Antioxidant and Antimicrobial Activities, and Phenolic Compounds of Selected Inula species from Turkey

Article *in* Natural product communications · April 2013

Source: PubMed

CITATIONS
15

READS
452

6 authors, including:

Alper Gökbulut
Ankara University
30 PUBLICATIONS **74** CITATIONS
SEE PROFILE

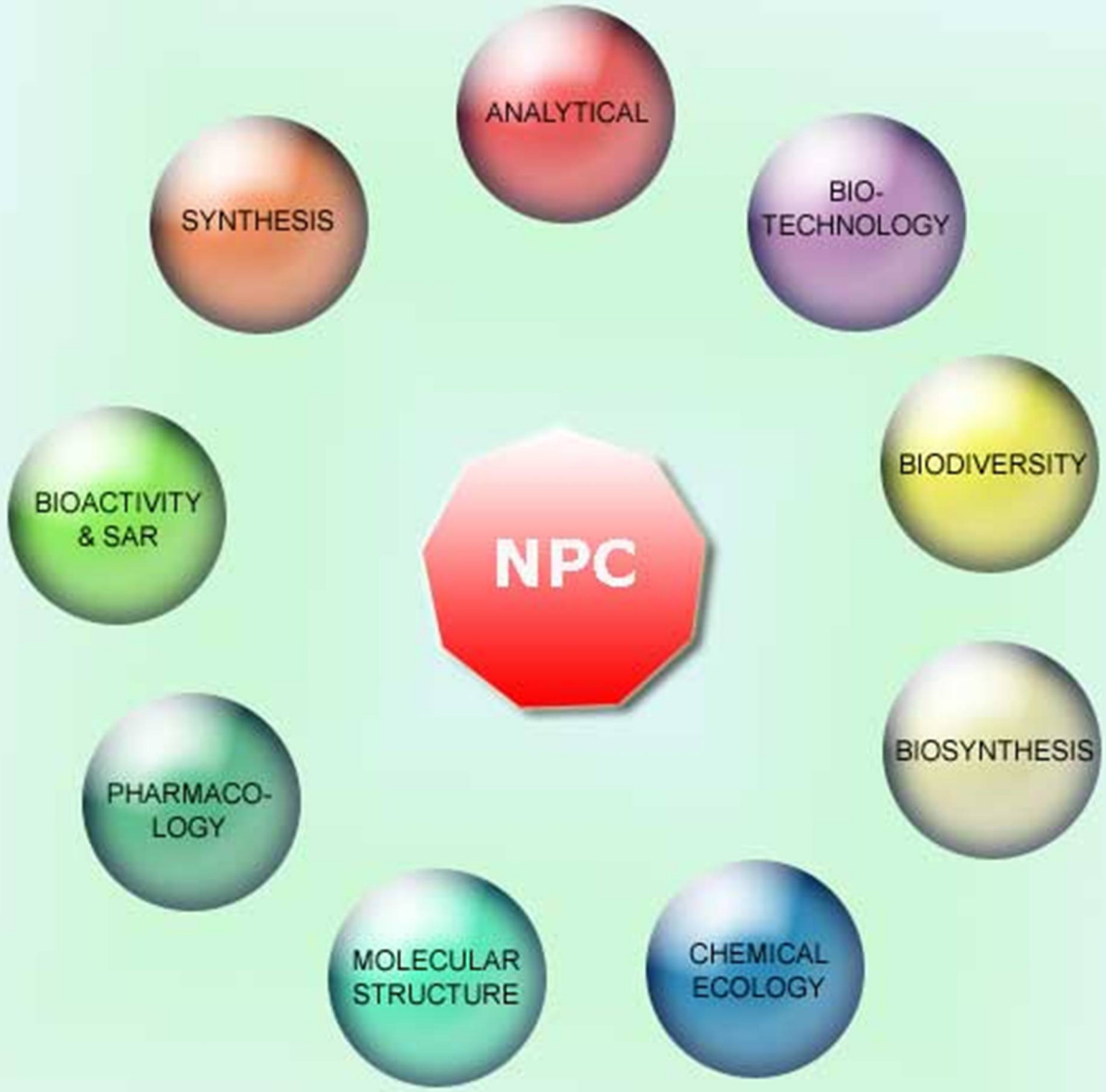
Basri Satilmis
Inonu University
11 PUBLICATIONS **42** CITATIONS
SEE PROFILE

Kadir Batcioglu
Inonu University
35 PUBLICATIONS **562** CITATIONS
SEE PROFILE

Engin Sarer
Ankara University
30 PUBLICATIONS **219** CITATIONS
SEE PROFILE

NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research



Volume 8. Issue 4. Pages 427-552. 2013
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

NPC Natural Product Communications

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site http://www.naturalproduct.us.

