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Original article

Promising medicinal plant *Inula viscosa* L.: Antiproliferative, antioxidant, antibacterial and phenolic profiles

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Summary. *Introduction. Inula viscosa* (L.) Aiton (sticky fleabane) is popular medicinal plant in the family Asteraceae. It has been used in traditional medicine in the treatment of cancer, diabetes, hypertension, bronchitis, tuberculosis, wounds, skin diseases, infertility, lung and gastro-duodenal disorders. *Methods.* Aim of this study was to evaluate *in vitro* antiproliferative, antioxidant and antibacterial activities of aqueous and methanol extracts of aerial parts of *I. viscosa* and their phenolic constituents. Antiproliferative activity was evaluated against human breast adenocarcinoma (MCF-7) and human brain cancer (T98-G) cell lines using MTT assay. Antioxidant activity was revealed by using 2,2-diphenyl-1-picrylhydrazil (DPPH) method. Total phenol and flavonoid were determined by using Folin-Ciocaltaeu and aluminum chloride ($AlCl_3$) colorimetric method, respectively. The disc diffusion assay was used to screen for antibacterial activity against 10 bacteria. Phenolic constituents were detected by High Performance Liquid Chromatography-Diode-Array Detector (HPLC-DAD) via chosen ten phenolic molecules (gallic acid, caffeic acid, rutin, luteolin, kaempferol, rosmarinic acid, myricetin, quercetin, coumarin and apigenin). *Results.* Methanol extract of *I. viscosa* demonstrated better antiproliferative activity than aqueous extract against MCF-7 and T98-G cell lines. Strong DPPH radical scavenging activity was observed with both extracts. Total phenol and flavonoid content of methanol extract were twice as much as aqueous extract. Only Gram-positive bacteria (*S. aureus*, *S. epidermidis* and *S. pyogenes*) were inhibited by both extracts of *I. viscosa*. HPLC-DAD analysis of phenolic compounds revealed that *I. viscosa* was significant source of kaempferol. *Conclusion: I. viscosa* showed promising antibacterial, antioxidant and anticancer activities, and further studies should be conducted to isolate the active components.

Key words: antibacterial, anticancer, antioxidant, antiproliferative, extraction, *Inula viscosa*

Introduction

Inula viscosa (L.) Aiton (syn. *Dittrichia viscosa* [L.] Greuter), commonly known as 'false yellowhead, sticky fleabane, aromatic inula, yapışkan andız otu in Turkish' is a perennial Mediterranean plant in the family Asteraceae. It is suffrutescent, viscid and strong smelling plant of bushy growth (1). *I. viscosa* has been used traditionally as a remedy for wounds, skin diseases, rheumatic pains, lung disorders, diabetes, gastro-duodenal problems, hypertension, cancer, bronchitis, tuberculosis and infertility (2-10). It has been widely used for therapeutic purposes in folk medicine as anthelmintic, antipyretic, antiseptic, anti-inflammatory, antiphlogistic, balsamic, expectorant, anti-scabies, diuretic, anti-anemic and muscle relaxant (2-10). *I. viscosa* contains some bioactive compounds including guaianolides, sesquiterpenes, sesquiterpenes acids, triterpenoids, azulenes, lactones, flavonoids, costic acid and essential oils (10-17). Cytotoxic (8, 15, 17-22), antimicrobial (9, 19, 22-26), antioxidant (7, 25-28), antihypertensive (30), hypoglycemic (5), hypolipidemic

(5), abortifacient and anti-implantation (4) activities of *I. viscosa* have been investigated with experimental studies in the literature.

The objective of this study was to reveal the biological activities (antibacterial, antioxidant and antiproliferative) and phenolic contents of *I. viscosa*, a Turkish medicinal plant.

Material and Methods

Plant material and extraction

Aerial parts of *I. viscosa* were obtained from Hasköy, Akhisar, Manisa/Turkey in August 2015. Plant was identified by using "Flora of Turkey and the East Aegean Islands" (1) and voucher specimens (AUT-2232) were stored at the Department of Biology, Abant Izzet Baysal University (AIBU), Bolu, Turkey. Upper parts of *I. viscosa* including flowers, leaves and young stem were dried in a room and then grounded. Plant material was extracted with distilled water and pure methanol (Merck). For aqueous extraction, 150 ml boiling water was poured over 15 g of dried plant material and allowed to steep for 10 minutes. Aqueous extraction was prepared according to folkloric usage of *I. viscosa* as described by Hasan Alemdar and Lütfü Acet (personal communication, August 15, 2015). The aqueous extract was filtered and then lyophilized. For methanol extraction, 15 g of dried plant sample was extracted with 150 ml methanol at 40°C in a water bath for 18 h, and methanol was evaporated under vacuum. Yields of the extracts were calculated using the formula "Yield (%) = Weight of extract (g)/powdered plant sample (g) X 100".

