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In vitro investigation of antimicrobial, enzyme inhibitory and free radical scavenging activities of *Inula salicina* L.

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Abstract

In this study, *in vitro* biological activities and total phenol/flavonoid contents of methanol extract (ISM) and its hexane (ISH), chloroform (ISC), ethyl acetate (ISEA) and aqueous methanol (ISAM) fractions obtained from aerial parts of *Inula salicina* were investigated. ISEA showed the highest antioxidant activity against DPPH and ABTS radicals with an IC_{50} value of 0.014 mg $ml^{-1}$for both assays. ISEA exhibited a good anti-inflammatory activity with an IC_{50} value of 0.060 mg ml^{-1}. ISEA was found to exhibit a moderate level of antidiabetic activity against α amylase enzyme with an IC_{50} value of 0.290 mg ml^{-1}. ISEA and ISM presented low and moderate inhibitory activity against acetylcholinesterase and butyrylcholinesterase enzymes with IC_{50} values of 0.577 and 0.279 mg ml^{-1}, respectively. ISC with MIC values of 78 and 156 µg ml^{-1} displayed a significant antimicrobial activity against *Staphylococcus aureus* and *S. epidermidis*, respectively. Almost all extracts had moderate effect against *Candida* species. The highest total phenolic and flavonoid contents were determined in ISEA with 574.8 mg GAE (gallic acid equivalent) g^{-1} extract and 30.48 mg QE (quercetin equivalent) g^{-1} extract, respectively. These results showed that ISEA had a good antioxidant and anti-inflammatory activity with moderate α-amylase and butyrylcholinesterase inhibitory activity. Also, ISC exhibited a significant antimicrobial activity against *Staphylococcus* species.

Keywords

Asteraceae, Biological activity, Extracts, Fractions, *Inula salicina*

Introduction

The *Inula* genus is a perennial plant that spreads in Europe and East Asia (Konıshı et al., 2002). The genus *Inula* belongs to the Asteraceae family and consists of 28 species and 33 taxa (Anonymous, 2021). *Inula salicina* is a species with stem erect, roughly pubescent, 30-60 cm high. Flower heads are borne alone at the apex of the stem and measure 2.5-4 centimeters (0.98-1.57 inches) in diameter. Each head contains 35-70 yellow ray flowers containing 100-250 yellow disc flowers (Davis, 1975).

The genus *Inula* is a well-known medicinal plant among the people and it is used in folk medicine in the treatment of respiratory tract diseases such as asthma, bronchitis and pertussis, digestive disorders, urinary tract infections and also skin diseases (Stojanović-Radić et al., 2012). On the other hand, some *Inula* species in Turkey are used as cholagogue, diuretic, antitussive, expectorant, tonic, appetizing, against hemorrhoids, for wound healing and in the treatment of colds, bronchitis and stomach ailments (Sen et al., 2019). Also, the flowering aerial parts of *Inula salicina* are traditionally consumed as an herbal tea in Spain (Tardio et al., 2006).

Numerous biological activity studies are being conducted on *Inula* species. Antiproliferative (Dorn et al., 2006), antioxidant, anti-inflammatory, antidiabetic and antimicrobial activities are a few of the biological activities studied on *Inula* species. Also, essential oil of *Inula* species have antibacterial, antifungal (Cafarchia et al., 2002; Deriu et al., 2008), anti-inflammatory (Sen et al., 2019), antidiabetic (Sen et al., 2019) and antioxidant (Jallali et al., 2014; Sen et al., 2019) activities. The active constituents of the genus *Inula* are mainly flavonoids, terpenoids (sesquiterpene lactones and dimers, diterpenes, and triterpenoids) and essential oils (Tavares and Seca, 2019;; Trendafilova et al., 2020).

Total phenol content of *Inula salicina* was previously investigated by Sevindik et al. (Sevindik et al., 2020), while no study on *in vitro* anti-Alzheimer, antidiabetic, anti-inflammatory, antimicrobial and antioxidant activities (DPPH and ABTS radical scavenging activities) along with total flavonoid content of *Inula salicina* extracts have been reported until now. In this context, the present study aims to comprehensively evaluate the biological activities of *Inula salicina* extracts with different activity assays together with their total phenol and flavonoid content.

