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Antibacterial Activity of Some Turkish Plant Hydrosols [1]

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Summary

The in vitro antibacterial activity of the hydrosols of (distilled plant water) twenty plant samples (Thyme, sumach, clove, nettle, angelica, acacia, oak, sage, juniper, rosemary, echinacea, green tea, basil, myrtle, walnut, laurel, mint, strawflower, daisy, hypericum) were tested on Aeromonas hydrophila, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens which play role especially at spoilage of freshwater fish. Thyme and clove were effective against all bacteria. Consequently, it is likely that, these plant hydrosols may be used as antimicrobial agents to prevent the deterioration of food products.

Keywords: *Hydrosol, Antibacterial effect, Aeromonas hydrophila, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens*

Türkiye'de Yetişen Bazı Bitkilere Ait Hidrosollerin Antibakteriyel Etkisi

Özet

Bu çalışmada, 20 bitki örneğinden elde edilen hidrosollerin (Kekik, sumak, karanfil, ısırgan, melek otu, akasya, meşe, adaçayı, ardıç, biberiye, ekinezya, yeşil çay, reyhan, mersin, ceviz, defne, nane, altın otu, papatya ve sarı kantaron), özellikle tatlı su balıklarının bozulmasında rol oynayan Aeromonas hydrophila, Escherichia coli, Pseudomonas aeruginosa ve Pseudomonas fluorescens üzerine in vitro antibakteriyel etkisi araştırılmıştır. Kekik ve karanfil, incelen tüm bakterilere karşı etkin bulunmuştur. Sonuç olarak bu bitki hidrosollerinin gıdaların bozulmaya karşı korunmalarında antimikrobiyel ajanlar olarak kullanılabilecekleri kanaatine varılmıştır.

Anahtar sözcükler: *Hidrosol, Antibakteriyel etki, Aeromonas hydrophila, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens*

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INTRODUCTION

Antimicrobial agents, including food preservatives have been used to inhibit foodborne bacteria and extend the shelf life of processed food. Many naturally occurring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens [1]. Spices and their essential oils are the most efficient natural antioxidants and antimicrobial agents have long been used to preserve food [2]. The leafy part of plants such as sage, thyme, oregano and savory belonging to the Labiatae family have been added to meat, fish and food products for years. Being natural foodstuffs, they appeal to consumers who tend to question the safety of synthetic additives. It has been suggested that some synthetic chemicals convert some ingested materials into toxic substances or carcinogens by increasing the activity of microsomal enzymes [3]. Also, some chemicals require caution in handling since they are corrosive and their vapours can irritate the eyes and respiratory tract. On the contrary, herbs and their derivatives such as extracts and decoctions possessing antimicrobial activity might have beneficial effects, but cause no health problems to the handler and consumer [4]. Recently, there has been considerable emphasis on studies involving essential oils, extracts and decoctions of spices on inhibiting the growth of microbes. But there is a limited number of researches on the inhibitory effect of spice hydrosols (distilled spice water).

The objective of this study was to investigate the antimicrobial activity of thyme, sumach, clove, nettle, angelica, acacia, valonia oak, sage, juniper, rosemary, echinacea, green tea, basil, myrtle, walnut, laurel, mint, strawflower, daisy, and hypericum hydrosols against *A. hydrophila, P. aeruginosa, P. fluorescens* and *E. coli* which play role at spoilage of freshwater fish.

MATERIAL and METHODS

Bacterial cultures

The four bacteria used as test organisms were as follows: *A. hydrophila* 95080, *E. coli* 97010, *P. aeruginosa* 97020, *P. fluorescens* 589. They were provided by Refik Saydam National Type Culture Collection, Ankara–Turkey.

Plant samples

Plant samples were bought from herbalists in Turkey and identified botanically at the Biology Department of Cumhuriyet University. The commercial and scientific names of plants which used in this study are given in *Table 1.*

Table 1. *Plant materials from which hydrosols were obtained*
Tablo 1. *Hidrosollerin elde edildiği bitkisel materyaller*

