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Full Length Research Paper

Antioxidant and antimicrobial activities of *Solidago virgaurea* extracts

Hülya Demir[1], Leyla Açık[2]*, E. Burcu Bali[2], L. Yasemin Koç[2] and Gönül Kaynak[3]

[1]Gazi University, Atatürk Meslek Yüksekokulu, Department of Chemistry, Ankara-Turkey.
[2]Gazi University, Faculty of Arts and Science, Department of Biology, Ankara-Turkey.
[3]Uludag University, Faculty of Arts and Science, Department of Biology, Bursa-Turkey.

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Solidago virgaurea **is an herbaceous perennial plant of the family Asteraceae. It has been traditionally used to terat urinary tract, nephrolithiasis and prostate. Our objective was to determine the antioxidant activities, reducing powers, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scaenging activities and antimicrobial activities of metanol and hot water extracts of *S. virgaurea* from Bursa region in Turkey. The highest antioxidant activity was found in the metanol extract. The reducing power of the metanol extract was the highest, but its reducing power was markedly lower than that of ascorbic acid. The highest DPPH radical-scavenging activity was found in the metanol, with 50% DPPH radical scavenging at a concentration of 74.66 µgml^{-1} dried metanol extract, while at the same concentration of dried water extract the value was 22.16%. Metanol extract of *S. virgaurea* showed antimicrobial activities against *Staphylococcus aureus*, *Enterobacter fecalis*, *Escherichia coli* and *Bacillus cereus.* The MIC valaues against bacteria 50 µgml^{-1}. It was found that metanol and water extracts of *S. virgaurea* show antioxidant activity. It has been reported that there is an inverse relationship between dietary intake of antioxidant-rich foods and the incidence of a number of human diseases, therefore these results are interesting. This plant, which may contain both polar and apolar antioxidant compounds, could be a potential source of natural antioxidants.**

Key words: *Solidago virgaurea*, antioxidant activity, reducing power, DPPH radical scavenging, antimicrobial activity.

INTRODUCTION

Free radicals were a major interest for early physicists and radiologists and much later were found to be a product of normal metabolism. Today, we well know that radicals cause molecular transformations and gene mutations in many types of organisms. Oxidative stres is well-known to cause many diseases (Storz and Imlay, 1999), and scientists in many diffent disciplines, have become more interested in natural sources which could provide active components to prevent or reduce its impact on cells (Ulubelen et al., 1995; Yan et al., 2002).

Lipids containing polyunsaturated fatty acids are readily oxidised by molecular oxygen and such oxidation proceeds by a free radical chain mechanism (Squadriato and Peyor, 1994). When oxygen is supplied in excess or its reduction is insufficient, reactive oxygen species (ROS), such as superoxide anions, hydroxyl radicals and hydrogen peroxide, are generated (Aruoma, 1999). In living organisms, ROS and RNS can form in different ways. Normal aerobic respiration, stimulated polymorphonuclear leucocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells. Free radicals can cause lipid peroxidation in foods that leads to their deterioration (Fridovich, 1986; Alho and Leinonen, 1999).

ROS and RNS may cause DNA damage that could lead to mutation. In addition, ROS and RNS have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer (Tanizawa et al., 1992; Duh, 1998). When produced in excess, ROS

*Corresponding author. E-mail: leylaacik@gmail.com.

can cause tissue injury, whilst tissue injury can itself cause ROS generation. Nevertheless, all aerobic organisms, including human beings, have antioxidant defences that protect against oxidative damage, and numerous damage removal and repair enzymes to remove or repair damaged molecules. However, the natural antioxidant mechanisms can be inefficient, hence dietary intake of antioxidant compounds becomes important (Duh, 1998; Terao et al., 1994; Espin et al., 2000; Halliwell, 1994).

In order to prolong the storage stability of foods and to reduce damage to the human body, synthetic antioxidants are used for industrial processing. But according to oxicologists and nutritionists, side effects of some synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are of concern. For example, these substances can show carcinogenic effects in living organisms (Branien, 1975; Ito et al., 1983). From this point of view, governmental authorities and consumers are concerned about the safety of food and about the potential effects of synthetic additives on health.

