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Chemical Composition and Antimicrobial Activities of Cold-Pressed Oils Obtained From Nettle, Radish and Pomegranate Seeds

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Abstract

Aim of study: The aim of this study is to put forward the antimicrobial activity of cold pressed oils obtained from seeds of nettle (*Urtica dioica*), radish (*Raphanus sativus*) and pomegranate (*Punica granatum*).

Material and Methods: Oils of these seeds were analysed for their antibacterial and antifungal activities by the disk diffusion and MIC tests against fifteen microorganisms, *Staphylococcus epidermidis* DSMZ 20044, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* SL 1344, *Salmonella kentucky*, *Salmonella infantis*, *Salmonella enteritidis*, *Pseudomonas fluorescens* P1 ATCC 13075, *Pseudomonas aeruginosa* DSMZ 50071, *Klebsiella pneumoniae*, *Escherichia coli* ATCC 25922, *Enterococcus faecium*, *Enterococcus faecalis* ATCC 29212, *Enterobacter aerogenes* ATCC 13048, *Candida albicans* DSMZ 1386 and *Bacillus subtilis* DSMZ 1971. The results were compared against 11 standard antibiotics, which are cefazolin, clindamycin, chloramphenicol, ciprofloxacin, amoxicillin/clavulanic acid, sulfamethoxazole/trimethoprim, ceftriaxone, gentamicin, ampicillin, cephalothin, cefuroxime and vancomycin. The extracts were also chemically analysed by using GC-MS.

Main results: As a result, radish oil is observed to be active against all microorganisms with the highest activity, where nettle oil is active against all microorganisms except for *S. epidermidis*. The lowest activity was observed in pomegranate oil.

Highlights: The results of the study clearly puts forward that oils obtained from nettle, radish and pomegranate could have a possible medicinal use.

Keywords: Chemical Composition, Cold-Pressed Oil, Antimicrobial Activity, Nettle, Radish, Pomegranate

Isırgan, Turp ve Nar Tohumlarından Soğuk-Sıkım Yöntemi ile Elde Edilen Yağların Kimyasal Bileşimi ve Antimikrobiyal Aktiviteleri

Öz

Çalışmanın amacı: Bu çalışmanın amacı ısırgan (*Urtica dioica*), turp (*Raphanus sativus*) ve nar (*Punica granatum*) tohumlarından elde edilen soğuk sıkım yağların antimikrobiyal etkilerinin ortaya koymaktır.

Materyal ve Yöntem: Bu tohumların yağları, *Staphylococcus epidermidis* DSMZ 20044, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* SL 1344, *Salmonella kentucky*, *Salmonella infantis*, *Salmonella enteritidis*, *Pseudomonas fluorescens* P1 ATCC 13075, *Pseudomonas aeruginosa* DSMZ 50071, *Klebsiella pneumoniae*, *Escherichia coli* ATCC 25922, *Enterococcus faecium*, *Enterococcus faecalis* ATCC 29212, *Enterobacter aerogenes* ATCC 13048, *Candida albicans* DSMZ 1386 ve *Bacillus subtilis* DSMZ 1971 gibi 15 mikroorganizmaya karşı disk difüzyon metodu ve MİK testi kullanılarak, antimikrobiyal aktiviteleri açısından araştırılmıştır. Sonuçlar sefazolin, klindamisin, kloramfenikol, siprofloksasin, amoksisilin/klavulanik asit, sülfametoksazol/trimetoprim, seftriakson, gentamisin, ampisilin, sefalotin, sefuroksim ve vankomisin olmak üzere 11 standart antibiyotik ile karşılaştırılmıştır. Ekstraktlar ayrıca GC-MS kullanılarak kimyasal olarak analiz edilmiştir.

Sonuçlar: Sonuç olarak, turp yağının en yüksek aktiviteyi göstererek bütün mikroorganizmalara, ısırgan yağının ise *S. epidermidis* hariç bütün mikroorganizmalara etkili olduğu gözlenmiştir. En düşük aktivite ise nar yağında gözlenmiştir.

