melanin production. Tyrosinase enzyme also plays an important role in the formation of neuromelanin in the human brain. Neuromelanine formation can cause dopamine neurotoxicity and neurodegeneration associated with Parkinson's disease. Tyrosinase inhibitors are also widely used in the treatment of neurodegeneration (Kim and Uyama, 2005).

It was aimed to determine the inhibitory properties of α-amylase, α-glucosidase, tyrosinase, acetylcholine esterase enzymes and essential oil content of *Thermopsis turcica* in this study.

2. Material and Methods

2.1 Plant Material

The plant was collected from the Eber Lake (31 ° 14 'East and 38 ° 36' North) in Afyon province in May-August 2019 for the research. The plant has been collected during flowering and fruiting periods in order to identify the plant. It was identified by Prof. Dr. Mustafa Kargıoğlu.

2.2 Preparation of Plant Extract

Mixtures of the *Thermopsis turcica* parts such as flower, leaves, branches and stems are used. It was cut into small pieces and dried in the shade at room temperature. Ethyl acetate (TTEA), water (TTW), methanol (TTM), diethyl ether (TTDE), hexane (TTH) and acetone (TTA) were chosen as solvents to prepare the extracts. The extracts obtained with the Soxhelet device were filtered through a filter paper and the solvents were removed with a rotary evaporator. The extracts prepared were stored in capped, dark colored glass bottles at +4 ºC and enzyme inhibition activities were determined.

2.3 Enzyme Inhibition Analysis

The α-amylase enzyme inhibition activity of the plant was determined by the Caraway-Somogyi iodide/potassium iodide (IKI) method (Yang et al., 2012). Phosphate buffer was added to the stock extract solution and then α-amylase solution was added. 10 minutes pre-incubation was applied. Starch solution was added first and then HCl was added. The absorbances were read at 630 nm by adding a color separator prepared from potassium iodide/iodine crystals. The α-amylase enzyme carries out its activity by hydrolyzing the α-(1→4) bonds of the molecule of starch, which is its substrate. Acarbose was used as the standard substance. The standard curve was drawn by applying the same procedures to the solutions of acarbose at different concentrations (50 µmol mL, 100 µmol mL^{-1}, 250 µmol mL^{-1}, 500 µmol mL^{-1} and 1000 µmol mL^{-1}). α-amylase inhibitory activities were calculated as acarbose equivalent (mmol ACAE/g extract) and % amylase inhibition.

The plant's α-glucosidase enzyme inhibition activity was determined by Palanisamy et al., (2011). The extract solutions, α-glucosidase, glutathione, and p-NPG (4-Nitrophenyl β-D-glucuronide) prepared in potassium buffer were incubated for 15 minutes at 37 ° C. It is a p-nitrophenyl colored compound and gives absorbance at 400 nm. Likewise, enzyme-free blanks and control samples containing solvents instead of samples were prepared. The reaction is stopped by adding Na_2CO_3. Acarbose was used as the standard substance. The standard curve was drawn by applying the same procedures to the solutions of acarbose at different concentrations (50 µmol mL^{-1}, 100 µmol mL^{-1}, 250 µmol mL^{-1}, 500 µmol mL^{-1} and 1000 µmol mL^{-1}). α- glucosidase inhibitory activities were calculated as acarbose equivalent (mmol ACAE/g extract) and % glucosidase inhibition (Palanisamy et al., 2011)

Ellman method was applied to determine the AChE enzyme inhibition activity of the plant. The enzyme converts acetyl thiocholine to thiocholine. Thiocholine reacts with DTNB (5,5-dithio-bis-2-nitrobenzoic acid) to form yellow colored TNB (5-thio-2-nitrobenzoic acid). The absorbance of the TNB is measured. AChE enzyme solution prepared from DTNB and Tris HCl buffer was added onto the extracts and incubated. Acetyl thiocholine iodide (ATCI) substrate was then added and incubated at room temperature for 10 minutes. Absorbances were measured at 405 nm (Ellman et al., 1961). Galantamine was used as the standard substance. The standard curve was drawn by applying the same processes to the solutions of Galatamine at different concentrations. Acetylcholine esterase inhibitory activity was calculated as galantamine equivalent (mg GALAE/g extract) and % acetylcholine esterase inhibition.

