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Anticancer, Antioxidant, Antimicrobial and Enzyme Inhibitory Activities of *Inula aucheriana*

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ABSTRACT

The *Inula aucheriana*, of the Asteraceae family, is widespread in Turkey. The aim of this study was to investigate different biological properties and thereby reveal the pharmacological potential of this plant, which is already known to be used in the treatment of various diseases,. Following the preparation of 80% ethanol extract from *I. aucheriana,* qualitative and quantitative methods were used to investigate the chemical composition (Q-TOF analysis), antioxidant (spectrophotometric analysis), inhibitory enzyme (Ellman's method), antimicrobial (MIC concentration value), and anti-cancer (XTT analysis) activities. The results showed that 80% ethanol extract from *I. aucheriana* was a potent antioxidant, with anti-cancer and enzyme inhibitor effects. In the chemical composition analysis, the primary compound of the extract was determined to be luteolin (32.55%). *I. aucheriana* extract was seen to have AChE and BChE inhibition, , and when compared with the reference drug, the extract was determined to have an inhibitory effect an enzyme called α-glucosidase. Besides, relatively high tyrosinase enzyme inhibition was also detected. The extract significantly showed antiproliferative activity on the MDA-MB-231 cells at 0.0625 mg/mL and higher concentrations,for 24 hours in a a dose-dependent manner.This study is the first to evaluate enzyme inhibitory effect and antioxidant activity of *I. aucheriana.*

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Inula aucheriana'nın Antikanser, Antioksidan, Antimikrobial ve Enzim İnhibitör Aktiviteleri

ÖZET

Türkiye'de yaygın olarak görülen *Inula aucheriana*, Asteraceae familyasına ait bir bitkidir. Çeşitli hastalıkların tedavisinde kullanıldığı bilinen bu bitkinin farklı biyolojik özelliklerinin araştırıldığı bu çalışma ile bitkinin farmakolojik potansiyelinin ortaya çıkarılması amaçlanmaktadır.*I.aucheriana*'nın %80 etanol ekstraktının, kimyasal bileşimi (Q-TOF analizi ile), antioksidant özellikleri (spektrofotometrik analizi ile), enzim inhibitör aktiviteleri (Ellman's yöntemi ile), antimikrobiyal aktivitesi (MIC konsantrasyon değeri ile) ve antikanser aktivitesi (XTT analizi ile) nitel ve nicel yöntemler kullanılarak araştırıldı.*I.aucheriana*'nın %80 etanol ekstraktının güçlü bir antioksidan, antikanser ve enzim inhibitörü olduğu tespit edildi. Kimyasal bileşim analizinde ekstraktın ana bileşiği luteolin (%32.55) olarak belirlendi. *I. aucheriana* ekstraktının AChE ve BChE inhibisyonuna sahip olduğu ortaya konuldu. Ayrıca *Inula* ekstraktının, referans ilaç ile karşılaştırıldığında α-glukosidaz enzimi açısından inhibitör etkiye sahip olduğu belirlendi. Bununla birlikte oldukça yüksek tirozinaz enzim inhibisyonu gösterdi. Ekstrakt, MDA-MB-231 hücrelerinde

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0.0625 mg/ml ve daha yüksek konsantrasyonlarda 24 saat boyunca inkübe edildiğinde,önemli ölçüde antiproliferatif aktivite gösterdi. Bu çalışma, *I. aucheriana*'nın enzim inhibitör aktivitesi ve antioksidan aktivitesinin ilk araştırmasıdır.

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INTRODUCTION

The genus *Inula*, which has approximately 100 species globally, is a plant in the Asteraceae family, and it grows densely in the Central Anatolia region of Turkey (Öztürk and Çetin, 2013). *Inula*, which is named "andız otu" in Turkish, is a medicinal plant used in the treatment of various diseases, especially sleep disorders, menstrual disorders, intestinal diseases.

Plants belonging to the genus *Inula* are known to have antimicrobial, anticonvulsant, antiproliferative, antioxidant and hepatoprotective properties. This genus is also rich in secondary metabolites (Khan et al., 2010; Moghadam et al., 2012; Kaur et al., 2014; Ekbatan et al., 2019). Many diseases, especially cancer, neurological disorders, diabetes and cardiovascular diseases, may develop due to a high level of oxidative stress in the body,. These diseases can be prevented by inhibiting free radicals that cause oxidative stress in the body through antioxidants (Gupta et al., 2014; Motor et al., 2014). High antioxidant activity has been detected in many species of *Inula* (Čanadanović-Brunet et al., 2002; Bai et al., 2005; Al-Fartosy, 2011).

