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The Use of Essential Oils of *Origanum rotundifolium* as Antimicrobial Agent Against Plant Pathogenic Bacteria

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Abstract: In this study, the antibacterial effect of *Origanum rotundifolium* Boiss's essential oil was evaluated against 20 plant pathogenic bacteria. The essential oil (EO) of *O. rotundifolium* was hydrodistilled from wild *O. rotundifolium* plants that grown in Eastern Anatolia region of Turkey. Then, 10 chemical compounds of EO were identified via gas chromatography/mass spectrometry (GC-MS). The main compounds were determined as thymol (40.86 %-GC), carvacrol (43.62 %), *p*-cymene (5.95 %) and borneol (2.49 %). According to the results of the study, the essential oil of *O. rotundifolium* exhibits considerable antibacterial effect against pathogenic bacteria that cause significantly crop loss.

Key words: *O. rotundifolium*, Plant pathogen, Antibacterial activity, GC-MS, thymol, carvacrol, *p*-cymene.

Introduction

Plant diseases causing crop loss are major problem for global food production as old as the history of human-beings. Because, agricultural plants are continually exposed by many factors present in their area like fungi, viruses, and especially plant pathogenic bacteria ^{1,2}. Despite all the plants have many mechanisms and metabolites for defending against microbial diseases, there is sometimes no possibility to control severe plant diseases caused by bacteria ³. Nowadays, the plant diseases

caused by bacteria are generally controlled in a short time with synthetic substances or antibiotics that have been used since the 1950s ⁴. Streptomycin and oxytetracyclin were used the most commonly. But, with revealing of their undesirable effects of the synthetic substances and antibiotics on the environment owing to their longstanding biodegradation in the ecosystems and their several serious side effects on mammalian health owing to accumulation in the food chain, the use of antibiotics like streptomycin is already

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forbidden in most countries of the European Union^{2, 4, 5, 6}. Therefore, nowadays the researchers have focused to find alternative harmless substances such as plants extracts and essential oils to overcome the significant crop loss caused by plant pathogenic bacteria^{4, 7}. *O. rotundifolium* is a member of *Origanum* genus belonging to the Lamiaceae (Labiatae) family. This plant known in Anatolia as ‘Mercan kösk’ is widely used as food additives and folk medicine because of its rich essential oil composition. Due to the chemical composition of its EO, it shows various biological activities such as antioxidant, antibacterial properties that have been linked compounds such as thymol, carvacrol, borneol, terpinen chemotype and spathulenol^{2, 8, 9}. In the present study, it was aimed to identified the chemical constituents of hydrodistilled *O. rotundifolium*’s EO via GC-MS and to evaluate its antibacterial activity against plant pathogen bacteria which have not been studied in previous.

Experiments

Preparation of plant material

O. rotundifolium plants were harvested during the flowering stage in July 2009, from the eastern part of Erzurum city of Turkey. Prof. Dr. Yusuf Kaya from the Department of Biology of Erzurum Ataturk University was approved the identification of plant materials. Herbarium samples of *O. rotundifolium* plants have been kept in herbarium of Science Faculty, Ataturk University, Erzurum.

Isolation of the essential oil

Plant samples were dried in a canopy room. The aerial parts (leaves, flowers and stems) of the plant were powdered with blender and then subjected to water distillation for 2-3 h in a Clevenger-type apparatus (Thermal Laboratory Equipment, Turkey). The EO was stored at +4°C for further studies.

Determination of the chemical compounds

The chemical compounds of the EO was identified through a Thermofinnigan Trace GC/Trace DSQ/A1300, (E.I Quadrapole) equipped with a SGE-BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm). All the conditions of GC-MS analyses were set according to Cetin *et al.*, 2009. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards’, Wiley 7N library data of the GC-MS system and literature data. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature^{10, 11, 12}.

