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Turkish Journal of Weed Science

Aratırma Makale / Research Article

Antioxidant Activities and Enzyme Inhibition Potentials of *Hypericum perforatum* L. Ethanol Extracts

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ABSTRACT

This study aimed to investigate the antioxidant activities, enzyme inhibition potentials, total phenolic and flavonoid contents of *Hypericum perforatum* L. ethanol extracts. The phenolic content of the plant extract was determined using the Folin-Ciocalteu reagent. The antioxidant activities of the plant extract were determined based on the iron-reducing power and scavenging potentials of the DPPH radical. Furthermore, chelating ability, superoxide dismutase and in vitro anti-inflammatory activity, tyrosinase α-glucosidase, and cholinesterase inhibition assays were determined by spectrophotometric techniques. The plant extract's total phenolic and flavonoid contents were 8.828 mg GAE/g and 10.366 mg CTE/g extract, respectively. *H. perforatum* ethanolic extract exhibited scavenging of 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) with an SC_{50} value of 0.469 mg/mL and superoxide anion radicals with IC_{50} values of 0.338 mg/mL. Ferric-ion chelating (%4.7 in 0.5 mg/mL), anti-inflammatory activity (%25.12 in 0.2 mg/mL), acetylcholinesterase inhibition (%18 in 0.2 mg/mL), butyrylcholinesterase inhibition (%15 in 0.2 mg/mL), tyrosinase inhibition (%35 in 0.5 mg/mL), and α-glucosidase inhibition (%35 in 0.2 mg/mL) activities of the plant extract were determined.

Keywords Anti-inflammatory activity, Antioxidant activity, enzyme inhibition potentials, ferric-ion chelating

1. INTRODUCTION

Hypericum perforatum is a persistent species widely grown throughout the World and it is included in the class of folk remedies because it has a therapeutic effect on many diseases. *H. perforatum* contains many bioactive molecules with a wide variety of pharmacological effects, especially antioxidant activity (Marah et al., 2022). *H. perforatum* L., which belongs to the Clusiaceae family, is distributed with approximately 450 species in the world, while the *Hypericum* genus is represented with 89 species in the flora of Turkey. Turkey's climate is very suitable for *H. perforatum*, also known as “kılıç otu’’ and ‘‘Sarı Kantaron’’ (Düzgüner and Erbil, 2020).

The therapeutic properties of St. John's Wort, mentioned in alternative and modern medicine, vary depending on many external factors, such as climatic conditions, geographical differences, altitude, harvest time, and drying method. These conditions directly affect the plant's oil content, phenolic compounds, flavonoids, antioxidant metabolites, and anthocyanin components (Gül et al., 2023; Guedes et al., 2012). There are many *Hypericum* species in Turkey, but for the reasons we have listed, all *H. perforatum* L. species have different effects due to their different contents (Gül et al., 2023). Therefore, phytochemical

studies with extract from plant collected from different locations than those studied before will be valuable. Due to the increasing need for raw materials that can be the source of organic bioactive compounds, there has been a lot of interest in this plant species recently.

Free radicals containing one or more unpaired electrons, which are inevitable to form in living systems after the normal metabolic process, are very active chemically and can oxidize nucleic acids, proteins and lipids, disrupt their chemical structures and produce negative results in their metabolism. However, the presence of these radicals and reagents above the normal level causes harmful processes such as tissue damage, cell death, premature aging, cancer, cardiovascular diseases and neurological disorders that develop as a result of oxidation of biomolecules (Güzel et al., 2019). These developments, after increased free radical formation, lead to oxidative stress, which is associated with multiple inflammatory conditions. Diseases caused by inflammation include cancer and cardiovascular diseases, as well as neurological diseases such as Alzheimer's and Parkinson's, and diabetes (Huang et al., 2021). In addition, it is known that medicinal plants rich in secondary metabolites are promising candidates for drug development for many chronic diseases by playing an active role on biochemical reactions by delaying vital metabolic pathways or inhibiting enzymes (Mettupalayam Kaliyannan Sundaramoorthy and Kilavan Packiam, 2020).

This study has stated that antioxidant activity tests reveal the ability of *H. perforatum* L. to scavenge free radicals and determine the total amounts of phenolics and flavonoids, which are thought to be the primary source of this activity. It was also designed to investigate the potential of inhibition on various clinical enzymes to investigate the plant's potential to be used as an active drug substance. Furthermore, the anti-inflammatory activity of the plant sample was revealed. Thus, it is aimed to reveal the relationship between enzyme inhibition effect and phenolic compounds or antioxidant activity.

