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Original article

FATTY ACID PROFILE AND ANTIMICROBIAL EFFECT OF THE SEED OILS OF *URTICA DIOICA* AND *U. PILULIFERA*

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Abstract

In present study, our goal was to investigate the fatty acid composition of the seed oils obtained from Urtica dioica and U. pilulifera cultivated in Turkey by capillary gas chromatography-mass spectrometry (GC-MS). Linoleic acid was found to be the main fatty acid (44.29 % for U. dioica and 62.99 % for U. pilulifera), followed by oleic acid (34.93 % for U. dioica and 21.91 % for U. pilulifera). Trace amount of linolenic acid (0.55 %) was analyzed only in U. pilulifera. These two oils were also tested against Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus, Bacillus subtilis, and Enterococcus faecalis for their antibacterial activity along with Candida albicans and C. parapsilosis for their antifungal activity by microdilution method.

Key Words: *Urtica dioica, Urtica pilulifera, Urticaceae, Fatty acid, GC-MS, Antimicrobial activity*

Urtica dioica ve *U. pilulifera* Tohum Yağlarının Sabit Yağ Profili ve Antimikrobiyal Aktivitesi

Bu çalışmada, amacımız Türkiye'de kültürü yapılan Urtica dioica ve U. pilulifera tohumlarından elde edilen sabit yağların kompozisyonlarını kapiller gaz kromatografisi-kütle spektrometresiyle incelemektir. Başlıca yağ asiti olarak linoleik asit (U. dioica'da % 44.29 ve U. pilulifera'da % 62.99), bunu müteakip oleik asit (U. dioica'da % 34.93 ve U. pilulifera'da % 21.91) olarak bulunmuştur. Eser miktarda linolenik asit (% 0.55) ise sadece U. pilulifera'da analiz edilmiştir. Her iki yağın, aynı zamanda Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus, Bacillus subtili, ve Enterococcus faecalis'e karşı antibakteriyal aktivitelerinin yanısıra, Candida albicans ve C. parapsilosis'e karşı antifungal aktiviteleri de mikrodilüsyon yöntemi ile test edilmiştir.

Anahtar Kelimeler: *Urtica dioica, Urtica pilulifera, Urticaceae, Yağ asidi, GC-MS, Antimikrobiyal aktivite*

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INTRODUCTION

The genus *Urtica* (Urticaceae) is represented by five species (*U. dioica* L., *U. haussknechtii* Boiss., *U. membranacea* Poiret in Lam., *U. pilulifera* L., and *U. urens* L.) in Turkey (1). Among them, *Urtica dioica* L., known as "stinging nettle, common nettle, or greater nettle" in English and "ısırgan" in Turkish, is a medicinal herb with a long history of use. While the greater nettle (*Urtica dioica* L.) is grown throughout the temperate regions of Europe and Asia, the roman nettle (*U. pilulifera*) bears its female flowers in little compact and globular heads.

Out of five *Urtica* species distributed throughout Turkey, three of them are widely used against rheumatism and cancer in the form of decoction (2-4). The leaves or herbs of *U. dioica* L. and *U. urens* L. or its hybrids are collected during their flowering seasons and consumed traditionally as an infusion three times a day. Seeds of *U. pilulifera* L., another traditional species of the genus *Urtica* in Turkey, are also commonly used internally by mixing its powder with honey or as an infusion (1, 2, 5).

On the other hand, nettle's main plant chemicals include lectins, plastocyanins, glycoproteins, carotenoids, fatty acids, sterols, flavonoids, polysaccharides, terpenes, and lignans (6-19). It has been proved to be one of the most important medicinal plants with its long-standing use as an anti-inflammatory aid for rheumatism and arthritis, which has been confirmed with clinical research (20). The last area of the studies on nettle focuses on its usefulness for prostate inflammation (prostatitis) and benign prostate hyperplasia (BPH) (21).