Plant pathogenic bacterial strains

The *O. rotundifolium*’s EO was evaluated in respect to antibacterial activity against 20 pathogenic bacterial strains for many commercial plants. The bacteria shown in Table 1 were isolated from commercial fruits and vegetables, which they have typical bacterial disease symptoms. They were

Table 1. Plant pathogenic bacterial species used in the study

Bacteria	Host	Strain No
<i>Agrobacterium tumefaciens</i>	Apricot	AA-685
<i>Bacillus pumilus</i>	Apricot	AA-479
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	Tomato	AA-703
<i>Enterobacter intermedius</i>	Cherry	AA-184
<i>Erwinia caratovora</i> subsp. <i>caratovora</i>	Tomato	AA-687
<i>Erwinia chrysanthemi</i>	Apricot	AA-58
<i>Pseudomonas cichorii</i>	Peach	AA-234
<i>Pseudomonas corrugate</i>	Tomato	AA-684
<i>Pseudomonas fluorescens</i>	Apricot	AA-616
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	Cherry	AA-218
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	Apricot	AA-637

table 1. (continued).

Bacteria	Host	Strain No
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	Apricot	AA-638
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	Apricot	AA-647
<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>	Beans	AA-652
<i>Pseudomonas syringae</i> pv. <i>pisi</i>	Peach	AA-237
<i>Pseudomonas syringae</i> pv. <i>tabaci</i>	Apricot	AA-704
<i>Pseudomonas syringae</i> pv. <i>tomato</i>	Cherry	AA-220
<i>Ralstonia solanacearum</i>	Apricot	AA-116
<i>Xanthomonas axonopodis</i> pv. <i>campestris</i>	Pepper	AA-705
<i>Xanthomonas vesicatoria</i>	Tomato	AA-683

identified via a microbial identification system (MIS) ¹³. The isolated and identified bacterial cultures were preserved in Luria broth and 30 % glycerol solutions at -80°C prior to use.

Antimicrobial activity

Disc diffusion assay

Two-fold serial dilutions of the EO was made by diluting 10 % DMSO to prepare a decreasing concentration between 500 µl/ml to 7.81 µl/ml. Antibacterial activity was determined by using disc diffusion assay. 100 µl of suspension containing 10⁸ cfu/ml of bacteria spread on tryptic soy agar (TSA) medium by a sterile swab ¹⁴. The discs (6 mm in diameter) were impregnated with 10 µl of the EO and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dilute the EO. Positive controls were prepared using the antibiotics as indicated in Table 2. The bacterial cultures were incubated at 27°C for 48 h. Antimicrobial activity was determined via measurement the zone of inhibition. Each test assays were repeated triplicate ^{4,11,12}.

Micro dilution assay

The minimal inhibition concentration (MIC) values were determined for the bacteria determined as sensitive to the EO in disc diffusion assay. The inocula of bacteria were prepared from 12 h broth cultures and cultures were adjusted to 0.5 McFarland Standard turbidity. The EO concentrations were prepared between 500 µl/ml to 7.81 µl/ml and tested in 10 ml sterile test tubes con-

taining tryptic soy broth (TSB). MIC values of the EO against bacterial strains were determined based on a micro-well dilution method ^{15, 16}. The 96-well plates were prepared by dispensing into each well 95 µl of tryptic soy broth and 5 µl of the inoculum. Then 100 µl from EO at all the prepared concentrations were added into the wells. Negative control was prepared as the last well containing 195 µl tryptic soy broth without EO and 5 µl of the bacteria. The concentrations of Maxipime (Bristol-Myers Squibb) between 500-7.81 µl were prepared in TSB and used as positive control. The plate was mixed on plate shaker at 300 rpm for 20 s, and incubated at 27°C for 24 h. Bacterial growth was determined by absorbance at 600 nm using the EL×800 universal microplate reader. The EO was tested against all the bacteria for three times. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms ^{12,17}.

