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Chemical Composition and Antibacterial Activity of Essential Oils Isolated from Medicinal Plants against Gall Forming Plant Pathogenic Bacterial Disease Agents

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ABSTRACT
The aims of this study were to determine chemical composition of essential oils from different plant species and their antibacterial activities against gal-forming plant pathogenic bacterial disease agents *Rhizobium radiobacter*, *Pseudomonas savastanoi* pv. *savastanoi* and *P.savastanoi* pv. *nerii*. The chemical compositions of essential oils were identified by GC/MS. The major constituents of the essential oils were carvacrol in *Thymbra spicata* var. *spicata* (66.88%) and *Origanum syriacum* (79.8%), thymol in *Thymus serpyllum* (41.03%), geranial in *Thymus sipyleus* (13.72%) and *Melissa officinalis* (30.4), 4-terpineol in *Origanum majorana* (31.67%), linalool in *Ocimum basilicum* (30.23%), carvone in *Mentha spicata* (55.58%), 1,8 cineole in *Lavandula stoechas* var. *stoechas* (35.5%), *Laurus nobilis* (35.5%) and *Rosmarinus officinalis* (18.47%), camphor in *Salvia officinalis* (24.59%) and *trans*-anethole in *Foeniculum vulgare* (82.8%) essential oils, respectively. Based on inhibition zone diameter values, essential oils showed very strong antibacterial activities against *P.savastanoi* pv. *savastanoi* (7.0-44.67 mm), followed by *R.radiobacter* (9.6-37.67 mm) and *P.savastanoi* pv. *nerii* (6.33-18.33 mm). Essential oils of plants belong to Lamiaceae family were generally found to be more efficient than those belong to Lauraceae and Apiaceae families. The essential oils of *O.syriacum, T.serpyllum* and *T.spicata* var. *spicata* were found to be the most promising essential oils displaying the highest antibacterial activities against all tested bacterial species. The findings of the present study revealed that essential oils have a potential to be used as antibacterial agents against gall forming bacterial disease agents.

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Tıbbi Bitkilerden Elde Edilen Uçucu Yağların Kimyasal Bileşimi ve Gal Oluşturan Bitki Patojeni Bakteriyel Hastalık Etmenlerine Karşı Antibakteriyel Etkinlikleri

ÖZET
Bu çalışmanın amacı, farklı bitki türlerinden elde edilen uçucu yağların kimyasal bileşimini ve gal (ur) oluşturan bitki patojenik bakteriyel hastalık etmeni *Rhizobium radiobacter*, *Pseudomonas savastanoi* pv. *savastanoi* ve *P.savastanoi* pv. *nerii*'ye karşı antibakteriyel etkinlerini belirlemektir. *Thymbra spicata* var*. spicata, Thymus serpyllum, Thymus sipyleus, Origanum syriacum, Origanum majorana, Ocimum basilicum, Mentha spicata, Melissa officinalis, Lavandula stoechas* var. *stoechas, Rosmarinus officinalis, Salvia officinalis, Laurus nobilis* ve *Foeniculum vulgare* uçucu yağlarının kimyasal bileşenleri GC/MS ile tanımlanmıştır. Carvacrol *Thymbra spicata* var*. spicata* (% 66.88) ve *Origanum syriacum* (% 79.8), thymol *Thymus serpyllum* (% 41.03), geranial *Thymus sipyleus* (% 13.72) ve *Melissa officinalis* (30.4), 4-terpineol *Origanum majorana* (31.67 %), linalool *Ocimum basilicum* (% 30.23), carvone *Mentha spicata* (% 55.58), 1,8 cineole *Lavandula stoechas* var. *stoechas* (% 35.5), *Laurus nobilis* (% 35.5) ve *Rosmarinus officinalis* (% 18.47), camphor *Salvia officinalis* (% 24.59) ve *trans*-anethole ise *Foeniculum vulgare* (% 82.8)

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uçucu yağlarının ana bileşenleri olarak belirlenmiştir. Engelleme zon çapı değerlerine dayanarak, uçucu yağları *P.savastanoi* pv. *savastanoi* (7.0-44.67 mm), ardından *R.radiobacter* (9.6-37.67 mm) ve *P.savastanoi* pv. *nerii* (6.33-18.33 mm) ye karşı oldukça güçlü antibakteriyel etkinlik göstermiştir. Lamiaceae familyasına ait bitkilerin uçucu yağlarının genellikle Lauraceae ve Apiaceae familyalarına göre daha etkili olduğu bulunmuştur. *O.syriacum, T.serpyllum* ve *T.spicata* var. *spicata*, test edilen tüm bakteri türlerine karşı en yüksek antibakteriyel aktiviteleri gösteren umut verici uçucu yağlar olarak belirlenmiştir. Bu çalışmanın bulguları uçucu yağların gal oluşturan bakteriyel hastalık etmenlerine karşı antibakteriyel ajan olarak kullanılma potansiyeline sahip olduğunu göstermiştir.