Antiproliferative activity

Human cancer cell lines and culture conditions

Human breast adenocarcinoma (MCF-7) and human brain cancer (T98-G) cell lines were used in the experiments (American Type Culture Collection; ATCC, Manassas, VA, USA). Dulbecco's minimum essential medium (DMEM) with Earle's salts (Mediatech Cellgro®, Herndon, USA) supplemented with 10% (v/v) FBS (heat inactivated fetal bovine serum, Hyclone®, Logan, USA) and 1% penicillin (100 U/ml) / streptomycin (100 µg/ ml) (Mediatech®, Cellgro, VA) was used as growth medium for both cell lines. Cancer cell lines were incubated at 37 °C in a humidified environment containing 5% CO_2. After harvesting of the cells by the treatment of tripsin/EDTA solution at 80-90% confluence, viable cells were counted and plated in 100 µl of medium/well in 96-well plates (Corning®).

Cell viability assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay was performed for the determination of cytotoxic activity of plant extracts (31-32). Different concentrations of each extracts (100 µl) in 0.5% Dimethyl sulfoxide (DMSO, Sigma-Aldrich®) were applied on cells (1×10^4 cells/well in 96 well plates). After 24 h incubation, the culture medium was removed and replaced with 90 µl of fresh culture medium. 10 µl MTT solution (5 mg/ml) in phosphate buffered saline (PBS, pH 7.4) was transferred into each well. After incubation for 4 h at 37 °C in 5% CO_2, 100 µl/well of DMSO was added to all samples for dissolving of formazan that is the final product of MTT reaction. Then they were incubated for a night at 37 °C in 5% CO_2. After incubation, absorbance of viable cells was determined spectrophotometrically in a Multiscan FC microplate photometer reader at 570 nm. Each experiment was carried out three times in triplicate. The relative cell viability (%) related to control wells containing cell culture medium without samples was calculated as:

$$100 \times A_{570\ nm}\,(\text{sample}) / A_{570\ nm}\,(\text{control}).$$

Antioxidant activity

Free radical scavenging activity

Free radical scavenging activity of aqueous and methanolic extracts of *I. viscosa* was determined spectrophotometrically by measuring the decrease in absorbance of 2,2-diphenyl-1-picrylhydrazil (DPPH·, Sigma-Aldrich Chemie®, Steinheim, Germany) at 517 nm, according to the method described by Brand-Williams et al. (33). Briefly, 1 ml of 0.15 mM DPPH radical in methanol was mixed with 3 ml of the extracts or reference solution, ascorbic acid as a natural antioxidant, at the different concentrations (25, 50, 100 and 200 µg/ml). After 30 minutes incubation in the dark at room temperature, measurement was made for the decrease in the absorbance at 517 nm with Hitachi U-1900, UV-VIS Spectrophotometer 200V against

blank sample (methanol). All analyses were made in triplicate. The DPPH• scavenging capacity of *I. viscosa* extracts was calculated using the following equation: DPPH· Scavenging Effect (% inhibition) = [(A0–A1/A0) x 100] where A0 is the absorbance of the control reaction, and A1 is the absorbance in the presence of tested *I. viscosa* extracts.

Determination of total phenolic content

The total phenolic contents in *I. viscosa* extracts were determined with the Folin Ciocalteu assay using the method of Marinova et al. (34). Extracts were prepared at 1mg/ml concentration with distilled water. Gallic acid was used as a standard phenolic compound. The standard calibration curve was prepared using 25, 50, 100, 150, 200 and 400 mg/l gallic acid. Briefly, 20 µl of each standard solution, sample, or blank (water) was mixed with 1.58 ml distilled water and 100 µl Folin-Ciocalteu reagent (Sigma®) and then mixed well. After 2 minutes, 300 µl of 20% Na_2CO_3 solution was added and was shaken very well. The mixture was incubated at 20 ^{0}C, in the dark, for 2 hours. The absorbance of each solution was measured at 765 nm against the blank using the spectrophotometer. The amounts of total phenolic compounds in *I. viscosa* extracts were expressed as micrograms of gallic acid equivalent/1g plant extracts, using an equation that was obtained from a standard gallic acid graph (R^2: 0.9944). All analyses were made in triplicate.