Materials and Methods

Plant material

Aerial parts of plant were collected at their flowering period from the Hanönü district of Kastamonu Turkey and kept in a dark and cool place until extraction. The plant was identified by Dr. İsmail Şenkardeş, a botanist of the Faculty of Pharmacy, University of Marmara. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE No:19871).

Extraction

About 15 g of dried aerial parts of *Inula salicina* were extracted with 8×200 ml EtOH, using an ultrasonic bath. After filtration and evaporation, the methanol extract (ISM) was dissolved in 30 ml 60% aqueous methanol, and subjected to solvent-solvent partition between *n*-hexane (5×50 ml), chloroform (3×50 ml), and ethyl acetate fraction (2x50 ml). The *n*-hexane, chloroform, ethyl acetate fractions and aqueous methanol fraction of *Inula salicina* obtained by this method were coded as ISH, ISC, ISEA and ISAM, respectively. Extraction yields have been summarized in Table 1. All extracts were stored under refrigeration for further analysis.

DPPH radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity of extract and fractions was determined by the method of Zou et al. (Zou et al., 2011).

ABTS radical-scavenging activity

2,2′-Azino-bis[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) radical cation scavenging activity assay was carried out according to the method described by Zou et al. (Zou et al., 2011).

In vitro anti-lipoxygenase activity

The anti-lipoxygenase activity was evaluated with slight modifications according to the method described by Phosrithong et al. The method was adapted to the 96 well transparent microplate (Phosrithong and Nuchtavorn, 2016; Yıldırım et al., 2019; Iduğ et al., 2022).

α-amylase inhibitor activity

The α-amylase inhibitor activity was evaluated with slightly modified method of Ramakrishna et al. The method was adapted to a 96-well microplate format (Ramakrishna et al., 2017; Sen et al., 2019).

Cholinesterase inhibitory activity

Acetylcholinesterase and butyrylcholinesterase inhibitory activities of extract and fractions were determined by the method of Im et al. (Im et al., 2016).

In vitro antimicrobial activity

Antimicrobial activity against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153 and *Candida albicans* ATCC 1023, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* ATCC 750 were determined by the microbroth dilutions technique using the Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2006; CLSI, 2008). Ciprofloxacin and fluconazol were used as reference antibimicrobials for bacteria and yeast, respectively of standardization of the assay. The MIC values of the ciprofloxacin and fluconazole were within the accuracy range in CLSI throughout the study (CLSI, 2014). The antimicrobial activity of extract/fractions were performed according to Bitis et al. (2017).

Determination of total phenolic contents (TPC)

Total phenolic contents of *Inula salicina* extract/fractions were measured using Folin–Ciocalteau reagent. The assay was adapted to the 96 well microplate format (Gao et al., 2000; Yıldırım et al., 2019).

Determination of total flavonoid contents (TFC)

Total flavonoid content was determined following a method by Zhang at al. The assay was adapted to the 96 well microplate format (Yıldırım et al., 2019; Zhang et al., 2013).

Results and Discussion

The antioxidant activity of the extract/fractions was investigated by two methods; DPPH radical scavenging activity, ABTS radical scavenging activity. DPPH is a purple colored radical that transforms into a yellow non-radical form in the presence of a powerful antioxidant molecule. This color change occurs when the DPPH radical takes up a hydrogen from the antioxidant molecule (See et al., 2017). ABTS radical cation decolorization analysis is a method for screening the antioxidant activities of molecules and can be applied to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids and plasma antioxidants. The preformed radical monocation of 2,2′-Azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) is produced by oxidation of ABTS with potassium persulfate and is reduced in the presence of hydrogen donating antioxidants (Re et al., 1999).