2. MATERIAL AND METHODS

Preparation of Hypericum perforatum extract

The flowering aerial parts of *Hypericum perforatum* L. were collected from different sites (Gülyalı, Perşembe, and Fatsa boroughs) and the center of Ordu province of Turkey in August 2018. Altitude of the investigated area was 6 m for and center of the Ordu province, 9 m for Perşembe, 1 2m for Gülyalı, and 13 m for Fatsa. After the samples were first lyophilized, they were homogenized with a blender. The homogenized plant samples (60 g) were dissolved in 300 ml of ethanol for 72 h at room temperature in a shaking incubator (Kolören et al., 2019; Buhian et al., 2016). Afterward, the plant extract was evaporated to remove the resolvent, and the dry residue was thawed in distilled water. The final concentration of plant extract was 30 mg/ml. Different dilutions of *H. perforatum* L. extract (5, 10, 15, 20, 25, and 30 mg/ml) were made through serial dilution with distilled water (Kolören et al., 2019).

Determination of total phenolic and flavonoid contents

The amount of the total phenolics in the ethanol extract of the plant sample was determined by using Folin–Ciocalteu reagent, and the result was expressed as mg of gallic acid equivalent (GAE) (Singleton and Rossi, 1965). Quantitatively, the total flavonoid amount was calculated as catechin equivalents (CTE) by following the aluminum chloride method using the catechin calibration curve (Kim et al., 2003).

DPPH free radical scavenging activity

The scavenging efficiency of the extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was calculated using the absorbance measurement results at 517 nm after the tubes contained the same amount of DPPH radical, and the extract at different concentrations was kept in the dark for 30 minutes (Sànchez-Moreno et al., 1998).

The % scavenging activity values were calculated using the equation below, considering the absorbance value obtained for each concentration and the absorbance value of the tube content containing only sample solvent and DPPH solution, without any sample.

Scavenging activity (%) = ($Absorbance_{control}$ − $Absorbance_{sample}$)/($Absorbance_{control}$) x 100

The extract concentration that scavenges half of the DPPH radicals in the reaction medium was calculated (SC_{50}; mg/mL) from the line equation of the graph obtained by plotting the % scavenging values calculated in the case of each tested concentration of the sample against the concentration

Fe^{2+} chelating potential

The ability of the extract to chelate with Fe^{2+}, another antioxidant activity evaluation method, was

expressed by the degree of inhibition on the complexation of ferrozine and iron ions. For this purpose, after incubation of 0,5 mg/mL part of the extract with two mM $FeCl_2.4H_2O$ solution at room temperature for 30 minutes, five mM ferrozine solution was added to this mixture, and interaction was ensured again for 10 minutes. After incubation, the absorbance of the tube containing the tested sample was recorded at 562 nm against the blank prepared without the sample. The same procedures were performed with 0.005 mg/mL EDTA instead of the extract, and the result obtained was used for comparison (Dinis et al., 1994).

Superoxide dismutase activity

The superoxide dismutase activity of the extract was evaluated as its capacity to inhibit the light reduction of nitroblue tetrazolium (NBT) in the reaction mixture (Beauchamp and Fridovich). The tube contents were prepared in phosphate buffer (50 mM; pH 7.8) to contain varying concentrations of the sample, 0.1 mM EDTA, 13 mM methionine, 75 µM NBT and two µM riboflavin. The reactions were performed for 10 min by placing the tubes under the fluorescent source (24 W) and terminated by removing the tubes from the light source. The photo-reduction of NBT was recorded spectrophotometrically at 560 nm and compared with the blank sample, which had buffer instead of sample. The result was compared with the test result for ascorbic acid performed under the same conditions.

In vitro *anti-inflammatory activity assay*

The bovine serum albumin (BSA) anti-denaturation assay is a suitable screening assay for identifying the biological potential of natural products for therapeutic drug research. Hence, the anti-inflammatory efficiency of the alcoholic extract was tested. BSA, denatured by heating, was used as a reactive agent for this spectrophotometric assay based on the inhibition of albumin denaturation. The test mixture was prepared in phosphate-buffered saline (PBS) buffer, which was formed to contain 137 mM NaCl, 2.7 mM KCl, ten mM Na_2HPO_4, and 1.8 mM KH_2PO_4 and made ready by adjusting the pH to 6.3 with HCl. The reaction mixture containing 0.8% BSA and the extract to be tested or ibuprofen used as a standard at 0.2 mg/mL concentrations was kept at 37 °C for 20 minutes and then at 71 °C for 15 minutes. The mixture was allowed to cool, and the resultant turbidity was measured at 660 nm against the blank tube prepared by adding only the solvent instead of the sample (Williams et al., 2008).