Determination of total flavonoid

The total flavonoid contents of *I. viscosa* extracts were determined with aluminum chloride ($AlCl_3$) colorimetric assay using the method of Marinova et al. (34). Extracts were prepared at 1250 and 500 mg/ml concentrations in ethanol. Catechol was used as a reference flavonoid. The standard calibration curve was prepared using 12.5, 25, 50, 100, 150, 200 and 400 mg/ml catechol. Briefly, 500 µl of each extract, standard solution, or blank (ethanol) was mixed with 2 ml distilled water and 150 µl of 5% $NaNO_2$. After 5 min, 150 µl of 10 % $AlCl_3$ was added and at 6 min, 1000 µl of 1M NaOH was added to the mixture. Immediately, the reaction tube was diluted to volume of 5 ml with the addition of 1200 µl of distilled water and thoroughly mixed. The mixture solutions were left in the dark at room temperature for 30 minutes. Absorbance of the mixture was determined at 510 nm versus a blank. The total flavonoid content of *I. viscosa* samples were expressed as mg catechol equivalents (CE)/1g plant extracts. Samples were analyzed in three replications.

Antibacterial bioassay

Antibacterial activity was evaluated using disc diffusion assay according to the method described by Yıldırım et al. (35). Three Gram-positive bacteria [*Streptococcus pyogenes* (ATCC® 19615), *Staphylococcus aureus* (ATCC® 25923) and *Staphylococcus epidermidis* (ATCC® 12228)] and seven Gram-negative bacteria [*Escherichia coli* (ATCC® 25922), *Pseudomonas aeruginosa* (ATCC® 27853), *Salmonella typhimurium* (ATCC® 14028), *Serratia marcescens* (ATCC® 8100), *Proteus vulgaris* (ATCC® 13315), *Enterobacter cloacae* (ATCC® 23355) and *Klebsiella pneumoniae* (ATCC® 13883)] were used for the experiment. Turbidity of each broth culture of bacteria was adjusted with saline (0.5 McFarland) and Mueller Hinton agar plates were inoculated using cotton swabs. Filter-sterilized extracts were loaded on sterile filter paper discs (Whatman®) and then located into inoculated plates. Erythromycin, ampicillin, carbenicillin, tetracycline and chloramphenicol (Bioanalyse®) were used as positive control, and negative control was DMSO. Diameter of inhibition zones was determined after 16 to 18 hours at 37°C. All experiments were repeated three times.

Determination of phenolic compounds
Sample preparation for HPLC analysis

Dried *I. viscosa* extracts (100 mg) were dissolved in 1 ml HPLC grade methanol. All standards (gallic acid, caffeic acid, rutin, luteolin, kaempferol, rosmarinic acid, myricetin, quercetin, coumarin and apigenin-Sigma®) were prepared at 1 mg/ml in HPLC grade methanol and mixed together to obtain standard curve with eight different concentrations (1, 5, 10, 20, 40, 60, 80 and 100 mg/l). Filtration was performed using 0.2-µm GHP Acrodisc (25 mm) (Pall Corporation®) into 2-ml HPLC vials for all tested materials.

HPLC-DAD analysis

HPLC system (VWR-Hitachi LaChrom Elite®) containing a Hitachi L-2455 Diode-Array Detec-

tor (DAD), Hitachi L-2130 Pump, Hitachi L-2200 Autosampler, Hitachi column oven L-2300 (25 °C) and Venusil XBP C18 column (Bonna-Agela Technologies®particle size 5 µm, 4.6 x 250 mm) was used for the analysis of aqueous and methanolic extracts of *I. viscosa*. Flow rate and injection volume were 1ml/min and 20 µl, respectively. A gradient elution containing solvent (A) acetonitrile (ACN) and solvent (B) 0.1% acetic acid was used. The chromatograms were obtained at 280 nm.

Data analysis

Analysis of variance (ANOVA) and Duncan's Multiple Range Tests using SPSS vers. 15 (SPSS Inc., Chicago, IL, USA) were used for analysis.

Results and Discussion

Aqueous and methanol extracts of *I. viscosa* were evaluated for their antiproliferative, antioxidant and antibacterial activities (Table1, 2, 3 and 4). Phenolic constituents of *I. viscosa* were also determined (Table 5). Yields (%) of aqueous and methanol extracts of *I. viscosa* were 19.6 % and 15 %, respectively.