S. virgaurea is an herbaceous perennial plant of the family Asteraceae. It is grown as a garden flower with many different cultivars. It flowers profusely in late summer (Thiem and Goslinska, 2002). It has been traditionally used to terat urinary tract, nephrolithiasis and prostate. Antimicrobial, antimycotic, anti-inflammatory, analgetic, anticancerogenic, sedative, and hypotensive activities for naturally growing plant have been also reported (Thiem and Goslinska, 2002).

In the pesent study the antioxidant activities, reducing powers and DPPH radical-scavenging activities of metanol and hot water extracts of *S. virgaurea* were determined. It was also interesting to find out whether it possesses antimicrobial activity and whether there is any connection between the antioxidant and antimicrobial activities of these extracts.

MATERIALS AND METHODS

Preparation of extracts and their solutions

S. virgaurea young shoots with leaves were harvested in October in the Bursa-Uludağ Kaplıcakaya around and left on bench to dry. A dried sample (10 g) was chopped into small pieces using a blender. Metanol extraction was performed in a soxhlet apparatus until the refluxed solvent became colourless. Extraction was followed by filtration through Whatman No 1 filter paper and evaporation of the filtrate to dryness at 30°C in the Büchi V-700 rotary vacuum evaporator.

The dry residue was mixed with 150 ml of metanol in a screw-capped Erlenmeyer flask and placed on a Nüve SL 350 shaker (Nüve, Ankara, Turkey) to obtain an metanol extract. Extraction was repeared until the solvent became colourless; 200 ml of metanol was used in total. The combined extracts were filtered through Whatman No 1 filter paper and evaporated to dryness at 40°C in the Büche V-700 rotary vacuum evaporator.

The residue obtained after filtration was left in a dark place at room temperature to dry, then extracted with 150 ml of boiling distilled water. This extract was filtered and the filtrate was freeze-dried in a Labconco 117 freeze-dryer at 5 m Hg and -50°C. The dried samples of all the extracts were stored under nitrogen at 4 °C until use.

For antioxidant activity measurements, dried extract solutions were prepared by dissolving 20 mg of dried exctract in 20 ml of solvent. Although the same solvent were used for all the assays, concentrations differed from assay to assay as described below. There was no detectable effect of the solvents on any measured activity, as established by control experiments in which solvents containing no extract were used in the assays.

In all cases, three independent experiments, each with duplicate measurements, were performed. The results shown are the means of these measurements.

Reducing power

This was determined as described previously (Yıldırım et al., 2000). Briefly, the extract (50 – 500 µg) in 1 ml of the correspoding solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10 gl^{-1}), then the mixture was incubated at 50°C for 30 min. Afterwards, 2.5 ml of trichloro-acetic acid (100 gl^{-1}) was added to the mixture, which was then centrifuged at 2000 x g for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (1gl^{-1}) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

DPPH radical-scavenging activity

This was determined according to the Blois (Blois, 1958) method with a slight modification. Brifty, 1 ml of a 0.781 mM solution of DPPH radical in methanol was mixed with 3 ml of extract solution in metanol (containing 50 – 400 µg of dried extract), and after 30 min the absorbance was measured at 517 nm. This activity is given as % DPPH radical scavenging calculated according to the following equation: % DPPH radical scavenging = [(control absorbance – extract adsorbance)/control absorbance] x100

Antioxidant activity

This was determined according to the thiocyanate method. Briefly, a 0.02 M linoleic acid (Fluka) emulsion was prepared by mixing 0.28 g of linoleic acid with an equal amount of Tween 20 (Fluka) in 50 ml of 0.02 M, pH 7.0 Phosphate-buffered saline. Afterwards, the required amount of extract solution (containing 50 – 150 µg of dried extract) was mixed with 2.5 ml of 0.02 M linoleic acid emulsion and the final volume was adjusted to 5 ml with phosphate-buffered saline (0.02 M, pH 7.0) in a test tube. The mixtue was then incubated in darkness at 37°C. The amount of peroxide was determined, by reading the absorbance at 500 nm after colouring with $FeCl_2$ and thiocyanate, at intervals during incubation (Yen and Chen, 1995).