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INTRODUCTION

Bacterial disease caused by different disease agents are responsible for huge economic losses in variety agriculturally important crop species in several agricultural commodities worldwide. Major diseases caused by different bacterial species are soft rots, wilts, galls and overgrowths, leaf spots and blights, scabs and cankers. Bacterial galls, also known as knots, are characterized by tumorous overgrowths produced on the stems, leaves, and roots of diverse economically relevant trees and shrubs infected with certain bacterial phytopathogens and constitute an important group of plant diseases that cause serious reductions in crop yields and considerable economic losses in nurseries and orchards such as stone-fruits, pome fruits, olive and oleander in several countries (Agrios, 2005). Formations of galls and knots on stems, twigs and leaves were incited by phytohormones produced by these bacterial isolates (Caballo-Ponce et al., 2017). Bacterial crown gall disease caused by *Rhizobium radiobacter* (Syn. *Agrobacterium tumefaciens*) is considered as the main bacterial disease of fruit rootstocks worldwide (Pulawska, 2010; Li et al., 2019). The olive knot disease caused by *Pseudomonas savastanoi* pv. *savastanoi* and the oleander knot disease caused by *Pseudomonas savastanoi* pv. *nerii* are one of the most widespread bacterial diseases of olive, pomegranate, myrtle, jasmine, Fontanesia and oleander plants worldwide (Young, 2004; Bella et al., 2006; Mirik et al., 2011; Mirik and Aysan, 2011; Ramos et al., 2012; Bozkurt et al., 2014). The bacterium also cause considerable deformations on the inflorescences and seed pods, and/or reduces blooming and eventual death of the plant (Caballo-Ponce et al., 2017). Olive knot disease is considered as an economically important problem for olive crops because of its effect on vegetative growth, olive yield, and olive oil qualities such as an unpleasant smell and a bitter, rancid taste (Godena et al., 2012; Mirik and Aysan, 2011).

Control of plant bacterial disease is generally preferred by the use of copper based synthetic pesticides and antibiotics (such as streptomycin). The use of copper compounds prevent bacterial multiplication but not always adequate control of bacterial disease (Nguyen et al., 2018). Disease control is difficult because of a copper resistance to the pathogens and lack of commercially acceptable resistant fruit cultivars (Rhouma et al., 2008). Frequent use of copper compounds and antibiotics against plant pathogenic bacteria has also led to the selection of resistant bacterial population against to chemicals used (Duman and Soylu, 2019). The high cost of frequent use of pesticides, development of pesticides/antibiotic resistant bacterial isolates, official restriction on the use of antibiotics against bacterial pathogens in European countries, including Turkey, and the public interest of environmental consideration raise the need to find environmentally friendly method(s) as novel alternative approaches to prevent tumor formation caused by these disease agents. Recent studies focused on plant-derived natural bactericides and their possible applications in agriculture to control plant bacterial diseases are being recently intensified. The antimicrobial activities of essential oils, extracts and their major constituents from a wide range of aromatic plants species have been investigated against the comprehensive range of microorganisms including bacteria, fungi and viruses (Burt, 2004; Bakkali et al., 2008).

The most important major essential oil bearing plant are usually dominated within the certain plant families such as Lamiaceae, Apiaceae, Lauraceae. Lamiaceae. Lamiaceae family comprises very diverse group of aromatic herbs and shrubs including lavender, oregano, thyme, peppermint, sage, and marjoram (Nieto, 2017; Mamadalieva et al., 2017). Apiaceae family contains a widely distributed group of annual, biennial and perennial plants, including dill, caraway and anise (Pannek et al., 2018). The Lauraceae family hosts over 2500 species such as bay leaf, cinnamon, avocado and sassafras (Rohwer, 1993). Observations in the eastern Mediterranean region of

Hatay province, Turkey indicate that there exist veld types with a rich composition of indigenous aromatic and medicinal plant species belonging Lamiaceae, Lauraceae and Apiaceae families. Although, very few studies were conducted to assess efficacy of essential oils from the medicinal plants, including those used in this study against crown gall disease agent *R. radiobacter* (Basim et al., 2000; Ateeq-ur-Rehman et al., 2009; Schollenberger et al., 2018), to the best of our knowledge there is no research has been conducted on antibacterial activities of essential oils from aromatic plants used in this study against the gall forming bacterial species causing bacterial knot, gall and tumor by *P. savastanoi* pv. *savastanoi* and *P. savastanoi* pv. *nerii* on woody plants.