To Subscribe: Natural Product Communications is a journal published monthly. 2013 subscription price: US$2,395 (Print, ISSN# 1934-578X); US$2,395 (Web edition, ISSN# 1555-9475); US$2,795 (Print + single site online); US$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

NPC Natural Product Communications

2013
Vol. 8
No. 4
475 - 478

Antioxidant and Antimicrobial Activities, and Phenolic Compounds of Selected *Inula* species from Turkey

Alper Gökbulut[a*], Onural Özhan[a], Basri Satılmış[b], Kadir Batçıoğlu[b], Selami Günal[c] and Engin Şarer[a]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06100, Ankara, Turkey*
[b]*Department of Biochemistry, Faculty of Pharmacy, İnönü University, 44280, Malatya, Turkey*
[c]*Department of Microbiology, Faculty of Pharmacy, İnönü University, 44280, Malatya, Turkey*

gokbulut@pharmacy.ankara.edu.tr

Received: September 27th, 2012; Accepted: February 12th, 2013

Three *Inula* species, *I. viscosa*, *I. helenium* ssp. *turcoracemosa* and *I. montbretiana*, collected from different locations of Anatolia were investigated for their antioxidant and antimicrobial potential, and their total phenolic content and phenolic composition. Antioxidant activities of various extracts of the plant parts were measured using DPPH radical scavenging and ABTS assays. Antimicrobial potential of methanol extracts of the plant parts was determined by the agar dilution method against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida tropicalis*. All the extracts were more active against Gram-positive bacteria and yeasts than Gram-negative bacteria. The extracts exhibited antioxidant and antimicrobial activities in different concentrations. Total phenolic concentration of the extracts was estimated with Folin-Ciocalteu reagent using gallic acid as standard. The total phenolic content varied widely in different parts of the three tested *Inula* species, ranging from 21.1 ± 0.8 to 190.9 ± 6.1 mg GAE/g extract. Phenolic components, such as chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin and kaempferol were quantified by HPLC-DAD in the methanol extracts of the *Inula* species. It was obvious that the antioxidant and antimicrobial properties of the plants were due to the phenolics.

Keywords: *Inula* sp., DPPH, ABTS, Phenolic compounds, RP-HPLC.

The genus *Inula*, consisting of almost 100 species throughout the world, is distributed predominantly in the Mediterranean region. Several species are attributed to have anti-inflammatory, antitussive, bactericidal, antiproliferative, antidiabetic and hepatoprotective activities and the known chemical constituents of the genus are mono-, sesqui- and diterpenes, flavonoids and glycolipids [1a-1h]. *I. viscosa* (L.) Aiton is a well-known perennial medicinal herb that grows wild in Turkey, Spain, Italy, Portugal, Bulgaria and the Middle East. In the Mediterranean area, *I.viscosa* has been used for years in folk medicine for its anti-inflammatory, antipyretic, antiseptic, antiphlogistic activities and for the treatment of diabetes [2a-2d]. *I. helenium* L. is a widely occurring perennial herb in Europe and East Asia. It is an important medicinal plant and preparations of its roots are used in folk medicines against asthma, bronchitis, lung disorders, tuberculosis, indigestion, chronic enterogastritis, and infectious and helminth diseases [3a-3c]. *I. montbretiana* DC. is a rhizomatous, perennial herb widespread in Anatolia and known to possess antiprotozoal activity [4]. A restricted number of studies have been performed on *Inula* species collected from Turkey, especially *I. montbretiana*. We report here, for the first time, the antioxidant activity of this species, with the main focus on phenolic compound profile.