Plant name	Botanical name	Family	Part used
Thyme	*Thymus serpyllum*	*Labiatae*	Leaves
Sumach	*Rhus aromaticus*	*Anacardiaceae*	Fruits
Clove	*Syzygium aromaticum*	*Myrtaceae*	Buds
Nettle	*Urtica dioica*	*Urticaceae*	Leaves
Angelica	*Angelica archangelica*	*Umbelliferae*	Leaves
Acacia	*Robinia pseudoaccacia*	*Fabaceae*	Bark
Oak	*Quercus ithaburensis*	*Fagaceae*	Fruits
Sage	*Salvia officinalis*	*Labiatae*	Leaves
Juniper	*Juniperus communis*	*Cupressaceae*	Seeds
Rosemary	*Rosmarinus officinalis*	*Lamiaceae*	Leaves
Echinacea	*Echinacea purpurea*	*Asteraceae*	Flowers
Green tea	*Camelia sinensis*	*Theaceae*	Leaves
Basil	*Ocimum basilicum*	*Lamiaceae*	Leaves
Myrtle	*Myrtus communis*	*Myrtaceae*	Leaves
Walnut	*Juglans regia*	*Juglandaceae*	Leaves
Laurel	*Laurus nobilis*	*Lauraceae*	Leaves
Mint	*Mentha longifolia*	*Lamiaceae*	Leaves
Strawflower	*Helichrysum plicatum*	*Asteraceae*	Flowers
Daisy	*Anthemis nobilis*	*Asteraceae*	Flowers
Hypericum	*Hypericum perforatum*	*Clusiaceae*	Flowers

Preparation of hydrosols

A 50 g sample of each plant was ground in an omnimixer. The hydrosols of ground plant were obtained after 1 h in steam distillation apparatus with 500 ml distilled water (1:10 w/v). Then, the oil was removed. Hydrosols were kept in sterile bottles under refrigerated conditions until use.

Determination of antibacterial effect

Stock culture of A. hydrophila was grown in BHI (Brain Heart Infusion Broth, Oxoid CM 225) at 25°C for 18 h. The other bacteria were grown in the same medium at 30°C for 18 h. Final cell concentrations were 10^6-10^7 cfu/ml. All of the hydrosols were dispensed separately to

sterile test tubes. Tubes containing physiological saline inoculated with test microorganisms were used as controls. For the determination of any microbial growth, 5 ml of each treatment solution was inoculated to BHI, and incubated at 30°C for 18 h. Then, 100 µl of the enriched samples was spread over PCA (Plate Count Agar, Oxoid CM 463) plates and incubated at 30°C for 48 h.

To evaluate the antibacterial activity of the treatment solutions against the test bacteria, the tubes containing 10 ml of treatment solution and the control tubes containing 10 ml of physiological saline were inoculated with 10 µl of overnight (18 h) broth culture of the test microorganisms and kept at 20±2°C for 60 minutes. At 10, 30 and 60th min of the treatment period, 2 ml of each inoculated tube content were transferred to sterile empty test tubes and neutralised, with the exception of the controls, using 0.1 or 0.01 NaOH solution. One ml of each sample was pour plated using PCA. Appropriate serial dilutions of the remaining samples were prepared in PW (Pepton Water, Oxoid CM 9), and 100 µl of each dilution was spread over Aeromonas Medium Base (Ryan) (Oxoid CM 833) with Ampicillin Selective Supplement (Oxoid SR 136) for *A. hydrophila* Pseudomonas Agar Base (Oxoid CM 559) with C-F-C Supplement (Oxoid SR 103) for *P. aeruginosa* and *P. fluorescens,* VRBA (Violet Red Bile Lactose Agar, Oxoid CM 107) for *E. coli*. The Aeromonas plates were incubated at 25°C for 48 h, CFC plates were incubated at 30°C for 48 h and VRBA plates were incubated at 37°C for 48 h. The colonies grown on the plates were enumerated and the counts were converted to log 10 cfu/ml. Three replications of the experiment were made. The data obtained were statistically analyzed.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA). Differences among the mean values of various treatments were determined by the Tukey's Post Hoc Test. The significance was defined at P<0.05. Statistical analysis were made using SPSS 9.05 programme.

RESULTS

In this study, hydrosols of thyme, clove and rosemary showed a significant antimicrobial activity against *A. hydrophila* (P<0.05). Some of the other extracts reduced the counts of tested bacteria with the exception of nettle, angelica, acacia, oak, echinacea, green tea, basil, walnut and daisy *(Table 2)*. *P. fluorescens* was not inhibited by acacia, oak, green tea, basil, walnut, strawflower and daisy *(Table 3)*. However, the initial count of *P. aeruginosa* was not only significantly reduced by thyme, clove and rosemary but also affected by juniper, myrtle and mint *(Table 4)*. *E. coli* was the most resistant strain in the test bacteria and any of plant hydrosols could inhibit its growth except thyme and clove *(Table 5)*. The test bacteria counts remained constant in the physiological saline tubes at the end of the 60 min. Clove and thyme hydrosols were the most effective agents reduced the count of all test strains to below countable level (1 cfu/ml) followed by the rosemary.

