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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF HERBAL TEAS

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ABSTRACT
Teas made from 18 Hungarian medicinal plants were investigated for their total phenolic content and antioxidant effect as radical scavenging activity using the Folin-Ciocaltau and DPPH assays. Antimicrobial effect of the herbal teas was measured by agar diffusion method.
In total, the results showed that the smallflower hairy willowherb (*Epilobium parviflorum*) had the highest antioxidant effect among the plants studied. Teas made from fennel (*Foeniculum vulgare*) and bean pods (Phaseoli legumen) have the lowest activity. Only four herbal teas showed some antimicrobial activity: the smallflower hairy willowherb (*Epilobium parviflorum*), common agrimony (*Agrimonia eupatoria)*, spearmint (*Mentha crispa*) and bean pods. At smaller concentrations the relationship found between total phenolic content and radical scavenging activity was linear but with increasing phenol content the antioxidant activity remained the same.

Keywords: medical plants, antioxidant activity, antimicrobial activity, aqueous extract, total phenols

INTRODUCTION

Oxidative stress plays an important role in the development of cancer and heart diseases. Antioxidant compounds of plants can help to protect our health from oxidative damage. Polyphenols found in medical plants, work as naturally antioxidants (AOSHIMA ET AL., 2007; CONDRAT ET AL. 2009). Herbs are also a rich source of antimicrobial agents (SOUZA ET AL., 2005). In this study teas made from 18 Hungarian medicinal plants were investigated for their antioxidant and antimicrobial activity. The goal was to find a relationship between the total phenol content and the antioxidant capacity, as well as to compare results of the antioxidant and antimicrobial measurements.

MATERIAL AND METHOD

Plant materials

The following dried Hungarian herbs were purchased from a Hungarian grower: Bean pods (*Phaseoli lengumen*), Black elder (*Sambucus nigra*) – flowers, Buckthorns (*Frangula alnus*) – cortex, Celery (*Apium graveolens*) – leaves, Common agrimony (*Agrimonia eupatoria*) – aerial part of the plant, Common chicory (*Cichorium intybus*) – aerial part and roots, Dandelion (*Taraxacum officinale*) – leaves and roots, European dewberry (*Rubus caesius*) – leaves, Fennel (*Foeniculum vulgare*) - fruit, Field horsetail (*Equisetum arvense*) – aerial part of the plant, Hedge bedstraw (*Galium mollugo*) – aerial part of the plant, Lemon balm (*Melissa offininalis*) – leaves, Maize silks (*Maydis stigma*), Smallflower hairy willowherb (*Epilobium parviflorum*) – aerial part of the plant, Small-leaved lime (*Tilia cordata*) – leaves, Spearmint (*Mentha spicata*) – leaves, Yellow bedstraw (*Galium verum*) – aerial part of the plant, Yellow sweet clover (*Melilotus officinalis*) – aerial part of the plant.

Test organisms and media
Gram-positive bacteria: *Bacillus cereus* and *Bacillus subtilis*
Gram-negative bacteria: *Escherichia coli* and *Pseudomonas putida*
Yeasts: *Saccharomyces cerevisiae* and *Geotrichum candidum*
Molds: *Aspergillus niger, Penicillium chrysogenum, Fusarium spp*.
The Gram-positive bacteria were cultured on Tryptone Glucose Yeast Extract Agar (TGE), the Gram-negative bacteria on Luria-Bertani (LB) medium, and yeasts and molds on Malt Extract Agar (MEA).

Brewing herbal teas
Herbal teas were prepared by addition of 200 ml boiling distilled water to 2 g of dried herbs. After 20 min incubation teas were filtered through filter paper, cooled to room temperature and used for the chemical analysis. A small volume of the teas (5 ml) was sterile filtered through a membrane filter (0.45 μm) and stored in a refrigerator until the antimicrobial tests were done.