In the current work, the antioxidant and antimicrobial potential of *I. viscosa*, *I. montbretiana* and *I. helenium* ssp. *turcoracemosa* were evaluated, along with their total phenolic content; some of the phenolic compounds of these species were also determined.

Phenolic compounds contribute to the overall antioxidant potential of plants, so that the methanol extracts of the aerial parts and roots of the *Inula* species were analyzed for their total phenolic content by the Folin Ciocalteu method. Values varied widely in different parts of the three tested *Inula* species, ranging from 21.1 ± 0.8 to 190.9 ± 6.1 mg GAE/g extract (Table 1). The highest amount of total phenolics was found in *I. helenium* herb and *I. montbretiana* root, followed by *I. viscosa* herb and root. A close relationship was observed between antioxidant activity of the methanol extracts and the total phenolic contents. In parallel, the lowest total phenolic content was determined for *I. helenium* root, for which the highest IC_{50} value was obtained.

Table 1: Yields and total phenol contents of methanol extracts of *Inula* species.

Parts of the plants	yields, %	Phenolic contents (mg GAE/g extract)[a]
I. viscosa herb	15	176.9 ± 7.8
I. viscosa root	21	177.1 ± 3.6
I. montbretiana herb	10	119.4 ± 7.4
I. montbretiana root	17.5	190.9 ± 6.1
I. helenium herb	16.5	189.6 ± 3.9
I. helenium root	20.5	21.1 ± 0.8

[a]mean ± SD (*n*=3)

DPPH radical scavenging and ABTS assays were used to evaluate the antioxidant potential of the *Inula* species. Regarding the IC_{50} values obtained by both methods, all the extracts showed antioxidant activity in various concentrations (Table 2). Nearly all the ethyl acetate extracts had low antioxidant activity, with high IC_{50} values, in comparison with the water and methanol extracts. The methanol extract of *I. helenium* flowers showed significant antioxidant activity with both methods. According to the IC_{50} values gained from the DPPH radical scavenging activity method, the water extract of *I. viscosa* flowers (IC_{50}: 0.28 ± 0.03 mg/mL), and the methanol extract of *I. helenium* flowers (IC_{50}: 0.14 ± 0.06 mg/mL) exhibited the highest antioxidant activities. With regard to the IC_{50} values obtained from the ABTS assay, water extracts of *I. helenium* flowers (IC_{50}: 0.05 ± 0.02 mg/mL) and *I. viscosa* flowers (IC_{50}: 0.17 ± 0.03 mg/mL), and the methanol extract of *I. helenium* flowers (IC_{50}: 0.15 ± 0.04 mg/mL) showed the highest antioxidant capacities. All the extracts had higher IC_{50} values than Trolox (IC_{50}: 0.04 mg/mL for DPPH assay; 0.04 mg/mL for ABTS assay).

Table 2: IC_{50} values of water, methanol and ethyl acetate extracts of *Inula* species according to DPPH and ABTS assays.

	Water extract		Methanol extract		Ethyl acetate extract	
	DPPH IC_{50} (mg/mL)[a]	ABTS IC_{50} (mg/mL)	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)
I. viscosa flower	0.28 ± 0.03	0.17 ± 0.03	0.36 ± 0.04	0.47 ± 0.07	0.99 ± 0.09	0.55 ± 0.02
I. viscosa leaf	0.47 ± 0.03	0.21 ± 0.07	0.42 ± 0.02	0.50 ± 0.09	1.05 ± 0.11	0.65 ± 0.09
I. viscosa root	1.07 ± 0.09	0.23 ± 0.03	0.40 ± 0.08	0.50 ± 0.01	2.90 ± 0.13	1.17 ± 0.09
I. montbretiana flower	2.34 ± 0.12	0.44 ± 0.06	0.40 ± 0.01	0.80 ± 0.05	1.97 ± 0.09	0.84 ± 0.06
I. montbretiana leaf	1.07 ± 0.06	0.42 ± 0.01	0.50 ± 0.02	0.95 ± 0.11	1.44 ± 0.06	1.49 ± 0.15
I. montbretiana root	2.24 ± 0.04	0.25 ± 0.08	0.23 ± 0.03	0.62 ± 0.08	4.32 ± 0.18	1.18 ± 0.09
I. helenium ssp. flower	1.14 ± 0.11	0.05 ± 0.02	0.14 ± 0.06	0.15 ± 0.04	2.21 ± 0.16	2.10 ± 0.19
I. helenium ssp. leaf	0.49 ± 0.05	0.19 ± 0.009	0.23 ± 0.03	0.39 ± 0.06	10.81 ± 0.95	4.95 ± 0.21
I. helenium ssp. root	18.42 ± 1.07	2.88 ± 0.07	2.98 ± 0.08	1.52 ± 0.08	144.5 ± 2.11	2.03 ± 0.09