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes are cholinesterases that act on thiocholine to release acetylcholine, which aids neurosynaptic transmission. In cases where these enzymes are inhibited, acetylcholine in the synaptic space is more effective in signal transmission. AChE and BChE inhibitors have been used for many years to treat Alzheimer's and Myasthenia gravis diseases (Orhan et al., 2004; Zhao et al., 2013; Mehndiratta et al., 2014). However, the side effects of these synthetic drugs have led researchers to seek natural AChE and BChE inhibitors. The results of studies of different *Inula* species enzyme inhibition activity are promising. However, no such study has been found for the *I. aucheriana* species as yet (Trendafilova et al., 2020).

Cancer is one of the diseases with the highest mortality rate worldwide. Multifactorial causes affecting the mechanism of the disease are of great importance for the clarification of the treatment process. Medicinal and aromatic plants are often used in the treatment of cancer. Therefore, studies of these plants with anti-cancer properties, have increased recently. In vitro studies of *Inula* on different cancer cell lines (such as Burkitt's lymphoma, lung cancer, liver cancer) have shown that this plant has anti-cancer properties (Cui et al., 2018; Wang et al., 2019; Virdis et al., 2020).

According to the data obtained from previous studies, many *Inula* species, except *I. aucheriana*, were found to be rich in biological activity (Gökbulut et al., 2013). However, a comprehensive study on the biological activities of 80% ethanol extract of *I. aucheriana* has not been conducted. Therefore, in this study, it was aimed to investigate the anti-cancer, antimicrobial, antioxidant and enzyme inhibitory activities of this species.

MATERIAL and METHODS

The aerial parts of the plants in full flowering periods were collected from a natural area (Yozgat-3446408 E, 3948346 N, 1216 m) on 05.07.2017. The collected fresh aerial parts were dried at room temperature. Species identification of the collected plants was made in Yozgat Bozok University Biology Department. The experiments in this study were repeated three times with random selection in Sivas Cumhuriyet University Faculty of Pharmacy laboratory in 2019.

Preparation of Extracts

The aerial parts of the plants were dried and ground (Blue House). Taking 10 g of the resulting dry plant, it was mixed with 50 mL of 80% ethanol and shaken intermittently for 48 hours. Then it was filtered with Whatmann filter paper No.1. The filtrate was intensified to dryness under reduced pressure on a rotary evaporator at 40°C, and this procedure was performed three times.

The Chemical Composition

The extracts prepared were stored in 10 ml of ethanol for three days, then mixed with the help of a magnetic fish at 500 rpm for 10 minutes for complete dissolution. The resulting 100 µL extract was mixed by vortexing with a 900 µL of Methanol: Water: Formic acid (80: 20: 0.1) solution. It was then centrifuged for 30 minutes at 10000 rpm and 4°C. The upper phase was taken into a vial and passed through a 45 µm filter. Liquid passing through the filter was

injected into the device. A Q-TOF (Agilent Accurate Mass Q-TOF LC-MS 6530) device and Poroshell 120 SB-C18 (2.7µm 4.6x100mm) type column were used for analysis. The samples were measured at 30 °C for 55 minutes in water / acetonitrile mobile phases with a flow rate of 0.6 ml / min. The obtained data were evaluated with the Agilent Metlin database.

In vitro Antioxidant Activity

The 2,2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging activity of the extract was evaluated according to the Blois (1958) method with few alterations. The 2, 2′ - azinobis (3 - ethylbenzothiazoline - 6 – sulfonic acid) (ABTS) radical scavenging activity was evaluated using the Re et al. (1999) method with minor modifications. The total phenolic content (TPC) was specified with the spectrophotometric method(Clarke et al., 2013) and clarified as gallic acid equivalents and the total flavonoid content (TFC) was assigned with the Molan and Mahd (2014) aluminum chloride colorimetric method. The TFC was stated as milligram-catechin equivalent per gram of dry weight of the extract.