Önemli vurgular: Çalışmanın sonuçları, açık bir şekilde, ısırgan otu, turp ve nardan elde edilen yağların destekleyici sağlık ürünü olarak ve ilave çalışmalarla medikal amaçlı olarak da kullanılabileceğini ortaya koymaktadır.

Anahtar Kelimeler: Kimyasal Kompozisyon, Soğuk Pres Yağ, Antimikrobiyal Aktivite, Isırgan, Turp, Nar

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Introduction

Using natural plant derived products against diseases is as old as human history. Today scientists are trying to analyse the anti-infective potential of these herbal remedies (Abbasi, Khan, Ahmad, Jahan & Sultana, 2010). These studies triggered the development of several antimicrobial agents rooted from natural products (Andrews, 2003; Ates and Erdogrul, 2003; Altuner, Cetin & Cökmüs, 2010a and b).

Today it is generally proposed that there is a remarkable progress in human medicine as a result of these scientific studies, but today especially in the countries, which are developing, still several diseases related to bacteria, virus and fungus are causing severe problems for the public health due to the extensive antibiotic resistance of microorganisms (Okeke et al., 2005; Cos, Vlietinck, Vanden Berghe & Maes, 2006).

"The World Health Report" which was published in 2007 by WHO (World Health Organization) declared that the antibiotic resistance due to the microbial evolution possibly have a great impact on human beings for the next century. And this underlined idea was mentioned as the most potential serious problem for the public health (WHO, 2007; Syed, Syed, & Oh, 2010; Altuner et al., 2011a and b).

With the aim of preventing to spread these microorganisms, which are resistant to commercially used antimicrobials, characterization of new antimicrobial agents is proposed as the major aim for the scientists (Özkinali, Şener, Gür, Güney & Olgun, 2017; Canlı, Şimşek, Yetgin & Altuner, 2017).

Nettle (*Urtica dioica*) is commonly distributed in North America and Europe, and found in some parts of Asia and North Africa too. In some areas in the world some naturalized groups are also known to be present. Nettle plants are known to be collected for several reasons since ancient times, which are still being collected not only for culinary purposes but also for healing diseases too. In addition to that these plants are also known to have several medicinal properties, such as extracts of nettle leaves are still commonly used for several purposes. In addition, the leaves covered with needle like hairs are applied to skin against rheumatism traditionally. It was previously proven that leaves of this plant can be used against inflammation. Also the root extract can be used in BPH (benign prostate hyperplasia) (Kew Science, 2017a).

Radish (*Raphanus sativus*) is known to be rooted from East-Mediterranean lands. It is known that this plant was one of the main foods produced in Egypt about 2.000 BC's. Today radish cultivars are found in different ecological environments. There are several records showing that starting from very early times of human history this plant has been cultivated. The taproot is mostly consumed without cooking. Black radishes are also commonly used especially in Eastern-European foods (Kew Science, 2017b).

There are several studies showing that pomegranate (*Punica granatum*) is originated from the lands falling into today's North India and Iran, and it is shown that cultivation of this plant has been started in Mediterranean area very long time ago. Pomegranate plant has several uses, such as production of tannins to treat leathers, inks and dyes, and also for juice. In addition, it is also used for the production of different medicines against several diseases. It is also worth to mention that in many cultures pomegranate, especially pomegranate fruit, is accepted to be the symbol of fertility (Rieger, 2017).

There are several ways of uses especially for the oil obtained from pomegranate seed, such as against aging of the skin, reversing the damage in the skin and healing the wounds by triggering cell regeneration, protecting skin from UV and thus decreasing the risk of skin cancer, controlling oil and acne production of the skin, calming psoriasis and eczema, soothing redness and irritation of the skin, improving the health of the scalp and hairs (Health Beckon, 2014).

In this study, cold-pressed seed oils of *Urtica dioica, Raphanus sativus* and *Punica granatum* are tested for their antimicrobial activity by MIC and the disk diffusion tests. In addition to that the chemical composition of these oils were determined through a GC-MS analysis.

Materials and Method

Plant samples

Urtica dioica, *Raphanus sativus* and *Punica granatum* seeds were purchased from a local company (Özşen Lokman Hekim).