[a] mean ± SD (*n*=3)

Antimicrobial potential of methanol extracts of the plant parts was determined by the agar dilution method against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida tropicalis*. All plant extracts exhibited antibacterial and anticandidal activities, with MIC values ranging from 50 to 800 µg/mL (Table 3). In general, all the extracts were more active against the Gram-positive bacteria and yeasts than the Gram-negative bacteria. *I. viscosa* root extract had the highest inhibitory activity against all the microorganisms, with MIC values varying from 50 to 100 µg/mL. The yeast strain, *C. tropicalis*, was considerably more sensitive to all the tested extracts. The Gram-positive bacterium *E. faecalis* was very sensitive to *I. viscosa* root and *I. montbretiana* flower extracts, with MIC values of 50 µg/mL. The Gram-negative bacterium, *E. coli*, was sensitive to *I. viscosa* root (MIC: 100 µg/mL), *I. montbretiana* flower (MIC: 200 µg/mL) and *I. helenium* root (MIC: 200 µg/mL) extracts.

Table 3: Minimal inhibitory comcentrations (MIC in µg/mL) of methanolic extracts of *Inula* species

	Ec	*Pa*	*Sa*	*Ef*	*Ca*	*Ct*
I. viscosa flower	800	400	200	200	200	200
I. viscosa leaf	400	400	200	200	200	200
I. viscosa root	**100**	200	**50**	**50**	100	**50**
I. montbretiana flower	200	200	100	**50**	100	**50**
I. montbretiana leaf	400	400	100	200	200	100
I. montbretiana root	400	400	100	100	200	100
I. helenium ssp. flower	800	800	200	400	400	200
I. helenium ssp. leaf	400	400	100	200	200	100
I. helenium ssp. root	200	400	100	100	100	**50**
Ampicillin	3.12	-	3.12	1.56	-	-
Ciprofloxacine	1.56	3.12	0.39	0.78	-	-
Fluconazole	-	-	-	-	3.12	3.12

Ec: E. coli, Pa: P. aeruginosa, Sa: S. aureus, Ef: E. faecalis, Ca: C. albicans, Ct: C. tropicalis.

In a previous study, it was indicated that one of the most active of the twenty plant extracts tested against *C. albicans* was *I. viscosa.* Also, *I. viscosa* water and ethanol extracts were found to be active against *E. coli* and *P. aeruginosa* [5a]. In parallel, our results revealed that *I. viscosa* methanol extracts were significantly active against both *C. albicans* and *C. tropicalis*, and against all the tested bacteria, especially the root extract. In another study, the methanol extract of the aerial parts of *I. viscosa* from Tunisia showed lower antibacterial activity against *S. aureus* (MIC: 625 µg/mL) compared with our results (MIC: 200 µg/mL). Also, it was found to be inactive against *E. coli*, *P. aeruginosa* and *E. faecalis*, contrary to our results [5b]. Oskay and Sarı investigated some medicinal plants from Turkey and indicated that *I. viscosa* leaves demonstrated significant anticandidal activity [5c]. Our results confirm this, and also show the root extract of *I. viscosa* to be more active against *Candida* species than the leaf and flower extracts.