A low IC_{50} value shows the high activity. As shown in Table 1, ISEA and ISM with IC_{50} values of 0.014 and 0.019 mg ml^{-1} were found to be superior to other *I. salicina* extracts for the DPPH radical scavenging activity assay. ISH with an IC_{50} of 0.639 mg ml^{-1} was found to have lowest antioxidant activity for DPPH assay. The DPPH radical scavenging powers of the extracts in decreasing order were as follows: ISEA > ISM > ISAM > ISC > ISH (Table 1). In ABTS radical scavenging assay, ISEA with an IC_{50} value of 0.014 mg ml^{-1} was better than other extracts, while ISH with an IC_{50} value had the lowest activity of 0.406 mg ml^{-1}. The ABTS radical scavenging

powers of the extracts in decreasing order were as follows: ISEA > ISAM > ISM > ISC > ISH (Table 1). While data on DPPH and ABTS radical scavenging activity of this plant are not available in the literature, there are studies on different *Inula* species. Chendouh et al. carried out the antioxidant analysis of the ethyl acetate fraction of the methanol extract obtained from the dry leaves of *Inula viscosa*. According to the results, the free radical scavenging capacity of the IvE (*Inula viscosa* ethyl acetate) fraction was found to be IC_{50} 14.1 µg ml^{-1} and 24.2 µg ml^{-1} against DPPH and ABTS radicals, respectively (Brahmi-Chendouh et al., 2019). Ivanova et al. found that the methanol extract of the leaves and flowers of the *Inula britannica* had an equivalent of 15.5 mg and 44.4 mg trolox per g dry weight of plant while chloroform extract of the leaves and flowers of this plant was 1.6 and 1.1 mg trolox equivalent per dry plant, respectively (Ivanova et al., 2017). Gökbulut et al. evaluated the DPPH and ABTS radical scavenging activities of water, methanol and ethyl acetate extracts of leaf, flowers and roots of *Inula viscosa, I. montbritania* and *I. helenium*. According to DPPH radical scavenging activity results, the aqueous extract of *I. viscosa* flowers, methanol extract of the roots of *I. montbritania* and methanol extract of flowers of *I. helenium* showed the best antioxidant activity with IC_{50} values of 0.28 mg ml^{-1}, 0.23 mg ml^{-1} and 0.14 mg ml^{-1}, respectively. According to ABTS radical scavenging activity results, the aqueous extract of *I. viscosa* flowers, aqueous extract of roots of *I. montbritania* and aqueous extract of flowers of *I. helenium* showed the best antioxidant activity with IC_{50} values of 0.17 mg ml^{-1}, 0.25 mg ml^{-1} and 0.05 mg ml^{-1}, respectively (Gökbulut et al., 2013). In a study performed by Bucchini et al. was investigated the antioxidant activity of hexane, dichloromethane and methanol extracts of *I. crithmoides* in terms of DPPH radical scavenging activity. According to their results, hexane (IC_{50}: 0.57 mg ml^{-1}) and methanol (IC_{50}: 0.59 mg ml^{-1}) extracts exhibited the highest antioxidant activity (Bucchini et al., 2015). In another study by Mahmoudi et al., it was found that methanol extract of *I. viscosa* had antioxidant activity with IC_{50} values 23.33 and 16.75 mg ml^{-1} against DPPH and ABTS radicals, respectively (Mahmoudı et al., 2016). In the present study, ISEA with IC_{50} values of 14 µg ml^{-1} (for both) against DPPH and ABTS radicals and ISM with an IC_{50} value of 19 µg ml^{-1} against DPPH radicals showed significant antioxidant activity compared to standards ascorbic acid and trolox. The results obtained from the present study were close (Chendouh et al. and Gökbulut et al.) to or better than the results of other studies.

ISEA exhibited good anti-lipoxygenase activity with an IC_{50} value of 0.060 mg ml^{-1} compared to the standard (IC_{50} for indomethacin: 0.022 mg ml^{-1}). Also, ISH with an IC_{50} of 0.220 mg ml^{-1} showed the lowest anti-lipoxygenase activity (Table 1). To the best of our knowledge, this is one of the first studies to investigate the antilipoxygenase activity of *Inula salicina* extracts. Also, there is only one study in the literature on *Inula crithmoides*, a different *Inula* species. In the study conducted by Bucchini et al., it was reported that hexane, dichlorometane and methanol extracts of the aerial parts of *Inula crithmoides* showed anti-inflammatory activity against lipoxygenase enzyme with 13.48, 951.37 and 97.45 µg ml^{-1} IC_{50} values (Bucchini et al., 2015). In the current study, ISH, ISC and ISM with IC_{50} values of 220, 97 and 174 µg ml^{-1} exihibited antilipoxygenase activity against lipoxygenase enzyme. When the activity of the ISC (since it has similar polarity to dichloromethane) was compared with the result found by Bucchini et al. for the dichloromethane extract, the ISC was found to have a much higher anti-lipoxygenase activity.