Since plants are proven to be the highly rich sources of essential fatty acids (EFAs), in our search on finding alternative sources for fatty acids, the present study was undertaken to evaluate fatty acid profile of two *Urtica* species (*U. dioica* L. and *U. pilulifera* L.) cultivated in Turkey by both gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The oils were also screened against the ATCC strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis* for their antibacterial activity as well as *Candida albicans* and *C. parapsilosis* for their antifungal activity by microdilution method.

EXPERIMENTAL

Plant materials

The seeds of *Urtica dioica* and *U. pilulifera* were obtained by cultivation using organic fertilizer at the experimental farm of Faculty of Agriculture, Selçuk University, Konya, Turkey. The samples were deposited at the Herbarium of Faculty of Pharmacy of Gazi University (*U. dioica* GUE 2599; *U. pilulifera* GUE 2600).

Oil extraction

The seeds of the abovementioned plant species were accurately weighed and ground in a grinder in the presence of anhydrous sodium sulfate. The powdered seeds were extracted with *n*-hexane (Merck) continuously in a Soxhlet apparatus for 8 hours. After extraction, the solvent was concentrated *in vacuo* at 40 °C by evaporating and the oil yields of the seeds belonging to two *Urtica* species (*U. dioica* and *U. pilulifera*) were calculated by total seed weight as 22.59 % and 25.66 % (*w*/*w*), respectively.

Saponification and methyl esterification

Afterward, the seed oils were independently placed in 25 ml-volumetric flasks, saponified by adding 12 ml of 0.5 N methanolic sodium hydroxide and were heated on a steam bath until the fat globules disappeared. Then, 2 ml of BF_3/MeOH (Sigma-8.01663.0100) were added and the mixture was boiled for 2 minutes. After cooling down at room temperature, each solution was added up to 25 ml with saturated sodium chloride solution and the fatty acid methyl esters (FAMEs) were obtained (22). Prior to GC-MS analysis, the FAMEs were dissolved in *n*-hexane. 1 µl of each oil sample was injected and analyzed by both GC and GC-MS. The analysis was performed in triplicate and mean values of relative percentages for each fatty acid were calculated.

GC-MS conditions

GS-MS analysis was carried out on Agilent 6890N Network GC system combined with Agilent 5973 Network Mass Selective Detector (GC-MS). The capillary column used was an Agilent 19091N-136 (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm). Helium was used as carrier gas at a flow rate of 3.3 ml/min with 1 µl injection volume. Samples were analyzed with the column held initially at 100 °C for 1 min after injection, then increased to 170°C with 10°C/min heating ramp without hold and increased to 215°C with 5°C/min heating ramp for 5 min. Then the final temperature was increased to 240°C with 10°C/min heating ramp for 10.5 min. The injections were performed in split mode (30:1) at 250°C. Detector and injector temperatures were 260°C and 250°C, respectively. Pressure was established as 50.0 psi. Run time was 35 min. Temperature and nominal initial flow for flame ionization detector (FID) were set as 250°C and 3.1 ml/min, correspondingly. MS parameters were as follows: scan range (*m/z*): 35-450 atomic mass units (AMU) under electron impact (EI) ionization (70 eV).

Identification of the peaks

Fatty acid components of the two seed oil samples were determined by comparing of their retention times and mass weights with those of authentic samples of the respective fatty acids obtained by GC as well as the mass spectra from the Wiley and Nist database searches by GC-MS. As authentic samples; FAME mix Supelco-1891-1AMP [containing palmitic acid methyl ester 16:0, stearic acid methyl ester 18:0, oleic acid methyl ester 18:1, linoleic acid methyl ester (18:0; cis 9,12), linolenic acid methyl ester (18:3; cis 9,12,15)], and arachidonic acid methyl ester (20:4) (Sigma-A9298) were used.

Microbiological studies

The oils were dissolved in ethanol:hexane (1:1) by using 51% tween 80 solution at a final concentration of 1024 µg/ml, sterilized by filtration using 0.22 µm Millipore (MA 01730, USA), and used as stock solutions. Standard antibacterial powders of ampicilline (AMP, Fako), ofloxacine (OFX, Hoechst Marion Roussel) as well as standard antifungal powders of ketoconazole (KET, Bilim) and fluconazole (FLU, Pfizer) were obtained from their respective manufacturers and dissolved in phosphate buffer solution (AMP, pH: 8.0, 0.1 mol/l), DMSO (KET), in water (FLU and OFX). Stock solutions of the agents were prepared in medium according to the recommendations of National Committee for Clinical Laboratory Standards (23).