Determination of total polyphenols (TP)
TP was determined by the Folin-Ciocalteu assay. After the appropriate dilution 1 ml of the tea was mixed with 1 ml ethanol (96 v/v%), 5 ml distilled water and 0,5 ml of Folin-Ciocalteu's reagent (50 %). After 5 min, 1 ml of aqueous sodium carbonate solution (5 %) was added to the mixtures and were incubated at room temperature in dark for 1 hour. The absorbance was measured at 725 nm by an UV/VIS spectrophotometer (Philips PU8740). For the calibration curve gallic acid solution (50 μg/ml) was used. The polyphenol concentration was expressed in mg gallic acid equivalent (GAE)/g dried herb (AOSHIMA ET AL., 2007; MILIAUSKAS ET AL., 2004).

Determination of DPPH radical scavenging activity
In the assay 5-fold dilutions of herbal teas were used. Three ml of 100 μM DPPH (2,2-diphenil-1-picrylhydrazil) solution was added to 0.5 ml of the diluted samples and these mixtures were incubated in dark at room temperature for 30 min. To the control, instead of the tea sample, 0.5 ml ethanol was added. After 30 min changes in color (from violet to yellow) were measured at 517 nm. Radical scavenging activity was calculated by the following equation:
DPPH• scavenging activity (%) = $((A_c - A_s)/A_c) \times 100$,
where: A_c was the absorbance of the control sample and A_s was the absorbance of the tea sample (MILIAUSKAS ET AL., 2004; AOSHIMA ET AL., 2007).

Determination of antimicrobial activity
The agar well diffusion assay was used. One ml of 18-22 h old suspensions of bacteria and yeasts, and 0.1 ml of spore suspension of 72 hours old mold cultures (the spores from the agar were washed with 10 ml sterile distilled water) were seeded on the appropriate medium by the spread plate method. After drying, three 8 mm wells were prepared by a sterile cork-borer. Then 0.1 ml of each tea was added into the wells. Streptomycin (bacteria) and nystatin (yeasts and molds) were used as positive control and sterile distilled water as negative control. The plates were incubated at appropriate temperatures for 24 h. The antimicrobial effect was determined by measuring the diameter of the inhibition zones (PAREK AND CHANDA, 2007; MAHESH AND SATISH, 2008).

RESULTS

The highest polyphenol content and antioxidant activity was measured in smallflower hairy willowherb tea (83.45 mg GAE/g dried herb and 83.9 %) followed by dewberry leaf tea. Teas made from fennel (1.95 mg GAE/g and 2.1 %) and bean pods had the lowest activity (*Figures 1* and *2*). Linear relationship was found between total phenol content >20 mg GAE/g and DPPH scavenging activity but at higher phenol content the radical scavenging activity didn't changed showing saturation (*Figure 3*).