To determine the active principles responsible for the significant antioxidant and antimicrobial potential of the species, the amounts of seven phenolic compounds in the methanol extracts of different parts of *Inula* species were determined by RP-HPLC. Retention times, the equations obtained from calibration curves, test range, LOD and LOQ values are given in Table 4 and quantification results in Table 5. The results revealed that all the investigated plant parts contain chlorogenic and caffeic acids in various amounts. Especially, *I. viscosa* root extract was found to be rich in chlorogenic acid, while *I. helenium* flower extract contained caffeic acid in higher amount than the other extracts. *I. montbretiana* flower extract had a high content of luteolin when compared with the other *Inula* extracts. Due to the restricted number of studies on *I. montbretiana*, this finding was thought to be important, especially for presenting a distinction between the *Inula* species. While myricetin was only determined in *I. helenium* flower extract in small amount, all the investigated flavonoids were absent from all the root extracts. The higher antioxidant potential of the methanol extracts of *I. helenium* flowers could be explained with regard to the presence of significant amounts of caffeic acid and rutin. In parallel, the presence of most of the investigated phenolics could be responsible for the high antioxidant activities of the extracts of different parts of *I. viscosa*, especially the significant amount of chlorogenic acid. In a previous study, *I. helenium* root was investigated for its phenolic composition and while hydroxycinnamic acids were determined in significant amounts, flavonoids were not detected. The caffeic acid content was especially high compared with our results, which could be due to the different extraction procedure used [6].

In conclusion, in this study, the total phenolic content and qualitative and quantitative analysis of phenolic compounds, as well as the antioxidant and antimicrobial activities of various extracts from different parts of three *Inula* species have been investigated. In general, all the plant extracts exhibited antioxidant, antibacterial and anticandidal activities in different concentrations. The screening of the phenolic compounds of the methanol extracts showed that the investigated parts of the *Inula* species were rich in antioxidant and antimicrobial compounds, and, therefore, the tested medicinal plants are antibacterial and anticandidal agents and could be used in the treatment of various ailments caused by bacteria and yeasts. To the best of our knowledge, while both the antioxidant activity and the phenolic compound profile of *I. montbretiana* have not been studied before, only a restricted number of activity and analytical studies have been carried out on the other two investigated *Inula* species. Our results of antimicrobial and antioxidant assays also justified and partially supported the previous literature data and the popular usage of the tested plants.

Experimental

Chemicals and reagents: DPPH (D9132) and ABTS Antioxidant Assay Kits (CS0790) were purchased from Sigma (Germany). Chromatographic grade double-distilled water, HPLC grade methanol, acetonitrile and analytical grade trifluoroacetic acid were

Table 4: Linear relationships between peak areas and concentrations.

Analyte	Retentiom time (min)	Standard curve[a]	r	Test range (µg/mL)	LOD[b] (µg/mL)	LOQ[c] (µg/mL)
Chlorogenic acid	6.5	*y*=20692*x*-64.979	0.9996	0.25-515	0.075	0.250
Caffeic acid	8.1	*y*=50521*x*-10.69	0.9989	0.22-103	0.067	0.220
Rutin	10.3	*y*=13776*x*-12.522	0.9999	0.20-500	0.060	0.200
Myricetin	13.3	*y*=52997*x*-24.262	0.9999	0.41-100	0.120	0.410
Quercetin	16.5	*y*=33078*x*-21.421	0.9997	0.28-400	0.085	0.280
Luteolin	16.9	*y*=40356*x*-13.308	0.9999	0.18-100	0.053	0.180
Kaempferol	19.5	*y*=28901*x*-3.4329	0.9998	0.27-100	0.081	0.270

[a] y=peak area; x:=concentration of analyte (mg/mL); [b] LOD= limit of detection S/N:3 (*n*=9); [c] LOQ= limit of quantification S/N:10 (*n*=9)

Table 5: The contents of phenolic compounds in methanol extracts of different parts of *Inula* species.

Species	Content (w/w,%)[a]						
	Chlorogenic acid	Caffeic acid	Rutin	Myricetin	Quercetin	Luteolin	Kaempferol
I. viscosa flower	0.61±0.03	0.010±0.001	0.074±0.005	nd	0.039±0.0002	0.044±0.003	0.030±0.003
I. viscosa leaf	0.17±0.02	0.015±0.0003	0.110±0.0004	nd	0.021±0.0003	0.054±0.002	nd
I. viscosa root	**0.82±0.05**	0.013±0.0002	nd[b]	nd	nd	nd	nd
I. montbretiana flower	0.34±0.03	0.020±0.003	0.027±0.001	nd	0.007±0.0001	**0.166±0.002**	nd
I. montbretiana leaf	0.18±0.002	0.012±0.0001	nd	nd	nd	0.029±0.0001	nd
I. montbretiana root	0.32±0.002	0.009±0.0004	nd	nd	nd	nd	nd
I. helenium flower	0.45±0.03	**0.046±0.0006**	**0.240±0.003**	0.0038±0.0004	0.054±0.001	0.010±0.0001	0.003±0.0001
I. helenium leaf	0.54±0.04	0.016±0.0001	0.095±0.015	nd	nd	nd	nd
I. helenium root	0.14±0.008	0.003±0.0001	nd	nd	nd	nd	nd

