International Journal of Secondary Metabolite
2023, Vol. 10, No. 4, 525–534
https://doi.org/10.21448/ijsm.1272860
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

Inula viscosa L. (Asteraceae): A study on its antimicrobial and antioxidant activities, chromatographic fingerprinting profile

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Abstract: Food products contaminated with pathogens and spoiled not only lead to a decrease in the quality and quantity of food products but also contribute to the spread of diseases, which are increasingly becoming a public health problem in both developed and developing countries. Due to the multiple resistance of these pathogens to antibiotics, the search for natural products with antimicrobial properties is becoming increasingly important. *Inula viscosa* has been used as a medicinal plant for a long time in many Mediterranean countries. The aim of this study was to investigate the antimicrobial effects of *I. viscosa* extracts against foodborne pathogens and their non-enzymatic antioxidant potential. Antimicrobial activity was measured using the disc diffusion method. Additionally, plant extracts were tested against 2,2-Diphenyl-1-picrylhydrazyl and 2,2'-azinobis (3-ethyl-benzothiazoline 6-sulfonate) radicals for antioxidant activity. *Inula viscosa* showed the highest antibacterial activity against *Bacillus subtilis* with the methanol extract (19 mm zone diameter), while the lowest activity was observed against *Salmonella* Typhimurium, with inhibition zone diameters of 7 mm. The highest antioxidant activity was recorded as 77.5% for the DPPH• method and 73.8% for the ABTS• method. In conclusion, this plant can be considered a natural antimicrobial and antioxidant agent against foodborne pathogens, and it is a promising candidate for large-scale experiments.

ARTICLE HISTORY

Received: Mar. 29, 2023
Revised: July 18, 2023
Accepted: Aug. 28, 2023

KEYWORDS

Inula viscosa,
Food pathogens,
Antimicrobial activity,
Antioxidant activity,
Thin layer chromatography.

1. INTRODUCTION

It is estimated that annual global food losses reach up to 40% due to various factors, including spoilage by microorganisms (Gonelimali *et al*., 2018). Bacteria, yeast, and molds are among the common types of microorganisms responsible for the spoilage of a significant number of food and food products (Lianou *et al*., 2016). Foodborne illnesses are another common food safety problem caused by the consumption of contaminated food products, which is a major safety concern for public health (Kirk *et al*., 2017).

It is known that products obtained from both the above-ground and underground parts of plants are used in traditional medicine in our country (Günter *et al*., 2020). Today, in addition

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e-ISSN: 2148-6905 / **© IJSM 2023**

to meeting basic needs, bioactive compounds from plants are used in various fields such as the pharmaceutical industry, chemistry, cosmetics, and agricultural control (Birch & Bonwick, 2018; Daliu *et al*., 2018; Aguiar *et al*., 2019; Reque & Brandelli, 2021). Furthermore, due to the side effects of synthetic drugs, especially bacterial resistance to them, the importance of natural plant-derived ingredients has increased even more today (Çolak *et al*., 2020).

The *Inula* L. genus, consisting of nearly 100 species worldwide, is predominantly distributed in the Mediterranean region (Gökbulut *et al*., 2013). *Inula viscosa* L. is a perennial herbaceous species belonging to the Asteraceae family and is known as "kanserotu" in Türkiye (Mohti *et al*., 2020). This plant has been traditionally used for its anti-inflammatory, antipyretic, and antiseptic properties (Özhan *et al*., 2013), as well as in the treatment of diabetes, cancer, hypertension, bronchitis, tuberculosis, and various other disorders in traditional medicine for many years (Fontana *et al*., 2007; Ozkan *et al*., 2019).

In this study, the aim was to determine the antioxidant activity as well as the antimicrobial activity of *Inula viscosa* against food-spoilage pathogens.