The tyrosinase inhibitory activity was carried out according to the dopachrome method using L-DOPA as a substrate. The extracts were prepared in 50% DMSO. Phosphate buffer and tyrosinase enzyme solution were added on it and incubated. After L-DOPA was added and incubated again for 10 minutes, absorbances were read at 492 nm (Orhan et al., 2012; Çakmak et al., 2017). Kojic acid was used as the standard substance. The standard curve was drawn by applying the same processes to the solutions of kojic acid at different concentrations. Tyrosinase inhibitory activity was calculated as kojic acid equivalent (mg KAE /g extract) and % tyrosinase inhibition. The following formula was used to find% inhibition in all enzyme inhibition assays.

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{extract}) / Abs_{control}] \times 100$$

Absextract: Absorbance of the inhibitor-containing extracts and standard substances.
Abscontrol: Absorbance of the 100% active control without inhibitor.

2.4 Determination of Essential Fatty Acid Amount and Its Components

Hydrodestilation process was applied using Clevenger device to determine the amount and components of essential oils. The plant was enclosed in a glass flask and essential oils were obtained under reflux with distilled water. The amount of essential oil was determined and percentage yields were calculated in terms of mass/mass by using pressurized nitrogen gas. Then the extracts obtained were analyzed in GC-MS (Agilent) with suitable solvents. The Helium (Flow rate: 0.7 mL) was used as carrier gas in the analysis. The column temperature was programmed to increase from 60 ºC to 220 ºC with an increase of 4 ºC $minute^{-1}$, and kept at the same temperature for 10 minutes, then increase again 4 ºC $minute^{-1}$ to 240 ºC. The separated components were identified by the comparison method with Wiley 9-nist 11 mass spectral database data by calculating the peak areas.

2.5 Statistical analysis

Samples taken from the extracts were analyzed in triplicate and recorded in order to determine the results. Statistical calculations of the results were carried out using the SPSS 18 program. The analysis of the data has been expressed as "mean ± standard deviation". One-way variance analysis (ANOVA) was applied to the absorbance values measured by spectroscopy in order to determine the statistical differences between enzyme inhibition results.

Differences between absorbance data were determined using LSD and DUNCAN tests and $p<0.05$ values were considered statistically different.

3. Results and Discussion

Diabetes mellitus is a serious metabolic disease that threatens public health. Patients diagnosed with Type 2 diabetes are initially administered medical nutrition therapy and an exercise program. Antidiabetic drugs are also used to provide glycemic control in Type 2 diabetes. Those that increase insulin secretion, increase insulin sensitivity, inhibit glucose absorption; α-glucosidase enzyme inhibitors (acarbose) are used atidiabetic agents. α-amylases are the enzyme that breaks down maltodextrin, malto-oligosaccharides and glucose by hydrolyzing glucosidic bonds in starch and glycogen. It has been reported that α-amylase inhibitors may be effective in the treatment of Type 2 diabetes, since α-amylase inhibition causes carbohydrate tolerance, feeling of satiety, weight loss and prolonged gastric emptying (Gerrard et al., 2000, Conforti et al., 2005). α-glucosidase, another enzyme important in carbohydrate digestion, is a membrane-bound enzyme in the small intestine epithelium and hydrolyzes oligosaccharides and disaccharides to glucose units. α-glucosidase is involved in the last step of digestion of carbohydrates, so inhibitors of this enzyme prolong the time of complete digestion of carbohydrates and delay their digestion (Kim and Uyama, 2005). Therefore, α-glucosidase inhibitors can suppress postprandial blood glucose levels by slowing down the hydrolysis and absorption of carbohydrates. α-glucosidase inhibitors cause a decrease in glucose absorption rate and increased postprandial blood glucose ratio, than α-glucosidase inhibition gains importance in the regulation of Type 2 diabetes (Hamdan and Afifi, 2004). The inhibition activity of the α-amylase enzyme of the species was shown as acarbose equivalent in Table 1 and as % inhibition activity in Table 2. It is seen that diethyl ether and acetone extracts of the plant have higher inhibition of % α-amylase than other extracts and especially diethyl ether extract is not statistically different from the% inhibition of standard substance acarbose ($p> 0.05$) and has an effect close to % inhibition of acarbose.