Popularity of *I. viscosa* as a remedy for cancer in Turkish media has been increasing in recent years (36). Two crude extracts of *I. viscosa* were tested *in vitro* against two human cell lines, namely, MCF-7 (breast cancer cell line) and T98-G (glioblastoma cell line) by the MTT assay (Table 1). Glioblastoma is the most common and aggressive type of brain tumor. Shape of dose-response curves exhibited a significant inhibition of cell growth in dose-dependent manner (Fig. 1 and 2). Methanol extracts of *I. viscosa* showed better antiproliferative activity than aqueous extracts against MCF-7 and T98-G cells, with IC_{50} values of 179.5 ± 2.0 µg/ml and 121.1 ± 3.0 µg/ml, respectively. Aqueous extract of *I. viscosa* was not as effective as methanol extract. IC_{50} values of aqueous extract were more than 200 µg/ml (Table 1). A plant extract is usually regarded as interesting if IC_{50}< 100 µg/ml for *in vitro* cytotoxic activity (37). Crude methanol extracts of *I. viscosa* showed noticeable cyotoxic activity against T98-G cells with IC_{50} value of 121.1 ± 3.0 µg/ml. Generally, methanol extracts were more active on tested cell lines. Methanol extracts of plant materials may contain more active components such as terpenoids and polyphenols including flavonoids and tannins. Numerous studies on animal models have shown

Table 1. *In vitro* cytotoxic activities of *I. viscosa* extracts on MCF-7 and T98 cell lines. Results were presented as a mean of IC_{50} concentration ± SE for three independent determinations.

	IC50 (µg/mL)	
Treatments	**MCF-7**	**T98**
I. viscosa-Water	> 200	> 200
I. viscosa-MeOH	179.5 ± 2.0	121.1 ± 3.0

Table 3: Total phenolic and flavonoid content of *I. viscosa* extracts. Data were presented as a mean number of phenolic/flavonoid content ± standard error (SE).

Extracts	**Total Phenolics mg GAE/g dry extract**	**Total Flavonoids mg CE/ g dry extract**
I. viscosa-Water	59.2 ± 0.0100	68.5 ± 0.0001
I. viscosa-MeOH	107.0 ± 0.0001	158.35 ± 0.0002

Table 2. % inhibition of DPPH by *I. viscosa* extracts. Data were presented as a mean number of % DPPH inhibition ± standard error (SE).

Treatments		**% DPPH Inhibition (± SE)**									
		Concentrations									
	IC_{50} (µg/ml)	**5 µg/ml**	**10 µg/ml**	**20 µg/ml**	**30 µg/ml**	**40 µg/ml**	**50 µg/ml**	**60 µg/ml**	**80 µg/ml**	**100 µg/ml**	**120 µg/ml**
Ascorbic acid	5.7	14.85 ± 0.0000	97.01 ± 0.0003	96.64 ± 0.0003	96.81 ± 0.0003	96.68 ± 0.0003	96.85 ± 0.0000	96.85 ± 0.0005	95.47 ± 0.0003	97.09 ± 0.0000	96.93 ± 0.0003
I. viscosa-Water	23.2	10.61 ± 0.0000	18.77 ± 0.0003	31.81 ± 0.0005	62.81 ± 0.0005	66.37 ± 0.0003	79.41 ± 0.0001	90.02 ± 0.0003	92.41 ± 0.0008	93.78 ± 0.0003	90.83 ± 0.0003
I. viscosa-MeOH	20.4	0.36 ± 0.0003	21.11 ± 0.0003	48.52 ± 0.0005	66.41 ± 0.0003	84.25 ± 0.0005	94.18 ± 0.0005	94.9 ± 0.0003	94.22 ± 0.0006	94.38 ± 0.0003	93.78 ± 0.0003

Table 4. Antibacterial activity of *I. viscosa* extracts. Data were presented as a mean diameter of inhibition zones ± standard error (SE). Means with the same letter within columns are not significantly different at $P>0.05$.