In vitro Enzyme Inhibition Assay

The butyrylcholinesterase and acetylcholinesterase inhibition assay was applied pursuant to the Ellman's method as defined in our previous study (Ellman et al.,1961; Ergül et al., 2019). As reported by Kumar et al. (2012), the α-glucosidase inhibition method was applied. The alpha-amylase inhibition activity of the extract was investigated using the method reported by Kumar et al. (2013). The positive control used in both the a-glucosidase and the a-amylase inhibition method was acarbose.

Antimicrobial Activity

Microdilution broth method

The bacteria used in this study were *Bacillus cereus*, *Staphylococcus aureus, Escherichiacoli*, and *Pseudomonas aeruginosa*. Two yeast strains, *Candida albicans* and *Candida tropicalis* were also used. The minimum inhibitory concentration (MIC) of the *I.aucheriana* ethanol extract was determined in accordance with the broth microdilution method of Eloff (1998). Mueller-Hinton broth (Accumix®AM1072) for bacteria and Sabouraud Dextrose Broth (Himedia ME033) for *Candida sp.* were used as the culture medium (CLSI, 2002; 2012).

The extract was dissolved in dimethylsulfoxide (DMSO) (50 mg/mL). 90 µl of media were applied to the first row of the microplates and 50 µL to the remaining wells. Wells to which 100 µL of broth was added were used as growth controls. To the first row of the microplate, 10 µL of the extract, at a concentration of 2.5-0.004 mg/mL, was added and serial double dilutions were prepared. The fungi and bacteria suspensions (50 µL) were put into the prepared samples. The final inoculation size was 5×10^5 CFU/mL in the bacteria wells and $0.5\text{-}2.5\times10^3$ CFU/mL in the *Candida sp.* wells (CLSI, 2002;2012). The MIC concentration of the extract was defined as the lowest concentration inhibiting discernible growth of bacteria and yeast after overnight incubation at 37 °C.

Cell Culture and Reagents

MDA-MB-231 (HTB-26TM, Human Breast Cancer Cell Line) and L-929 (CCL-1TM, Mouse Fibroblast Cell Line) were obtained from ATCC, (Manassas, VA, USA). The cells were maintained in DMEM medium (Gibco Life Technologies, USA), which was completed with 10% (v/v) FBS (Biochrom, Berlin) and 1% pen/strep (Gibco Life Technologies, USA). The cells were incubated at 5% CO_2 humidified atmosphere and 37°C until 80-90% confluence was reached.

Cell Viability Assay

The aim of this study was to specify the cytotoxic effect of *I. aucheriana* ethanol extract on MDA-MB-231 and L929 cells for 24 hours. The cells were treated with an increased concentration of 0.0625 - 0.125 – 0.25 – 0.5 - 1 mg/mL of extract and the IC_{50} value was calculated. The ethanol extract of *I. aucheriana* was diluted in phenol red-free Dulbecco's Modified Eagle's Medium (DMEM) before treatment. The growing cells were seeded into 96-well microplates at a density of 1.5×10^4 cells per well in 100 µL complete culture medium and were allowed to adhere overnight. . These cells were then incubated with increasing concentrations of the ethanol extract of *I. aucheriana* (0.0625, 0.125, 0.25, 0.5, 1 mg/mL) for 24 hours. The cell proliferation was assigned using the XTT assay kit (BIOTIUM, Inc) according to the user's guide. Briefly, 50 µL XTT labelling mixture (to prepare the activated XTT kit solution, the activation reagent and the XTT solution were mixed in a 5:1 ratio) was placed on each well to identify metabolically active cells, and the plates were then cultured at 37°C for another 4h. The absorbance was evaluated using a spectrophotometer (ELISA reader; Thermo, Germany) at 450 nm. All experimental studies were conducted in three independent stages, and the cell proliferation results were described as a percentage of control (100% of viability).

Statistical Analysis

The statistical significance for the assays was assigned using GraphPad Prism 7 (GraphPad Software, Inc.). The obtained data were subjected to the ANOVA test. A value of $p \leq 0.01$ was considered statistically significant.

RESULTS

The Chemical Composition

When the data obtained were evaluated in terms of the proportional values among the phenolic compounds of the *Inula* plant, the highest phenolic value was determined as "Luteolin" with 32.55%, "Apigenin" with 21.66%, "Diosmetin" with 19.82% and 16.6% followed by Quercetin 3-methyl ether phenolics (Table 1).