2. MATERIAL and METHODS

2.1. Plant Material

Inula viscosa was collected from the C2 region (Mugla) of Türkiye (37°01'29.40"N; 28°30'13.79"E) in 2020. The plant material was identified by Dr. Olcay Ceylan, and the plant is stored in the herbarium of Mugla Sitki Kocman University, Department of Biology, Türkiye. The plant was identified according to Flora of Turkey (Davis (1965-1988; Grierson, 1975).

2.2. Preparation of Plant Material

The samples were washed 2-3 times in running water and once in sterile distilled water. Plant parts were air-dried and then powdered using a shredder. All materials were stored at room temperature until sample preparation, and then they were kept at 4°C until needed for analysis.

2.3. Plant Extraction

The air-dried and powdered samples were extracted with water, methanol, and ethanol using a Soxhlet apparatus. After the extracts in organic solvents were evaporated, they were preserved in their respective solvents in small, sterile, opaque bottles and stored in a refrigerator until they were needed.

2.4. Microorganisms

There are 6 foodborne pathogens used in the study, all of which are bacteria. These bacteria include *Bacillus subtilis* RSKK245, *Staphylococcus aureus* RSKK2392, *Salmonella* Typhimurium RSKK19, *Enterococcus faecalis* ATCC8093, *Yersinia enterocolitica* NCTC11174, and *Listeria monocytogenes* ATCC644. Bacterial cultures were grown in Mueller-Hinton Broth (Merck) medium for 24 hours at 37°C. The microorganisms were obtained from the American Type Culture Collection, National Collection of Type Cultures, and the Refik Saydam National Culture Collection.

2.5. Cultivation

Microorganisms were grown in Mueller-Hinton Broth (MHB, Merck) medium by incubating them at their respective temperatures for 24 hours. Active cultures grown for 18 hours were used for all experiments. The turbidity of all microbial cultures was adjusted to 0.5 McFarland.

2.6. Determination of in vitro Antimicrobial Activity

Antimicrobial activity studies were performed using the disk diffusion method (Bauer & Kirby, 1966). Plant extracts (400 mg/ml) were tested by the disk diffusion method, and cultures were

incubated on Mueller-Hinton Agar plates (MHA, Merck) for 24 hours at their respective temperatures. The turbidity of bacterial cultures was adjusted to 0.5 McFarland. Chloramphenicol (30 µg) antibiotic was used as a positive control.

2.7. Determination of the Minimum Inhibitory Concentration (MIC)

The broth dilution method was tested as defined in the CLSI standards (CLSI, 2003; CLSI, 2006). This test was adjusted for the final concentrations of each extract, which were 13.000; 6.500; 3.250; 1.625; and 812.5 µg/mL.

2.8. Determination of Non-Enzymatic Antioxidant Activity

In this study, 2,2-diphenyl-1-picrylhydrazyl (DPPH*) and 2,2'-azino-bis (3 ethyl benzo thiazoline-6-sulfonic acid) (ABTS*) radical scavenger activities were used to assess the antioxidant activities of plant extracts (Brand-Williams *et al*., 1995; Re *et al.*, 1999). The results of the trials are reported in mM trolox (TE) per milligram of dry weight.

2.9. Thin Layer Chromatography Analysis for Components of Plant Extracts

This assay was conducted using thin-layer chromatography (TLC) on aluminum sheets coated with silica gel 60F254 (Merck). The extracts (5 spots) were applied to TLC plates and run in a chloroform:methanol mixture (4.5:0.5, v/v). Each plant extract was spotted onto a plate using volumetric micropipettes, along a virtual line positioned 50 mm from the bottom edge of the plate (6.7 x 0.4 cm). The spots were applied at 10-mm intervals. After the chromatogram was developed, the plates were dried, and the spots were visualized sequentially (Zingales, 1967; Zingales, 1968).

3. RESULTS

In our study, which examined the antimicrobial activities of *Inula viscosa* extracts against foodborne pathogens, the zone diameters observed against the tested microorganisms are presented in Table 1. The highest activity was achieved with the methanol extract against *Bacillus subtilis* RSKK245, resulting in a zone diameter of 19 mm (Table 1).