Treatments	Mean Diameter of Inhibitory Zones (mm ± SE)									
	S. Auerus	*S. Epidermidis*	*S. Pyogenes*	*S. Marcescens*	*S. Typhimurium*	*P. Aeruginosa*	*P. Vulgaris*	*K. Pneumonia*	*E. Cloacae*	*E. Coli*
I. viscosa-Water	14.0 ± 0.3^{d}	10.4 ± 0.2^{e}	16.8 ± 0.4^{f}	-	-	-	-	-	-	-
I. viscosa-MeOH	14.0 ± 0.3^{d}	16.4 ± 0.2^{d}	16.8 ± 0.4^{f}	-	-	-	-	-	-	-
Ampicillin	34.5 ± 0.2^{a}	29.0 ± 1.1^{c}	50.5 ± 1.4^{b}	-	27.0 ± 0.0^{b}	-	23.0 ± 0.0^{c}	7.5 ± 0.2^{d}	26.5 ± 0.2^{c}	20.0 ± 0.0^{c}
Carbenicillin	36.0 ± 0.0^{a}	33.0 ± 0.0^{b}	54.0 ± 1.7^{a}	22.5 ± 1.4^{b}	27.0 ± 0.0^{b}	22.0 ± 0.0^{a}	29.0 ± 0.0^{a}	-	32.0 ± 0.5^{a}	28.0 ± 0.0^{a}
Chloramphenicol	24.5 ± 0.8^{c}	32.3 ± 0.7^{b}	36.0 ± 0.5^{c}	24.0 ± 0.5^{a}	28.5 ± 0.8^{a}	8.5 ± 0.2^{c}	26.0 ± 0.0^{b}	23.0 ± 0.0^{b}	26.5 ± 0.8^{c}	27.0 ± 0.0^{b}
Erythromycin	28.5 ± 0.8^{b}	37.0 ± 0.0^{a}	44.5 ± 0.2^{c}	7.0 ± 0.0^{d}	9.0 ± 0.5^{d}	8.0 ± 0.0^{c}	11.0 ± 0.0^{d}	11.5 ± 0.8^{c}	-	11.0 ± 0.0^{d}
Tetracycline	28.5 ± 0.8^{b}	-	41.5 ± 0.8^{d}	18.0 ± 1.1^{c}	21.0 ± 0.5^{c}	12.5 ± 0.2^{b}	28.5 ± 0.2^{ab}	24.5 ± 0.2^{a}	28.5 ± 0.8^{b}	26.5 ± 0.2^{bc}
DMSO	-	-	-	-	-	-	-	-	-	-

that polyphenols limit the development of cancers, cardiovascular diseases, neurodegenerative diseases, diabetes, and osteoporosis (9, 38). Antiproliferative potential of *I. viscosa* may be explained by high content of kaempferol in our study (Table 5). Merghoub et al. (18) reported a significant cytotoxic effect of methanol extract of *I. viscosa* on the human cervical cancer cell lines SiHa and HeLa with MTT assay. Talib and Mahasneh (19) showed the cytotoxicity of ethanol extract of *I. viscosa* flowers against Vero cell line using the MTT assay with IC_{50} value of 202.43 µg/ml. They also reported the antiproliferative activity of methanol fraction of *I. viscosa* flowers against MCF-7 with IC_{50} value of 15.78 µg/ml (20). Talib et al. (9) documented the antiproliferative activities of four flavonoids (nepetin, 3,3'-di-*O* methylquercetin, hispidulin, and 3-*O*-methylquercetin) that were isolated from *I. viscosa*. The most potent compound was nepetin, with IC_{50} values of 5.87 and 11.33 µg/ml against MCF-7 and Hep-2 (larynx carcinoma) cell lines, respectively. Hispidulin also exhibited noticeable antiproliferative activity against MCF-7 and Hep-2 cell lines with IC_{50} values of 10.35 and 19.50 µg/ml, respectively. IC_{50} values were 10.11 µg/ml for 3,3'-di-*O* methylquercetin and 11.23 µg/ml for 3-*O* methylquercetin against MCF-7 cells. They pointed out that methylated quercetins obtained from *I. viscosa* gave rise to anticancer and antimicrobial prop-

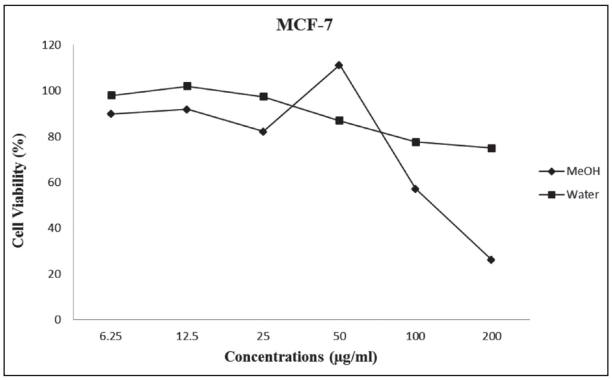

Figure 1. Antiproliferative effects of *I. viscosa* extracts on MCF-7 cells.