Oil extraction

The oil was obtained through a cold-press production (MP-001 Screw Press, Turkey). One kilogram of each seed was pressed, filtered and allowed to stand overnight. After 24 hours the upper clear layer of oil was separated through a separation funnel. Obtained oils were kept in cold (4°C) and dark until used in test. The yield percentage for all plant samples were found to be 50% (w/w) for *U. dioica*, 30% (w/w) for *R. sativus* and 10% (w/w) for *P. granatum*.

Microorganisms

Several Gram positive and Gram negative microorganisms were selected to analyse the activity of the oils. The fifteen microorganisms used in this study are *Staphylococcus epidermidis* DSMZ 20044, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* SL 1344, *Salmonella kentucky*, *Salmonella infantis*, *Salmonella enteritidis*, *Pseudomonas fluorescens* P1 ATCC 13075, *Pseudomonas aeruginosa* DSMZ 50071, *Klebsiella pneumoniae*, *Escherichia coli* ATCC 25922, *Enterococcus faecium*, *Enterococcus faecalis* ATCC 29212, *Enterobacter aerogenes* ATCC 13048, *Candida albicans* DSMZ 1386 and *Bacillus subtilis* DSMZ 1971.

Inoculum

Microorganisms used in this study were cultured in line with their requirements as stated in some previous studies (Altuner and Çetin, 2009; Altuner and Canli, 2012; Canlı Altuner & Akata, 2015).

For inoculum, microorganisms were suspended in sterile physiological saline solution (Canlı, Altuner, Akata, Türkmen & Üzek, 2016; Canlı, Yetgin, Akata & Altuner, 2016a and b; Canlı, Yetgin, Akata & Altuner, 2017a) and to adjust equal the number of the colonies in the solution, 0.5 McFarland standard was used (Hammer, Carson & Riley, 1999; Altuner, Akata & Canli, 2012a and b).

Disk diffusion (DD) method

For DD test previously mentioned methodologies were used (Andrews, 2003; Altuner, Ceter & Islek, 2010; Canli et al, 2014). Petri dishes having 9 mm dimensions, which contain 25 mL of Mueller Hinton Agar were used in order to standardize the work (Ilhan, Savaroğlu, Çolak, Iscen & Erdemgil, 2006; Canlı, Yetgin, Akata & Altuner, 2016c). Five and 15 microliters of oils were transferred to sterile antibiotic disks (SAD) and then they were kept at 40 °C for 24 h in aseptic conditions (Canlı, Akata & Altuner, 2016). Microorganism suspensions were inoculated to agar medium (Mueller Hinton) and left in aseptic conditions for 2-3 minutes before applying oil loaded disks as described in the previous studies Altuner and Akata (2010). Inhibition zones were defined in mm by the method mentioned by Altuner, Canli & Akata (2014).

Determination of MIC

The MIC values for all oil samples were identified as stated previously (Balouiri, Sadiki & Ibnsouda, 2016). The concentration range was between 100 to 0.195 µg/mL.

Determination of chemical composition by GC-MS

For determination of the chemical composition of oil samples the methodology mentioned previously was used (Canlı, Yetgin, Akata & Altuner, 2017b).

Controls

Empty SAD was used as negative controls for disk diffusion test and sterilized broth medium for MIC test. In addition, microorganisms were inoculated in Mueller Hinton broth in order to control the viability of each microorganism.

As positive controls eleven standard antibiotics, which are cefazolin, clindamycin, chloramphenicol, ciprofloxacin, amoxicillin / clavulanic acid, sulfamethoxazole / trimethoprim, ceftriaxone, gentamicin, ampicillin, cephalothin, cefuroxime and vancomycin are used.

Statistics

The tests were replicated three times. All the results given were mentioned as the mean

of the parallels. The statistical analysis were conducted as mentioned by Altuner (2011) with a *p* value of 0.05.

Results and Discussion

According to the GC-MS results the most abundant component determined for *U. dioica, R. sativus* and *P. granatum* oils are found to be 34%, 20% and 19% in scanning of essential oils and 14%, 35% and 32% in scanning of fatty acid, respectively and the list of all main components for *U. dioica, R. sativus* and *P. granatum* oils are presented in Table 1 and Table 2.