Table 1. The chemical composition of the 80% ethanol extract of *I.aucheriana*
Çizelge 1. *I.aucheriana'nın %80 etanol ekstraktının kimyasal bileşimi*

No	R.T.	Phenolic composition	% Area
1	6.49	3,4-Dihydroxybenzoic acid	1,456394
2	17,6	Chlorogenic acid, iso chlorogenic acid	3,020271
3	20.14	Rutin	0.656758
4	22.15	Dicaffeoyl quinic acid isomers	4.241029
5	25.57	Luteolin	32.55262
6	25.63	Quercetin 3-methyl ether	16.5999
7	26.7	Apigenin	21.6573
8	26.9	Diosmetin	19.81573

Antioxidant Activity

ABTS and DPPH Radical Scavenging Activity

The in vitro antioxidant activities (ABTS and DPPH radical scavenging activities, total phenolic and flavonoid contents) of *I.aucheriana* in 80% ethanol extract were tested. The obtained data were compared with the reference substance butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). The extract had the lower IC_{50} value of DPPH radical scavenging activity (the IC_{50} value of 317.28±0.012 µg/mL) than the standard BHA (the IC_{50} value of 4.1±0.01 µg/mL). Similarly, the standard BHT (1.95±0.018 µg/mL) showed higher ABTS radical scavenging activity than the *I.aucheriana* 80% ethanol extract with the IC_{50} value of 237.4±0.008 µg/mL. However, it can be said that the values are close to the reference substance and the 80% ethanol extract of *I. aucheriana* has strong antioxidant activity (Figure 1).

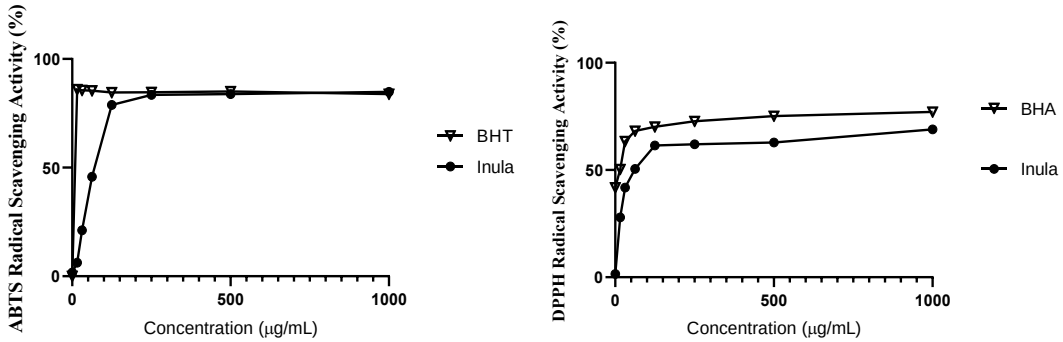


Figure 1. ABTS and DPPH radical scavenging activity of *I.aucheriana*
Şekil 1.*I.aucheriana'nın DPPH ve ABTS radikal süpürme aktivitesi*

Total Flavonoid and Total Phenol

When the total flavonoid and total phenol in 80% ethanol extract from *I.aucheriana* was examined, the total flavonoid content was found to be 94.36±1.9 mg CE/g, and the total phenolic content was 265.56±11.25 mg GAE/g (Figure 2). The total flavonoid and total phenol contents of *I.aucheriana* were found to be quite high. The phenolic compounds are the most important among the phytoconstituents in terms of antioxidant activity value.

The Enzyme Activities

The enzyme activities of 80% ethanol extract obtained from *I. aucheriana* were investigated (Table 2).

Butyrylcholinesterase–Acetylcholinesterase Inhibition Assay

The butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) inhibitory activities of the ethanol 80% extract of *I.aucheriana* were evaluated (Table 2). When the obtained results were compared with the reference drug (galantamine hydrobromide used for the treatment of Alzheimer's disease) (93.87±0.56% and 89.89±0.01 for % AChE and BChE inhibition, respectively), the ethanol 80% extract of *I.aucheriana* (75.94±0.09% and 78.63±0.02%, respectively) was seen to have AChE and BChE inhibition.