The inhibition activity of the α-glucosidase enzyme of the plant extracts is shown in Table 1 and Table 2 as acarbose equivalent and % inhibition. It was found that methanol extract showed the closest % α-glucosidase activity to acarbose. The relationship between % α-glucosidase enzyme inhibition of the extracts is as TTW<TTH<TTDE<TTEA<TTA<TTM. The plant extracts appear to inhibit these enzymes responsible for carbohydrate hydrolysis. In particular, the standard substance acarbose had an effect on the α-amylase enzyme. It depends on the reduction of carbohydrate absorption of polyphenols in the structure of plants, improvement of β cell function, stimulation of insulin secretion, modulation of enzymes exposed to antioxidant activity. The inhibitory effect of phenolic compounds such as flavonoids, tannins and phenolic acids against α-glucosidase and α-amylase, the enzymes responsible for digesting carbohydrates, is known. Flavonoids, tannins and phenolic acids contained in *Thermopsis turcica* may have an inhibitory effect on α-glucosidase and α-amylase enzymes. Aksoy et al., (2013) was observed that radical scavenging effect of acetone and methanol extracts of *Thermopsis turcica*. They stated that the radical scavenging effect is related to the total amount of phenolic substances. They showed that the radical scavenging effect and total phenolic substance content of *Thermopsis turcica* in acetone extract is high (Aksoy et al., 2013). In another study, the effects of ethanolic and aqueous extracts of *Thermopsis turcica* on MDA, GSH, SOD, CAT, and plasma AOA in rat blood and tissue samples were investigated. It has been determined that aqueous extracts of the plant have positive effects on GSH, decreasing MDA values in blood, liver and kidney tissue samples (Çelik and Küçükkurt, 2016). They stated that it is an effective against lipid peroxidation and supports the antioxidant system. There is an increase in free radical activity in diabetes. In addition, there is a relationship between hyperglycemia and hyperlipidemia and the level of lipid peroxides in diabetic patients. It is known that lipid peroxidation plays an important role in the emergence of many diseases and that lipid peroxidation is increased in both types of diabetes (Çakatay et al., 2000). Studies have reported that free oxygen radicals and lipid peroxidation significantly increase in rats with diabetes and diabetic patients and oxidative stress plays a role in the etiology and progression of diabetes. It is known that molecules with antioxidant properties have important effects on the correction of oxidative stress, protein glycation and glucose metabolism in diabetes (Pitkanen et al., 1992). It can be thought that *Thermopsis turcica* may be antidiabetic because it contains phytochemicals that support the antioxidant system, prevents free radical formation and lipid peroxidation, and inhibits carbohydrate digestive enzymes, especially α-glucosidase.

Acetylcholine (ACh) is one of the most important neurotransmitters. ACh molecules are hydrolyzed by the acetylcholine esterase (AChE) enzyme. As a result of the reaction, the degradation products enter the presynaptic cell. ACh is then regenerated and so the chain of events continues (Kiss and Vizi, 2001). Alzheimer's is a progressive and chronic neurodegenerative disease that occurs in the brain. The symptomatic treatment protocol of Alzheimer's is to increase the synaptic amount of acetylcholine in the brain by inhibition of AChE, the enzyme responsible for hydrolysis of acetylcholine (McGeer and McGeer, 2003).

AChE inhibitors such as tacrine, galantamine and donepezil are used in the symptomatic treatment of Alzheimer. Physostigmine has been used as an acetylcholine esterase inhibitor. Physostigmine is an alkaloid isolated from *Physostigma venenosum* L. (Fabaceae). *Thermopsis turcica* used in this study is a species of legume (Fabaceae) family. Galantamine was chosen as the standard substance in the study. The inhibitory effect of galantamine equivalent AChE of different extracts of the species and the inhibitory effect of AChE % are given in Table 1 and Table 2. AChE inhibition activity of *Thermopsis turcica* methanol extract was found to be statistically significant ($p<0.05$) higher than other extracts. However, it appears that even methanol extract (61.76±0.86%) is not as effective as galantamine (85.86±1.44%). Cholinesterase inhibitors that bind to the peripheral anionic region of the enzyme, act in two ways as that inhibit both the cholinergic system and amyloid β-peptide aggregation originating from this region. Galantamine, on the other hand, binds to nicotinic receptors allosterically, modulating nicotinic receptors to increase acetylcholine release (Morgana et al., 2004).