GC-MS results showed that the major essential oils of *U. dioica* oil contains (Z,Z)-9,12-octadecadienoyl chloride (4.88%), 9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- (11.25%), linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)- (3.00%), cis-9-hexadecenal (33.81%), tridecanedial (9.56%), hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (13.45%), (Z,Z)-9,12-octadecadien-1-ol (5.81%) as the main components as essential oils; *R. sativus* contains 9,12-octadecadienoyl chloride, (Z,Z)- (3.01%), 9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- (7.55%), di-(9-octadecenoyl)-glycerol (3.53%), cis-9-hexadecenal (58.65%), hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (18.25%) and *P. granatum* contains (Z,Z)-9,12-octadecadienoyl chloride (7.85%), 9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- (13.69%), di-(9-octadecenoyl)-glycerol (3.06%), cis-9-hexadecenal (61.77%). The main essential oil components of the oils obtained from three plant samples are given in Table 1.

Table 1 clearly shows that cis-9-hexadecenal is the most abundant essential oil in all by three plant oils.

Table 1. The major essential oils

U. dioica	**%**	***R. sativus***	**%**	***P. granatum***	**%**
cis-9-hexadecenal	33.81	cis-9-hexadecenal	58.65	cis-9-hexadecenal	61.77
hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	13.45	hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	18.25	9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	13.69
9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	11.25	9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	7.55	(Z,Z)-9,12-octadecadienoyl chloride	7.85
tridecanedial	9.56	di-(9-octadecenoyl)-glycerol	3.53	di-(9-octadecenoyl)-glycerol	3.06
(Z,Z)-9,12-octadecadien-1-ol	5.81	9,12-octadecadienoyl chloride, (Z,Z)-	3.01		
(Z,Z)-9,12-octadecadienoyl chloride	4.88				
Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)-	3.00				

Table 2. The major fatty acids

U. dioica	**%**	***R. sativus***	**%**	***P. granatum***	**%**
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	38.87	13-docosenoic acid, methyl ester	25.40	methyl-9.cis.,11.trans.t,13.trans.-octadecatrienoate	55.93
9-octadecenoic acid (Z)-, methyl ester	14.34	6-octadecenoic acid, methyl ester, (Z)-	17.22	9-octadecenoic acid (Z)-, methyl ester	6.42
cyclohexanecarboxylic acid, decyl ester	22.18	11-Eicosenoic acid, methyl ester	8.54	tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-cis-1,10	6.40
Methyl 5,11,14-eicosatrienoate	9.79	9,12-octadecadienoic acid (Z,Z)-, methyl ester	8.30	(Z,Z)-9,12-octadecadienoic acid methyl ester	5.75
hexadecanoic acid, methyl ester	4.32	di-(9octadecenoyl)-glycerol	4.14	di-(9-octadecenoyl)-glycerol	4.62

GC-MS results showed that the major fatty acids of *U. dioica* oil contains hexadecanoic acid, methyl ester (4.32%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (38.87%), 9-octadecenoic acid (Z)-, methyl ester (14.34%), Methyl 5,11,14-eicosatrienoate (9.79%), cyclohexane carboxylic acid, decyl ester (22.18%); *R. sativus* contains 9,12-octadecadienoic acid (Z,Z)-, methyl ester (8.30%), 6-octadecenoic acid, methyl ester, (Z)- (17.22%), 11-Eicosenoic acid, methyl ester (8.54%), di-(9octadecenoyl)-glycerol (4.14%), 13-docosenoic acid, methyl ester (25.40%) and *P. granatum* contains (Z,Z)-9,12-octadecadienoic acid methyl ester (5.75%), 9-octadecenoic acid (Z)-, methyl ester (6.42%), methyl-9.cis.,11.trans.t,13.trans.-octadecatrienoate (55.93%), di-(9-octadecenoyl)-glycerol (4.62%), tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-cis-1,10 (6.40%). The main fatty acid components of the oils obtained from three plant samples are given in Table 2.

Table 2 clearly presents that the most abundant fatty acids of each plant oil is different, which are 9,12-octadecadienoic acid (Z,Z)-, methyl ester (38.87%) for *U. dioica*, 13-docosenoic acid, methyl ester (25.40%) for *R. sativus* and methyl-9.cis.,11.trans.t,13.trans.-octadecatrienoate (55.93%) for *P. granatum*.