α-Amylase and α-Glucosidase Inhibition Assay

Acarbose was used as the reference drug for the inhibitory effects against α-amylase and α-glucosidase, which are are related to the antidiabetic activity enzyme. According to obtained data, the α-amylase and α-glucosidase inhibitory effect of *I.aucheriana* in the ethanol 80% extracts were determined as 53.26±0.12 and 18.07±0.03, respectively (Table 2). When the extract was compared with the reference drug (57.56±0.52% and 58.40±0.63% for the α-glucosidase and α-amylase, respectively), the 80% ethanol extract of *I.aucheriana* was seen to have an inhibitory effect in terms of α-glucosidase.

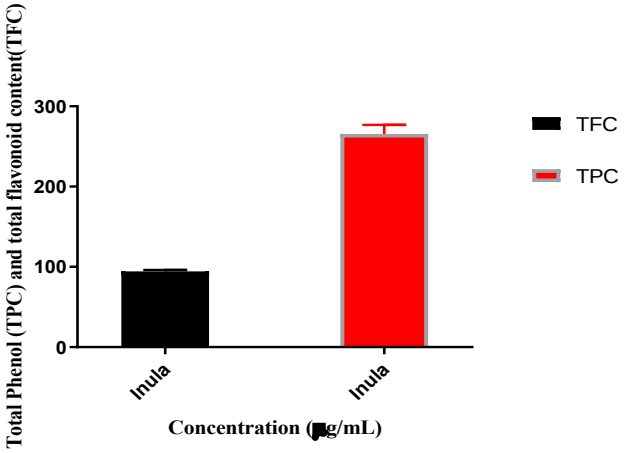


Figure 2. Total phenol and flavonoid content of 80% ethanol extract of *I.aucheriana.*
Şekil 2. *I. aucheriana'nın% 80 etanol ekstresinin toplam fenol ve flavonoid içeriği*

Table 2. Enzyme inhibition activity of 80% ethanol extract obtained from *I. aucheriana* and reference standards (at concentration of 2 mg/mL).
Çizelge 2. *Referans standartların ve I.aucheriana'nın % 80 etanol ekstresinin enzim inhibisyon aktivitesi (2 mg /mL konsantrasyonda).*

Extracts	Anticholinesterase Activity		Antidiabetic Activity		Skin Whitening
	AChE	BChE	α-Glucosidase	α-Amylase	Tyrosinase
80% ethanol extract of *I.aucheriana*	75.94±0.09	78.63± 0.02	53.26±0.12	18.07±0.03	59.21±0.08
Reference Drugs					
Galanthamine Hydrobromide	93.87± 0.56	89.89±0.01			
Acarbose			57.56±0.52	58.40±0.63	
Kojic Acid					56.42 ± 1.59

Tyrosinase Inhibition Assay

Kojic acid was used as the reference drug for the tyrosinase inhibition assay. When the % inhibitory activities of *I.aucheriana* in the 80% ethanol extract were compared with the positive control drug kojic acid (56.42±1.59%), the extract was seen to have very high Tyrosinase inhibition activity (59.21±0.08%) (Table 2).

Antimicrobial Activity

The antimicrobial activities of *I. aucheriana* ethanol extract against *C. tropicalis* and *C. albicans* and *B.cereus, E. coli, P. aeruginosa, S. aureus* were determined using the microdilution technique at the concentration range 0.312 to >2.5mg/mL (Table 3).

Table 3. The antimicrobial activity values of *I. aucherana* ethanol extract
Çizelge 3. *I.aucheriana'nın etanol ekstresinin antimikrobiyal aktivite değerleri*

	Micro-organisms and MIC values (mg/mL)					
	E.coli	*S.aureus*	*P.aeruginosa*	*B.cereus*	*C.albicans*	*C.tropicalis*
	ATCC 25922	ATCC 29213	ATCC 27853	ATCC 11778	ATCC 10231	DSM 11953
I. aucheriana	2.5	0.312	2.5	2.5	>2.5	2.5

The reference values of MIC were taken according to Holetz et al (2002). In the light of these data, it was found that the ethanol extract of *I. aucheriana* showed moderate antimicrobial activity on the *S. aureus* strain.