Table 1. Antimicrobial activities of *Inula viscosa* against food pathogens (400 mg/ml)

Microorganisms	Inhibition zone diameter (mm)						
	EE	ME	AE	E	M	A	C
Bacillus subtilis RSKK245	16	19	17	-	-	-	12
Staphylococcus aureus RSKK2392	9	12	8	-	-	-	15
Salmonella Typhimurium RSKK19	8	8	7	-	-	-	22
Enterococcus faecalis ATCC8093	-	8	-	-	-	-	22
Listeria monocytogenes ATCC7644	9	10	8	-	-	-	22
Yersinia enterocolitica NCTC11174	8	9	-	-	-	-	20

EE: Ethanol Extract; ME: Methanol Extract; AE: Aqueous Extract; E: Ethanol; M: Methanol; A: Water; C: Chloramphenicol; (-): No inhibition

Table 2 contains the MIC values of the various solvents obtained from the broth dilution method for the plant used in the study. The lowest MIC value, 3250 µg/mL, was determined for the methanol extract of *Inula* against *Bacillus subtilis* RSKK245 (Table 2).

Table 2. Minimum inhibitory concentrations of *Inula viscosa* against food pathogens (µg/ml)

Microorganisms	Minimum inhibitory concentration		
	EE	ME	AE
Bacillus subtilis RSKK245	13000	3250	6500
Staphylococcus aureus RSKK2392	13000	6500	_
Salmonella Typhimurium RSKK19	13000	13000	_
Enterococcus faecalis ATCC8093	NT	13000	NT
Listeria monocytogenes ATCC7644	13000	6500	_
Yersinia enterocolitica NCTC11174	_	13000	NT

EE: Ethanol Extract; ME: Methanol Extract; AE: Aqueous Extract; NT: not tested; (-): No activity could be detected at values up to 13000 µg/ml

The antioxidant activities of *Inula viscosa* were determined using the DPPH and ABTS methods. In this study, the radical scavenging activity of the ethanol extract was 77.5%, that of the methanol extract was 76%, and that of the aqueous extract was 58.7%. Therefore, the DPPH activity was determined as follows: ethanol > methanol > water (Table 3).

Table 3. DPPH radical scavenging activities of *Inula viscosa*.

Plant (400mg/ml)	EE		ME		AE	
	SA	TE	SA	TE	SA	TE
Inula viscosa	77.5	2.3	76.3	2.3	58.7	2.08

EE: Ethanol Extract; ME: Methanol Extract; AE: Aqueous Extract; SA: Scavenging activity (%); TE: Trolox equivalent (mM trolox (TE)/mg dry weight)

When analyzing the ABTS radical scavenging activity data, which is another antioxidant analysis, the ethanol, methanol, and water extracts of *Inula viscosa* were found to be 72%, 71.1%, and 73.8%, respectively (Table 4).

Table 4. ABTS radical scavenging activities of *Inula viscosa*.

Plant (400mg/ml)	EE		ME		AE	
	SA	TE	SA	TE	SA	TE
Inula viscosa	72.3	2.18	71.1	2.15	73.8	2.2

EE: Ethanol Extract; ME: Methanol Extract; AE: Aqueous Extract; SA: Scavenging activity (%); TE: Trolox equivalent (mM trolox (TE)/mg dry weight)

Thin-layer chromatography is usually conducted for a better identification of bioactive compounds. In the present study, the plates were run in chloroform:methanol (4.5:0.5) and developed with E, M, and S, which indicate ethanol, methanol, and aqueous extracts, respectively (Figure 1). TLC studies revealed the presence of at least 5 compounds in the methanol, ethanol, and aqueous extracts. The three components were separated in the ethanol extract of the plant, while only one component was separated in the methanol and water extracts of *Inula viscosa*. It was observed that among the three solvents, ethanol was the most effective in extracting the maximum number of secondary metabolites (Figure 2).