ISEA with an IC_{50} value of 0.290 mg ml^{-1} showed the highest anti-α-amylase activity when compared to other extracts but lower than that of the reference compound acarbose (Table 1). Although there is no study on *α*-amylase inhibitory activity of *Inula salicina* extracts, two studies on extracts from different *Inula* species have been previously reported. In a comprehensive study on α-amylase inhibitory activity of many plants by Kim et al., methanol extracts of the above-ground parts of *Inula britannica* and *I. helenium* against alpha amylase enzyme did not show any inhibitory activity with 0% and -49% percent inhibition rates at a concentration of about 500 µg ml^{-1}, respectively (Kim et al., 2002). In another study investigating the antidiabetic activity of aqueous, methanol and ethyl acetate extracts of flowers, leaves and roots of *Inula helenium* subsp. *turcoracemosa, I. montbretiana, I. peacockiana, I. thapsoides* subsp. *thapsoides* and *I. viscosa*, alpha amylase inhibitory activities of the extracts were observed to range from 0.38 to 39.94 percent at a concentration of 3 mg ml^{-1} (Orhan et al., 2017). In present study, α-amylase inhibitory activities (IC_{50} values) of *Inula salicina* extracts were found to vary between 0.290-1.748 mg ml^{-1}. The results were better compared to previous studies.

All tested extracts were weak inhibitors of AChE with IC_{50} values between 0.577 and 3.603 mg ml^{-1} in comparison with galantamine (IC_{50}: 0.032 mg ml^{-1}) used as positive control. However, ISM with an IC_{50} of 0.279 mg ml^{-1} demonstrated good inhibition for BchE in comparison with galantamine (IC_{50}: 0.190 mg ml^{-1}) used as positive control (Table 1). No studies on *in vitro* anti-Alzheimer activities of *Inula salicina* extracts have been reported so far, but there is only two previous study on AChE inhibitory activities of different *Inula* species. Also, this is the first study on BChE inhibitory activities of *Inula* species. In an experiment performed by Trendafilova et al. in which the anti-Alzheimer activity of the of *I. conyza* flowers and leaves, *I. ensifolia* flowers, *I. aschersoniana* var. *aschersoniana* flowers, *I. oculus-christi* flowers, *I. bifrons* flowers, *I. germanica* flowers were examined with the aid of acetylcholinesterase enzyme, all extracts tested at a concentration of 3 mg ml^{-1} were weak inhibitors of AChE with an inhibition of between 5% and 17%. It was the methanol extract of *I. ensifolia* flowers, with 17% inhibition of AChE, that showed the highest activity among the extracts (Trendafilova et al., 2020). In another study, Abuhamdah et al. reported that ethanol extract of *I. viscosa* exhibited less than 50% inhibition on AChE enzyme at a concentration of 0.5 mg ml^{-1} (Abuhamdah et al., 2014). In the current study, almost all extracts showed higher AChE inhibitory activity than previous studies.

Total phenolic and flavonoid contents of extracts were calculated as gallic acid and quercetin equivalents per g dried extract, respectively. Among all the extracts studied, the highest total phenolic and flavonoid amounts were found in ISEA (574.8 and 201.40 mg g^{-1}, respectively). As shown in Table 2, the total amount of phenolics and