Cytotoxicity Assay

The ethanol extract of *I. aucheriana* considerably inhibited cell growth on the MDA-MB-231 cells at 0.0625 mg/mL and higher concentrations for 24 h in a dose-dependent manner (Figure 3). The IC_{50} value of the extract was calculated as 0.123 mg/mL. The extract did not show significant cytotoxicity on the L929 cell line at the IC_{50} concentrations.

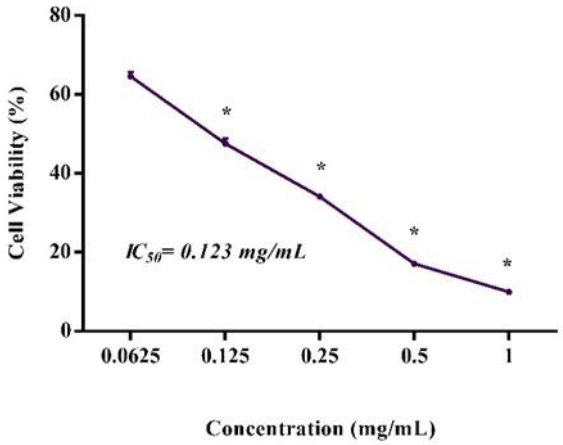


Figure 3. Cytotoxicity was determined by XTT assay. MDA-MB-231 cells treated with 0.0625 to 1 mg/mL of *I.aucheriana* ethanol extract for 24 h. Data are representative of the mean ± SEM of three separate experiments performed in triplicate.

Şekil 3. *Sitotoksisite, XTT testi ile belirlenmiştir. 0.0625 ila 1 mg mL I. aucheriana etanol ekstraktı ile 24 saat muamele edilen MDA-MB-231 hücrelerine ait veriler görülmektedir. Veriler, üç kez yapılan üç ayrı deneyin ortalama ± standart sapma oranını temsil etmektedir.*

DISCUSSION

In this study, different biological activities were investigated for more effective use of the medicinal plant, *Inula aucheriana.* The antioxidant and antimicrobial activity, enzyme inhibitory activity and cytotoxicity of 80% ethanol extract of this species were tested.

Eight different chemical components were obtained from 80% ethanol extract of *I. aucheriana* using the Q-TOF method. Luteolin was determined to be the main component at 32.55% (Table 1). In a study by Gökbulut et al. (2013), the chemical content of different species of the Inula plant was examined, and luteolin was found at a significantly high rate in the methanol extract obtained from *I. montbretiana* flowers, but was low in other species. In other studies conducted of different Inula species, one of the species with the richest luteolin content shares similar properties with *I.aucheriana* (Gökbulut et al., 2013;Ozkan et al., 2019). In a review article prepared by Lin et al.(2008), it was revealed that luteolin has many biological properties such as anti-cancer, antioxidant, anti-inflammatory and anti-allergy effects. In this respect, the phenolic compound content of *I.aucheriana* can be understood to be important.

According to the results obtained from the comparison of total flavonoid, total phenol levels with reference values (ABTS and DPPH radical scavenging activities), it was seen that *I. aucheriana* was a powerful antioxidant. Studies have indicated that antioxidant levels are high in different species of the Inula genus *(I. helenium, I. graveolens L. and I. britannica)* (Čanadanović-Brunet et al., 2002; Khan et al.,2010; Al-Fartosy et al., 2011;Kaur et al., 2014). Moreover, in another study conducted on six different Inula species, DPPH radical scavenging activity of these species and DPPH radical scavenging activity of 80% ethanol extract of *I. aucheriana*, which was examined in this study, were compared. It was found that *I.aucheriana* has much higher antioxidant activity compared to the other six species (Trendafilova et al., 2020). When the enzyme inhibitory activity was examined, it was determined that galantamine hydrobromide which was used as a reference drug in Alzheimer's disease, has an inhibitory property close to its inhibition on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).