α-Glucosidase inhibition assay

Extract from St. John's Wort plant at a concentration of 0.2 mg/mL was incubated with α-glucosidase (from *Saccharomyces cerevisiae*) enzyme solution for 20 min at 37 °C in 0.1 M phosphate buffer (pH 6.8). After 20 min, the reaction was started with the addition of 4-Nitrophenyl α-D-glucopyranoside (pNPG) used as a substrate, and incubation at 37 °C was continued for 30 minutes. To stop the reaction, 0.1 N of Na_2CO_3 (50 µL) was added, and the final absorbance was measured at 405 nm using a spectrophotometer (Pistia-Brueggeman & Hollingsworth, 2001). The same procedure was performed for the 0.014 mg/mL concentration of the antidiabetic drug acarbose, and a comparison was made.

Cholinesterase inhibition assay

The inhibitory activities of the extract on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were evaluated using electric eel AChE and BuChE from equine serum. The reaction mixture was first prepared to contain 0.2 M 5,5′-dithiol-bis-(2-nitrobenzoic) acid (DTNB) and 0.2 M enzyme solution in the presence of 0.2 mg/mL extract as an inhibitor or galantamine as a standard inhibitor and incubated for 15 min at 25 °C. The reaction was then initiated by adding 0.2 M of each substrate (acetylthiocholine iodide or butyrylthiocholine chloride). The hydrolysis of the substrate was monitored by the release of the yellow 5-thio-2-nitrobenzoate anion because of the reaction of DTNB with thiocholine, catalyzed by enzymes at 412 nm (Ellman et al., 1961).

Tyrosinase inhibition assay

Mushroom tyrosinase was first incubated with 0.5 mg/mL extract at 25 °C for 10 minutes in 50 mM phosphate buffer (pH 6.8) to examine the tyrosinase inhibition of the extract. Then, L-DOPA was added to this mixture, and the enzymatic reaction was followed by monitoring the change in absorbance at 475 nm due to DOPA chrome formation over time. Kojic acid at a concentration of 0.05 mg/mL was used as a standard inhibitor for comparison (Kasangana et al., 2015). The following equation was used to calculate the inhibition percentages in all tests.

$$\text{Inhibition ratio (\%)} = (\text{Absorbance}_{control} - \text{Absorbance}_{sample}) / (\text{Absorbance}_{control}) \times 100$$

Triplicate analysis was used in all the assays, and the results are expressed as the mean ± standard

deviation (SD) using SPSS software package programme.

3. RESULTS AND DISCUSSION

Total phenolic and flavonoid contents in the extracts of H. perforatum

In this study, the total phenolic and flavonoid content values of the extract prepared from St. John's Wort plant collected around Ordu province were calculated as 8.828 mg GAE/g and 10.366 mg CTE/g extract, respectively (Table 1). In a previous study, the average phenolic content values of *H. perforatum* collected from different locations of the Eastern Black Sea region were reported to be in the range of 15.82-45.22 mg GAE/g (Gül et al., 2023). It has been reported that the phenolic content values calculated by testing the fresh flowering plants of the *H. perforatum* L. plant, which grows naturally in the Akçatekir Plateau of the Pozantı district of Adana province, during the flowering periods by applying different extraction techniques, varied between 0.9388-0.2944 mg GAE/g sample (Burunkaya et al., 2021). In a study revealing that harvest time affects secondary metabolites and antioxidant activity, the highest flavonoid value was calculated as 160 mg KTE/ g sample in *H. perforatum* from Poland extracts prepared with different extraction solvents after drying different plant parts with different techniques (Makarova et al., 2021).

Reactive oxygen species play a role in the development of Alzheimer's disease by causing age-related degeneration of neurons. However, although the deficiency of antioxidant compounds plays a role in neurodegenerative disorders, many findings have reported that antioxidants can reduce or prevent neuronal death in Alzheimer's disease. Therefore, there is a growing interest in naturally occurring antioxidant compounds, especially polyphenols, in plants to reduce oxidative damage and prevent neurodegenerative disorders (Ersoy et al., 2020).

Table 1. Phenolic and flavonoid content, antioxidant, and anti-inflammatory activity values compared with standards.