Table 1. Inhibitory properties of extracts prepared with different solvents on α-amylase, α-glucosidase, acetylcholine esterase, tyrosinase enzymes

Çizelge 1. Farklı çözücülerle hazırlanan ekstraktların α-amilaz, α-glukozidaz, asetilkolin esteraz, tirozinaz enzimlerini inhibe edici özellikleri

	α-amylase inhibition (mmolACAE/g extract)	α-glucosidase inhibition (mmol ACAE/g extract)	Acetylcholine esterase inhibition (mg GALAE/g extract)	Tyrosinase inhibition (mg KAE/g extract)
TTEA	0.57±0.015^{a}	1.07±0.030^{c}	1.47±0.036^{b}	92.03±1.028^{c}
TTW	0.59±0.021^{ab}	0.60±0.027^{a}	1.61±0.025^{c}	89.43±0.706^{a}
TTM	0.60±0.006^{b}	1.63±0.040^{e}	3.97±0.050^{e}	90.84±0.385^{e}
TTDE	0.80±0.021^{d}	0.87±0.020^{b}	2.74±0.025^{d}	90.50±0.639^{b}
TTH	0.58±0.021^{ab}	0.61±0.025^{a}	2.69±0.020^{d}	88.15±1.062^{a}
TTA	0.74±0.015^{c}	1.51±0.030^{d}	1.39±0.015^{a}	101.28±0.948^{d}

* Values are mean ± standard deviation; n = 3. $^{a, b, c, d, e}$: Different letters in the same column represent statistically significant differences ($p<0.05$) among different extracts. ACAE: Acarbose equivalent; GALAE: Galanthamine equivalent; KAE; Kojic Acid Equivalent. TTEA: *Thermopsis turcica* ethyl acetat extract; TTW: *Thermopsis turcica* water extract; TTM: *Thermopsis turcica* methanol extract; TTDE; *Thermopsis turcica* diethyl ether extract; TTH: *Thermopsis turcica* hexzane extract; TTA: *Thermopsis turcica* acetone extract.

Tyrosinase is a copper-containing enzyme that determines skin and hair color. It takes part in melanin biosynthesis. Treatments for tyrosinase enzyme inhibition are preferred in skin diseases related to melanin hyperpigmentation. Inhibition of tyrosinase activity and expression is the main goal in skin whitening agent development studies. For this reason, many active phytochemicals are isolated and used from natural sources (Slominski et al., 2005; Yamauchi and Mitsunaga, 2016). Tyrosinase activity is determined by methods such as the determination of oxygen consumption, the use of nucleophilic reagents that capture o-quinones and produce chromophoric structures, determination of oxidation of quinones using reducing structures such as ascorbic acid, and direct measurement of o-quinones or their products (Munoz et al., 2006). Tyrosinase is also problematic of the pigmentation of neurons in the substantia nigra region of the brain, which is rich in dopamine, through the tyrosine-tyrosinase enzymatic pathway. In this pathway, it provides the formation of some neurotoxic metabolites as a result of the oxidation reaction catalyzed by 5-S-cysteinyl-dopamine. This may lead to dopamine toxicity in Parkinson's patients. Therefore, the use of tyrosinase inhibitors in Parkinson's patients is a current approach. Phytochemicals with inhibitory activity against tyrosinase have been determined in the studies. Flavonoids (such as campherol, quercetin and morine) have been shown to be inhibitory to tyrosinase (Kim and Uyama, 2005). One of the synthetic inhibitors frequently used to inhibit tyrosinase enzyme activity is kojic acid. It is a strong tyrosinase inhibitor and acts by chelating with copper (Maeda and Fukuda, 1991). Finding the toxic effects of long-term use of synthetic inhibitors has accelerated the studies to determine alternative natural inhibitors. Kojic acid was used as a standard substance in

the study. The tyrosinase inhibitory effect of kojic acid equivalent of different extracts of the species in Table 1 and the % inhibitory effect in are given in Table 2. The tyrosinase inhibition activity of acetone extract of *Thermopsis turcica* was found to be statistically significantly higher ($p < 0.05$) than other extracts. However, acetone extract (71.07±1.567%) was found to be statistically significantly lower ($p<0.05$) than kojic acid (87.12 ± 1.26%).