[a] mean ± SD (*n*=3); [b] nd=not detected.

used for HPLC analysis. Folin Ciocalteu reagent and the following phenolic compounds were purchased from Sigma (Germany): gallic acid (G7384), chlorogenic acid (C3878), caffeic acid (C0625), rutin (R5143), myricetin (M6760), quercetin (Q4951), luteolin (L9283), and kaempferol (K0133). All other chemicals were analytical grade and obtained from either Sigma or Merck.

Plant materials: *Inula* species were collected in their flowering stages from Anatolia. *I. montbretiana* DC. and *I. helenium* (L.) ssp. *turcoracemosa* were collected near Ankara, and *I. viscosa* (L.) Aiton from Isparta. Voucher specimens have been deposited in the Herbarium of Ankara University Faculty of Pharmacy under the herbarium codes of AEF 25191, AEF 25193 and AEF 25534, respectively.

Extraction: Five g of dried and milled flowers, leaves and root of plant samples were extracted with water, methanol and ethyl acetate (100 mL each) by magnetic stirrer for 1 h (50ºC, 250 rpm). After filtration, the organic phases were evaporated completely in a rotary evaporator (Buchi-R200), and the water extracts were freeze-dried. The crude extracts were used for antioxidant activity tests. For antimicrobial activity measurements, methanol extracts of the different plant parts were used, obtained using the same procedure. For HPLC analysis, 200 mg of dried and milled flowers, leaves and roots were extracted with methanol, using a magnetic stirrer, for 6 h (50^{o}C, 250 rpm). The extract was then filtered, made up to 10.0 mL in a volumetric flask with methanol, passed through a 0.45 µm filter, and injected into the HPLC system**.**

Determination of total phenolic content: The total phenolic content of the extracts was determined spectrophotometrically using a modified Folin Ciocalteu method [7a]. The reduction of the reagent, which resulted in the formation of a blue colour, was recorded at 765 nm. One hundred µL of the methanol extract of each plant part (2 mg/mL) was mixed with 7.9 mL of distilled water. Folin Ciocalteu reagent (500 µL) was added and the contents of the flask shaken vigorously. After 8 min, 1.5 mL of 20% Na_2CO_3 was added. After 2 h incubation at room temperature, the absorbance was measured at 765 nm with a Shimadzu spectrometer. Gallic acid was used as standard. All measurements were performed in triplicate, and the average values were used to express the mg of gallic acid equivalents (GAE)/g dry extract.

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity: The capacity to scavenge the stable free radical DPPH was monitored according to the modified method of Barros *et al.* [7b]. Various concentrations of extracts (0.25 mL) were mixed with 2.75 mL of methanolic solution containing DPPH radical. The mixture was shaken vigorously and left to stand for 10 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The radical scavenging activity (Inh%) was calculated as a percentage of DPPH discoloration using the equation: Inh% = $[(A_{DPPH}-A_s) / A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract was added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. Trolox (Sigma, Germany) was used as standard.

ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] assay: The ABTS method was performed using a modified method of Miller and Rice-Evans [7c] with an antioxidant assay kit supplied from Sigma. Myoglobin working solution (20 µL) and ABTS working solution (150 µL), prepared by mixing 3% hydrogen peroxide solution (25 µL) and ABTS solution (10 mL), were added to various concentrations of extracts (10 µL). After an incubation period of 5 min, stop solution (100 µL) was added to the media and endpoint absorbance values were recorded at 405 nm. The antioxidant activity (Inh%) was calculated as a percentage value by using the equation: Inh% = $[(A_0-A_s)/ A_0] \times 100$, where A_S is the absorbance gained at the end of the process with sample extract, and A_0 is the absorbance of the control. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. Trolox was used as the standard.