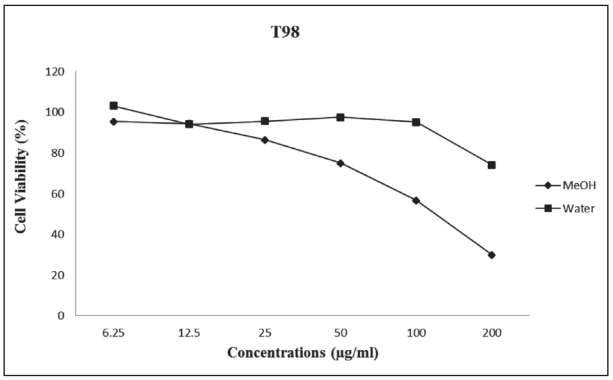

Figure 2. Antiproliferative effects of *I. viscosa* extracts on T98 cells.

erties (9). Benbacer et al. (8) showed that methanol extract of *I. viscosa* exhibited strong cytotoxic activities against human cervical cancer SiHa and HeLa cell lines with IC_{50} values of 54 µg/ml and 60 µg/ml, respectively. Messaoudi et al. (22) evaluated the cytotoxic activities of ethanol and ethyl acetate extracts of *I. viscosa* on two human breast cancer cell lines, MCF-7 and MDA-MB231. Ethanol and ethyl acetate extracts showed the best activities with IC_{50} values of 195.42 µg/ml and 112.20 µg/ml, respectively. Tomentosine, inuviscolide and isocostic acid in *I. viscosa* were strongly related to cytotoxic activity (22). Merghoub et al. (18) investigated the antiproliferative activity of *I. viscosa* leaf extracts on human cervical HeLa and SiHa cancer cells. *I. viscosa* extracts significantly inhibited cell growth in a dose-dependent manner in the tested cancer cell lines. Especially, hexanic extract and dichloromethane fraction showed strong cytotoxicity with IC_{50} values of 6 µg/ml and 22.04 µg/ml for HeLa and SiHa cell lines, respectively. Çelik and Aslantürk (15) demonstrated that high concentration of *I. viscosa* leaf extracts showed cytotoxic and genotoxic activity on the root meristem cells of *Allium cepa*.

DPPH antioxidant assay was used to evaluate the free radical scavenging activities of various antioxidant substances using DPPH radical as a substrate. The reduction capability of antioxidants on the DPPH radical was determined by the decrease in its absorbance at 517 nm. Reaction between antioxidants and free radicals causes the decline in absorbance of DPPH radical. The antioxidant activity of methanol and water extracts of *I. viscosa* was evaluated by free radical scavenging activity (DPPH), total phenolic content and total flavonoid content (Table 2 and 3). Generally, both extracts of *I. viscosa* revealed close free radical scavenging activity. Methanol extract of *I. viscosa* showed better radical scavenging activity than aqueous extract having IC_{50} value of 20.4 µg/ml and 23.2 µg/ml, respectively (Table 2). In DPPH assay, *I. viscosa* extracts showed a concentration dependent free radical scavenging activity (%) by scavenging DPPH radical. Free radical scavenging activity rose when concentrations advanced from 5 to 60 µg/ml for methanolic extract and from 5 to 100 µg/ml for aqueous extract. Although methanol extract exhibited the highest DPPH radical scavenging activity (94.9 %) at 60 µg/ml concentration, aqueous extract showed the highest activity (93.78 %) at 100 µg/ml concentration (Table 2). Similar to our result, Mahmoudi et al. (27) reported IC_{50} value of methanol extract of *I. viscosa* leaves was 23.33 µg/ml for DPPH assay. Gökbulut et al. (26) evaluated the antioxidant capacity of flowers, leaves and roots of *I. viscosa* using water, methanol and ethy acetate as extraction solvents. Water extract of *I. viscosa* flowers exhibited the highest DPPH radical scavenging activity with IC_{50} value of 0.28 mg/ml. Danino et al. (7) isolated a polyphenolic antioxidant, 1,3-dicaffeoylquinic acid, from the leaves of *I. viscosa*, exhibiting a significantly stronger ability to inhibit DPPH radicals than trolox and caffeic acid. Chahmi et al. (29) reported the IC_{50} value of ethanol extract of *I. viscosa* aerial parts from Morocco as 0.18 g/l for DPPH scavenging activity.