Antimicrobial activities

Two technique were used to test the microbial activity of the *S. virgaurea* methanol exract agar disc diffussion (İlhan et al., 2006) and agar well difussion technique (Parekh and Chanda, 2007) against reference bactrial strains *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli*

ATCC 35218, *Escherichia coli ATCC* 25922, *Bacillus subtilis* ATCC 29213, *Bacillus cereus* NRLL B-3008, *Enterobacter fecalis* ATCC 292112 and five different clinical *Klebsiella pnemonia* isolates resistance to cloramfenicol. The strain *Candida albicans* and *Candida tropicalis* were also used for testing.

Agar well diffusion

The metanol extract of *S. virgaurea* was carried out by the agar diffusion method (İlhan et al., 2006). Cultures (mentioned above) were grown exponential phase in nutrient broth at 37°C for 18 h and adjusted to final concentration 10^8 cfu/ml by diluting fresh cultures and comparison to McFarland density. Medium was prepared and mixed with culture suspension and poured into plates. The wells with 6.0 mm diameter were made. The test compound was added to the well. The plates were incubated at 37 °C for 24 h. The diameter of inhibition zone was measured in mm.

Diffussion method

The metanol extract of *S. virgaurea* was carried by the diffusion method (Parekh and Chanda, 2007). 100 µl of bacteria (containing 10^8 CFU/ml) were incubated in nutrient broth agar at 37°C for 24 h (30°C 48 h for fungi). The dics (6 mm) was saturated with 10 µl of the extract allowed to dry for two hours at 4°C and was introduced on the top inoculated agar plate. After incubation the diameters of inhibition zones were measured in mm. Gentamycin and Amphicilin were used as positive controls. All the experiment were repeated three times.

MIC

The minimol inhibitory concentration (MIC) the *S. virgaurea* metanol extract was determined based on a microdilution method (Bayati and Sulaiman, 2008). The dissolvent extract were diluted to the highest concentration to be tested, 50 µl of NB was distrubuted. 2nd to the tubes. A volume of 100 µl from methanol extract was pipetted into the 1st tube and then 50 µl of dilution was transferred from the 2nd to the 12th tubes. In brief, seven tubes were prepared by pipeting into each tubes 95 µl of Mueller Hinton Broth and 5 µl of inocula. 100 µl from the exract (100 mg/ml) was added into the first tube. Then, 100 µl from their serial dilutions was transferred into the other tubes. The final volume in each tubes was 200 µl. Negative and positive control were included. After shaking tubes were incubated at 37°C for 24 h (bacteria) and at 30°C for 48 h for fungi. Microbial growth was determined.

RESULTS AND DISCUSSION

Reducing power

The reducing powers of the methanol and water extracts increased as the amount of extract increased (Figure 1). To compare the reducing powers of these extracts with that of a known reducing reagent (Meir et al., 1995), the reducing powers of 500 µg extracts (corresponding to a final concentration of 50 μgml^{-1}) and ascorbic acid were measured. As can be seen in Table 1, the reducing powers of all the extracts were much lower than that of ascorbic acid, although they were higher than that of the control. The reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of its potantial antioxidant activity (Meir et al., 1995). However, we have previously found that this may not always be the case (Yıldırım et al., 2000).

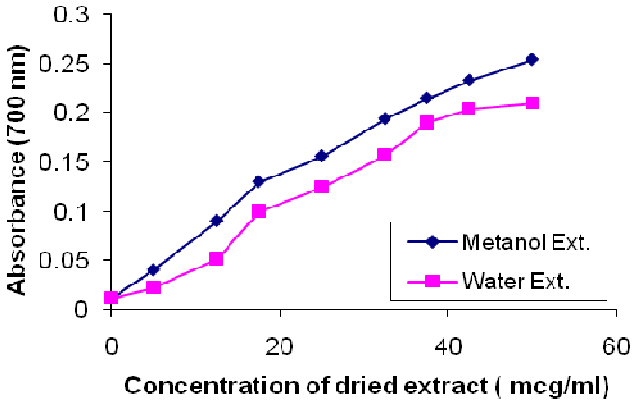

Figure 1. Comparison of reducing powers of methanol and water extracts of metanol and water extracts of *S. virgaurea.* Results are means of three different experiments in each of which two measurements were made.

