TOTAL PHENOLIC CONTENT, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SOME MEDICINAL PLANTS

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ABSTRACT

Crude extracts from *Inula aucherana, Fumaria officinalis, Crocus sativus, Vicum album, Tribulus terestris, Polygonatum multiflorum, Alkanna tinctoria and Taraxacum officinale* were screened for their *in vitro* antioxidant and antimicrobial properties. Total phenolic content of extracts from these plants were also determined. β-carotene bleaching assay and Folin-Ciocalteu reagent were used to determine total antioxidant activity and total phenols of plant extracts. Antimicrobial activity was determined by using disk diffusion assay. Antioxidant activity and total phenolic content varied among plants used and *Viscum album* and *Crocus sativus* had the highest antioxidant (82.23%) and total phenolic content (42.29 mgGAE/g DW), respectively. The methanol extracts from *Vicum album* and *Alkanna tinctoria* showed antimicrobial activity against 9 out of 32 microorganisms, however extract from *Inula aucherana* showed antimicrobial activity against 15 out of 32 microorganisms. The results provided evidence that the studied plant might indeed be potential sources of natural antioxidant and antimicrobial agents.

Keywords: Antioxidant activity, antimicrobial activity, medicinal plants, methanol extract.

INTRODUCTION

Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Mothana and Lindequist, 2004, Bajpai *et al*., 2005; Wojdylo *et al*., 2007).

Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity) (Chopra *et al*., 1992; Bruneton, 1995). The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs.

Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Scientific experiments since the late 19th century have documented the antioxidant properties of some spices, herbs, and their components (Zaika, 1988; Bajpai *et al*., 2005). Many studies reported the activities of spices and herbs on food borne pathogenic microorganisms (Arora and Kaur, 1999; Yano *et al.*, 2006).

Inula aucherana, Fumaria officinalis, Crocus sativus, Vicum album, Tribulus terestris, Polygonatum multiflorum, Alkanna tinctoria and Taraxacum officinale has been widely used in traditional medicine in Turkey as spice or herb so a long time. Among them, particularly *Crocus sativus* as known Safran in Turkish has special importance in Turkish consumer for not only for medicinal use but also as flavor agent (Baytop, 1984).

The purpose of the present study was to investigate the antioxidant and antimicrobiyal properties of *Inula aucherana, Fumaria officinalis, Crocus sativus, Vicum album, Tribulus terestris, Polygonatum multiflorum, Alkanna tinctoria and Taraxacum officinale*. In this paper we report the results of such studies in order to orient future investigations towards the finding of new, potent and safe antioxidant and antimicrobial compounds.

MATERIALS AND METHODS

Chemicals

All of the chemicals used in this work were purchased from Sigma-Aldrich (USA) and Merck (Germany) companies. The chemicals were analytical degree

Plant material

Aerial parts of *Inula aucherana, Fumaria officinalis, Crocus sativus, Vicum album, Tribulus terestris Polygonatum multiflorum, Alkanna tinctoria and Taraxacum officinale* were collected from different natural habitats of the country. Voucher specimens were

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deposited in the Food Engineering Department, Ataturk University, Erzurum-Turkey. The sampled materials were chopped, dried and powdered (40-mesh). Then materials (400 g) were extracted in a Soxhlet with methanol (MeOH) at 72 h. The extracts were filtered using Whatman filter paper (No:1) and then concentrated in vacuo at 40 ^{0}C using a Rotary evaporator. The residues obtained were stored in a freezer at − 80^{o}C until further tests.

Biological materials

Total 32 microorganisms were used in this study. The microorganisms maintained on Nutrient Agar (Merck, Darmstadt, Germany) were supplied by Microbiology Laboratory of Agricultural Faculty of Ataturk University, Erzurum, Turkey. The food-associated microorganisms were selected because they are frequently reported in foods.

Determination antioxidant activity and total phenolics

In β-Carotene-linoleic acid assay, antioxidant capacity of methanol extracts is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Barriere *et al*., 2001).

A stock solution of β-carotene/linoleic acid (Sigma-Aldrich) was prepared as follows. First, 0.5 mg of β-carotene was dissolved in 1 ml of chloroform (HPLC grade), then 25 µl of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated using a vacuum evaporator. Then 100 ml of distilled water saturated with oxygen (30 min at 100 ml/min) was added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and 350 µl portions of the extracts (2 g/l in ethanol) were added before incubating for 48 h at room temperature. The same procedure was repeated with butylated hydroxyanisole (BHA) and butylated hydroxytolune (BHT) at the same concentration and a blank containing only 350 µl of ethanol. After the incubation period the absorbance of the mixtures were measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHA, BHT and the blank.