When the α- amylase and α- glucosidase inhibition levels of *I.aucheriana* were examined, it was observed that they gave very similar results to acarbose, which was the reference drug, in determining the α - glucosidase inhibition level. However, the α- amylase

inhibition level was not high. In a previous study, three different extracts (MeOH, Aqueous and EtOAc) were prepared in five different Inula species (*I. helenium ssp. turcoracemosa, I. viscosa, I. thapsoides, I. peacockiana, I. montbretiana*), α - glucosidase inhibition levels were examined, and it was reported that the highest inhibition of α - glucosidase was in MeOH extract of *I. helenium* species (88.69% at 3000 µg / mL). When this study is compared with these previously published data, it can be said that α-glucosidase inhibition in 80% EtOH extract of *I.aucheriana* is more appropriate (53.26 ± 0.12 at 2000 µg / mL) (Orhan et al., 2017). In addition, the α-amylase inhibition level of *I. aucheriana* was found to be higher than that of the other five species. Even if different solvents were used, *I. aucheriana* appears to inhibit α- amylase and α- glucosidase at higher levels than other species. This indicates that this strain has high antidiabetic activity and could be a drug for diabetic patients.

The elevation of tyrosinase enzyme constitutes a potential danger in respect of the formation of dermatological disorders and skin cancer. Therefore, inhibition of tyrosinase enzyme may be clinically valuable in dermatological treatments (Hashemi and Emami, 2015). When the tyrosinase inhibition activity of *I. aucheriana* was examined, it showed a higher rate of inhibitory activity compared to the reference drug kojic acid (Table 2). Interestingly, contrary to these findings, different Inula species (*I. ensifolia* L., *I. oculus-christi L., I. conyza* (GRIESS.) DC, *I. aschersoniana* JANKA *var. Aschersoniana, I. germanica* L.*,* and *I. bifrons* L.) MeOH extract has shown low inhibition against the tyrosine enzyme (Trendafilova et al. 2020). Another study found that *I. crithmoides* species was a moderate tyrosinase inhibitor compared to kojic acid (Jdey et al., 2017). Although the high anti-thyrosinase activity of *I. aucheriana* suggests significant potential for dermatological treatment methods, it is still unknown whether different mechanisms increase this effect. There is a need for further studies in this direction.

When the effect of 80% ethanol extract of *I.aucheriana* on different bacterial and fungal strains was examined, it was found that it showed moderate antimicrobial activity on the *S. aureus* strain (Table 3). Similarly, it has been reported that antimicrobial activities show moderate effect in *I.helenium* and *I.montbretiana* species. However,in contrast, it has been suggested that *I. viscosa* species exhibit a more effective antimicrobial activity than other species (Gökbulut et al., 2013; Diguţăet al.,2014). These data show that the species belonging to this genus have different antimicrobial properties.

One of the most common research areas of medicinal plants today is cancer research. Using medicinal plants as active ingredients instead of synthetic drugs can have more effective results on metabolism. When the cytotoxic effect of *I.aucheriana* was examined on healthy fibroblast cell line L-929 and breast cancer cell line MDA-MB-231, it was determined that cancer cells were effectively inhibited (IC_{50}: 0.123 mg / mL) within 24 hours. However, there was no significant inhibition on healthy cells. In previous studies, sesquiterpene lactones, one of the secondary compounds of *I. aucheriana* was isolated and its cytotoxic effect on different cell lines (HepG-2, MCF-7 and A-549) was investigated, with results showing that all cell lines were effectively inhibited (Gohari et al. 2015). The results of both studies with *I. aucheriana* seem to support each other. However, in a study conducted by Trendafilova et al.(2020) of 6 different Inula species, it was stated that the lung cancer cell line A549 and the healthy kidney cell line MDCK II showed low cytotoxic properties. The cytotoxic effect of the *I.viscosa* species has been investigated in four different cell lines (MCF-7, C6, MG63, and L929), and it has been reported that the MCF-7 breast cancer cell line has a high rate of cytotoxic effect and the L929 cell line has a low cytotoxic effect (Hepokur et al., 2019). The different plant ingredients can explain the different cytotoxic effects of different species. These question marks can be eliminated by focusing on biochemical and genomic analyses on this issue.

CONCLUSION

Considering all these data, the pharmaceutical importance of *I. aucheriana* cannot be denied. In terms of being a versatile plant with antidiabetic, anti-hyperpigmentation, antioxidant and antiproliferative activity, it can be seen as a potential pharmaceutical plant for the treatment of many diseases, especially Alzheimer's, diabetes and dermatological diseases. The results of more comprehensive studies with the active ingredients of this plant will strengthen the possibility of using *I.aucheriana* to treat various diseases in the future.

Author Contributions

All the authors contributed equally to this study.

Statement of Conflict of Interests

The authors declare that they have no conflict of interests.

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