The results for disk diffusion test of *U. dioica*, *R. sativus* and *P. granatum* oils are given in Table 3 and the results for standard antibiotic disks are given in Table 4 too.

The MIC values observed for *U. dioica*, *R. sativus* and *P. granatum* are given in Table 5.

According to the results, *U. dioica* seed oil was observed to be active all bacteria and fungi except for *S. epidermidis* and the MIC values were observed to be between 12.5 and 25 µg/mL. Although an activity was observed against *P. aeruginosa* in disk diffusion test, no MIC value can be identified. This could be due to *U. dioica* seed oil presented a static activity, rather than cidal activity. In order to understand this, further analysis should be conducted.

R. sativus seed oil was observed to be active all bacteria and fungi, and the MIC values were observed to be between 12.5 and 25 µg/mL.

P. granatum showed to be active against *S. epidermidis*, *S. aureus*, *S. kentucky*, *S. enteritidis*, *P. fluorescens*, *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *E. faecium* and *E. aerogenes* and the MIC values for these bacteria were observed to be 25 µg/mL, where no activity was observed against *B. subtilis*, *S. typhimurium*, *S. infantis*, *E. faecalis* and *C. albicans*.

Some studies were also previously studied the activity of *U. dioica*, *R. sativus* and *P. granatum* against several microorganisms. But only a minute amount of them are the antimicrobial activity of seed oils.

Kan et al. (2009) studied *U. dioica* seed oils and determined the antimicrobial activity and the fatty acid profile too. The yield percentage in this study was 22.59%, where 30% in our study. This difference could possibly base on the oil extraction method used. Kan et al. (2009) extracted the oil from seeds by using Soxhlet apparatus with *n*-hexane as an extraction solvent, but we have extracted oils by direct cold pressing.

Kan et al. (2009) determined the activity of *U. dioica* seed oil against 10 microorganisms. They have found a MIC value of 32 µg/mL for both *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212. In our study we determined a 25 µg/mL of MIC value for the same strains. This difference is possibly related to higher yield due to application of cold pressing. This method might be effective in increasing the active substances during extraction.

On the other hand, they also tested the antimicrobial activity of *U. dioica* seed oil against *B. subtilis*, *C. albicans*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*, and the MIC values for these microorganisms were identified to be 32 µg/mL, 16 µg/mL, 16 µg/mL, 16 µg/mL and 32 µg/mL respectively.

In our study we found the MIC values for these microorganisms as 25 µg/mL for *B. subtilis*, 12.5 µg/mL for *C. albicans*, 25 µg/mL for *E. coli*, 12.5 µg/mL for *K. pneumoniae* and no MIC value for *P. aeruginosa*. The main reason for this difference depends on the difference in the strains used in both studies.

As for other antimicrobial studies of different type of *U. dioica* extracts, such as leaf and stem extracts, previously presented that this plant is active against *C. albicans*, *S. enteritidis*, *Salmonella gallinarum*, *Salmonella agalactiae*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *Lactobacillus plantarum* and *E. coli* (Kelet, Bakırel, Ak & Alpmar, 2001; Gülçin, Kührevioğlu, Oktay & Büyükokuroğlu, 2004; Kukrić et al., 2012). But since the extracts are different it is no use to compare these results with our results.

Some researchers also presented the activity of *R. sativus* in the literature, but nearly all of them are the activity of crude juices or extracts of different parts such as leaves, seeds and roots, since both the juices and leaves, seeds and roots are known to be used as medicine all over the world (Chopra, Nayar & Chopra, 1986; Kritikar and Basu, 1987; Bin Sina, 1987; Ahmad, Hasan, Chishti & Ahmad, 2012).

Table 3. Disk diffusion test results for 5 µL and 15 µL of cold press oils obtained from *U. dioica*, *R. sativus* and *P. granatum* seeds (Inhibition zones in mm).