Figure 1. Thin-layer-chromatography of extracts made from *Inula viscosa*

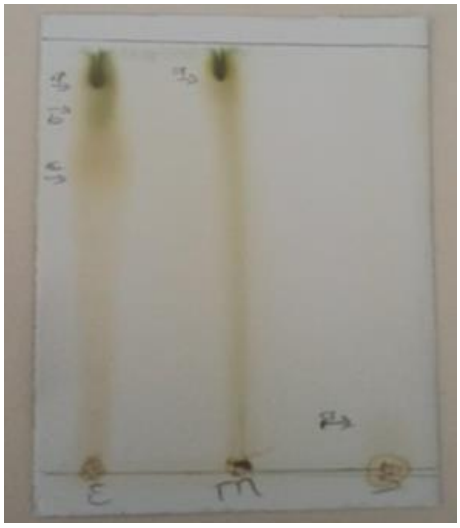
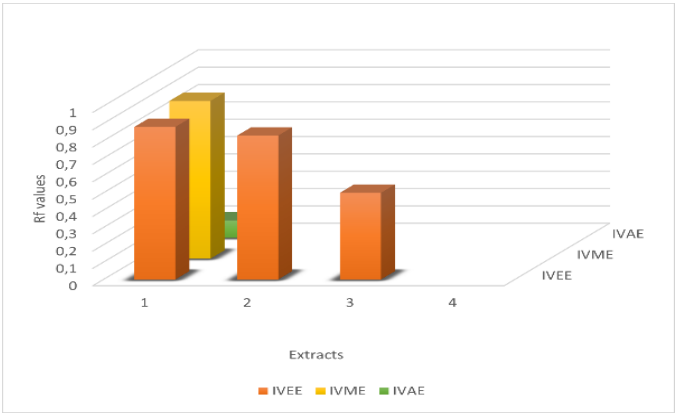


Figure 2. The Rf values of components of *Inula viscosa*



IVEE: Ethanol extract, IVME: Methanol extract, IVAE: Aquoeus extract of *Inula viscosa*

4. DISCUSSION and CONCLUSION

Najefi *et al*. (2011) examined the antimicrobial activities of the phenolic and non-phenolic fractions of *Inula viscosa*. In their study, they tested these fractions against *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Salmonella* Entritidis. The zone diameters of the total extract (480 mg/ml) against these bacteria were 15 mm, 19 mm, and 17 mm, respectively. Ozkan *et al.* (2019) tested *Inula viscosa* for antimicrobial activity, using 10 different bacteria in their study. According to the study, the inhibition zone diameter of the methanol extract was 12 mm for *Staphylococcus aureus*. In another study, different extracts of *Citrus aurantium* flowers were tested against various bacteria, including *Listeria monocytogenes*, *Salmonella aureus*, *Bacillus cereus*, and *S.* Typhimurium. The zone diameters for these bacteria were 22 mm, 24 mm, 26 mm, and 16 mm, respectively (Değirmenci and Erkurt, 2020). Tomar and Yıldırım (2019) investigated the antimicrobial and antioxidant activities of *Beta vulgaris* against some foodborne pathogens. Researchers reported that the water extract exhibited high antibacterial activity against *Listeria monocytogenes* (17 mm zone). The data in the literature support our study.

Mohti *et al*. (2020) reported the antimicrobial, antioxidant, and phenolic compounds of *Inula viscosa*. The researchers found that the MIC value against bacteria was 250 µg/ml. In another study, researchers investigated three *Inula* species and tested them against six different

microorganisms. According to the findings obtained at the end of the study, it was reported that the MIC values of *Inula viscosa* varied between 50 µg/ml and 800 µg/ml against these bacteria (Gökbulut *et al*., 2013). Sassi *et al*. (2007) reported the antimicrobial and antioxidant potentials of some medicinal plants collected from Tunisia. Looking at the results obtained in the study, they found that the MIC value of *Inula viscosa* methanol extract was 625 µg/ml for *Staphylococcus aureus*. Talib *et al*. (2012) examined compounds isolated from *Inula viscosa* for their antimicrobial effects. In this study, the plant did not show antibacterial activity against *S. aureus*. However, the plant was effective against other bacteria and yeast. As can be understood from all the data provided in the literature, these studies are in accordance with the data we obtained in our study and support them.