Results and discussion

The EO of *O. rotundifolium* showed antibacterial activity against all tested bacterial strains (7-45 mm inhibition zone). MIC values showed by the EO of *O. rotundifolium* were in the range of 7.81 - 62.5 µl/ml. The results are given in Table 2. *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Clavibacter michiganensis* subsp. *michiganensis*, *Enterobacter intermedius*, *Erwinia caratovora caratovora*, *Erwinia chrysanthemi*, *Pseudomonas corrugata*, *Pseudomonas fluorescens*, *Pseudomonas*

Table 2. Antibacterial activities of the essential oil of *O. rotundifolium*

Bacterium	Concentration							MIC	Negative control DMSO	Positive control Standard antibiotic discs
	500 µl	250 µl	125 µl	62.5 µl	31.25 µl	15.63 µl	7.81 µl			
<i>Agrobacterium tumefaciens</i>	48	48	47	13	13	10	8	7.81	-	29 (SCF)
<i>Bacillus pumilus</i>	47	10	10	10	9	8	7	7.81	-	23 (OFX)
<i>Clavibacter michiganensis</i> ssp. <i>michiganensis</i>	48	47	47	47	11	11	9	7.81	-	26 (SCF)
<i>Enterobacter intermedius</i>	16	14	12	11	10	9	8	7.81	-	26 (SCF)
<i>Erwinia caratovora caratovora</i>	48	48	48	48	12	11	9	7.81	-	30 (OFX)
<i>Erwinia chrysanthemi</i>	47	47	47	11	10	8	8	7.81	-	25 (SCF)
<i>Pseudomonas cichorii</i>	10	9	8	8	7	-	-	31.25	-	25 (OFX)
<i>Pseudomonas corrugate</i>	48	47,5	47,5	12	12	11	10	7.81	-	26 (OFX)
<i>Pseudomonas fluorescens</i>	45	10	9	9	8	8	7	7.81	-	10 (OFX)
<i>Pseudomonas syringae</i> pv. <i>syringae</i> ***	48	48	48	11	10	10	9	7.81	-	25 (OFX)
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	8	8	8	8	-	-	-	62.5	-	20 (OFX)
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	8	8	8	7	-	-	-	62.5	-	20 (OFX)
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	8	7	7	7	7	-	-	31.25	-	20 (OFX)
<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>	14	11	10	9	7	7	-	15.63	-	24 (OFX)
<i>Pseudomonas syringae</i> pv. <i>pisi</i>	14	10	10	9	8	8	-	15.63	-	24 (OFX)
<i>Pseudomonas syringae</i> pv. <i>tabaci</i>	10	9	8	8	7	-	-	31.25	-	23 (OFX)
<i>Pseudomonas syringae</i> pv. <i>tomato</i>	11	10	10	10	9	9	8	7.81	-	24 (OFX)
<i>Ralstonia solanacearum</i>	48	48	48	47	15	10	10	7.81	-	22 (SCF)
<i>Xanthomonas axonopodis</i> pv. <i>campestris</i>	45	15	13	10	8	-	-	31.25	-	20 (SCF)
<i>Xanthomonas vesicatoria</i>	45	15	12	8	7	7	7	7.81	-	21 (SCF)

*DMSO; Dimethyl sulfoxide (10 %); ** OFX, ofloxacin (10 µg/disc)

SCF, sulbactam (30 µg/disc) + cefoperazone (75 µg) (105 µg/disc) were used as positive reference standard antibiotic discs (oxid)

*** from different host (cherry)

syringae pv. *syringae* (from cherry), *Pseudomonas syringae* pv. *tomato*, *Ralstonia solanacearum* and *Xanthomonas vesicatoria* were determined the most sensitive microorganism (MIC value 7.81) against to EO. The other sensitive microorganisms were *Pseudomonas syringae* pv. *phaseolicola* and *Pseudomonas syringae* pv. *pisi* (MIC value 15.63); *Pseudomonas cichorii*, (1) *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *tabaci* and *Xanthomonas axonopodis* pv. *campestris* (MIC value 31.25). MIC values of 2 different strains of *Pseudomonas syringae* pv. *syringae* isolated from apricot were determined higher level than the others (MIC value 62.5). Additionally, it was observed that *P. s.* pv. *syringae* strains had not equal sensitivity to the EO. *P. s.* pv. *syringae* strain from isolated cherry was more sensitive than *P. s.* pv. *syringae* strains. These results can be observed because of differences in host, virulent of pathogens, toxins produced by these pathogens.