	U. dioica		*R. sativus*		*P. granatum*	
	5 µL	15 µL	5 µL	15 µL	5 µL	15 µL
B. subtilis	-	7	8	9	-	-
C. albicans	8	9	10	11	-	-
E. aerogenes	8	9	9	10	-	7
E. faecalis	7	8	9	10	-	-
E. faecium	-	7	8	10	7	8
E. coli	7	8	7	8	7	8
K. pneumoniae	8	9	9	10	7	8
P. aeruginosa	-	7	7	8	-	7
P. fluorescens	7	8	9	10	7	8
S. enteritidis	7	8	8	9	7	8
S. infantis	7	8	9	10	-	-
S. kentucky	8	9	9	10	-	7
S. typhimurium	7	9	8	9	-	-
S. aureus	7	8	8	10	7	8
S. epidermidis	-	-	8	9	-	7

"-" implies no effect.

Table 4. The results for standard antibiotic disks (Inhibition zones in mm)

	CFZ	**CLI**	**CAM**	**CPR**	**AMC**	**SXT**	**CRO**	**GEN**	**AMP**	**CEF**	**CXM**	**VAN**
B. subtilis	44	34	37	36	56	42	38	30	41	36	44	20
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-
E. aerogenes	14	-	26	30	9	24	21	23	-	-	16	-
E. faecalis	14	-	19	19	28	29	-	13	14	-	-	15
E. faecium	40	30	11	28	43	34	31	28	32	24	33	26
E. coli	18	-	22	-	16	12	-	20	6	6	6	6
K. pneumoniae	-	-	22	30	9	6	6	22	6	6	6	-
P. aeruginosa	-	-	9	28	-	-	-	15	-	-	-	-
P. fluorescens	10	8	22	19	26	26	-	12	14	-	-	16
S. enteritidis	23	-	28	36	28	31	27	24	16	-	16	-
S. infantis	22	-	28	24	26	24	26	24	14	-	17	-
S. kentucky	22	-	29	34	26	27	30	13	15	-	19	-
S. typhimurium	22	-	27	35	26	21	27	23	13	-	14	-
S. aureus	31	24	21	22	30	27	16	24	25	22	29	16
S. epidermidis	37	35	33	34	45	32	26	25	24	26	32	21

"-": No activity observed, CFZ: Cefazolin, CLI: Clindamycin, CAM: Chloramphenicol, CPR: Ciprofloxacin, AMC: Amoxicillin/Clavulanic acid, SXT: Sulfamethoxazole/Trimethoprim, CRO: Ceftriaxone, GEN: Gentamicin, AMP: Ampicillin, CEF: Cephalothin, CXM: Cefuroxime, VAN: Vancomycin

Table 5. MIC values (µg/mL) for *U. dioica*, *R. sativus* and *P. granatum*

	U. dioica	*R. sativus*	*P. granatum*
B. subtilis	25	12.5	-
C. albicans	12.5	12.5	-
E. aerogenes	12.5	12.5	25
E. faecalis	25	12.5	-
E. faecium	25	12.5	25
E. coli	25	25	-
K. pneumoniae	12.5	12.5	25
P. aeruginosa	-	25	-
P. fluorescens	25	12.5	25
S. enteritidis	25	12.5	25
S. infantis	25	12.5	-
S. kentucky	12.5	12.5	25
S. typhimurium	12.5	12.5	-
S. aureus	25	12.5	25
S. epidermidis	-	12.5	25

"-" implies no effect.

Crude juice of *R. sativus* was found to have antimicrobial activity on *E. coli*, *Pseudomonas pyocyaneus*, *P. aeruginosa*, *Salmonella typhi*, *Salmonella thyphosa* and *B. subtilis* (Abdou, Abou-Zeid, El-Sherbeeny & Abou-El-Gheat, 1972; Caceres, 1987).