This research was conducted to evaluate chemical compositions and the antibacterial potential of essential oils from different medicinal plant species, belonging to Lamiaceae (such as *Thymbra spicata* var. *spicata*, *Thymus serpyllum, Thymus sipyleus, Origanum syriacum, Origanum majorana, Ocimum basilicum, Mentha spicata, Melissa officinalis, Lavandula stoechas var. stoechas, Rosmarinus officinalis, Salvia officinalis*), Lauraceae (*Laurus nobilis*) and Apiaceae (*Foeniculum vulgare*) families growing in the region, against gall forming bacterial disease agents *R. radiobacter, P. savastanoi* pv. *savastanoi* and *P. savastanoi* pv. *nerri*.

MATERIAL and METHODS

Plant Material and Isolation of Essential Oils

All plant materials used in this study were collected from the wild populations of each plant species growing in Hatay Province of Turkey and identified by Prof.Dr. I. Uremis. Voucher specimens of all plant species have been deposited in the herbarium of the MKU BİSAK. Essential oils from seeds of *F. vulgare* and air dried leaves of the rest of the plant species were ground to fine powder for essential oil extractions by steam distillation through Clevenger's apparatus for 2.5 h. After extraction, all essential oils were dried over anhydrous sodium sulphate and stored in amber glass bottles at 4°C until required.

Determination of Chemical Compositions of the Essential oils

The chemical compositions of the essential oils was analyzed using GC-MS (Thermo Scientific Trace Ultra) linked to a Thermo Scientific ISQ mass selective detector equipped with a TR-FAME MS (Crosslinked 5% Phenyl Methyl Siloxane) capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness). Helium (99.9%) was used as the carrier gas at a flow rate of 1 mL / min.

The ionization 22 energy was set at 70 eV, the mass range m/z was 1.2-1200 amu. Scan Mode was used for data collection. The MS transfer line temperature was 250°C, the MS ionization temperature was 220°C, the injection port temperature was 220°C, the column temperature was initially 50°C and the temperature was increased to 220°C with a rate of heat increase of 3°C/min. Identification of the spectra of major components of essential oils were determined by comparing their retention indices and mass spectra with authentic spectra present in the library (Wiley 9) by using Xcalibur program (Adams, 2001).

Isolation, Identification and Culture of Bacterial Isolates

The bacterial isolates of *R. radiobacter, P. savastanoi* pv*. savastanoi* and *P. savastanoi* pv*. nerii* were isolated from their respective infected host plants plum, olive and oleander growing in nurseries in Hatay province of Turkey (Figure 1). Bacterial isolates were identified by MALDI-TOF (Bruker Daltonics GmbH, Bremen, Germany) analysis as described by Aktan and Soylu (2020). All isolates were preserved on King Medium B Agar at 4°C. Inoculum suspensions were prepared by transferring a loopful of cells from the stock cultures to 10 mL flasks of Nutrient Broth which were incubated for 24 h at 26°C. The cultures were diluted with fresh appropriate broth to adjust concentration to 10^8 cfu ml^{-1} by dilution to give OD_{640} of 0.12. These suspensions were used as required.

Determination of Antibacterial Activity of the Essential oil

The *in vitro* antibacterial activity of the each essential oils was determined by using the disc diffusion technique in Petri dishes. The surface of Petri dishes with King Medium B (Merck, Darmstadt, Germany) was inoculated with 200 µL of bacterial suspension prepared as described previously. Sterile filter paper (Whatman No.1) discs (6 mm in diameter) containing 10 µl of the tested essential oils were placed on the centre of the agar surface. Disc containing the 10 µl sterile broth media was used as negative control. The lid of each individual Petri dish was replaced immediately to prevent eventual evaporation. After allowing 1 h at room temperatures for the essential oils to diffuse across the surface, the plates were sealed with sterile parafilm and incubated at 25°C for 48 hr. The antibacterial activity of oils and antibiotics was demonstrated by a clear zone of inhibition around the disc (Figure 2). The zone of inhibition was measured with the help of Vernier calipers.