Phenolics and flavonoids are powerful scavengers free radicals due to their hydroxyl groups. Total phenol and flavonoid contents of methanol extract of *I. viscosa* aerial parts including stem, leaves and flowers in our study [107 mg Gallic acid (GA) equivalent (E)/g and 158.35 mg Catechol (C) E/g dried extract, respectively] showed nearly twice as much as aqueous extract (59.2 mg GAE and 68.5 mg CE/g dried extract) (Table 3). There is a correlation among DPPH radicals scavenging activity, total phenol and flavonoids for both extracts. Methanol extract showed higher DPPH radical scavenging activity, total phenol and flavonoid content in our study (Table 2 and 3). In another studies, Gökbulut et al. (26) reported the total phenol content of methanol extract of *I. viscosa* aerial part as 176.9 ± 7.8 mg GAE/g dry extract. Mahmoudi et al. (27) documented that total phenol and flavonoid content of methanol extract of *I. viscosa* leaves were found to be 103 mg GAE/g dry extract and 99 mg CE/g dry extract, respectively. Trimech et al. (28) successively extracted roots and aerial parts of *I. viscosa* with solvents of increasing polarity (diethyl ether, ethyl acetate and finally methanol). They found that ethyl acetate extract of stem had the highest phenol (24.18 GAE/g dry extract) and aerial parts (flowers, leaves and stem) of *I. viscosa* exhibited strong DPPH radical scavenging activity (0.02-0.05 mg ferulic acid equivalents/ml). Chahmi et al. (29)

reported the total phenol and flavonoid contents of ethanol extracts of *I. viscosa* aerial parts from Morocco were 170-274 mg GAE/g dry extract and 44 µg QE (quercetin equivalent)/mg dry extract, respectively. Amrouche et al. (39) provided that solid–liquid extraction using water, as a solvent instead of organic solvents was more efficient method for the recovery of antioxidant polyphenols from leaves of Algerian *I. viscosa*. Conversely, methanolic extract of *I. viscosa* included more total phenol and flavonoid content than aqueous extract in our current study (Table 3).

Antibiotics (positive controls) generally showed antibacterial activity to our tested pathogens. Since final concentrations of all extracts were adjusted with DMSO, it was used as a negative controls and no inhibition was observed with it (Table 4). Only Gram-positive bacteria (*S. aureus*, *S. epidermidis* and *S. pyogenes*) were sensitive to aqueous and methanol extracts of *I. viscosa* (Table 4). The best antibacterial activity was obtained with both extracts of *I. viscosa* against *S. pyogenes* (16.8 mm) (Table 4). Methanol extract also demonstrated better antibacterial activity against *S. epidermidis* (16.4 mm). *I. viscosa* extracts did not exhibit antibacterial activity against Gram-positive bacteria. Similarly, in another study, no inhibition was observed with petroleum ether,chloroform and ethanol extracts of *I. viscosa* against Gram-negative bacteria (25). Sensitivity of Gram-positive bacteria may come from single layer cell wall structure. On the other hand, Gram-negative cell wall has multi-layered and complex structure. Contrary to our results, some studies showed the sensitivity of Gram-negative bacteria against *I. viscosa* extracts. For example, Oskay and Sarı (23) and Oskay et al. (24) reported that ethanol extract of *I. viscosa* showed broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria, and multi-drug resistant human pathogens. *P. vulgaris* and *S. marcescens* were sensitive to ethanol extract of *I. viscosa* (23). Talib and Mahasneh (19) demonstrated that *P. aeruginosa*, *S. typhimurium* and *E. coli* were inhibited by ethanol extract of *I. viscosa* flowers. According to Gökbulut et al., (26), *E. coli* and *P. aeruginosa* were vulnerable to methanol extracts of *I. viscosa* flowers, leaves and roots. Aqueous and ethanol extracts of *I. viscosa* aerial parts collected from Palestinian area inhibited *P. vulgaris* and *K. pneumonia* (3). Talib et al. (9) reported that *Salmonella typhimurium* and *Bacillus cereus* were inhibited by two *I. viscosa* flavonoids 3-*O*-methylquercetin and 3,3'-di-*O*-methylquercetin. Strong antibacterial activity against Gram-positive bacteria may be attributed to high content of kaempferol and quercetin in our this study (Table 5). Noticeable antibacterial activities of *I. viscosa* against *S. aureus*, *S. epidermidis,* and *S. pyogenes* may explain why this plant has been used in traditional medicine in the treatments of wounds, skin diseases, lung and gastroduodenal problems (2-4, 6).

Quantification of the chosen phenolic in aqueous and methanol extracts of *I. viscosa* was con-

Table 5. Identified phenolic compounds and their amounts in *I. viscosa* extracts. Data were presented as a mean number of quantities of phenolic compound ± standard error (SE).