flavonoids in the extracts ranged from 40.62 to 574.80 mg gallic acid equivalents and 10.22 to 201.40 mg quercetin equivalents per dried extract, respectively (Table 2). There is only one study by Sevindik et al. (2020) on the total phenolic compound content of *I. salicina* (58.54 µg GAE ml^{-1}) while no study on total flavonoid content of this species has been found in the literature (Sevindik et al., 2020). However, few studies were performed previously on total phenol and flavonoid contents of different *Inula* species. Mahmoudi et al. investigated the total phenol and flavonoid amounts of the methanol extract of the aerial parts of *I. viscosa* and found to be 103 mg gallic acid equivalent (GAE) and 84,92 mg catechin equivalent (CE) per g extract, respectively (Mahmoudı et al., 2016). In another study conducted by Jallali et al., it was determined that 80% aqueous acetone extract of *I. crithmoides*, collected from two different Tunisian regions, were 14.1 and 6.7 mg GAE g^{-1} dry weight for total phenol contents and 6.7 and 5.6 mg CE g^{-1} dry weight for total flavonoid contents, respectively (Jallali et al., 2014). Ivanova et al. revealed that total phenolic content in the methanol extract of *I. britannica* flowers was 7.9 mg of gallic acid equivalent g^{-1} of dried weight (Ivanova et al., 2017). In a study conducted with various *Inula* species (*I. viscosa* [herb and root], *I. montbretiana* [herb and root], *I. helenium* [herb, root]), it was observed that total phenolic contents of methanol extracts of *Inula* species ranged between 21.1 and 190.9 mg GAE per g extract (Gökbulut et al., 2013). In another study, among three different extracts of the aerial parts of the *Inula crithmoides*, methanol extract had the highest amount of phenolic compound with a value of 15.52 mg g^{-1} dry extract (Bucchini et al., 2015). In previous studies, the total phenol and flavonoid contents of methanol extracts of *Inula* species were generally investigated. In our current study, the total phenol and flavonoid contents of the methanol extract obtained from *I. salicina* were found to be 143.8 mg GAE (gallic acid equivalent) g $extract^{-1}$ (9.10 mg GAE g dry g^{-1} of dried weight) and 83.92 mg QE (quercetin equivalent) g $extract^{-1}$ (5.31 mg QE g dry g^{-1} of dried weight), respectively. These results were close to or better than most results of the previous study. At the same time, the total phenol (574.8 mg GAE g $extract^{-1}$ or 6.32 mg GAE g dry g^{-1} of dried weight) and flavonoid content of ISEA (201.4 mg QE g $extract^{-1}$ or 2.22 mg QE g dry g^{-1} of dried weight) was found to be significantly higher than previous studies.

Table 1. Biological activities of *I. salicina* extracts

Extracts*, **/ Standards	Yield (%)	Antioxidant activity		Anti-inflammatory activity	Antidiabetic activity	Anti-Alzheimer activity	
		ABTS radical scavenging activity	DPPH radical scavenging activity	Anti-lipoxygenase activity	α-amylase inhibitory activity	Acetyl-cholinesterase inhibitory activity	Butyryl-cholinesterase inhibitory activity
		IC_{50} (mg ml^{-1})					
ISM	6.33	0.125±0.001 [d]	0.019±0.000 [a]	0.174±0.002 [e]	0.768±0.002 [d]	2.974±0.014 [c]	0.279±0.004 [b]
ISH	0.97	0.406±0.040 [e]	0.639±0,007 [f]	0.220±0.000 [f]	1.748±0.192 [f]	3.603±0.032 [d]	1.114±0.001 [d]
ISC	0.27	0.133±0.001 [d]	0.105±0.001 [d]	0.097±0.001 [c]	0.333±0.001 [c]	2.862±0.008 [c]	1.027±0.005 [d]
ISEA	1.10	0.014±0.000 [a]	0.014±0.000 [a]	0.060±0.002 [b]	0.290±0.001 [b]	0.577±0.012 [b]	0.474±0.006 [c]
ISAM	4.04	0.107±0.001 [c]	0.093±0.001 [c]	0.127±0.003 [d]	0.781±0.001 [e]	3.458±0.022 [d]	3.890±0.002 [e]
Ascorbic acid		0.015±0.000 [a]	0.018±0.000 [a]				
Trolox		0.013±0.000 [a]	0.015±0.000 [a]				
Butylated hydroxyanisole		0.017±0.001 [a]	0.057±0.000 [b]				
Butylated hydroxytoluene		0.027±0.001[b]	0.214±0.015 [e]				
Indomethacin				0.022±0.000 [a]			
Acarbose					0.006±0.000 [a]		
Galantamine						0.032±0.001 [a]	0.190±0.001 [a]