Tests	***H. perforatum* extract**	**Standards**
Total Phenolic Content (mg GAE/g extract)	8.828±0.653	
Total Flavonoid Content (mg CTE/g extract)	10.366±0.966	
DPPH radical scavenging activity (SC_{50}; mg/mL)	0.469±0.100	0. 0072±0.0011 for ascorbic acid
SOD (IC_{50}; mg/mL)	0.338±0.063	0.0024±0.0005 for ascorbic acid
Ferric ion chelating activity (%)	4.7±1.2 for 0.5 mg/mL	25.37±2.23 for 0.005 mg/mL EDTA
Anti-inflammatory activity (%)	25.12±1.6 for 0.2 mg/mL	38±4.56 for 0.2 mg/mL ibuprofen

Antioxidant activities

Evaluating the antioxidant activity by applying more than one antioxidant method and considering the various oxidation means in the environment is imperative. In this context, the antioxidant properties of the ethanol extract were investigated using three methods: DPPH free radical scavenging, superoxide dismutase, and metal chelating activity. The SC_{50} value of the *H. perforatum* extract, which we investigated in this study, was found to be 0.469 mg/mL, which expresses the amount sufficient to scavenge half of the DPPH radical in the reaction medium (Table 1).

Similarly, it was determined that the extract could scavenge half of the superoxide radicals formed by the effect of fluorescent light in the reaction medium at a concentration of 0.338 mg/mL. Suppose these findings are compared with the standard antioxidant ascorbic acid values. In that case, it can be seen from Table 1 that the DPPH radical scavenging efficiency of the tested sample is 65 times weaker. The extreme scavenging efficiency of superoxide is 14 times weaker than that of ascorbic acid, and activities of this magnitude can be considered moderate. SOD is an important enzyme that removes free radicals by converting superoxide radicals to hydrogen peroxide and plays an essential role in human oxidation-antioxidant balance (Oguntibeju et al., 2010). SOD is thought to play a crucial role in the

homeostasis of reactive oxygen species. Many studies have reported that SOD-encoding gene expression in nontransgenic plants reduces oxidative stress (Gill et al., 2015). Therefore, to obtain enzymatic antioxidant data, the superoxide radical scavenging activity of our tested extract was determined. In many studies in the literature, it has also been shown that the *H. perforatum* plant can prevent possible damage due to SOD activity (Cakır et al., 2016; Mohanasundari et al., 2006).

Many metal ions play essential roles in many biological and environmental systems, including the metal Fe required for mitochondrial electron transport and oxygen delivery in plants. However, if the concentrations of these biological metals exceed the recommended limits in the body, they pose a high potential risk to organisms (Zhang et al., 2022). Substances that act as iron chelators can activate tissue iron by forming soluble, stable complexes that can be excreted in feces and urine. Iron-related complications in humans are reduced by procedures known as chelation therapy. For example, such substances improve the quality of life and overall survival in some diseases, such as thalassemia (Fathi & Ebrahimzadeh, 2013).

For this reason, it is crucial that iron can be removed from the environment by components with antioxidant capacity. In the metal chelating activity test, ferrozine can quantitatively form complexes with Fe^{2+}. However, the extract with antioxidant activity added to the medium prevents this formation, and there is a decrease in the expected color depending on the strength of the antioxidant activity (Safari et al., 2020). 0.5 mg/mL of the tested sample can chelate only 4.7% of Fe^{2+}, while 0.005 mg/mL of EDTA, used as a positive control, causes 25.37% discolouration. If these numerical values are interpreted, it can be said that there is a difference of approximately 500 times; that is, our extract has a weak chelation activity. Similar studies on *H. perforatum* collected from Uludag environs reported that the chelating activity was relatively low compared to EDTA. The IC_{50} value given for the scavenging efficiency of DPPH radicals of the same species in the literature is 0.029 mg/mL (Ersoy et al., 2020). An *H. perforatum* sample, which has a low chelation activity of approximately 115 times compared to EDTA, was also collected from Iran (Fathi & Ebrahimzadeh, 2013).

Enzyme inhibition potentials

It is known that substances with antioxidant effects can inhibit or delay the oxidation process by preventing the initiation or propagation of oxidizing chain reactions. In this way, they have an essential role in preventing a wide variety of serious chronic diseases, including Alzheimer's and cancer. It should be emphasized that there is a significant correlation between damage caused by oxidative stress and Alzheimer's disease (AD) (Ersoy et al., 2020).