Statistical Analysis

All *in vitro* antibacterial experiments were performed twice with five replicate plates of each essential oil for each bacterium. The data were subjected to analysis of variance (ANOVA) by using SPSS statistic program (Version 17.0) and the significant differences between concentrations were determined by means of Duncan's

Figure 1. Typical disease symptoms, shown as gals and knots (arrows), caused by *R. radiobacter* on plum and apple crown (**A, B**), *P. savastanoi* pv. *savastanoi* on olive (**C**) and *P. savastanoi* pv. *nerri* on oleander (**D**) branches.

*Şekil 1. R. radiobacter tarafından erik (**A**) ve elma (**B**) fidanlarının kök boğazında, P. savastanoi pv. savastanoi tarafından zeytin (**C**) ve P. savastanoi pv. nerri tarafından zakkum (**D**) dalları üzerinde oluşturduğu tipik gal ve ur (ok) şeklindeki hastalık belirtileri.*

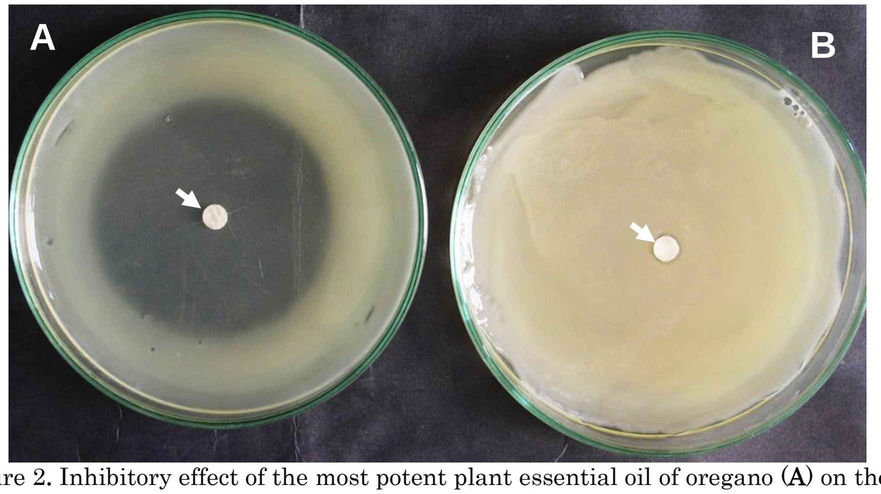

Figure 2. Inhibitory effect of the most potent plant essential oil of oregano (**A**) on the growth of *R. radiobacter*. Note clear zone of inhibition around the filter disc (arrow) containing 10 µl of the essential oils. (**B**) shows no inhibition caused by filter disc containing sterile broth medium.

*Şekil 2. Test edilen uçucu yağlar arasında en etkili olan Origanum uçucu yağının (**A**) R. radiobacter gelişiminin engelleme etkisi. 10 µl uçucu yağı içeren filtre kağıt diskinin etrafında ortaya çıkan şeffaf engelleme bölgesi (ok) açıkça görünmektedir. (**B**) Steril besi yeri içeren diskin herhangi bir engelleme etkinliğinin olmadığını gösterir.*

Multiple Range Test (P≤0.05).

RESULTS and DISCUSSIONS

The essential oil of medicinal and aromatics plans has been reported to contain a wide variety of compounds, such as phenolics, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites, which possess antimicrobial and antioxidant activities (Burt, 2004; Bakkali et al., 2008).

The chemical compositions of essential oils from seeds and leaves of each plant species were identified by gas chromatograph/mass spectrometer (GC/MS). Based on GC-MS analysis, total numbers of compounds determined in *T. sipyleus*, *R. officinalis, M. spicata, L. nobilis, O.basilicum, O. majorana, M.officinalis, S.officinalis, T. spicata* var. *spicata, L. stoechas* var. *stoechas*, *O. syriacum, T. serpyllum* and *F. vulgare* essential oils were 53, 43, 39, 39, 37, 37, 33, 32, 25, 25, 24, 17, 17, respectively (Table 1). Among the determined components, carvacrol was determined as the most abundant compound for *T. spicata* var. *spicata* (66.88%) and *O. syriacum* (79.8%), thymol for *T. serpyllum* (41.03%), geranial for *T. sipyleus* (13.72%) and *M. officinalis* (30.4), 4-terpineol for *O. majorana* (31.67%), linalool for *O. basilicum* (30.23%), carvone for *M. spicata* (55.58%), 1,8-cineole (eucalyptol) for *L. stoechas* var. *stoechas* (35.5%), *L. nobilis* (35.5%) and *R. officinalis* (18.47%), camphor for *S. officinalis* (24.59%) and *trans*-anethole for *F. vulgare* (82.8%) essential oils, respectively (Table 1).