Standard and the isolated strains of the following bacteria including *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 10145), *Proteus mirabilis* (ATCC 7002), *Klebsiella pneumoniae* (RSKK 574), *Acinetobacter baumannii* (RSKK 02026), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), and *Bacillus subtilis* (ATCC 6633) for antibacterial activity, as well as standard strains of *Candida albicans* (ATCC 10231) and *C. parapsilosis* (ATCC 22019) for antifungal activity were employed. Mueller-Hinton Broth

(Difco) and Mueller-Hinton Agar (Oxoid) for the bacteria, Sabouraud liquid medium (Oxoid) and Sabouraud dextrose agar (SDA) (Oxoid) for fungi and culture suspensions were prepared using microdilution method (24) as described in our earlier publication (25).

Accordingly, the medium RPMI-1640 with *L*-glutamine was buffered at pH 7 with 3-[*N*-morpholino]-propansulfonic acid (MOPS). Before the test, strains of bacteria and fungus were cultured on media and passaged at least twice to ensure purity and viability at 35°C for 24 to 48h. The bacterial suspensions used for inoculation were prepared at 10^5cfu/ml by diluting fresh cultures at McFarland 0.5 density (10^8 cfu/ml). The fungus suspension was prepared by the spectrophotometric method of inoculum preparation at a final culture suspension of $2.5x10^3$ cfu/ml. Media were placed into each well of the 96-well microplate. Oil solutions at 1024 µg/ml were added into first raw of microplates and two-fold dilutions of the compounds (512-0.25 µg/ml) were made by dispensing the solutions to the remaining wells. Culture suspensions (10 µl) were inoculated into all the wells. The sealed microplates were incubated at 35 °C for 24 hs and 48 hs in a humid chamber. The lowest concentration of the oils that completely inhibit macroscopic growth was determined and minimum inhibitory concentrations (MICs) were calculated.

RESULTS AND DISCUSSION

Table 1 summarizes the results of fatty acid composition of the seed oils of *U. dioica* and *U. pilulifera*. Both of the oils were dominated by linoleic acid (44.29 % for *U. dioica* and 62.99 % for *U. pilulifera*), followed by oleic acid (34.93 % for *U. dioica* and 21.91 % for *U. pilulifera*). Trace amount of linolenic acid (0.55 %) was detected only in *U. pilulifera*.

Table 1. Fatty acid composition of the seed oils of *U. dioica* and *U. pilulifera*

Fatty acids	Relative percentages of the fatty acids		
	Retention time (R_t)	*U. dioica*	*U. pilulifera*
Palmitic	22.4	12.52±0.24	9.74±0.14
Stearic	36.1	7.52±0.17	4.79±0.26
Oleic	38.5	34.93±0.71	21.91±0.37
Linoleic	40.5	44.29±0.48	62.99±0.29
Linolenic	44.3	-	0.55±0.03
Eicosanoic	46.9	1.09±0.07	-
Total	-	100.35	99.98

On the other hand, all of the oils tested herein had equal antibacterial activity against all bacteria between 16-32 µg/ml, whereas they were less effective against the isolates at 64 µg/ml (Table 2). However, the oils showed comparable antifungal properties against *Candida albicans* and *C. parapsilosis* at 16 µg/ml, where the references, ketoconazole and fluconazole, had the MIC values of 1 and 2 µg/ml, respectively.