There are many reports about the chemical compositions, antimicrobial and antioxidant activities of the essential oils obtained from various *Origanum* species and their commercial applications.¹⁸ According to the literature, the extracts or EO of *O. rotundifolium* possess antagonistic activity against a lot of bacteria^{2,19,20}. The previous studies have been demonstrated that *Origanum* species have shown a stronger antibacterial activity against tested bacteria. Our data generally confirmed previous findings. However, the EO of *O. rotundifolium* have never been tested against plant pathogenic bacteria. According to our knowledge, this study is the first report about inhibitory activity of the EO against plant pathogenic bacteria. Besides, this research exhibited that the average of inhibition zones of the EO were more effective and powerful than antibiotics used as positive control even at low concentration. Thus, it is thought that the findings of the present study are valuable for further investigations to discover new biopesticide.

According to the recent studies, there was a significant correlation between main compounds and biological activities of plant essential oils. Oregano essential oils with high concentrations of thymol and carvacrol usually inhibit pathogenic

bacteria^{11,21,22}. The results of the research suggest the possibility of using the selected essential oils or their main compounds such as carvacrol and thymol as natural food preservatives, because some of these possess strong antibacterial activities. Up to now, there are a lot of studies that previously cited *Origanum* species have shown that are rich in carvacrol and thymol^{2,23}. The results of this study generally confirmed the findings of previous studies. The EO of *O. rotundifolium* were also characterized by high amounts of thymol and carvacrol, with small variations in this study. The results presented in Table 3. On the other hand, in this study, the chemical composition of the essential oil has been determined as 10 compounds. The determined compounds and their amounts from analyzed essential oil are that; thymol (40.86 %), carvacrol (43.62 %) and p-cymene (5.95 %) have been high observed. According to previous studies, the essential oils of *O. rotundifolium* from different localities in Turkey have been found to differ qualitatively and quantitatively^{8,9,11,20,24,25}. Goze *et al.* examined the composition of essential oils of *O. rotundifolium*⁹. There is no concordance between the essential oils identified by our study and Goze *et al.* and the compounds identified and found major components of their study were as follows: borneol (15.99 %-GC and 28.21 %-FID), terpinen-4-ol (18.74 %-GC and 8.64 %-FID), and spathulenol (9.88 %-GC and 8.89 %-FID) on the other hand, other studies by Dikbas *et al.* has been characterized by the presence of carvacrol, while thymol was absent or detectable in small amounts and traces^{9,20}. Baser *et al.* have been observed *cis*-sabinene hydrate (21.5 %), linalyl acetate (7.5 %), α -terpineol (6.2 %), β -caryophyllene (6.1 %), and *trans*-sabinene hydrate (4.2 %) in essential oils isolation from *O. rotundifolium*⁸. Cetin *et al.* have been found that essential oil of *O. rotundifolium* contained mainly carvacrol (54.6 %), p-cymene (12.5 %), borneol (5.9 %) and thymol (3.5 %) together with linalool (1.8 %) and terpinene-4-ol (1.5 %)²⁶. These findings indicate that the compositions of the EO can be affected with several factors such as local, climatic, seasonal, experimental conditions, the influence of water stress, and the origin, the chemotype, chemi-

Table 3. Essential oil content of *O. rotundifolium*

No.	RI	RT	Components	(%)	Identification methods
1	1034	14.21	p-Cymene	5.95	GC, MS, RI
2	1067	15.74	γ -Terpinene	1.52	GC, MS, RI
3	1079	16.61	cis-Sabinenehydrate	0.56	GC, MS, RI
4	1172	21.58	Borneol	2.49	GC, MS, RI
5	1178	21.99	Terpinen-4-ol	0.63	GC, MS, RI
6	1289	27.34	Thymol	40.86	GC, MS, RI
7	1296	27.72	Carvacrol	43.62	GC, MS, RI
8	1419	32.23	β -Caryophyllene	2.44	GC, MS, RI
9	1442	33.05	Aromadendrene	0.22	GC, MS, RI
10	1579	39.58	Caryophyllene oxide	1.71	GC, MS, RI