Table 1. Major chemical compounds detected in the essential oils used in this study.
Çizelge 1. Çalışmada kullanılan uçucu yağlarda belirlenen ana kimyasal bileşenler

Essential oils (*Uçucu yağlar*)	**No. of compound identified** (*Belirlenen bileşen sayısı*)	**Major compounds[a] identified** (*Belirlenen ana bileşenler[a]*)
Thymus sipyleus Boiss.	53	geranial (13.72%), carvacrol (11.06%), thymol (10.17%)
Rosmarinus officinalis L.	43	1,8 cineole (18.47%), camphor (17.55%), α-pinene (16.38%)
Mentha spicata L.	39	carvone (55,58%), limonene (10,22%), dihydrocarveol (6.52%)
Laurus nobilis L.	39	1,8-cineole (35.5%), sabinene (15.0%), α-terpineyl acetate (14.2%),
Origanum majorana L.	37	4-terpineol (31.67%), γ-terpinene, (14.74%), α -terpinene (9.18%)
Ocimum basilicum L.	37	linalool (30.23%), cinnamic acid methyl ester (20.47%), 1,8-cineole (15.21%)
Melissa officinalis L.	33	geranial (30.4%), z-citral (23.58%), citronella (18.48%)
Salvia officinalis L.	32	Camphor (24.59%), Thujone (21.14%), 1,8 cineole (19.38%)
Thymbra spicata var. *spicata* L.	25	carvacrol (66,88%), cymol (12.18%), γ-terpinene (10.73%)
Lavandula stoechas L. var. *stoechas*	25	1,8-cineole (35.5%), camphor (20.2%), α-thujone (15.9%)
Origanum syriacum Ietswaart	24	carvacrol (79.8%), *p*-cymene (8.15%), γ-terpinene (4.7%)
Thymus serpyllum L.	17	thymol (41.03%), carvacrol (33.59%), γ-terpinene (12.08%)
Foeniculum vulgare Mill.	17	*trans*-anethole (82.8%), 4-allyl-anisole (6.5%), limonene (5.8%)

[a] Components showing a peak area of more than 5% relative to the total peak area on gas chromatography (GC) are listed in order of their highest relative peak area. Numbers are percentage of compound relative to total essential oil

The antibacterial activities of essential oils from each plant species were estimated by using the disc diffusion technique (Figure 2) and diameters of inhibition zones caused by each essential oils against bacterial disease agents were given in Table 2. The essential oils of different plant species displayed significantly varying levels of antibacterial activities. Significant differences in antibacterial susceptibility for each bacterial isolates were recorded depending on the plant species. Essential oils of plants belong to Lamiaceae family were found to be more efficient than those belong to Lauraceae and Apiaceae families. Based on inhibition zone diameter values, *P. savastanoi* pv. *savastanoi* was recorded as the most sensitive and *P. savastanoi* pv. *nerii* was the most resistant bacterial species against the majority of the tested essential oils, respectively. In general, essential oils of *T. serpyllum*, *T. spicata* var. *spicata* and *O. syriacum* showed the highest antibacterial activities against all tested bacterial species (Table 2). In the case for *P. savastanoi* pv. *savastanoi*, the highest inhibitory activities were also shown by *O. syriacum* (44.67 mm) and *T. serpyllum* (42.33 mm). The lowest inhibitory activity was shown by *F. vulgare* (7.0 mm). For *R. radiabacter*, the highest inhibitory activities were displayed by *O. syriacum* (37.67 mm) and *T. serpyllum* (36.33 mm). The lowest inhibitory activity was shown by *R. officinalis* (9.67 mm). The sizes of inhibition zones caused by each essential oils recorded for the most resistant disease agent *P. savastanoi* pv. *nerii* were significantly lower than that those recorded for *R. radiobacter* and *P. savastanoi* pv. *savastanoi* in essential oil amended petri dishes (Table 2). The highest size of inhibition zone recorded for *P. savastanoi* pv. *nerii* was recorded in *O. syriacum* essential oil added petri dishes (16.67 mm) which was significantly weaker than those recorded for *R. radiobacter* and *P. savastanoi* pv. *savastanoi* inoculated petri dishes.