Total phenolic constituents of plant extracts were performed employing the literature methods involving Folin-Ciocalteu reagent and gallic acid as standard (Slinkard and Singleton, 1977). Extract solution (0.1 ml) containing 1000 µg extract was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent were added and flask was shaken thoroughly. After 3 min, 3 ml of solution 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated to all standard gallic acid solutions (0–1000 mg, 0.1 ml^{-1}) and standard curve was obtained.

Antimicrobial activity

The antimicrobial activity of the extracts was carried out by disc diffusion test (Kim *et al*., 1995) using 100 µl of suspension containing 10^8 CFU/ml of bacteria spread on nutrient agar (NA) medium. Sterile 6 mm diameter filter paper discs were impregnated with 300 µg all the steril test material and placed onto nutrient agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. NV30 (30µg novobiocin/disc), SAM20 (10µg sulbactam + 10µg ampicillin/disc), CC2 (2µg clindamycin/disc), OFX10 (10 µg ofloxacin/disc), AMC30 (20µg amoxicillin + 10µg clavulanic asit/disc), KF30 (30 µg cephalothin/disc), TE30 (30µg tetracycline/disc), AZM15 (15 µg azithromycin/disc), AMP20 (20 µg/disk Amphotericin) used as positive reference standards to determine the sensitivity of one strain in each bacterial species tested. The inoculated plates with food-associated bacteria were incubated at 27°C for 24 h. The antibacterial activity was measured as the diameter (mm) of clear zone of growth inhibition. Five disc per plate

Table 1: Antioxidant activity and total phenolic content of plant extracts

Plants	Antioksidant activity (%)	Total phenolic content (mgGAE/g DW)
Inula aucherana	80.10bc	6.57bc
Fumaria officinalis	78.93bc	10.50bc
Crocus sativus	63.15cd	42.29a
Viscum album	82.23b	19.43b
Tribulus terestris L.	69.25c	14.43bc
Polygonatum multiflorum L.	75.85bc	4.07c
Alkanna tinctoria	79.80bc	11.57bc
Taraxacum officinale	43.05d	15.50bc
BHA (200 mg/l)	93.21a	-
BHT (200 mg/l)	90.71ab	-

The same letters in same column showed significant differences among plant species at *P*<0.01 statistical level.

and three plates were used, and each test was run in triplicate (Djipa *et al*., 2000).

STATISTICAL ANALYSIS

The experiment was a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan multiple range test at $P<0.01$ significant level.

RESULTS AND DISCUSSION

The total phenolic content of plant is shown in table 1. The differences in total phenolics content among plants used were statistically significant ($p<0.01$, table 1).

The total phenolic content of *Inula aucherana, Fumaria officinalis, Crocus sativus, Vicum album, Tribulus terestris Polygonatum multiflorum, Alkanna tinctoria* and *Taraxacum officinale* were in range of 4.04 mg GAE/g (*Polygonatum multiflorum* L.) to 42.29 mg GAE (*Crocus sativus*) per g dry weight basis. Earlier, a wide variation was observed on total phenolic content in different aromatic and medicinal plants were 6.80-32.10 mg gallic acid equivalents per g dry weight basis (Bajpai *et al*., 2005). Moreover, total phenolic content of *Polygonatum* species were found 1.27-8.69 mg gallic acid equivalents per g dry weight basis (Huang *et al*., 2008) which supports our findings.

It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health-benefical effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores (Vaya *et al*., 1997).

The antioxidant capacity by using β-carotene bleaching assay in plants used is shown in table 1. A statistical significant difference ($p<0.01$) was found among the samples. In β-carotene bleaching assay, all plant extracts showed moderate to high antioxidant capacity. The highest antioxidant capacity was observed in *Viscum album* (82.23%), followed by *Inula aucherana* (80.10%),