Essential fatty acids (EFAs), which cannot be synthesized by human being, are necessary for health and should be obtained through dietary intake. The human body needs EFAs to construct and repair cell membranes, enabling the cells to obtain optimum nutrition and expel harmful waste products. A primary function of EFAs, which support the cardiovascular, reproductive,

immune, and nervous systems, is the production of prostaglandins that regulate body functions such as heart rate, blood pressure, blood clotting, fertility, and play a role in immune system by regulating inflammation (26-29). On the other hand, there has been no previous study on the fatty acid composition of *U. pilulifera* growing in Turkey, whereas the seed oil of *U. dioica* of different origins has been so far documented. One study on the seed oil of stinging nettle grown in Spain revealed the oil composition to consist of palmitic (25.4 %), stearic (2.3 %), oleic (4.8 %), linoleic (22.7 %), and linolenic acids (6.6 %) (17), being quite dissimilar from the seed oil of its Turkish counterpart in this study.

Table 2. Antimicrobial activity of the seed oils of *U. dioica* (UD) and *U. pilulifera* (UP) as minimum inhibitory concentrations (MICs) (µg/ml).

	B. subtilis	**I_a**	***S. aureus***	**I_b**	***E. faecalis***	**I_c**	***P. aeruginosa***	**I_d**	***P. mirabilis***	**I_e**	***A. baumannii***	**I_f**	***K. pneumoniae***	**I_g**	***E. coli***	**I_h**	***C. albicans***	***C. parapsilosis***
UD	32	64	32	64	32	64	32	64	16	64	16	64	16	64	16	64	16	16
UP	32	64	32	64	32	64	32	64	16	64	16	64	16	64	16	64	16	16
AMP	0.5	16	1	16	0.25	1	-	-	2	32	2	32	2	32	2	32	-	-
OFX	1	32	0.5	-	1	8	1	8	0.12	4	0.12	4	0.12	4	0.12	8	-	-
KET	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
FLU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2

AMP: Ampicilline; OFX: Ofloxacine; KET: Ketoconazole; FLU: Fluconazole; - : No activity observed; I_a: Isolated strain of *B. subtilis*; I_b: Isolated strain of *S. aureus*; I_c: Isolated strain of *E. faecalis*; I_d: Isolated strain of *P. aeruginosa*; I_e: Isolated strain of *P. mirabilis*; I_f: Isolated strain of *A. baumannii*; I_g: Isolated strain of *K. pneumoniae*; I_h.

In another study, the fatty acid content of the *U. dioica* seed oil was found to be palmitic (9.0 %), oleic (14.6 %), linoleic (73.7 %), and linoleic (2.7 %) acids (30) in which linoleic acid content was much higher than that of our sample.

U. pilulifera was reported to be used against for treatment of skin diseases suggestive of dermatophyte infections in an ethnobotanical survey carried out in the Palestinian area (31).As for the antimicrobial studies on *Urtica* species, leaf and stem extracts of *U. urens*, which was reported to be ineffective against *S. aureus*, *Streptococcus pyogenes*, *E. coli*, and *P. aeruginosa* in South Africa, was found to be completely inactive against *Mycobacterium tuberculosis* (32,33). Another study performed on the antibacterial property of *U. dioica* growing in Turkey indicated that this species was active against *Salmonella enteritidis*, *S. gallinarum*, *S. agalactiae*, *K. pneumoniae*, *Staphylococcus aureus*, and *E. coli* except *Streptococcus dysgalactiae* (34). The water extract of *U. dioica* growing in Turkey was also reported to display moderate antimicrobial effect against the ATCC strains and their clinical isolates of nine bacteria and one fungus (*C. albicans*) by disk diffusion method (35). In a similar study, *U. gracilenta* growing in Iran was tested against *P. aeruginosa* and *P. fluorescens* by both micrudilution and disk diffusion method (36). While this plant was active against *P. aeruginosa* with the MIC value of 15 μg/ml and 10 mm of zone diameter, it exhibited no inhibitory effect on *P. fluorescens*.

To the best of our knowledge, this is the first report on the fatty acid composition of *U. pilulifera* seeds cultivated in Turkey, except for *U. dioica*. The present data underlines that seed oils of *U. dioica* and *U. pilulifera* have been proven to be the rich sources for linoleic acid in particular. However, it can be concluded that the variance among linoleic acid amounts in the seed oils of *U. dioica* samples of different origins may depend on climatic, soil type, and other environmental factors.

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