Researchers have reported that the antioxidant activity of *Inula viscosa* has different IC_{50} values (Danino *et al*., 2009; Chahmi *et al*., 2015; Mahmoudi *et al*., 2016; Salim *et al*., 2017; Kheyar-Kraouche *et al.,* 2018). Mitic *et al.* (2020) researched the antioxidant activity of *Inula oculuschristi* in a study. When examining the data they obtained at the end of the study, they reported that the radical scavenging activities of DPPH and ABTS were 57% and 82.7%, respectively. When the data obtained from the literature are compared with our study, it can be seen that the results support our analysis. Additionally, pharmacologically active compounds found in *I. viscosa* include phenolic acids, terpenes, and glycolipids (Fontana *et al*., 2007; Danino *et al*., 2009; Karamenderes & Zeybek, 2000; Andolfi *et al*., 2013). Hispidulin is a naturally occurring flavone present in several traditional Chinese medicinal herbs, including *I. viscosa* (He *et al*., 2011; Xie *et al*., 2015). These compounds discovered in *Inula* also explain the high antioxidant activity detected in our study.

Different Rf values of the compounds provide an idea about their polarity, which may also help in selecting a particular solvent system for further isolation of any compound from the plant extracts using chromatographic and spectroscopic techniques (Biradar & Rachetti, 2013). Compounds showing a high Rf value in the less polar solvent system have low polarity, while those with a low Rf value have high polarity (Talukdar *et al.*, 2010). In this study, 5 bioactive components with different Rf values were determined. These components have varying polarities (Figure 2).

The public health problem caused by microorganisms in foods has made this issue very significant. Antibiotics are used to eliminate and combat bacteria in foods, but their use can lead to bacteria developing resistance. This has highlighted the importance of focusing on naturally available plant-derived substances that bacteria cannot develop resistance to and that naturally inhibit or kill them.

In this study, the effectiveness of the *Inula* plant against these microorganisms was determined, and it is believed to be a strong candidate for use in food preservation due to its high antimicrobial and antioxidant activity. The present study confirms the presence of many important phytochemicals in *Inula viscosa* from Turkey. Observing the results of the chromatography analysis, it can be concluded that *Inula* produces many secondary metabolites of medicinal value. Therefore, the plant can be used as a source to produce phytochemicals using advanced techniques of extraction, screening, identification, and isolation. To consider the use of this product in phytotherapy, we need to further determine the composition of the aerial parts of the plant.

Acknowledgments

This study was partly supported by the Mugla Sıtkı Kocman University Scientific Research Projects Coordination Unit (No. 2013-101). The authors would like to thank Dr. Olcay Ceylan (Mugla Sıtkı Kocman University, Türkiye) for plant identification. This study was presented as an oral presentation at the 5th International Engineering and Natural Sciences Conference

(IENSC 2020) held from November 5 to 6, 2020, in Diyarbakır, Türkiye. It was published as an abstract paper in the proceeding book of the IENSC-2020.

Declaration of Conflicting Interests and Ethics

The authors declare no conflicts of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s). .

Authorship Contribution Statement

Gulten Okmen: Conception, Design, Supervision, Fundings, Analysis and/or Interpretation, Writing, Critical Review. **Kutbettin Arsalan**: Materials, Data Collection and/or Processing, Literature Review. **Ridvan Tekin**: Materials, Data Collection and/or Processing, Literature Review.

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