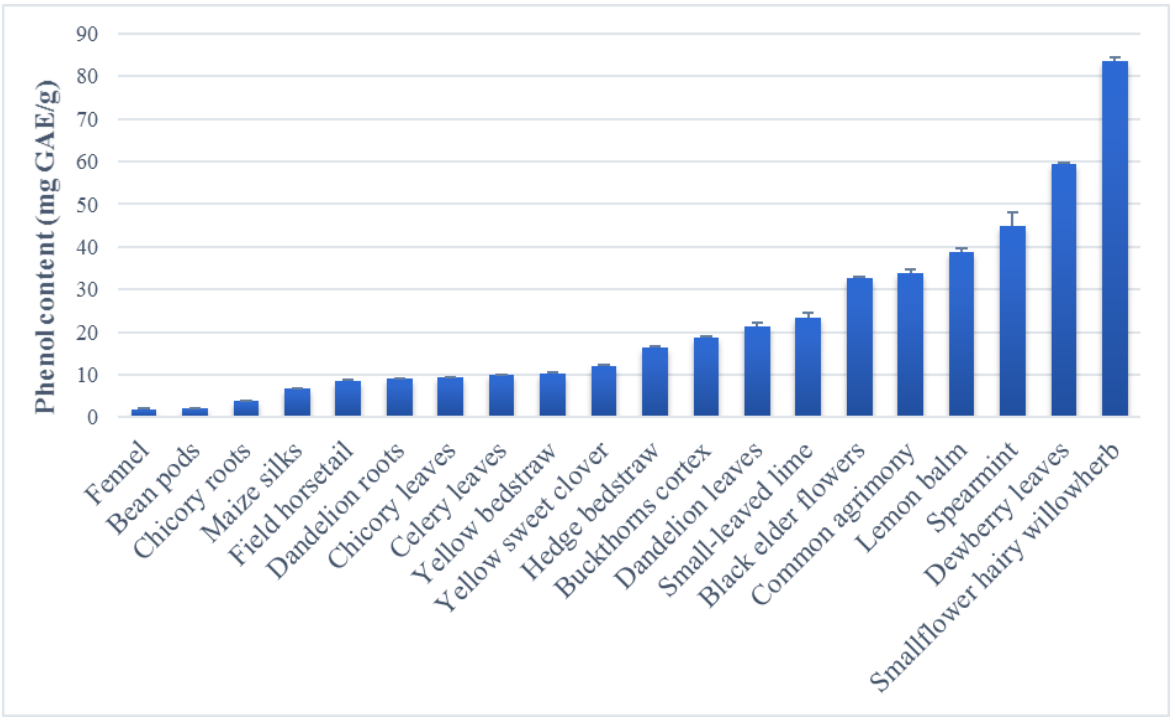

Figure 1. Total phenol content of the investigated herbal teas

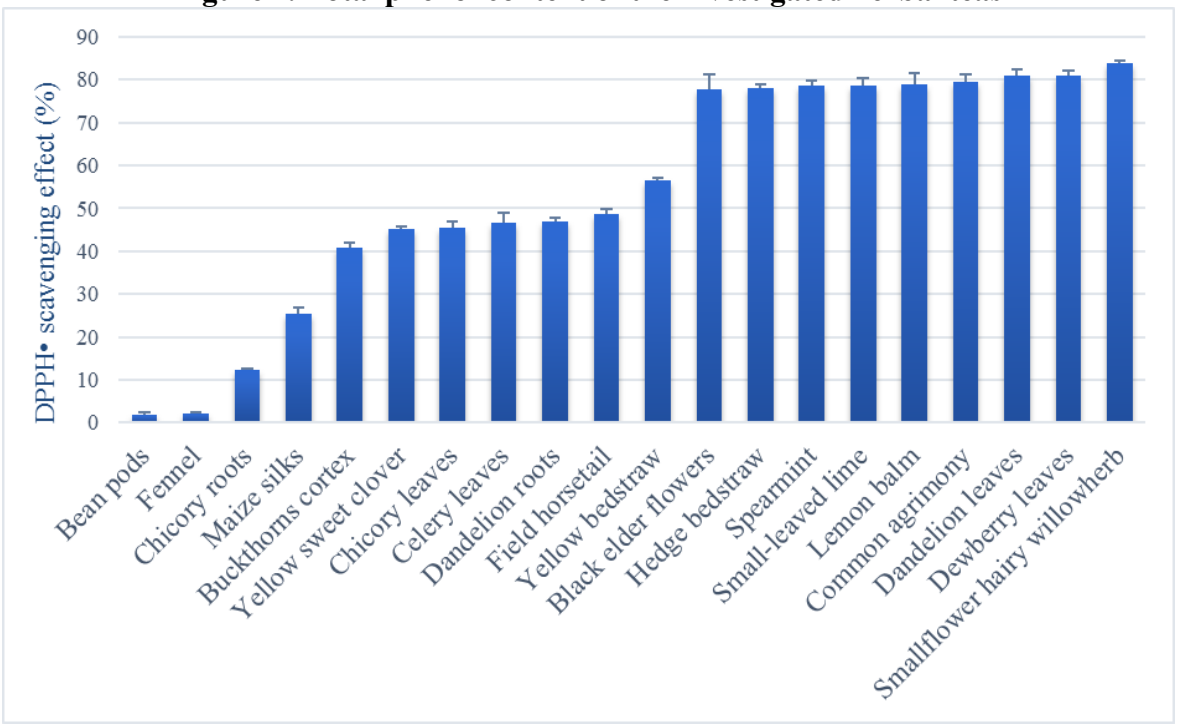

Figure 2. DPPH radical scavenging activity of the investigated herbs

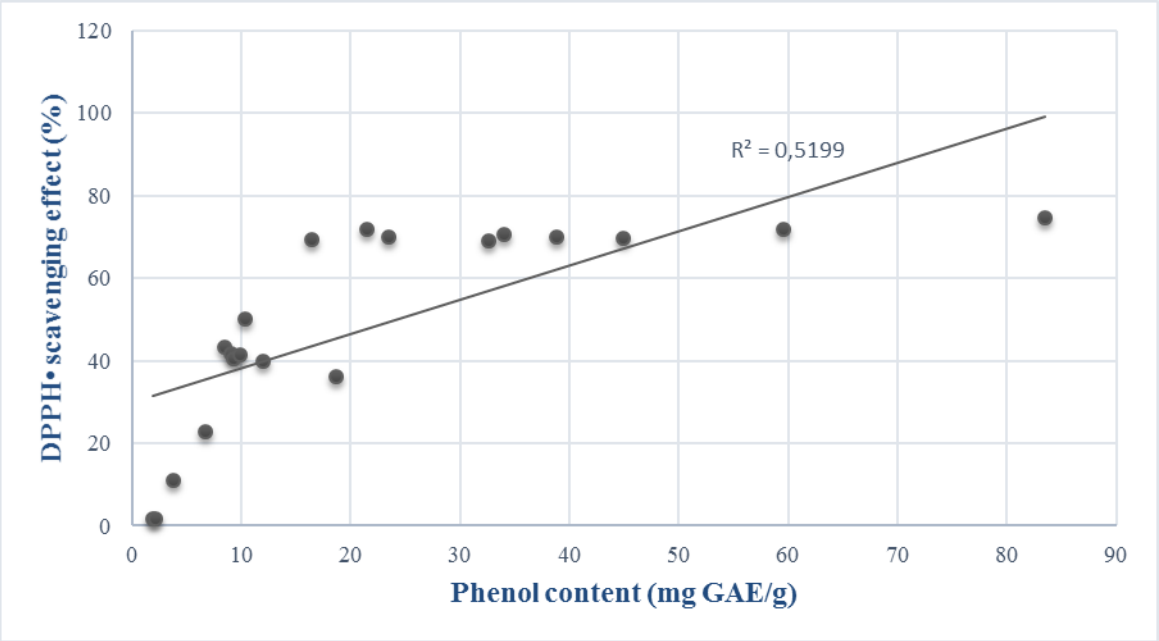

Figure 3. Relationship between radical scavenging activity and total phenol content

Only four teas showed some antimicrobial activity: the smallflower hairy willowherb on *B. subtilis (*inhibition zone (IZ) 12 mm), and *P. putida* (IZ 14 mm), common agrimony on *B. cereus* (IZ 12 mm), bean pods also on *B. cereus* (IZ 11 mm) and spearmint on *B. cereus* (IZ 9.33 mm). None of the herbs studied showed antifungal activity.

DISCUSSION

Our results show that there are large differences in the total phenol content of herbal teas, but half of the investigated herbs showed excellent antioxidant effect with about 80 % radical scavenging activity. It possibly means that not only phenols are responsible for the antioxidant effect; other heat stable agents play also a role. Considering the antimicrobial activity, probably a more concentrated tea or ethanol extract would be more effective. In total, the results showed that, in herbal teas different compounds could be responsible for the antioxidant and antimicrobial activity. Teas made from Hungarian herbs, in addition to the other beneficial effects, are great sources of antioxidants.

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