*Abbreviations: ISM, ISH, ISC, ISEA, ISAM show the methanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous methanol fractions of *Inula salicina*, respectively.
* The yields of extracts were calculated from the powdered dry plant.
** Each value in the table is represented as mean ± SD (n=3). The values with different letter superscripts in the same column indicate significant differences ($p<0.05$).

Table 2. Total phenol and flavonoid contents of *I. salicina* extracts

Extracts *	TPC (mg GAE/g extract) **	TFC (mg QE/g extract) **
ISM	143.80 ± 0.26 [c]	83.92 ± 1.08 [c]
ISH	40.62 ± 0.00 [a]	10.22 ± 0.12 [a]
ISC	166.20 ± 0.25 [d]	67.14 ± 0.86 [b]
ISEA	574.80 ± 8.44 [e]	201.40 ± 2.58 [d]
ISAM	126.10 ± 4.35 [b]	86.44 ± 1.11 [c]

* Abbreviations: ISM, ISH, ISC, ISEA, ISAM show the methanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous methanol fractions of *Inula salicina*, respectively.
** Total phenolic and total flavonoid contents were expressed as gallic acid equivalent (GAE) and quercetin equivalent (QE), respectively.
*** Each value in the table is represented as mean ± SD (n=3). The values with different letter superscripts in the same column indicate significant differences ($p<0.05$).

Saraiva et al. (2011) suggested that plant extracts with MIC values of < 100 µg ml^{-1} were considered to be highly active antimicrobial agents; those with MICs of 100 to 500 µg ml^{-1} were defined as active; those with MICs of 500 to 1000 µg ml^{-1} were defined as moderately active; those with MICs of 1000 to 2000 µg ml^{-1} were considered to have low activity; and those with MICs of > 2000 µg ml^{-1} were defined as inactive (Saraiva et al., 2011). Based on

this evaluation, ISC with MIC values of 78 and 156 µg ml^{-1} showed good an antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The extracts generally showed moderate antifungal activity against *Candida* sp., while they exihibited weak antibacterial activity against the bacteria tested (Table 3). So far, there is no study that demonstrated antimicrobial activity of *Inula salicina*, but there are many studies about antimicrobial activity of different *Inula* species. In one of these studies, Gökbulut et al. (2013) reported that methanol extracts of *I. viscosa* root (MIC: 100 µg ml^{-1}) against *E. coli; I. viscosa* root (50 µg ml^{-1}), *I. montbretiana* flower (100 µg ml^{-1}), *I. montbretiana* leaf (100 µg ml^{-1}), *I. montbretiana* root (100 µg ml^{-1}), *I. helenium* ssp. *turcoracemosa* leaf (100 µg ml^{-1}), *I. helenium* ssp. *turcoracemosa* root (100 µg ml^{-1}) against *S. aureus; I. viscosa* root (50 µg ml^{-1}), *I. montbretiana* flower (50 µg ml^{-1}), *I. montbretiana* root (100 µg ml-$^{1)}$, *I. helenium* ssp. *turcoracemosa* root (100 µg ml^{-1}) against *E. faecalis*; *I. viscosa* root (100 µg ml^{-1}), *I. montbretiana* flower (100 µg ml^{-1}), *I. helenium* ssp. *turcoracemosa* root (100 µg ml^{-1}) against *C. albicans; I. viscosa* root (50 µg ml^{-1}), *I. montbretiana* flower (50 µg ml^{-1}), *I. montbretiana* leaf (100 µg ml^{-1}), *I. montbretiana* root (100 µg ml^{-1}), *I. helenium* ssp. *turcoracemosa* leaf (100 µg ml^{-1}), *I. helenium* ssp. *turcoracemosa* root (50 µg ml^{-1}) against *C. tropicalis* had antimicrobial activity (Gökbulut et al., 2013). In another study by Gokbulut at al (2016), it was suggested that methanolic extracts of I. *thapsoides* ssp. *thapsoides* flower (MIC: 100 µg ml^{-1}), *I. thapsoides* ssp. *thapsoides* leaf (100 µg ml^{-1}), *I. thapsoides* ssp. *thapsoides* root (100 µg ml^{-1}) against *E. coli; I. peacockiana* flower (50 µg ml^{-1}), *I. thapsoides* ssp. *thapsoides* root (50 µg ml^{-1}) against *S. aureus; I. peacockiana* flower (50 µg ml^{-1}), *I. thapsoides* ssp. *thapsoides* root (50 µg ml^{-1}) against *E. faecalis; I. peacockiana* flower (100 µg ml^{-1}), *I. thapsoides* ssp. *thapsoides* root (50 µg ml^{-1}) against *C. albicans; I. peacockiana* flower (50 µg ml^{-1}), *I. thapsoides* ssp. *thapsoides* flower (100 µg ml^{-1}), *I. thapsoides* ssp. *thapsoides* leaf (100 µg ml^{-1}), *I. thapsoides* ssp. *thapsoides* root (50 µg ml^{-1}) against *C. tropicalis* had antimicrobial activity (Gökbulut et al., 2013). In another study, it was found that aqueous extract of *Inula oculus-christi* had antimicrobial activity against *Shigella boydii, Shigella dysanteriae, Bacillus subtilis, Klebsiella pneumoniae, S. aureus* and *Corynebacterium diphteriae* with MIC values of 36.00, 18.00, 36.00, 72.00, 36.00 and 72.00 µg ml^{-1}, respectively (Berk et al., 2000). In contrast to these studies, *Inula salicina* methanol extract was found to have weak antimicrobial activity. However, similar to these studies, *Inula salicina* chloroform extract was found to be effective against *S. aeurus* (Table 3).