Table 2. *The antibacterial activity of hydrosols against A. hydrophila*
Tablo 2. *Hidrosollerin A. hydrophila'ya karşı antibakteriyel etkisi*

Plant Name	Bacteria Counts (log cfu/ml) (mean±SD)		
	10th minute	30th minute	60th minute
Physiological saline	6.47±0.03	6.45±0.04	6.44±0.05
Thyme	0	0	0
Sumach	5.83±0.04	5.66±0.12	5.50±0.24
Clove	0	0	0
Nettle	6.44±0.02	6.40±0.03	6.43±0.06
Angelica	6.37±0.01	6.37±0.01	6.30±0.03
Acacia	6.36±0.09	6.26±0.03	6.21±0.01
Oak	6.17±0.05	6.11±0.04	6.06±0.04
Sage	6.21±0.01	6.02±0.03	5.93±0.07
Juniper	5.94±0.24	5.30±0.09	4.89±0.20
Rosemary	5.19±0.01	3.92±0.12	3.26±0.16
Echinacea	6.34±0.03	6.29±0.01	6.24±0.03
Green tea	6.45±0.06	6.32±0.02	6.32±0.02
Basil	6.44±0.01	6.42±0.01	6.39±0.01
Myrtle	6.11±0.12	5.70±0.03	5.35±0.18
Walnut	6.35±0.02	6.30±0.01	6.25±0.01
Laurel	5.99±0.10	5.78±0.04	5.34±0.08
Mint	6.00±0.10	5.81±0.07	5.72±0.04
Strawflower	6.11±0.04	6.08±0.04	5.87±0.04
Daisy	6.28±0.31	6.25±0.32	6.17±0.42
Hypericum	6.14±0.05	5.94±0.11	5.78±0.06

Table 3. *The antibacterial activity of hydrosols against P. fluorescens*
Tablo 3. *Hidrosollerin P. fluorescens'e karşı antibakteriyel etkisi*

Plant Name	Bacteria Counts (log cfu/ml) (mean±SD)		
	10th minute	30th minute	60th minute
Physiological saline	6.42±0.10	6.41±0.10	6.41±0.10
Thyme	0	0	0
Sumach	5.90±0.08	5.63±0.18	5.42±0.22
Clove	0	0	0
Nettle	6.13±0.08	5.99±0.12	5.97±0.12
Angelica	6.08±0.08	5.91±0.13	5.85±0.12
Acacia	6.19±0.04	6.18±0.04	6.16±0.06
Oak	6.11±0.06	6.09±0.08	6.08±0.07
Sage	5.74±0.06	5.58±0.07	5.55±0.05
Juniper	6.37±0.05	6.31±0.02	5.76±0.13
Rosemary	5.24±0.06	6.01±0.02	3.68±0.07
Echinacea	6.05±0.05	6.01±0.02	5.61±0.15
Green tea	6.22±0.08	6.19±0.08	6.00±0.09
Basil	6.35±0.09	6.33±0.10	6.18±0.10
Myrtle	6.05±0.19	5.82±0.05	5.66±0.15
Walnut	6.31±0.10	6.29±0.09	6.04±0.08
Laurel	5.78±0.04	5.68±0.08	5.58±0.05
Mint	6.00±0.06	5.93±0.12	5.91±0.66
Strawflower	6.27±0.23	6.28±0.03	6.26±0.04
Daisy	6.28±0.07	6.25±0.07	6.20±0.06
Hypericum	5.68±0.19	5.67±0.17	5.48±0.20

Table 4. *The antibacterial activity of hydrosols against P. aeruginosa*
Tablo 4. *Hidrosollerin P. aeruginosa'ya karşı antibakteriyel etkisi*