RI* - Retention index relative to n-alkanes on SGE-BPX5 capillary column

RT** - Retention times

GC - Identification was based on retention times of authentic compounds on SGE-BPX5 capillary column

MS - Tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data

RI - Identification was based on comparison of retention index with those of published data

cal polymorphism, and the stage of the plant materials^{11,12, 20, 21}.

In conclusion, the present results show that essential oil of *O. rotundifolium* may be used as potential antibacterial agents against plant pathogenic bacteria for the first time. Thus, the devel-

opment of natural antimicrobials will help to decrease the negative environmental effects of chemical compounds and antibiotics. However, further studies will need to be performed for determination of the safety and toxicity of the EO.

References

1. **Young, J.M., Bradbury, J.F., Vidaver, A.K. (1990).** The impact of molecular biological studies on the nomenclature of plant pathogenic bacteria. Plant Pathogenic Bacteria, Pts a and B: Proceedings of the 7th International Conference on Plant Pathogenic Bacteria, ed. Z. Klement, 659-661.
2. **Kotan, R., Cakir, A., Dadasoglu, F., Aydin, T., Cakmakci, R., Ozer, H., Kordali, S., Mete, E., Dikbas, N. (2010).** Antibacterial activities of essential oils and extracts of Turkish *Achillea*, *Satureja* and *Thymus* species against plant pathogenic bacteria. Journal of the Science of Food and Agriculture 90: 145-160.
3. **Kang, S.C., Bajpai, V.K., Dung, N.T., Suh, H.J. (2010).** Antibacterial Activity of Essential Oil and Extracts of *Cleistocalyx operculatus* Buds Against the Bacteria of *Xanthomonas* spp. Journal of the American Oil Chemists Society 87: 1341-1349.
4. **Yanmis, D., Gormez, A., Bozari, S., Orhan, F., Gulluce, M., Agar, G., Sahin, F. (2012).** Determination of chemical composition and antibacterial properties of essential oil of *Mentha longifolia* ssp. *longifolia* against phytopathogenic bacteria. Microbes in Applied Research, 531-535.
5. **McManus, P.S., Stockwell, V.O., Sundin, G.W., Jones, A.L. (2002).** Antibiotic use in plant agriculture. Annual Review of Phytopathology, 40: 443.
6. **Horvath, G., Kovacs, K., Kocsis, B. and Kustos, I. (2009).** Effect of Thyme (*Thymus vulgaris* L.) Essential Oil and Its Main Constituents on the Outer Membrane Protein Composition