Antimicrobial activity: Antimicrobial activities of the extracts were determined by using the agar dilution procedure outlined by the Clinical and Laboratory Standards Institute (CLSI) [8a, 8b]. Minimal inhibitory concentrations for each extract were investigated against standard bacterial strains: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853, all obtained from the American Type Culture

Collection (Rockville, Md.), and the yeasts *Candida albicans* and *C. tropicalis,* obtained from the Department of Microbiology, Faculty of Medicine, Ege University (Turkey). The stock solution of the extracts was prepared in dimethyl sulfoxide (DMSO), which had no effect on the microorganisms in the concentrations studied. All of the dilutions were made with distilled water. The concentrations of the tested extracts were 800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL. Ampicillin, Ciprofloxacine and Fluconazole from FAKO (Istanbul, Turkey) were used as reference compounds. A loopful (0.01 mL) of the standardized inoculum of the bacteria and yeasts (10^6 CFUs/mL) was spread over the surface of the agar plates. All the inoculated plates were incubated at 35°C and results were evaluated after 16-20 h of incubation for bacteria and 48 h for yeasts.

Analysis of phenolic compounds by RP-HPLC: The qualitative and quantitative analyses of the phenolic compounds in the extracts were performed according to the following procedure. The analysis was performed with a LC system consisting of a HP Agilent 1100 series quaternary pump, degasser and photodiode array detector. The samples were injected into a HP Agilent 1100 Autosampler with a thermostatted column compartment on a Phenomenex-Luna C_{18} column (5 µ, 250 mm X 4.6 mm) at 30°C. The system was controlled and data analysis was performed with Agilent ChemStation software. All the calculations concerning the quantitative analysis were performed with external standardization by measurement of the peak areas. Gradient elution was applied with a flow rate of 1 mL/min and column temperature was set to 30°C. The mobile phase was a mixture of trifluoroacetic acid 0.1% in water (solution A), trifluoroacetic acid 0.1% in methanol (solution B), and trifluoroacetic acid 0.1% in acetonitrile (solution C). The composition of the gradient was (A:B:C), 80:10:10 at 0 min, 60:25:15 at 5 min, 50:30:20 at 10 min, 40:40:20 at 15 min and 0:75:25 at 20 min. The duration between runs was 5 min. All solvents were filtered through a 0.45 µm Millipore filter before use and degassed in an ultrasonic bath. From each solution and sample 10 µL was injected into the column and the chromatograms were recorded from 200 to 400 nm. Standard solutions were analyzed and three-dimensional chromatograms (wavelength; time; absorbance) were obtained to select the optimum wavelength for detection of the phenolics with maximum sensitivity. Quantification was performed by measuring at 330 nm for caffeic and chlorogenic acids, 340 nm for luteolin, and 360 nm for rutin, myricetin, quercetin and kaempferol using a photo-diode array detector. The chromatographic run time was 20 min and the column void volume 1.60 min.

Acknowledgments: Authors wish to thank Prof. Dr Zeki Aytaç for identification of the plant species used in this work.

References

[1] (a) Zhao Y-M, Zhang M-L, Shi Q-W, Kiyota H. (**2006**) Chemical constituents of plants from the genus *Inula*. *Chemistry & Biodiversity*, ***3***, 371-384; (b) Topçu G, Öksüz S, Shieh H-L, Cordell GA, Pezzuto JM, Bozokjohansson C. (**1993**) Cytotoxic and antibacterial sesquiterpenes from *Inula graveolens*. *Phytochemistry*, ***33***, 407-410; (c) Marco JA, Sanz_Cervera JF, Manglano E. (**1993**) Chlorinated thymol derivatives from *Inula crithmoides*. *Phytochemistry*, ***33***, 875-878; (d) Zarga MHA, Hamed EM, Sabri SS, Voelter W, Zeller K-P. (**1998**) New sesquiterpenoids from the Jordanian medicinal plant *Inula viscosa*. *Journal of Natural Products*, ***61***, 798-800; (e) Shao Y, Bai N-S, Zhou B-N. (**1996**) Kaurane glycosides from *Inula britannica*. *Phytochemistry*, ***42***, 783-786; (f) Mosaddegh M, Moghadam MH, Ghafari S, Naghibi F, Ostad SN, Read RW. (**2010**) Sesquiterpene lactones from *Inula oculus-christi. Natural Product Communications*, ***5***, 511-514; (g) Blagojević PD, Radulović NS. (**2012**) Conformational analysis of antistaphylococcal sesquiterpene lactones from *Inula helenium* essential oil. *Natural Product Communications*, ***7***, 1407-1410; (h) Moghadam MH, Hajimehdipoor H, Saeidnia S, Atoofi A, Shahrestani R, Read RW, Mosaddegh M. (**2012**) Anti-proliferative activity and apoptotic potential of britannin, a sesquiterpene lactone from *Inula aucheriana*. *Natural Product Communications*, ***7***, 979-980.