Herbal sources used therapeutically can disrupt metabolic processes by inhibiting enzymes through biochemical reactions (Mettupalayam Kaliyannan Sundaramoorthy and Kilavan Packiam, 2020).

Acetylcholinesterase is the key enzyme that catalyzes the breakdown of certain choline ester compounds, the most important of which is acetylcholine, which functions as a neurotransmitter. Reversible acetylcholine inhibitors treat some neurodegenerative disorders, especially Alzheimer's disease, which affects memory and contributes to approximately 60% of dementia cases. During the development of Alzheimer's disease, the amount of acetylcholine decreases in the hippocampus and cortex regions of the brains of people who suffer from it. Therefore, the use of reversible acetylcholinesterase inhibitors for the treatment of Alzheimer's disease is auspicious. Butyrylcholinesterase is another cholinesterase found in the liver and plasma. An increased level of butyrylcholinesterase causes benign plaques to turn into malignant plaques that can eventually lead to neurodegeneration (Ersoy et al., 2020).

For this reason, we evaluated the inhibition potential of the cholinesterase enzyme to investigate whether the extract, which has moderate antioxidant activity, has a protective effect against Alzheimer's So, the inhibition degrees of the 0.2 mg/mL portion of the tested extract on both cholinesterases were examined and found to be almost equally effective (Table 2). Another study reported that the same amount of *H. perforatum* sample inhibited acetylcholinesterase by 39% and butyrylcholine esterase by approximately 80%. The anticholinesterase activity of the studied extracts is likely due to their rich phytochemical mixtures, including compounds known as cholinesterase inhibitors such as quercitrin, isoquercitrin, hyperoside, rutin, and chlorine. It has been previously presented in the literature that different concentrations of extracts prepared with different solvents show different degrees of inhibition of acetyl and butyryl choline esterase in samples of the same species collected from İzmir Ödemiş (Altun et al., 2013).

In addition, excessive activity of the tyrosinase enzyme, which plays a role in the

formation of neuromelanin in the human brain, causes dopamine neurotoxicity, leading to the development of Parkinson's disease, another neurodegenerative problem (Tusevski et al., 2018). The tyrosinase inhibition degree of the 0.5 mg/mL portion of St. John's Wort extract was 35%. In comparison, the inhibition degree of the 0.05 mg/ml portion of the standard tyrosinase inhibitor kojic acid was 89%. The tyrosinase inhibition potential of this species, whose phenolic content is widely studied in the literature, is attributed to the presence of chlorogenic acid, quercetin, rutin, hyperoside, quercitrin, kaempferol 3-O-rutinoside, flavonols and oligomeric procyanidins (Tusevski et al., 2018). Iwai et al. (2004) stated that the double bond at the C7 position of the caffeoyl group of chlorogenic acid leads to inhibition by forming a stable Schiff base with the primary amino groups of the enzyme active site.

In a study conducted on the water extract of *H. perforatum* L., collected from Manisa Province in the Aegean Region of Turkey, it was reported that 1 gram of the extract showed a tyrosinase inhibition potential equivalent to 28,89 mg of kojic acid (Sarikurkcu et al., 2020). This reported value is almost equivalent to that calculated in the present study.

The grounds that problems in insulin production affect the central nervous system, which modulates cognitive function, have led to the presentation of Alzheimer's disease as "Type 3 Diabetes". In other words, decreased insulin signaling and insulin resistance in the brain compete for an essential role in the pathogenesis of AD. This link has also been strengthened by clinical trials in humans demonstrating the contribution of some oral antidiabetic drugs to improving cognition (Mettupalayam Kaliyannan Sundaramoorthy and Kilavan Packiam, 2020).

Table 2. Degrees of inhibition (%) of varying amounts of extract and standard on the indicated enzymes.

Enyzmes	*H. perforatum* ethanolic extract	Standards
Acetylcholinesterase	%18 for 0.2 mg/mL	%50 for 0.027 mg/mL galantamine
Butrylcholinesterase	%15 for 0.2 mg/mL	%50 for 0.033 mg/mL galantamine
Tyrosinase	%35 for 0.5 mg/mL	%89 for 0.05 mg/mL kojic acid
α-Glucosidase	%35 for 0.2 mg/mL	%6 for 0.014 mg/mL acarbose

Diabetes mellitus is a critical public health problem that manifests itself with the deterioration of glucose metabolism caused by insulin secretion defects (Béjaoui et al., 2017).