Although it was previously mentioned that the *R. sativus* seeds contain quite high oil (Gutiérrez and Perez, 2004), there aren't much noteworthy studies about the antimicrobial activity of the oil obtained from radish seeds according to the best of our knowledge. Thus, it is not possible to compare our results regarding the antimicrobial activity of *R. sativus* to any other previous studies. Alternatively, one research conducted (Ahmad, Hasan, Chishti & Ahmad, 2012) could possibly extracted several fatty acids and essential oils from *R. sativus* seeds due to the extraction solvents used. They have used aqueous cold and hot solvents, benzene, chloroform, ethyl acetate, methanol and ethanol, and they applied agar well diffusion test to determine the antimicrobial activity against *Salmonella paratyphi* ATCC 9150, *S. typhi* ATCC 25241, *Shigella sonnie* ATCC 25931, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 6380, *K. pneumoniae* ATCC 27736 and *E. coli* ATCC 25922. As a result they have found that the diameter of inhibition zones for *E. coli* ATCC 25922 are 9.0 ± 1.4 mm, 10.0 ± 0.5 mm, 12.0 ± 0.5 mm, 12.5 ± 0.7 mm, 12.5 ± 0.7 mm and 14.5 ± 0.7 mm for ethyl acetate, chloroform, aqueous hot, methanol, benzene and ethanol extracts respectively. In our study we have found 8.0 mm inhibition zone as the highest for *R. sativus* oil against *E. coli* ATCC 25922.

On the other hand, Ahmad, Hasan, Chishti and Ahmad (2012) have observed that the diameter of inhibition zones for *S. aureus* ATCC 25923 are 9.0 ± 0.5 mm, 9.0 ± 0.5 mm, 10.0 ± 0.5 mm, 12.0 ± 0.5 mm, 13.5 ± 0.7 mm, 18.0 ± 0.5 mm and 19 ± 7.0 mm for aqueous cold, benzene, chloroform, aqueous hot, methanol, ethyl acetate and ethanol extracts respectively. In our study we have found 10 mm inhibition zone as the highest inhibition zone for *R. sativus* oil against *S. aureus* ATCC 25923.

There are three main reasons for these differences; (1) methods used to identify antimicrobial activity are different, namely disk diffusion test and agar well diffusion assay, (2) extraction method, therefore the composition of the extracts, are different and (3) the amount of extract tested in agar well diffusion assay was about 13 fold higher than our study.

There are more than hundred researches about the activities of *P. granatum*'s different parts such as fruit, fruit peels, seeds, etc., by using different extraction solvents (Singh, Chidambara Murthy & Jayaprakasha, 2002; Naz, Siddiqi, Ahmad, Rasool & Sayeed, 2007; Reddy, Gupta, Jacob, Khan & Ferreira, 2007; Altuner, 2011; Devi, Singh & Bhatt, 2011).

As for *U. dioica* and *R. sativus* there are not much studies about the antimicrobial activity of *P. granatum* seed oils.

In one of the studies Rustaiyan, Samiee, Kurabaslu and Taghizadeh et al. (2013) tested the antimicrobial activity of pomegranate seed oil against four microorganisms, namely *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *B. subtilis* and *Saccharomyces cerevisiae* ATCC 2365 by disk diffusion test. However, they didn't observe any antimicrobial activity against these four microorganisms. Instead we observed 8.0 mm inhibition zone as the highest inhibition zone against both for *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. This difference could possibly due to the difference of oil extraction processes used in these two studies. Rustaiyan et al. (2013) used hexane as extraction solvent and applied solvent extraction method previously described by AOAC (1990). This results may show that cold-pressing technique extracts antimicrobial substances more than solvent extraction method.

In another study Tanveer et al. (2016) studied the antimicrobial activity of pomegranate peel and seed extracts against *E. feacalis*, *E. coli*, *S. aureus* and *P. aeruginosa* by disk diffusion method. They observed inhibition zones of 7.12 mm for *E. coli*, 6.12 mm for *P. aeruginosa*, 6.25 mm for *S. aureus* and 7.12 mm for *E. feacalis* as a result of applying seed water extracts. In our study we observed higher inhibition zones for all microorganisms except for *E. feacalis*. This difference can be related with the difference in extraction method.

According to the results all three seed oils presented antimicrobial activity against *S. kentucky*, *P. aeruginosa*, *E. coli*, *E. faecium*, *E. aerogenes*, *K. pneumoniae*, *P. fluorescens*, *S. enteritidis* and *S. aureus*.

Intensive care units (ICU) are very serious places for especially nosocomial infections (NI). Thus, antimicrobial activity against any microorganism responsible for nosocomial infections in ICU would be very important.