[2] (a) Al-Dissi NM, Salhab AS, Al-Hajj HA. (**2001**) Effects of *Inula viscosa* leaf extracts on abortion and implantation in rats. *Journal of Ethnopharmacology*, ***77***, 117-121; (b) Baytop T. (**1984**) *Therapy with Medicinal Plants in Turkey.* Sanal Press, Istanbul, 167; (c) Yaniv Z, Dafni A, Friedman J, Palevitch D. (**1987**) Plants used for treatments of diabetes in Israel. *Journal of Ethnopharmacology*, ***19***, 145–151; (d) Fontana G, La Rocca S, Passannanti S, Paternostro MP. (**2007**) Sesquiterpene compounds from *Inula viscosa*. *Natural Product Research*, ***21***, 824-827.

[3] (a) Dorn DC, Alexenizer M, Hengstler JG, Dorn A. (**2006**) Tumor cell specific toxicity of *Inula helenium* extracts. *Phytotherapy Research*, ***20***, 970-980; (b) Konishi T, Shimada Y, Nagao T, Okabe H, Konoshima T. (**2002**) Antiproliferative sesquiterpene lactones from the roots of *Inula helenium*. *Biological & Pharmaceutical Bulletin*, ***25***, 1370-1372; (c) Ma X-C, Liu K-X, Zhang B-J, Xin X-L, Huang J. (**2008**) Structural determination of three new eudesmanolides from *Inula helenium*. *Magnetic Resonance in Chemistry*, ***46***, 1084-1088.

[4] Gökbulut A, Kaiser M, Brun R, Sarer E, Schmidt TJ. (**2012**) 9*β*-Hydroxyparthenolide esters from *Inula montbretiana* and their antiprotozoal activity. *Planta Medica*, ***78***, 225-229.

[5] (a) Ali-Shtayeh MS, Yaghmour RMR, Faidi YR, Salem K, Al-Nuri MA. (**1998**) Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. *Journal of Ethnopharmacology*, ***60***, 265-271; (b) Sassi AB, Harzallah-Skhiri F, Aouni M. (**2007**) Investigation of some medicinal plants from Tunisia for antimicrobial activities. *Pharmaceutical Biology*, ***45***, 421-428; (c) Oskay M, Sarı D. (**2007**) Antimicrobial screening of some Turkish medicinal plants. *Pharmaceutical Biology*, ***45***, 176-181.

[6] Wojdylo A, Oszmianski J, Czemerys R. (**2007**) Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, ***105***, 940-949.

[7] (a) Singleton VL, Orthofer R, Lamuela-Raventos RM. (**1999**) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, ***299***, 152–178; (b) Barros L, Baptista P, Ferreira ICFR. (**2007**) Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food and Chemical Toxicology*, ***45***, 1731-1737; (c) Miller NJ, Rice-Evans CA. (**1997**) Factors influencing the antioxidant activity determined by the ABTS (center dot+) radical cation assay. *Free Radical Research*, ***26***, 195-199.

[8] (a) Clinical and Laboratory Standards Institute (**2006**) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard-Seventh Edition, CLSI Document M7-A7, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania; (b) Clinical and Laboratory Standards Institute (**2002**) Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard-Second Edition, NCCLS document M27-A2 (ISBN 1-56238-469-4), NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Review/Account

Natural Product Communications
2013
Volume 8, Number 4

Contents

Continued Inside backcover