

Studies on Antioxidant and Antimicrobial Activities of the Aerial Parts of *Urtica dioica* L.

Urtica dioica L.'nın Topraküstü Kısımları Üzerinde Antioksidan ve Antimikrobiyal Aktivite Çalışmaları

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Abstract

In this study, aerial parts of *Urtica dioica* L. have been assessed for antioxidant activity and antimicrobial activity. Antioxidant activity was done according to the liposome TBA method. Antimicrobial activity was done according to the serial dilution method. The ethanol extract of the aerial parts of *U. dioica* showed considerable antioxidant activity by inhibition of lipid peroxidation (IC₅₀ 0.83 mg/mL). The dichlormethane extract showed weak antimicrobial activity (MIC > 50 µg/mL), but slightly better activity against the yeast *Candida albicans* (MIC 40 µg/mL). In contrast to some previous reports, the water, methanol and ethanol extracts displayed negligible antimicrobial effects.

Keywords: *Urtica dioica*, antioxidant activity, antimicrobial activity.

Introduction

Urtica dioica L. (Urticaceae) has been used in traditional medicine for centuries. Since the time of Dioscorides, the aerial parts of *U. dioica* have been used for their diuretic and laxative effects and more recently are used as medicinal teas having slimming or antirheumatic effects (Gunther 1968). In Turkish traditional medicine, *U. dioica* is used to treat stomach-ache, liver insufficiency, rheumatic pain and is also used against colds and cough (Sezik et al. 1997, Yeşilada et al. 1993 and 2001). In addition, this herb is used against cancer (Baytop 1999, Uzun et al. 2004).

The roots are also widely used to improve the life quality of elderly men having prostate disorders and studies on the root extract of *U. dioica* have shown the antiproliferative effect on the human prostate cancer cell lines (Barnes et al. 2002, Konrad et al. 2000, Bisset and Wichtl 1994).

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Several different principles, including, glucopyranosides, glycoprotein, protein, flavonol glycosides, carotinoids, as well as biologically-active compounds, such as amino acids, caffeoyl malic acid and quinic acid, essential oils, formic and acetic acid, histamine, tannins, mucilage, vitamins have been identified as contributing to the observed medicinal effects of *U. dioica* (Özen and Korkmaz 2003).

It was reported that the major compounds isolated from the methanolic extract of the aerial parts of *U. dioica* were determined as quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside and isorhamnetin-3-O-glucoside (Akabay et al. 2003). Seven flavonol glycosides, kaempferol-3-O-glucoside, and -3-O-rutinoside, isorhamnetin-3-O-glucoside, -3-O-rutinoside and -3-O-neohesperidoside have been isolated and identified from the methanolic extract of the flowers (Chaurasia and Wichtl 1987 b).

The antioxidant activity of *U. dioica* has been studied by different researches (Hudec et al. 2007, Avcı et al. 2006, İlhami et al. 2004, Özen and Korkmaz 2003). But there is limited data on the antimicrobial activity of *U. dioica* (İlhami et al. 2004).

The water extract of aerial parts of *U. dioica* collected from Erzurum, exhibited antimicrobial activity against all tested microorganisms except of *P. aeruginosa*. Also the same extract was investigated antioxidant activity by using different antioxidant tests including reducing power, free radical scavenging superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities It was found that the water extract of *U. dioica* had powerfull antioxidant activity (İlhami et al. 2004).

In this study the plant extracts obtained from aerial parts of *U. dioica* have been investigated for antimicrobial activity with serial dilution method and antioxidant activity with liposome TBA method.

Material and Methods

Plant material

The aerial parts of *Urtica dioica* L. were collected in July 2001 from Yoncalı-Kütahya and identified by Dr. Emine Akalın. A voucher specimen is deposited at the Herbarium of Faculty of Pharmacy, Istanbul University with Nr. ISTE: 83381. All aerial parts were left to dry at room temperature and then powdered.

10 g aerial parts of *U. dioica* were used for all extractions.

Preparation of extracts

Extraction for antioxidant activity

The aerial parts of *U. dioica* were percolated with 200 mL of ethanol and the extract taken to dryness under reduced pressure.

Extraction for antimicrobial activity

The antimicrobial activity tests were performed on extracts obtained from three separate 10.0 g batches of the aerial parts of *U. dioica* using methanol, ethanol and dichloromethane separately in a Soxhlet apparatus. 10.0 g of the same plant material was infused with 100 mL of distilled water for 30 min and the extract freeze-dried to give the aqueous extract.

Antioxidant activity test

Antioxidant activity assay was done according to the liposome TBA method using 0.5% w/v propyl gallate as a positive control which is assumed to give 100% antioxidant activity (Conforti et al. 2002). Eight concentrations (8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 mg/mL) were prepared from ethanol extract of

the aerial parts of *U. dioica*. The ethanol extract of the aerial parts of *U. dioica* show antioxidant activity by inhibition of lipid peroxidation.

The % inhibition of lipid peroxidation can be assessed by comparing the absorbance of full reaction mixture (FRM) with that of the extract test (ET), taking into account the absorbances of blank (B) and the extract alone (EA). The percentage inhibition of oxidation was calculated using the formula:

Inhibition %: $100 \times (\text{FRM} - \text{B}) - (\text{ET} - \text{B} - \text{EA}) / (\text{FRM} - \text{B})$

The IC₅₀ value (the concentration of the extract that inhibited 50% cells) was calculated from the data obtained GraphPad Prism software.