Table 3. Antimicrobial activities (MIC values, µg ml^{-1}) of *I. salicina* extracts

Extracts */	Microorganisms								
Standards	*S.a.*	*S.e.*	*E.c.*	*K.p.*	*P.a.*	*P.m.*	*C.a.*	*C.p.*	*C.t.*
ISM	1250	1250	1250	1250	1250	1250	625	1250	625
ISH	1250	-	-	-	-	-	625	625	625
ISC	78	156	-	-	-	-	-	-	625
ISEA	2500	2500	1250	-	1250	1250	625	625	625
ISAM	2500	2500	625	1250	1250	1250	625	625	625
Standards	0,25 **	-	-	-	-	-	0,5 ***		

* Abbreviations: ISM, ISH, ISC, ISEA, ISAM show the methanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous methanol fractions of *Inula salicina*, respectively. Also, *S.a., S.e., E.c., K.p., P.a., P.m., C.a., C.p.* and *C.t.* show *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* ATCC 750.
** ciprofloxacin, *** flukonazol
-: For MIC value < 2500 µg ml^{-1}

Conclusion

ISC and ISEA could be a new source of bioactive compounds with promising antioxidant, anti-inflammatory, and antimicrobial properties. These results confirm the traditional use (such as wound healing, treatment of asthma and bronchitis) of *Inula* species. However, it is primarily necessary to carry out bioactivity-directed isolation studies along with *in vivo* studies on these extracts. Also, traditional use of this species as an herbal tea may also have beneficial effects on health due to its antioxidant, antimicrobial and anti-inflammatory activities.

Compliance with Ethical Standards

Conflict of interest

No conflict of interest was declared by the authors.

Author contribution

All authors contributed extensively to the work presented in this paper. A.Y., A.Ş. and L.B. designed the study. İ.Ş. identified the plant. A.Y. and A.Ş. prepared *Inula salicina* extracts. A.Y., A.Ş., İ.Ş. and L.B. conducted a literature research related to *Inula salicina*. A.Y., A.Ş., M.H. and A.S.B.T. performed experiments. All authors discussed the results and implications and commented on the manuscript at all stages. All authors have approved the manuscript.

Ethical approval

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Data availability

Not applicable.

Consent for publication

Not applicable.

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