E. aerogenes is accepted as one of the Intensive Care Unit pathogens that will lead to significant mortality and morbidity. On the other hand the infection management for this microorganism is complicated since it may generate resistance to the drugs used against (Hidron, Edwards & Patel, 2008).

P. aeruginosa is accepted as a frequent reason for NI, especially in patients, which are critically ill and hospitalized. It is known that infections related to *P. aeruginosa* can be complicated and life-threatening (Obritsch, Fish, MacLaren & Jung, 2005).

S. aureus is one of other pathogen that is also responsible for NI in ICU (Richards, Edwards, Culver & Gaynes, 1999).

It was previously proven that Gram (-) microorganisms are resistant to antibiotics than Gram (+) microorganisms (Nikaido, 1998; Faucher and Avril, 2002). *K. pneumoniae* is one of the important Gram (-) strains, which also cause some severe infections in ICU (Villegas and Quinn, 2004).

Although in our study a high activity wasn't observed against *P. aeruginosa*, *S. aureus*, *E. aerogenes* and *K. pneumoniae* compared to standard antibiotic disks, the results can be accepted as noteworthy since all these pathogens are important in causing NI in ICU.

The composition of the oils extracted from *U. dioica*, *R. sativus* and *P. granatum* directly affects their antimicrobial activity.

According to GC-MS results 9,12-Octadecadienoic acid is found in all oil extracts with percentage of 38.87%, 8.30% and 5.75% in *U. dioica*, *R. sativus* and *P. granatum* respectively.

9,12-Octadecadienoic acid, which is also known as linoleic acid and its antimicrobial activity was defined in several previous studies. Tsuchida and Morishida (1995) determined the antimicrobial activity of linoleic acid by using several Gram negative and Gram positive strains. In this study it was presented that Gram positive strains are generally susceptible to linoleic acid.

9-octadecenoic acid is found in two of the oil extracts with percentage of 14.34% and 6.42% in *U. dioica* and *P. granatum* respectively.

9-octadecenoic acid is also known as oleic acid and several studies showed that it has potential of possessing antibacterial and antifungal activity (Kabara, Swieczkowski, Conley & Tuant, 1972; Dilika, Bremner & Meyer, 2000; McGraw, Jager & Van Staden,

2002; Seidel and Taylor, 2004). Dilika, Bremner and Meyer (2000) presented that oleic acids isolated from *Helichrysum pedunculatum* was active against several Gram (+) bacteria.

Hexadecanoic acid (palmitic acid) is found 4.32% in *U. dioica* oil according to GC-MS results. The antimicrobial activity of palmitic acid is also shown in some previous studies (Glover, Whittemore & Bryant, 1997; Risk, Harrison & Lewis, 1997; Bazes et al., 2009). A noteworthy activity of palmitic acid against both fungi and bacteria were previously reported (Altieri, Cardillo, Bevilacqua & Singaglia, 2007; Liu et al., 2008; Huang, George & Ebersole, 2011).

13-docosenoic acid (erucic acid) is found 25.40% of *R. sativus* oil according to GC-MS results. Bailey et al (1989) showed the activity of some erucic acid-glycolic acid derivatives against *Candida utilis*, *E. coli*, *S. aureus* and *Penicillum notatum*.

6-octadecenoic acid, which is known as petroselinic acid, is found 17.22% of *R. sativus* oil according to GC-MS results. According to some previous researches petroselinic acid has a considerable antimicrobial activity against several bacteria, yeast and mold species (Placek, 1963).

It is obvious that all oils extracted from *U. dioica*, *R. sativus* and *P. granatum* contains several fatty acids which are active as antimicrobial agents. The antimicrobial activity of these oils could be related to their fatty acid composition.

As a result, it can be concluded that there is a clear antimicrobial activity of all three seed oils against *E. aerogenes*, *E. faecium*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. fluorescens*, *S. enteritidis*, *S. kentucky* and *S. aureus*.

The antimicrobial activity of all oils against *E. aerogenes*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae* can be accepted as noteworthy since all these pathogens are important in causing NI in ICU.

On the other hand, the composition of the oils are promising for further researches, since they contain several fatty acids having antimicrobial nature. But further researches are needed to be conducted in order to analyse their activity mechanisms in details.

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