Table 2: Antimicrobial properties of plant extracts

Food borne-microorganisms	*Crocus sativus*		*Viscum album*		*Inula aucherana*		*Fumaria officinalis*		Positive control[b]
	Aqueous[a]	Methanolic[a]	Aqueous[a]	Methanolic[a]	Aqueous[a]	Methanolic[a]	Aqueous[a]	Methanolic[a]	
Acinetobacter lwoffi 2819	-	2	-	-	1	1	-	-	17 (AMC 30
Alcaligenes feacalis 0452	-	-	-	2	1	5	-	-	7 (SAM 20)
Bacillus cereus 6230	-	6	-	3	-	10	-	4	12 (CC 2)
Bacillus subtilis ATCC 6633	-	5	-	7	-	5	-	2	17 (NV 30)
Enterobacter cloacae 7418	-	-	-	-	-	-	-	-	10 (OFX 10)
Escherichia coli 1328	-	10	-	2	1	2	-	2	18 (CC 2)
Escherichia coli 1402	-	-	-	2	1	2	-	1	19 (CC 2)
Klebsiella pneumonia subsp. *ozanae* 5713	-	-	-	-	-	-	-	-	19 (CC 2)
Klebsiella pneumonia subsp. *pneumonia* 2124	-	2	-	-	-	-	-	-	13 (OFX 10)
Listeria monocytogenes 8353	-	4	-	-	-	2	-	2	24 (TE 30)
Proteus mirabilis 3242	-	-	-	-	-	-	-	-	12 (OFX 10)
Proteus vulgaris KÜKEM 1329	-	2	-	2	4	-	-	2	18 (AMC30)
Providencia alcaliaciens 3215	-	-	-	-	-	-	-	-	10 (AZM15)
Pseudomonas aeruginosa ATCC 9027	-	7	3	8	-	-	-	4	36 (TE 30)
Pseudomonas aeruginosa 3428	-	-	-	-	-	13	-	10	36 (TE 30)
Pseudomonas fluorescens 7324	-	-	-	-	-	-	-	-	31 (OFX 10)
Pseudomonas pseudoalcaligenes 3445	-	-	-	-	-	-	-	-	24 (OFX 10)
Pseudomonas putida 1617	-	-	-	-	-	-	-	-	16 (TE 30)
Salmonella typmiruim RSSK 95091	-	-	-	-	-	-	-	-	9 (OFX 10)
Staphylococcus aureus 7231	-	4	-	-	-	15	-	15	22 (KF 30)
Staphylococcus hominis 3221	-	-	-	-	-	2	-	-	24 (KF 30)
Streptococcus pyogenes ATCC 176	-	-	-	-	-	6	-	-	30 (NV 5)
Candida albicans ATCC 1223	-	2	-	-	-	-	-	-	15 (AMP 20)
Saccharomyces boulardii 6128	-	4	-	-	-	6	-	7	9 (AMP 20)
Saccharomyces cereviciae 6541	-	5	-	-	-	-	-	4	8 (AMP 20)
Aspergillus niger BC 102	-	-	-	-	-	-	-	-	21 (AMP 20)
Cladosporium herbarum	-	9	-	1	2	5	4	14	10 (AMP 20)
Paecilomyces variotii 108	-	-	-	-	-	10	-	7	19 (AMP 20)
Penicillium brevicompactum	-	-	-	-	-	-	-	-	14 (AMP 20)
Penicillium roquefortii BC 111	-	-	-	2	-	5	-	-	11 (AMP 20)
Penicillium roquefortii BC 113	-	-	-	-	-	-	-	-	8 (AMP 20)
Trichothecium roseum	-	-	-	-	-	-	-	-	32 (AMP 20)