Antioxidant activity results were means of four replicates.

Antimicrobial activity test

The antimicrobial assay was done according to the serial dilution method against *Bacillus subtilis* (NCTC 10073), *Staphylococcus aureus* (NCIMB 9515), *Pseudomonas aeruginosa* (NCIMB 10421) and *Escherichia coli* (NCTC 9002) (Özgen et al. 2003, NCCLS 2008). (18,19). Activity against the yeast *Candida albicans* (NCPF 3179) was also tested. All of the organisms were obtained from Kings College Culture Collection.

Each treatment was replicated three times.

Results and Discussion

In this study, antioxidant and antimicrobial activities of plant extracts of *U. dioica* collected from Kütahya were determined.

The ethanol extract of aerial parts of *U. dioica* possessed high antioxidant activity (91%) at 8 mg/mL concentration with an IC₅₀ value of 0.83 mg/mL.

The phenolic compounds may contribute directly to the antioxidative action. It is suggested that polyphenolic compounds may have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily are ingested from a diet rich fruits and vegetables. In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (İlhami et al. 2004, Pietta 2000, Yen et al. 1993)

The antioxidant activity is linked with a possible preventive effect against cancer (Atmani et al. 2009, Maraldi et al. 2009, Mattson et al. 2009). Therefore, any anticancer effect of the aerial parts of *U. dioica* are more likely due to its antioxidant, which might for prevent development of cancerous conditions induced by free radicals.

Antimicrobial activities results are shown in Table 1.

When the MIC values obtained from the antibacterial activity assay are compared, it is seen that the dichloromethane extract has the strongest activity against all microorganisms tested, even though this is rather weak (MIC > 75 µg/mL). The extracts all showed moderate activity against the yeast *C. albicans* (MIC 40 µg/mL). In contrast to previous report the water extracts displayed negligible antimicrobial effects. In our study the water extract showed antibacterial activity against *P. aeruginosa* with a MIC value of 620 µg/mL while no activity observed *P. aeruginosa* in a previous study.

Table 1. Antimicrobial activities (MIC in µg/mL) of extracts of the aerial parts of *U. dioica* ^a

Tested material	<i>B.s.</i>	<i>E.c.</i>	<i>P.a.</i>	<i>S.a.</i>	<i>C.a.</i>
MIC(µg/mL)					
Water extract	2500	1250	620	1250	40
Methanol extract	310	160	620	310	40
Ethanol extract	2500	620	310	1250	40
Dichloromethane extract	310	80	160	160	40
MIC(µg/mL)					
Tetracycline	10	10	10	10	-
Miconazole	-	-	-	-	10

B.s., *Bacillus subtilis*; *E.c.*, *Escherichia coli*; *P.a.*, *Pseudomonas aeruginosa*; *S.a.*, *Staphylococcus aureus*; *C.a.*, *Candida albicans*.

^aConcentration = 100 µg/mL extract in each well

In previous study was found that the water extract from the aerial parts of nettle possessed noticeable antimicrobial activity against *E. coli*, *Proteus mirabilis*, *Citrobacter koseri*, *S. aureus*, *Streptococcus pneumoniae*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Staphylococcus epidermidis* and *C. albicans* (with a zone diameter of 8, 8, 9, 8, 9, 9, 13, 11 and, 8 mm respectively) (İlhami et al., 2004). But it is difficult to compare the results of our study because of the different methods used for evaluation of antimicrobial activities.

Crude extracts from plants with a history of use in folk medicine have been screened in vitro for antibacterial activity by many research groups. The flavonoid compounds such as, quercetin and various quercetin glycosides and kaempferol and its derivatives with antibacterial activity have also been identified (Cushnie and Lamb 2005). The flavonoid compounds of *U. dioica* has been determined previously. Also several flavonoids quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside and isorhamnetin-3-O-glucoside were isolated as the major compounds from the methanol extract of the aerial parts of *U. dioica* (Akbay et al. 2003). In this study, the antimicrobial activity of different solvent extracts of *U. dioica* may be attributed to major flavonoid compounds.

Özet

Bu çalışmada, *Urtica dioica* L.'nin topraküstü kısımları antioksidan ve antimikrobiyal aktivite için değerlendirilmiştir. Antioksidan aktivite lipozom TBA metoduna göre yapıldı. Antimikrobiyal aktivite seri dilüsyon metoduna göre yapıldı. *U. dioica*'nın topraküstü kısımlarının etanol ekstresi lipid peroksidasyonunu inhibe ederek farkedilir derecede antioksidan aktivite göstermiştir (IC₅₀ 0.83 mg/mL). Diklormetan ekstresi zayıf antimikrobiyal aktivite göstermiştir (MIC > 50 µg/mL) fakat *Candida albicans* mantarına karşı aktivite biraz daha iyidir (MIC 40 µg/mL). Daha önce yapılan bazı çalışmaların tersine, su, metanol ve etanol ekstrahları önemsenmeyecek antimikrobiyal etki göstermiştir.

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