- of *Erwinia* Strains Studied with Microfluid Chip Technology. *Chromatographia* 70: 1645-1650.
7. **Costa, T.R., Fernandes, O.F.L., Santos, S.C., Oliveira, C.M.A., Liao, L.M., Ferri, P.H., Paula, J.R., Ferreira, H.D., Sales, B.H.N., Silva, M.D.R. (2000).** Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. *Journal of Ethnopharmacology* 72: 111-117.
 8. **Baser, K., Ozek, T., Tumen, G., Sezik, E. (1993).** Composition of the essential oils of Turkish *Origanum* species with commercial importance. *Journal of Essential Oil Research. JEOR (USA)*, 1993.
 9. **Goze, I., Alim, A., Tepe, A.S., Sokmen, M., Sevgi, K., Tepe, B. (2009).** Screening of the antioxidant activity of essential oil and various extracts of *Origanum rotundifolium* Boiss. from Turkey. *Journal of Medicinal Plants Research* 3: 246-254.
 10. **Bozari, S., Agar, G., Aksakal, O., Erturk, F.A., Yanmis, D. (2012).** Determination of chemical composition and genotoxic effects of essential oil obtained from *Nepeta nuda* on *Zea mays* seedlings. *Toxicology and industrial health* 29: 339-348.
 11. **Cetin, B., Cakmakci, S., Cakmakci, R. (2011).** The investigation of antimicrobial activity of thyme and oregano essential oils. *Turkish Journal of Agriculture and Forestry* 35:145-154.
 12. **Gormez, A., Bozari, S., Yanmis, D., Gulluce, M., Agar, G., Sahin, F. (2013).** Antibacterial activity and chemical composition of essential oil obtained from *Nepeta nuda* against phytopathogenic bacteria. *Journal of Essential Oil Research* 25:149-153.
 13. **Miller, I. and Berger, T. (1985).** Bacteria identification by gas chromatography of whole cell fatty acids. *Hewlett Packard Gas Chromatography Application Note*, Hewlett Packard Co., Alto, CA., pp 228-238.
 14. **Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Tenover, R.H. (1995).** *Manual of Clinical Microbiology*. (eds) American Society for Microbiology, Washington, D.C, 1482.
 15. **Zgoda, J.R. and Porter, J.R. (2001).** A convenient microdilution method for screening natural products against bacteria and fungi. *Pharmaceutical Biology* 39: 221-225.
 16. **Sonboli A, Gholipour A, Yousefzadi M. & Mojarad M. (2009).** Antibacterial Activity and Composition of the Essential Oil of *Nepeta menthoides* from Iran. *Natural Product Communications*. 4: 283-286.
 17. **Shafaghat, A. and Oji, K. (2010).** Nepetalactone Content and Antibacterial Activity of the Essential Oils from Different Parts of *Nepeta persica*. *Natural Product Communications*. 5: 625-628.
 18. **Chinou, I.B., Aligiannis, N., Kalpoutzakis, E., Mitaku, S. (2001).** Composition and antimicrobial activity of the essential oils of two *Origanum* species. *Journal of Agricultural and Food Chemistry*. 49: 4168-4170.
 19. **Aslim, B. and Yucel, N. (2008).** *In vitro* antimicrobial activity of essential oil from endemic *Origanum minutiflorum* on ciprofloxacin-resistant *Campylobacter* spp. *Food Chemistry*. 107: 602-606.
 20. **Cetin, B., Cakmakci, S., Cakmakci, R. (2011).** The investigation of antimicrobial activity of thyme and oregano essential oils. *Turkish Journal of Agriculture and Forestry*. 35: 145-154.
 21. **Nevas, M., Korhonen, A.R., Lindstrom, M., Turkki, P., Korkeala, H. (2004).** Antibacterial efficiency of Finnish spice essential oils against pathogenic and spoilage bacteria. *Journal of Food Protection*. 67: 199-202.
 22. **Bozin, B., Mimica-Dukic, N., Simin, N., Anackov, G. (2006).** Characterization of the volatile composition of essential oils of some lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *Journal of Agricultural and Food Chemistry*. 54: 1822-1828.
 23. **Sokmen, M., Serkedjieva, J., Daferera, D., Gulluce, M., Polissiou, M., Tepe, B., Akpulat, HA, Sahin, F., Sokmen, A. (2004).** *In vitro* antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*.

-
- Journal of Agricultural and Food Chemistry. 52: 3309-3312.
24. **Baser, K., Ozek, T., Tumen, G., Sezik, E. (1993).** Composition of the essential oils of Turkish *Origanum* species with commercial importance. Journal of Essential Oil Research: JEOR (USA).
 25. **Goze, I., Alim, A., Tepe, A.S., Sokmen, M., Sevgi, K., Tepe, B. (2009).** Screening of the antioxidant activity of essential oil and various extracts of *Origanum rotundifolium* Boiss. from Turkey. Journal of Medicinal Plants Research. 3: 246-254.
 26. **Dikbas, N., Bagci, E., Kotan, R., Cakmakci, R., Ozer, H., Mete, E., Erdogan, G. (2010).** Comparative antibacterial activities and chemical composition of some plants' oils against *Salmonella enteritidis*. Research on Crops. 11: 118-124.