Table 1. Comparison of reducing powers of ascorbic acid *S. virgaurea* extract.

Sample	Absorbance (700 nm)
Control	0.012 ± 0.002
Ascorbic acid	3.625 ± 0.003
Metanol extract	0.254 ± 0.002
Water extract	0.210 ± 0.008

500 µg of dried extract or ascorbic acid, corresponding to a final concentration of 50 $\mu g\ ml^{-1}$, was used. Control was test sample without extract or ascorbic acid. Results are given as mean ± standard deviation of three different experiments in each of which two measurements were made. High absorbance indicates high reducing power.

DPPH radical-scavenging activity

The DPPH radical-scavenging activities of the metanol and hot water extracts were concentration-dependent. At a concentration of 25 μgml^{-1} dried metanol or water extract the scavenging activities were 12.27 and 4.72% respectively, while at a concentration of 100 μgml^{-1} the respective activities were 30.67 and 64.26% (Figure 2).

Antioxidant activity

In the present study the antioxidant activities of metanol and hot water extracts of *S. virgaurea* were determined by the thiocyanate method, in which the amount of pero-

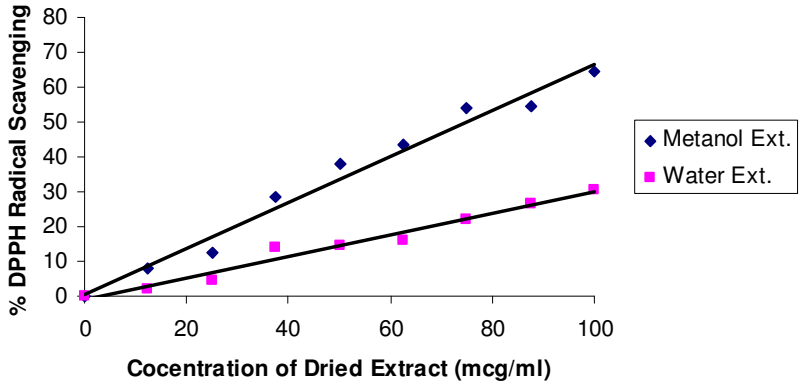

Figure 2. Comparison of DPPH radical-scavenging activities of metanol and water extracts of *S. virgaurea*. Results are means of three different experiments in each of which two measurements were made.

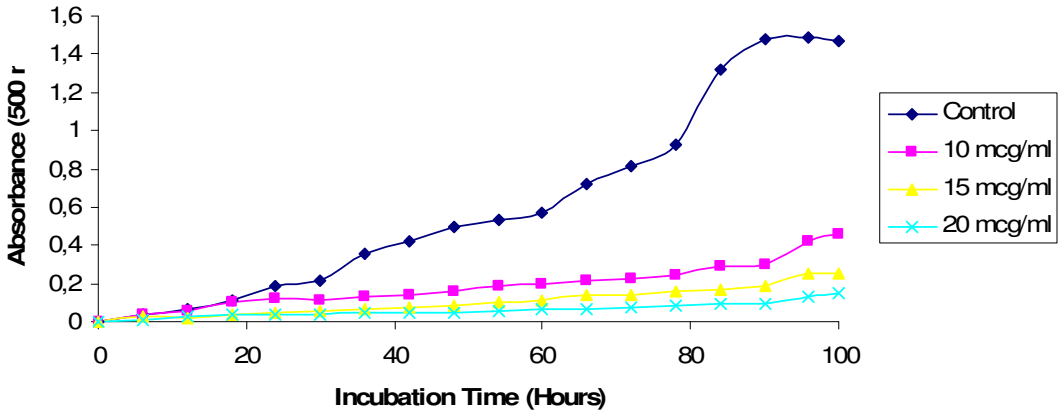

Figure 3. Antioxidant activity of methanol extracts of *S. virgaurea*. The indicated amounts of dried extract were present in 5 ml of linoleic acid emulsion (0.02 M, PH 7.0). The control was the linoleic acid emulsion without extract. Results are means of three differnt experiments in eac of which two measurements were made.