STANDART COMPOUNDS			**PLANT EXTRACTS (mg/g dry extract)**	
Name	**Peak number**	**RT (min)**	***I. viscosa* Aqueous extract**	***I. viscosa* MeOH extract**
Gallic acid	1	5.51	0.93±0.00	0.49±0.06
Caffeic acid	2	11.40	-	-
Rutin	3	11.84	0.87±0.01	0.48±0.04
Luteolin	4	12.33	-	-
Kaempferol	5	13.00	34.36±0.02	40.87±0.04
Rosmarinic acid	6	13.77	0.65±0.03	0.95±0.05
Myricetin	7	14.51	-	-
Quercetin	8	16.15	1.17±0.01	8.68±0.06
Coumarin	9	16.81	1.32±0.01	4.42±0.03
Apigenin	10	17.34	2.51±0.01	-

ducted with HPLC-DAD analysis and results were summarized in Table 5. The chromatogram of the used standards was shown in Fig. 3. According to HPLC analyses, kaempferol was dominant compound in both aqueous and methanol extracts of *I. viscosa* (34.36 mg/g and 40.87 mg/g, respectively). Quercetin (8.68 mg/g) and coumarin (4.42 mg/g) were also abundant in methanol extract (Table 5). On the other hand, apigenin were just found in water extract (2.52 mg/g). It was observed that *I. viscosa* was a good source kaemferol with noticeable amount. Generally, HPLC analysis showed that methanolic extract had higher phenolic contents than aqueous extract except gallic acid and rutin that were approximately twice as much as higher in methanol extract. Caffeic acid, luteolin and myricetin were not found in *I. viscosa* extracts (Table 5). On the other hand, Mahmoudi et al. (27) investigated the phenolic compounds in the leaves of *I. viscosa* and detected caffeic acid and luteolin using HPLC-PDA-ESIMS/MS analysis. In consistent with our study, Trimech et al. (28) identified quercetin in the ethyl acetate extract of flowers by LC-DAD/ESI(+)/MS analysis. According to Gökbulut et al. (26), methanol extract of *I. viscosa* leaves did not contain kaemferol that was found in small quantity in flower (0.030 mg/g) with HPLC-DAD analysis. In addition, *I. viscosa* flower and leaf included small amounts of quercetin (0.039 mg/g and 0.021mg/g, respectively). On the other hand, kaemferol and quercetin were found in high amounts in methanol extract of *I. viscosa* aerial part in our study (40.87 mg/g and 8.68 mg/g, respectively).

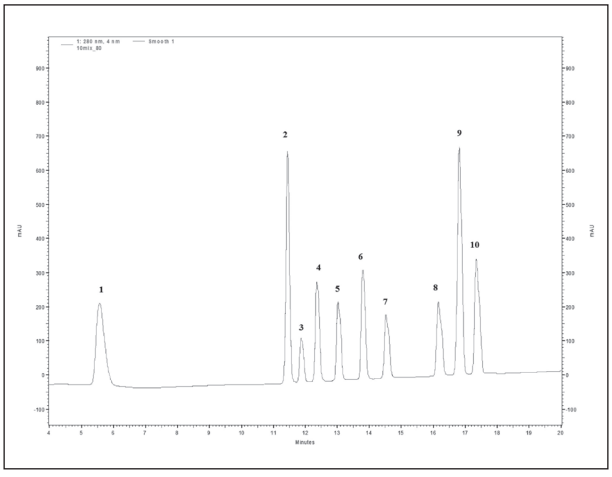

Figure 3. HPLC chromatogram of standards used for identification. 1. Gallic acid, 2. Caffeic acid, 3. Rutin hydrate, 4. Luteolin, 5. Kaempferol, 6. Rosmarinic acid, 7. Myricetin, 6. Quercetin, 7. Coumarin, 8. Apigenin.

Conclusion

Bioassays are valuable for the justification of the traditional usages of medicinal plants. Antiproliferative activity of *I. viscosa* against T98-G cell line was demonstrated for the first time. Strong antibacterial activity of *I. viscosa* was observed against Gram-positive bacteria. Traditional usage of *I. viscosa* as a remedy for wounds, skin diseases, lung and gastro-duodenal problems was justified with antibacterial results. It was demonstrated that *I. viscosa* has strong antioxidant activity (DPPH radicals scavenging activity, total phenolic and flavonoid content) and considerable amount of kaempferol. *I. viscosa* can be used as available source of natural antioxidant supplement. Increasing popularity of *I. viscosa* for the treatment of cancer in Turkish media should be verified with scientific studies. Anticancer potential of *I. viscosa* should be studied using different cancer cell lines in the future and further studies should focus on fractionation and isolation of the extracts obtained from aerial parts of *I. viscosa* in hopes of identifying active molecules.

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