Table 2. Antibacterial activities of plant essential oils on the growth of gall forming bacterial disease agents *P. savastanoi* pv. *savastanoi, R. radiobacter* and *P. savastanoi* pv. *nerii.*
Çizelge 2. Bitki uçucu yağlarının bakteriyel gal ve ur oluşturan hastalık etmenleri P. savastanoi pv. *savastanoi, R. radiobacter* ve *P. savastanoi* pv. *nerii gelişimi üzerine olan antibakteriyel etkinlikleri*

Essential oils (*Uçucu yağlar*)	**Inhibition zone (mm)[a]** (*Engelleme zonu (mm)[a]*)		
	P.s.* pv. *savastanoi	***R. radiobacter***	***P.s.* pv. *nerii***
Mentha spicata L.	12.00abB	20.00gC	10.00dA
Melissa officinalis L.	35.33dC	14.67deB	7.33abcA
Thymus serpyllum L.	42.33eB	36.33iB	18.33fA
Ocimum basilicum L.	11.33abA	17.33fB	9.67dA
Thymbra spicata var. *spicata* L.	32.33dB	32.33hB	16.33eA
Thymus sipyleus Boiss.	16.67bcC	13.33cdeB	8.33bcdA
Laurus nobilis L.	13.00abB	13.33cdeB	8.67cdA
Lavandula stoechas L. var. *stoechas*	13.67abC	10.33abB	7.00abcA
Foeniculum vulgare Mill.	7.00aA	12.00bcB	6.33aA
Rosmarinus officinalis L.	13.33abC	9.67aB	6.67abA
Salvia officinalis L.	9.67aB	13.00cdC	7.00abcA
Origanum majorana L.	21.33cC	15.33eB	8.67cdA
Origanum syriacum L.	44.67eC	37.67iB	16.67eA

[a]Inhibition zone includes diameter of disc (6 mm). ± indicates standard deviations of means. Means in the columns and rows followed by different small and capital letters are significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

The findings of the present study clearly revealed that essential oils have a great potential to be used as antibacterial agents against gall forming bacterial disease agents. Antibacterial activities of essential oils have been mainly investigated against human and food spoilage microorganisms. A relatively limited number of studies were, however, available in the literatures plant extracts or/and essential oils against phytopathogenic bacteria. Although, very few studies were conducted to assess efficacy of essential oils from the medicinal plants, including those used in this study (such as *M. spicata*, *T. spicata* var. *spicata* and *T. serpyllum*) against crown gall disease agent *R. radiobacter* (Basim et al., 2000; Ateeq-ur-Rehman et al., 2009; Schollenberger et al., 2018), to the best of our knowledge there is no research on the evaluation of the efficacy of essential oils used in the present study against gall forming bacterial species such as *P. savastanoi* pv. *savastanoi* and *P. savastanoi* pv. *nerii*.

Earlier studies have demonstrated the antibacterial activities of essential oils obtained from different plant species to retard and inhibit not only the growth of gal forming bacterial disease agent *R. radiobacter* (Basim et al., 2000; Iacobellis et al., 2005; Ateeq-ur-Rehman et al., 2009; Mohan et al., 2011; Melkani et al., 2011; Moghaddam et al., 2014; Badawy and Abdelgaleil, 2014; Schollenberger et al., 2018; Turker et al., 2018; Ben Hsouna et al., 2019) but also other plant pathogenic bacterial disease agents such as *Pseudomonas syringae* pv. *syringae* (Schollenberger et al., 2018; Moghaddam et al., 2014), *Xanthomonas arboricola* pv. *corylina* (Schollenberger et al., 2018), *Ralstonia solanacearum* (Moghaddam et al., 2014), *Pseudomonas syringae* pv. *lachrymans* (Moghaddam et al., 2014), *Pseudomonas tolaasii* (Moghaddam et al., 2014), *Xanthomonas oryzae* pv. *oryzae* (Moghaddam et al., 2014), *Xanthomonas citri* (Moghaddam et al., 2014), *Brenneria nigrifluens* (Moghaddam et al., 2014), *Pantoea stewartii* subsp. *indologenes* (Moghaddam et al., 2014), *Agrobacterium vitis* (Moghaddam et al., 2014), *Rhodococcus fascians* (Moghaddam et al., 2014), *Erwinia carotovora* var. *carotovora* (Basim et al., 2000;