xides formed in the emulsion during incubation is determined spectrophotometrically by measuring the absorbance at 500 nm. Peroxide oxidies Fe^{+2} to Fe^{+3} and then this ion reacts with SCN^- form the $FeSCN^{+2}$ complex, which has maximum absorbance at a wavelength of 500 nm.

The presence of lyophilised metanol or water extract at a concentration of 10 μgml^{-1} in the linoleic acid emulsion was able to reduce the formation of peroxides (Figures 3 and 4). Peroxide formation was measured up to 100 h of incubation, at which stage the absorbance of the control started to decline. However, to compare the antioxidant activities of metanol and water extracts, peroxide formation was also followed in samples containing 15 μgml^{-1} extract. To show any differences more clearly, the incubation period was extended to 120 h (Figure 5). However, like butylated hydroxytoluene (BHT), the water was able to prevent peroxide formation even for incubation. Hence the most effective antioxidant activity was shown by the metanol extract.

It has been reported that *S. Virgaurea* contains vitamin C and carotenids (Parekh and Chanda, 2007). Vitamin E is known as a reducing agent (Fiedorov, 1985) and may contribute to antioxidant activity by reducing the oxidised state of the phenolic antioxidant compounds. Thus these compounds may be regenerated, allowing their antioxidant capacity to increase (Davies, 1994). Carotenoids have been reported to show chain breaking antioxidant activity (Meir et al., 1995). These compounds are also effective singlet oxygen quenchers (Kalemba, 1998).

Phenolic compounds are called high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide free radicals and hydroxyl radicals (Davies, 1994). In addition, these compounds act as antioxidants by metal ion chelation(Mortensen and Skibsted, 1997).

Table 2. Antimicrobial activity of methanol extract of *S. virgaurea*.

Test organism	Stain No	Diameter of zone of inhibition (mm)	
		Control	Methanol
Staphylococcus aureus	ATCC 25923	30	15
Pseudomonas aeruginosa	ATCC 27853	25	-
Escherichia coli	*ATCC* 35218	24	-
Escherichia coli	*ATCC* 25922	24	10
Bacillus cereus	NRLL B-3711	30	15
Enterobacter fecalis	ATCC 292112	30	20
Klebsiella pnemonia	12	25	-
Klebsiella pnemonia	70	30	-
Klebsiella pnemonia	84	30	-
Klebsiella pnemonia	35	25	-
Klebsiella pnemonia	53	27	-

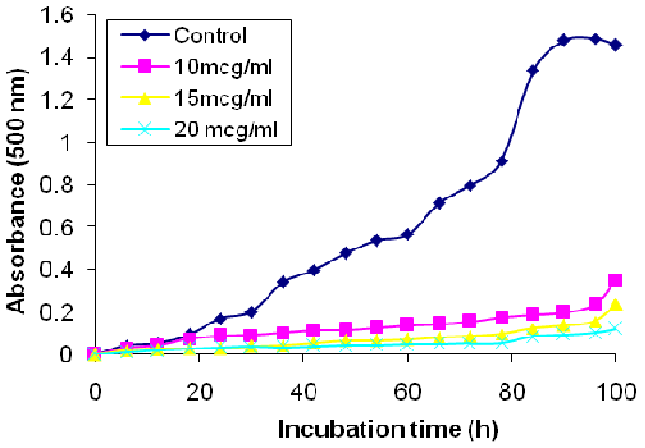

Figure 4. Antioxidant activity of water extracts of *S. virgaurea.* The indicated amounts of dried extract were present in 5 ml of linoleic acid emulsio (0.02 M, pH 7.0). The control was the linoleic acid emulsion without extract. Results are means of three different experiments in each of which two measurements were made.