Table 2. % Inhibition of extracts prepared with different solvents on α-amylase, α-glucosidase, acetylcholine esterase, tyrosinase enzymes

Çizelge 2. Farklı çözücülerle hazırlanan ekstraktların α-amilaz, α-glukozidaz, asetilkolin esteraz, tirozinaz enzimleri üzerinde % inhibisyonu

	α-amylase (% Inhibition)	α-glucosidase (% Inhibition)	Acetylcholine esterase (% Inhibition)	Tyrosinase (% Inhibition)
TTEA	53.09±0.781^{a}	68.25±1.218^{c}	41.20±2.244^{ab}	65.13±0.785^{bc}
TTW	69.10±1.689^{c}	51.76±1.473^{a}	42.52±1.155^{b}	60.17±1.172^{a}
TTM	70.63±1.736^{c}	78.36±1.236^{e}	61.76±0.863^{d}	62.48±2.234^{ab}
TTDE	89.87±1.125^{e}	58.91±1.280^{b}	50.26±1.913^{c}	65.91±1.753^{c}
TTH	61.12±1.953^{b}	52.62±0.585^{a}	49.33±1.871^{c}	60.14±1.631^{a}
TTA	85.18±1.636^{d}	75.04±1.130^{d}	39.30±0.355^{a}	71.07±1.567^{d}
Standart *	91.75±1.259^{e}	87.91±1.335^{f}	85.86±1.434^{e}	87.12±1.260^{e}

* Standard substance acarbose for α-amylase and α-glucosidase enzymes inhibition; galantamine for acetylcholine esterase enzyme inhibition; kojic acid for tyrosinase enzyme inhibition. Values are mean ± standard deviation; n = 3. $^{a, b, c, d, e}$: Different letters in the same column represent statistically significant differences ($p<0.05$) among different extracts. TTEA: *Thermopsis turcica* ethyl acetat extract; TTW: *Thermopsis turcica* water extract; TTM: *Thermopsis turcica* methanol extract; TTDE; *Thermopsis turcica* diethyl ether extract; TTH: *Thermopsis turcica* hexzane extract; TTA: *Thermopsis turcica* acetone extract

Essential oils are naturally occurring structures in many plants. Herbal essential oils have a very complex structure. Essential oils are used for medical purposes because they contain alcohols, esters, terpenes, aldehydes, coumarins (Sangwan et al., 2001, Carrapiso et al., 2002). Dry drug oil yield of *Thermopsis turcica* plant was determined by hydrodistilation and was found to be 0.04 %. The essential fatty acid content and relative percentages of the species are given in Table 3. The structure of 89.4 % of the essential fatty acid content has been defined. Palmitic acid (51.8 %), myristic acid (17.5 %) and hexahydrofarnesyl (5.3 %) are the most common structures in its structure. It also includes cosane in species structure. Cosanes are alkanes with a carbon number of 20-29.

Table 3. The essential oil content of the *Thermopsis turcica* and the percentage of essential oils

Çizelge 3. Thermopsis turcica'nın uçucu yağ içeriği ve uçucu yağların yüzdesi

Compounds	**Percentages (%)***	**Compounds**	**Percentages (%)***
Hexahydrofarnesyl acetone	5.3	Pentadecanoic acid	2.3
Tricosane	1.7	Nonacosane	2.9
Dodecanoic acid (Lauric acid)	1.3	Hexadecanoic acid (Palmitic acid)	51.8
Pentacosane	2.9	Octadecanioc acid (Stearic acid)	0.7
Fitol	2.4	(Z) -9-Octadecanoic acid (Oleic acid)	0.6
Tetradecanoic acid (Myristic acid)	17.5		
		Total	**89.4**

*Analysis of compounds presenting more than 0.5% was carried out.

4. Conclusion

The inhibition activities of *Thermopsis turcica*, α-amylase, α-glucosidase, acetylcholine esterase and tyrosinase enzyme and volatile fatty acid contents were determined in this study. It was observed that extracts of the plant obtained with different solvents did not show inhibitory properties compared to the standard substance against acetylcholine esterase and tyrosinase enzymes. It is thought that *Thermopsis turcica* may have antidiabetic effects due to its inhibitory activity against enzymes responsible for carbohydrate digestion. In addition, some polyphenols are absorbed directly from the stomach or small intestine and most of the polyphenols taken reach the large intestine after being metabolized in the intestinal microbiota before absorption. Some polyphenols are thought to stimulate the activity of bacteria in the digestive tract, prebiotic effect. Polyphenols from microbial transformation as well as gastric and intestinal absorption reach the liver via enterohepatic circulation and thus undergo Phase I and II biotransformation in the liver. Polyphenol metabolites formed in liver metabolism are absorbed into the blood, which will then be distributed through peripheral tissues to exhibit beneficial metabolic effects. Therefore, identification of polyphenol metabolites in blood and tissues is important for understanding the antidiabetic effects of polyphenols and for the development of nutraceuticals and functional foods in the future.

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