Plant Name	Bacteria Counts (log cfu/ml) (mean±SD)		
	10th minute	30th minute	60th minute
Physiological saline	6.13±0.64	6.11±0.63	6.10±0.64
Thyme	0	0	0
Sumach	5.54±0.52	5.38±0.50	5.00±0.49
Clove	0	0	0
Nettle	5.93±0.51	6.06±0.66	6.05±0.65
Angelica	6.07±0.60	6.06±0.59	6.01±0.62
Acacia	6.21±0.53	5.95±0.63	5.88±0.59
Oak	5.79±0.54	5.78±0.53	5.71±0.55
Sage	5.89±0.59	5.69±0.59	5.68±0.58
Juniper	6.01±0.59	4.56±0.70	4.51±0.69
Rosemary	5.17±0.05	3.71±0.45	3.10±0.29
Echinacea	6.02±0.58	6.00±0.58	5.94±0.58
Green tea	6.08±0.58	6.03±0.58	6.01±0.58
Basil	6.10±0.58	6.09±0.57	6.06±0.58
Myrtle	5.86±0.57	4.88±0.73	4.78±0.61
Walnut	6.02±0.58	5.97±0.56	5.91±0.57
Laurel	5.56±0.57	5.39±0.59	4.86±0.57
Mint	5.60±0.64	5.40±0.62	5.33±0.56
Strawflower	5.83±0.62	5.78±0.58	5.58±0.57
Daisy	6.06±0.55	6.06±0.54	6.05±0.54
Hypericum	5.87±0.35	5.51±0.57	5.36±0.56

Table 5. *The antibacterial activity of hydrosols against E. coli*
Tablo 5. *Hidrosollerin E. coli'ye karşı antibakteriyel etkisi*

Plant Name	Bacteria Counts (log cfu/ml) (mean±SD)		
	10th minute	30th minute	60th minute
Physiological saline	6.46±0.02	6.46±0.02	6.43±0.04
Thyme	0	0	0
Sumach	5.96±0.15	5.93±0.16	5.72±0.14
Clove	0	0	0
Nettle	6.14±0.06	6.06±0.01	6.11±0.09
Angelica	6.02±0.02	6.00±0.06	5.94±0.06
Acacia	6.19±6.20	6.15±0.01	6.13±0.01
Oak	6.11±0.00	5.96±0.03	5.83±0.02
Sage	5.96±0.03	5.88±0.08	5.68±0.14
Juniper	6.26±0.03	6.25±0.04	6.22±0.02
Rosemary	6.08±0.02	6.02±0.02	5.90±0.00
Echinacea	6.22±0.04	6.19±0.04	6.14±0.00
Green tea	6.01±0.08	6.01±0.09	5.97±0.08
Basil	6.23±0.01	6.19±0.01	6.16±0.03
Myrtle	6.01±0.02	5.96±0.05	5.85±0.07
Walnut	6.26±0.04	6.23±0.02	6.20±0.03
Laurel	5.94±0.06	5.89±0.05	5.54±0.08
Mint	6.30±0.02	6.26±0.01	6.23±0.00
Strawflower	6.26±0.02	6.24±0.03	6.16±0.01
Daisy	6.14±0.03	6.09±0.02	6.07±0.03
Hypericum	6.06±0.01	6.05±0.01	5.99±0.05

DISCUSSION

Many naturally occuring extracts like essential oils from edible and medicinal plants herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens [1]. It is known that the compositions of hydrosols and their antimicrobial effects depend on plant species and regional conditions [4].

Several studies have been conducted on the antimicrobial properties of herbs, spices and their derivatives such as essential oils, extracts and decoctions (5-10) but attention has not been focused intensively on studying antimicrobial effect of plant hydrosols. Abu-Shanab et al.[11] reported that while ethanolic, methanolic and hot water extracts of sage *(Salvia officinalis)*, thyme *(Thymus vulgaris)* and rosemary *(Rosmarinus officinalis)* had no inhibitoric effect, methanolic and ethanolic extracts of clove *(Syzygium aromaticum)* had antibacterial effect against *Pseudomonas aeruginosa*. In contrast to Abu-Shanab et al.[11], in the present study, thyme *(Thymus serpyllum)* and rosemary demonstrated antibacterial activity. Similarly while clove had the strongest antimicrobial effect, sage didn't show

this impact against *P. aeruginosa*.

In a study conducted by Sağdıç and Özcan [4], researchers investigated antibacterial activity of hydrosols against microorganisms including *E. coli* and they found that basil *(Ocimum basilicum)*, laurel *(Laurus nobilis)*, mint *(Mentha spicata)*, rosemary *(Rosmarinus officinalis)*, sage *(Salvia aucheri)* and sumach *(Rhus coriaria)* hydrosols were ineffective. Our results are in agree with that of their study.