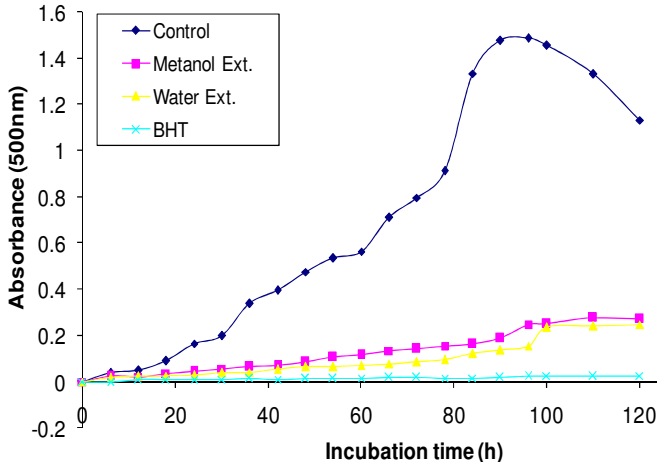

Figure 5. Comparison of antioxidant activities of metanol, water extracts of *Solidago virgaurea* with butylated hydroxytoluene (BHT). Each test sample contained 15 µg ml^{-1} dried extract or BHT in 5 ml of linoleic acid emulsion (0.02 M, pH 7.0). The control was the linoleic acid emulsion without extract. Results are means of three different experiments in each of which two measurements were made.

As briefly outlined above, various compounds may use different mechanisms to act as antioxidants. The aim of this study was to determine the total antioxidant activity of *S. virgaurea* extracts, and we did not attempt to identify the specific antioxidant mechanisms involved. We suggest that the main compounds responsible for antioxidant in the metanol and water extracts could be mainly phenolic compounds. We have to keep in mind that the total antioxidant activity of each of the extracts is the sum of the individual activities of each of the compounds present, and also that these compounds might have synergistic effects. Kalemba (1998) reported that the steam-distilled essential oil of *Solidago*. constituents are α-pinene, myrcene, α-pinene, limonene, sabinene and germacrene-D.

The 300 mg extract showed antimicrobial activities against *E. fecalis*, *S. aureus* and *B. cereus* (Table 2). The MIC valaues was 50 µgml^{-1} against bacteria which are tested. Maximum antibacterial activity was against *E. fecalis*. Methanol extract of *S virgaurea* did not showed any significant activity for *E. coli*, five *K. pnemonia* isolates and *P. aeroginosa*. The activity was effective against Gram positive bacteria compared to Gram negative bacteria. Thiem and Goslinska (2002) found that the extract of *S. virgaurea* from micropropagated showed a moderated antimicrobial activity.

Conclusions

In the present study it was found that metanol and water extracts of *S. virgaurea* show antioxidant activity. It has

been reported that there is an inverse relationship between dietary intake of antioxidant-rich foods and the incidence of a number of human diseases (Gordon, 1990; Hall and Cuppett, 1997; Nieto et al., 1993), therefore these results are interesting. This plant, which may contain both polar and apolar antioxidant compounds, could be a potential source of natural antioxidants.

Free radicals may play an important role in the origin of life and biological evolution, implicating their beneficial effects on organisms (Rice-Evans and Sampson, 1997). For example, oxygen radicals exert critical actions, such as signal transduction, gene transcription and regulation of solule guanylate cyclase activity in cells (Cook and Saman, 1996). Also, NO is one of the most widespread signalling molecules and participates in virtually every cellular and organ function in the body (Yıldırım et al., 2003). However, free radicals and other reactive species cause the oxidation of biomolecules (proteins, amino acids, lipid, and DNA), which leads to cell injury and death (McCord, 2000; Lander, 1997).

In addition to being consumed as a healthy vegetable, the compounds in *S. virgaurea* that are responsible for antioxidant activity could be isolated and then used as food additives to delay the oxidative deterioration of foods. This will be the subject of further studies.

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