Table 2 : continued

Food borne-microorganisms	*Crocus sativus*		*Viscum album*		*Inula aucherana*		*Fumaria officinalis*		Positive control[b]
	Aqueous[a]	Methanolic[a]	Aqueous[a]	Methanolic[a]	Aqueous[a]	Methanolic[a]	Aqueous[a]	Methanolic[a]	
Acinetobacter lwoffi 2819	-	3	-	4	-	2	-	-	17 (AMC 30
Alcaligenes feacalis 0452	-	10	4	11	-	10	-	2	7 (SAM 20)
Bacillus cereus 6230	1	9	1	5	1	11	1	6	12 (CC 2)
Bacillus subtilis ATCC 6633	-	10	-	2	-	8	-	2	17 (NV 30)
Enterobacter cloacae 7418	-	-	-	-	-	-	-	-	10 (OFX 10)
Escherichia coli 1328	-	2	-	-	-	1	-	1	118 (CC 2)
Escherichia coli 1402	-	1	-	1	-	-	-	1	19 (CC 2)
Klebsiella pneumonia subsp. *ozanae* 5713	-	-	-	-	-	-	-	-	19 (CC 2)
Klebsiella pneumonia subsp. *pneumonia* 2124	-	1	1	2	2	2	2	4	13 (OFX 10)
Listeria monocytogenes 8353	-	1	-	-	-	2	-	-	24 (TE 30)
Proteus mirabilis 3242	-	-	-	-	-	-	-	-	12 (OFX 10)
Proteus vulgaris KÜKEM 1329	1	2	-	1	-	2	1	2	18 (AMC30)
Providencia alcaliaciens 3215	-	-	-	11	-	-	-	3	10 (AZM15)
Pseudomonas aeruginosa ATCC 9027	-	-	-	-	-	-	-	-	36 (TE 30)
Pseudomonas aeruginosa 3428	-	-	-	-	-	-	-	-	36 (TE 30)
Pseudomonas fluorescens 7324	-	-	-	-	-	-	-	-	31 (OFX 10)
Pseudomonas pseudoalcaligenes 3445	-	-	-	-	-	-	-	-	24 (OFX 10)
Pseudomonas putida 1617	-	-	-	-	-	-	-	-	16 (TE 30)
Salmonella typmiruim RSSK 95091	-	-	-	-	-	-	-	-	9 (OFX 10)
Staphylococcus aureus 7231	-	5	-	-	-	-	-	-	22 (KF 30)
Staphylococcus hominis 3221	-	1	-	1	-	-	-	-	24 (KF 30)
Streptococcus pyogenes ATCC 176	-	5	-	-	-	5	-	-	30 (NV 5)
Candida albicans ATCC 1223	-	-	-	-	-	-	-	-	15 (AMP 20)
Saccharomyces boulardii 6128	-				-	-	-	-	9 (AMP 20)
Saccharomyces cereviciae 6541	-	-	-	-	-	-	-	-	8 (AMP 20)
Aspergillus niger BC 102	-	-	-	-	-	-	-	-	21 (AMP 20)
Cladosporium herbarum	-	-	-	3	-	-	-	7	10 (AMP 20)
Paecilomyces variotii 108	-	3	-	-	-	-	-	-	19 (AMP 20)
Penicillium brevicompactum	-	-	-	-	-	-	-	-	14 (AMP 20)
Penicillium roquefortii BC 111	-	-	-	-	-	-	-	-	11 (AMP 20)
Penicillium roquefortii BC 113	-	-	-	-	-	-	-	3	8 (AMP 20)
Trichothecium roseum	-	-	-	-	-	-	-	-	32 (AMP 20)

NV30 (30µg novobiocin/disc), SAM20 (10µg sulbactam + 10µg ampicillin/disc), CC2 (2µg clindamycin/disc), OFX10 (10 µg ofloxacin/disc), AMC30 (20µg amoxicillin + 10µg clavulanic asit/disc), KF30 (30 µg cephalothin/disc), TE30 (30µg tetracycline/disc), AZM15 (15 µg azithromycin/disc), AMP20 (20 µg/disk Amphotericin) used as positive standards antibiotic discs (oxoid).

Alkanna tinctoria (79.80%), *Fumaria officinalis* (78.93%), *Polygonatum multiflorum* (75.85%), *Tribulus terestris* (69.25%), *Crocus sativus* (63.15%) and *Taraxacum officinale* (43.05%), respectively. The antioxidant capacity of standard BHA and BHT was 93.21 and 90.71% (table 1). Previously the genus *Alkanna* were reported antioxidant-rich plants (Assimopulou *et al*., 2004).

There were no correlation between total phenolic content and antioxidant activity. Although some studies have demonstrated a correlation between phenolic content and antioxidant capacity (Yang *et al*., 2002), our result is in agreement with several the other findings. Bajpai *et al*. (2005) reported no correlation between total phenolic content and antioxidant capacities of a number of medicinal plant extracts. No correlation between total phenolic content and antioxidant capacity in our plant samples is possible owing to the presence of the following factors: the antioxidant capacity observed was not solely from the phenolic contents, but could possibly be due to the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments as well as the synergistic effects among them, which also contribute to the total antioxidant capacity. On the other hand, total phenolic content determined according to the Folin-Ciocalteu method is not an absolute measurement of the amount of phenolic materials. Different types of phenolic compounds have different antioxidant activities, which is dependent on their structure. The extracts possibly contain different type of phenolic compounds, which have different antioxidant capacities.

The results of antimicrobial activity of aqueous and methanolic extracts are shown in table 2. The methanolic extract had shown better antimicrobial activity compared to aqueous extract. The methanol extract of *Inula aucherana, Fumaria officinalis, Crocus sativus, Vicum* *album, Tribulus terestris, Polygonatum multiflorum, Alkanna tinctoria* and *Taraxacum officinale* were proved to possess considerable antimicrobial potentiality against a number of microorganisms (table 2).