Nascimento et al.[12] investigated inhibitoric effect of plant extracts on antibiotic resistant bacteria and they reported that thyme *(Thymus vulgaris)*, clove *(Caryophyllus aromaticus)* and basil *(Ocimum basilicum)* were effective against *P. aeruginosa* but they didn't affect *E. coli*. They also found that rosemary *(Rosmarinus officinalis)* and sage *(Salvia officinalis)* didn't have antibacterial effect against both of these two strains. Similarly, in this study thyme and clove inhibited *P. aeruginosa* but basil didn't demonstrate this activity. We also found that thyme and clove had antimicrobial effect against *E. coli*. This may be due to the differences in the methods applied or strains used.

Proestos et al.[13] showed that nettle *(Urtica dioica)* had strong antibacterial activity against *Staph. aureus, L. monocytogenes* and *B. cereus* and had weak antimicrobial effect against *P. putida* and *E. coli* O157:H7. However in the present study, nettle slightly reduced *P. fluorescens* at 30th min but didn't show antibacterial effect against other tested bacteria.

Consequently, some of the plant hydrosols investigated in this study exerted varying levels of antimicrobial effects against the four test bacteria. Thyme and clove hydrosols showed the highest antibacterial activity followed by rosemary. Our results suggest that the use of some plant hydrosols as antimicrobial agents may be exploitable to prevent deterioration of stored foods by bacteria, as long as the taste impact is acceptable in targeted foods. As a matter of fact, Oral et al.[14] represented *Enterobacteriaceae, Pseudomonas* and *Aeromonas* counts of fresh water fish which treated with the wild thyme hydrosol *(Thymus serpyllum)* were lower than that of controls after 20 days of refrigerated storage period. From the findings, the researchers indicated that wild thyme hydrosol substantially contributes to the extension of shelf life of Transcaucasian barb stored on ice, delaying spoilage while imparting a pleasant flavor to fish.

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REFERENCES

1. **Oussallah M, Caillet S, Saucier L, Lacroix M:** Antimicrobial effects of selected plant essential oils on the growth of a *Pseudomonas putida* strain isolated from meat. *Meat Sci,* 73, 236-244, 2006.
2. **Mahmoud BSM, Yamazaki K, Miyashita K, Shin II, Suzuki T:** A new technology for fish preservation by combined treatment with electrolyzed NaCl solutions and essential oil compounds. *Food Chem,* 99 (4): 656-662, 2006.
3. **Farag RS, Daw ZY, Hewedi FM, El-Baroty GSA:** Antimicrobial activity of some egyptian spice essential oils. *J Food Prot,* 52, 665-667, 1989.
4. **Sağdıç O, Özcan M:** Antibacterial activity of Turkish spice hydrosols. *Food Cont,* 14, 141-143, 2003.
5. **Deans SG, Svoboda KP:** Antimicrobial activity of summer savory *(Satureja hortensis L.)* essential oil and its constituents. *J Horticult Sci,* 64, 205-210, 1989.
6. **Dorman HJD, Deans SG:** Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J Appl Microbiol,* 88, 308-316, 2000.
7. **Hammer KA, Carson CF, Riley TV:** Antimicrobial activity of essential oils and other plant extracts. *J Appl Microbiol,* 86, 985-990, 1999.
8. **Hsieh PC, Mau JL, Huang SH:** Antimicrobial effect of various combinations of plant extracts. *Food Microbiol,* 18, 35-43, 2001.
9. **Özcan M, Boyraz N:** Antifungal properties of some herb decoctions. *Eur Food Res Technol,* 212, 86-88, 2000.
10. **Özcan M, Erkmen O:** Antimicrobial activity of the essential oils of Turkish plant spices. *Eur Food Res Technol,* 212, 658-660, 2001.
11. **Abu-Shanab B, Adwan G, Abu-Safiya D, Jarrar N, Adwan K:** Antibacterial activities of some plant extracts utilized in popular medicine in palestine. *Turk J Biol,* 28, 99-102, 2004.
12. **Nascimento GGF, Locatelli J, Freitas PC, Silva GL:** Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz J Microbiol,* 31, 247-256, 2000.
13. **Proestos C, Boziaris IS, Nychas G-JE, Komaitis M:** Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. *Food Chem,* 95, 664-671, 2006.
14. **Oral N, Gülmez M, Vatansever L, Güven A:** Application of antimicrobial ice for extending shelf life of fish. *J Food Prot,* 71 (1): 218-222, 2008.