Inhibiting the activities of α-amylase, which converts to disaccharides by acting on 1,4-glycosidic bonds in polysaccharides, and glucosidase, the enzyme that produces monosaccharides that cause an increase in blood glucose levels by acting on disaccharides, has become a promising approach for the treatment of diabetes. Type 2 diabetes can be ruled out by lowering the blood glucose level by using various natural and synthetic pharmaceuticals to inhibit these two key enzymes. However, synthetic inhibitors have side effects such as abdominal pain, diabetic ketoacidosis, and colon ulcerations. Therefore, it is thought that nutraceuticals, which hope to be obtained from plants of therapeutic importance, will be more advantageous for managing or treating metabolic disorders (Mettupalayam Kaliyannan Sundaramoorthy and Kilavan Packiam, 2020). To measure its potential to be evaluated as a nutraceutical, the degree of inhibition of the tested extract on the glucosidase enzyme was followed, and it was concluded that the extract's 0.2 mg/mL concentration inhibited the glucosidase enzyme by 45%. If this calculated result is compared with the 6% inhibition degree calculated for 14 µg/mL acarbose, it can be said that it is approximately two times lower. In a similar study conducted on *H. perfoliatum* collected from Tunisia, it was reported that 1 gram of the methanol extract of the plant showed inhibitory activity equivalent to 3.88 mmol acarbose (Béjaoui et al., 2017). Gulam et al. (2009) suggested that hyperforin, one of the known components of the plant, is responsible for antidiabetic activity.

Anti-inflammatory activity of H. perforatum L.

On the other hand, inflammation, which is one of the main reasons for the deterioration of organelle functions, leads to many diseases, such as aging and

cardiovascular and neurological diseases, and causes diabetes. Although steroid and nonsteroidal drugs are still used in the treatment of inflammation to prevent the development of such diseases, drug-active substances with anti-inflammatory potential are also widely investigated due to their severe side effects (Kelebekli et al., 2022). For this purpose, thermally provided protein denaturation under experimental conditions can be prevented at different doses by different inflammatory drugs, such as salicylic acid and phenylbutazone (Govindappa et al., 2015). Various plant extracts have been reported to show similar results (Sakat et al., 2010). The use of *Hypericum* preparations to treat general inflammatory conditions such as dermatitis and gastroenteritis, along with its many known uses, has led to further research into the anti-inflammatory activity of this genus (Kimberly and Diane, 2014). One of the easiest ways to observe the anti-inflammatory activity is to determine the degree of thermal degradation of bovine serum albumin in the presence and absence of the extract (Shah et al., 2017). After the spectrophotometric test, it was concluded that the extract at a concentration of 0.2 mg/ml suppressed the denaturation of BSA by 25.12%. At the same time, the same amount of the anti-inflammatory drug ibuprofen caused inhibition of only 38%, so our extract could be considered an effective anti-inflammatory agent almost as much as the standard drug. Many studies have shown that the flowering aerial parts of *H. perforatum* collected from Ankara Beypazarı, also the same species obtained from India, have anti-inflammatory activity in Turkey and around the world.

However, no example was found in which anti-inflammatory activity was demonstrated with the preferred method based on in vitro BSA denaturation in the current study (Kumar et al., 2001; Süntar et al., 2010).

4. CONCLUSIONS

In addition to the antioxidant and anti-inflammatory effects of the *H. perforatum* species collected from Ordu province, widely studied in the literature, the enzyme inhibition activities on which the treatment possibilities of important clinical diseases are based were determined.

It has been concluded that *H. perforatum*, which was found to be quite effective when compared to the standard anti-inflammatory agent ibuprofen, may be a potential drug raw material that can be tested with further studies to be used in the treatment of many diseases caused by inflammation. In addition, we can say that this extract, with its significant inhibition on cholinesterase enzymes, may be promising for patients suffering from depression accompanied by dementia.

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Geliş Tarihi/ Received: Nisan/April, 2023
Kabul Tarihi/ Accepted: Haziran/June, 2023

To Cite : Çol Ayvaz M., Aydoğdu G., Kolören Z., Kolören O. and Karanis P. (2023). Antioxidant Activities and Enzyme Inhibition Potentials of *Hypericum perforatum* L. Ethanol Extracts, Seeds. Turk J Weed Sci, 26(1):49-57.

Alıntı İçin : Çol Ayvaz M., Aydoğdu G., Kolören Z., Kolören O. and Karanis P. (2023). Antioxidant Activities and Enzyme Inhibition Potentials of *Hypericum perforatum* L. Ethanol Extracts, Seeds. Turk J Weed Sci, 26(1):49-57.