Badawy and Abdelgaleil, 2014), *Erwinia chrysanthemi* (Mohan et al., 2011), *Clavibacter michiganensis* subsp. *michiganensis* (Iacobellis et al., 2005; Soylu et al., 2009), *Erwinia amylovora* (Basim et al., 2000), *Pseudomanas viridiflava* (Basim et al., 2000), *Xanthomonas axonopodis* pv. *vesicatoria* (Basim et al., 2000), *Pseudomonas syringae* pv. *tomato* (Soylu et al., 2009), *Acidovorax citrulli* (Mengulluoglu and Soylu, 2012). The results of this investigation clearly showed that essential oils obtained from medicinal plants growing in the region have the antibacterial potential against all gall forming bacterial species. To the best of our knowledge this is the first research on the evaluation of the efficacies of essential oils used in this study against gall forming bacterial species such as *P. savastanoi* pv. *savastanoi* and *P. savastanoi* pv. *nerii*.

Recently, *Pteridium aquilinum* essential oil was reported to contain 32.86% of oxygenated monoterpenes, which are known for their very powerful antimicrobial activities against *E. amylovora, P. carotovorum* subsp. *carotovorum* and *P. savastanoi* pv. *savastanoi* (Bouchekouk et al., 2019). Furthermore, essential oils from *Verbena officinalis, Majorana hortensis* and *Salvia officinalis* plants were found to inhibit the growth of Gram positive and Gram negative plant pathogenic bacterial disease agents *Clavibacter michiganensis, Xanthomonas campestris*, *Pseudomonas savastanoi* and *P. syringae* pv. *phaseolicola* in a dose-dependent manner (Elshafie et al., 2016). Öksel and Mirik (2015) also tested the antibacterial effect of essential oils of thyme (*Thymus vulgaris*), bergamot (*Citrus bergamia*), garlic (*Allium sativum*), french lavender (*Lavandula stoechas*), clove (*Caryophyllus aromaticum*), eucalyptus (*Eucalyptus globus*) against *Pseudomonas savastanoi pv. savastanoi*. According to their results, essential oil of thyme was determined as the most effective essential oil which was followed by bergamot, lavender and eucalyptus essential oils respectively.

Essential oils of plants belonging Lamiaceae family were rich in phenolic compounds, which are believed to be responsible for the marked insecticidal, herbicidal and antimicrobial activity (Kordali et al., 2008; Jovanka et al., 2011; Mamadalieva et al., 2017; Kaya et al., 2018; Kachur and Suntres, 2019). The antimicrobial activities of the essential oils could be attributed to their hydrophobic nature that allows these compounds to penetrate microbial cells and cause alterations in its structure and functionality as reported (Yong et al., 2015; Kachur et al., 2019; da Silva et al., 2019; Lucas et al., 2012). Although the mode of action of essential oils used in this study against bacterial isolates is not studied, however the involvement of essential oil components may disrupt the cell membrane of the bacterium and change its permeability as reported by Lucas et al. (2012). Essential oils and their major components of oregano, thyme and thymus have been reported to induce rapid cell lysis of certain fungal and bacterial disease agents (da Silva et al., 2019; Kachur et al., 2019; Liu et al., 2019; Churklam et al., 2020). Because of essential oils and/or their main components such as carvacrol, thymol and linalool have been reported to possess fungicidal and bactericidal activities (Liu et al., 2019), essential oils or their component could serve as a bacterial disinfectant.

CONCLUSION

The possibility of controlling gal forming bacterial diseases with plant essential oils appears of particular interest considering the unavailability of commercial plant cultivars resistant to these disease agents. Findings of the present study suggest that the essential oils especially from thyme-like medicinal plants such as *Origanum, Thymbra* and *Thymus* spp. have a promising potential to be used as antibacterial agents and could be used to control plant bacterial disease caused by tumor-forming bacterial isolates of *R. radiobacter*, *P. savastanoi* pv. *savastanoi* and *P. savastanoi* pv. *nerii*. However further experiments are needed to obtain information regarding the economic aspects and antibacterial activities of essential oils *in vivo* without phytotoxic effects.

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Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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