The methanol extracts from *Vicum album* and *Alkanna tinctoria* showed antimicrobial activity against 9 out of 32 microorganisms, *Polygonatum multiflorum* 10 out of 32 microorganisms, *Taraxacum officinale* 11 out of 32 microorganisms, *Fumaria officinalis, Tribulus terestris* and *Crocus sativus* 13 out of 32 microorganisms and *Inula aucherana* showed antimicrobial activity against 15 out of 32 microorganisms (table 2).

The inhibition zones of the microorganisms sensitive to the methanol extract were 1-15 mm, respectively (table 1). The highest inhibition zone (15 mm) was observed in both *Inula aucherana* and *Fumaria officinalis* against *Staphylococcus aureus* (table 1). Methanolic extract of *Fumaria officinalis* had higher inhibition zone (14 mm) than positive control (10 mm) against *Cladosporium herbarum*. Methanolic extract of *Alkanna tinctoria* had higher inhibition zone (10 mm) than positive control (7 mm) against *Alcaligenes feacalis*. Methanolic extract of both *Tribulus terestris* and *Polygonatum multiflorum* had higher inhibition zone (10 and 11 mm) than positive control (7 mm) against *Alcaligenes feacalis* (table 2). These results are of interest since they have been obtained with methanol extracts and are not a pure product and it could be considered to have a good potency level.

Based on these results, it is possible to conclude that methanolic extracts of *Inula aucherana, Fumaria officinalis, Crocus sativus, Vicum album, Tribulus terestris, Polygonatum multiflorum, Alkanna tinctoria* and *Taraxacum officinale* had different level antioxidant and antimicrobial activity. The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to evaluate of possible synergism among extract components for their antioxidant and antimicrobial activity. Investigations are in progress to determine the degree of toxicity of these extracts.

REFERENCES

Arora DS and Kaur J (1999). Antimicrobial activity of spices. *Int J of Antimicrobial Agents,* **12**: 257-262.

Assimopoulou A.N, Boskou D and Papageorgiou VP (2004). Antioxidant activities of alkannin, shikonin and Alkanna tinctoria root extracts in oil substrates. *Food Chem.,* **87**(3): 433-438.

Bajpai M, Pande A, Tewari SK and Prakash D (2005). Phenolic contents and antioxidant activity of some food and medicinal plants. *International Journal of Food Sciences and Nutrition,* **56**(4): 287-291.

Barriere C, Centeno D, Lebert A, Leroy-Setrin S, Berdague JL and Talon R (2001). Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. *FEMS Microbiol. Lett.* **201**: 181-185.

Baytop T (1984). Medicinal Plants. IU Yayinlari No. 3255, Eczacilik Fak. No.40, 444s (in Turkish).

Bruneton J (1995). Pharmacognosy, Phytochemistry, Medicinal plants. France: Lavoisiler Publishing Co., pp.265-380.

Chopra RN, Nayer SL and Chopra IC (1992). Glossary of Indian Medicinal Plants, 3rd Edn. New Delhi: Council of Scientific and Industrial Research, pp.7-246.

Djipa CD, Delmee M and Quetin-Leclercq P (2000). Antimicrobial activity of bark extracts of *Syzygium jambos* L. *Journal of Ethnopharmacology,* **71**: 307-313.

Huang, WY, Cai YZ, Xing J, Corke H and Sun M (2008). Comparative analysis of bioactivities of four Polygonum species. *Planta Med.,* **74**: 43-49.

Kim J, Marshall MR and Wie C (1995). Antibacterial activity of some essential oil components gainst five foodborne pathogens. *Journal of Agriculture and Food Chemistry,* **43**: 2839-2845.

Mothana RAA and Lindequist U (2005). Antimicrobial activity of some medicinal plants of the island Soqotra. *J. of Ethnopharmacology* **96**: 177-181.

Slinkard K and Singleton VL (1977). Total phenol analyses: automation and comparison with manual methods. *Am. J. Enol. Viticult.* **28**: 49-55.

Vaya J, Belinky PA and Aviram M (1997). Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidative capacity toward LDL oxidation. *Free Radical Biol. Med.,* **23**(2): 302-313.

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

Yang JH, Lin HC, Mau JL (2002). Antioxidant properties of several commercial mushrooms. *Food Chem.,* **77**: 229-235.

Yano Y, Satomi M and Oikawa H (2006): Antimicrobial effect of spices and herbs on Vibrio parahaemolyticus. *Int. J. of Food Microbiol.,* **111**: 6-11

Zaika LL (1988). Spices and herbs: Their antimicrobial activity and its determination. *J. Food Safety,* **9**: 97-118.