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CHEMICAL COMPOSITIONS AND ANTIBACTERIAL ACTIVITIES OF ESSENTIAL OILS OF FIVE AROMATIC PLANTS AGAINST PLANT PATHOGENIC BACTERIAL DISEASE AGENTS

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ABSTRACT

In this present study, the antibacterial activity and the chemical composition of essential oils from five aromatic plants (*Helichrysum italicum*, *Inula graveolens*, *Cistus creticus*, *Echinacea purpurea* and *Hypericum perforatum*) growing in Hatay Province of Turkey were determined. The antibacterial activity of essential oils was tested by disc diffusion method against two different economically important plant bacterial disease agents such as bean halo blight disease agent *Pseudomonas syringae* pv. *phaseolicola* and potato soft rot disease agent *Pectobacterium carotovorum* subsp. *carotovorum*. The essential oils of five aromatic plants were analyzed by gas chromatography/mass spectrometry (GC/MS). According to GC-MS analysis, 50 components were identified in *H. italicum*, 42 components in *I. graveolens*, 48 components in *C. creticus*, 40 components in *E. purpurea* and 44 components in *H. perforatum*. Following GC-MS analysis, neryl acetate (27.17%) and α-pinene (12.3%) in *H. italicum*, fenchyl (bornyl) acetate (50.33%) and borneol (36.95%) in *I. graveolens*, α-pinene (19.44%) in *C. creticus*, caryophyllene oxide (16.94%), α-pinene (16.54%) and carvacrol (6.53%) in *E. purpurea* and α-pinene (32.15%) in *H. perforatum* were determined as main components. Based on inhibition zone, the highest antibacterial activities were displayed by *H. italicum* and *H. perforatum* EOs against *P. syringae* pv. *phaseolicola* (19.33-12.33 mm), respectively. The highest antibacterial activities against *P. carotovorum* subsp. *carotovorum* was shown by *H. perforatum* EO (14.33 mm) followed by *C. creticus* (10.67 mm) EO. Based on our results, the essential oil of aromatic plants collected from Hatay province has the potential to be applied against important plant bacterial disease agents.

Key words: *Antibacterial, essential oil, Pectobacterium carotovorum* subsp. *carotovorum, Pseudomonas syringae* pv. *phaseolicola.*

INTRODUCTION

Globally, plant diseases caused by some plant bacterial pathogens are a serious restriction and lead to significant yield losses in agriculture (Agrios, 2005). Depending on the infection rate and environmental conditions, annual yield loss caused by certain bacterial disease agents could reach up to 5-50% (Sundin et al., 2016). Nowadays, the major methods used to control plant bacterial diseases are mostly rely on cultural and/or chemical treatments such as copper compounds and antibiotics. The use of copper compounds prevent bacterial multiplication but not always adequate control of bacterial disease (Nguyen et al., 2018). The development of pesticides or antibiotic-resistant isolates, chemical residues on crops or food, government restrictions on antibiotic use, and public interest in environmental protection have led scientists to conduct various research efforts to find new environmentally friendly methods to combat bacterial diseases (Mengulluoglu & Soylu, 2012). Recent studies focused on plant-derived natural bactericides and their possible applications in agriculture to control plant bacterial

diseases are being recently intensified (Bozkurt et al., 2020). The antimicrobial properties of essential oils and their major constituents from numerous medicinal plants were investigated against a variety of bacterial and fungal plant diseases (Burt, 2004; Bakkali et al., 2008). The antibacterial activities of essential oils depend on their chemical components and the amount of each compound. The major component(s), structure, and also functional groups of the essential oils exhibit a significant role in determining their antimicrobial activity. Although antibacterial activity of essential oils from *Helichrysum italicum* (Dzamic et al., 2019; Mollova et al., 2020), *Inula graveolens* (Djenane et al., 2012; Sellem et al., 2020), *Cistus creticus* (Demetzos et al., 1997; Umarusman et al., 2019; Skoric et al., 2022), *Echinacea purpurea* (Sharifi-Rad et al., 2018) and *Hypericum perforatum* (Jianu et al., 2016; Moleriu et al., 2017; Metin & Bicer, 2020) plants have been reported in a variety of food and human pathogenic bacterial disease agents, to the best of our knowledge, no study has been performed on the use of essential oils from these medicinal and aromatic plants against the plant pathogenic bacteria.

The aim of this study was to investigate the chemical components and antibacterial properties of essential oils extracted from leaves of plants grown in Hatay province, including *Helichrysum italicum, Inula graveolens, Cistus creticus, Echinacea purpurea*, and *Hypericum perforatum*, against two different economically important bacterial plant diseases, namely the causal agent of bean halo blight *Pseudomonas syringae* pv. *phaseolicola* and *Pectobacterium carotovorum* subsp. *carotovorum*, the causal agent of potato soft rot, using the paper disc diffusion test.

MATERIALS AND METHODS

Essential oil isolations

All of the plants used in this study (*Helichrysum italicum*, *Inula graveolens*, *Cistus creticus*, *Echinacea purpurea* and *Hypericum perforatum*) were harvested from their wild populations located in Hatay Province of Turkey and identified by Prof. Dr. Ilhan Uremis. Voucher specimens of each plant species have been deposited in the herbarium of the HMKU BISAK Plant Herbarium and Microbial Culture Collection Centre, Hatay, Turkey. The dried leaves of each plant species were used to extract the essential oils. Leaves, stems and flower parts of each plant samples (100 g) were used for essential oil extraction procedure. Dried plant leaves that had been weighed was placed in a 1 L flask and distilled water was added until it completely covered the sample. Using distillation equipment of the Clevenger type apparatus, essential oils were extracted via steam-hydrodistillation (Bozkurt et al., 2020). The essential oils were stored in dark vial bottles at 4°C until analysis after being dried over anhydrous sodium sulphate.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of essential oils

An ISQ Single Quadrupole model gas chromatography-mass spectrometry device from Thermo Scientific was used to analyse the essential oils. A TR-FAME MS column (60 m x 0.25 mm, 0.25 m) and helium carrier gas were installed in the GC. The temperature of the MS transfer line was 250°C, the temperature of the MS ionization was 220°C, the mass spectra were recorded at 70 eV, and the mass range was 1.2-1200 m/z. The column and oven were initially 50°C in temperature, while the injection port was 220°C. The oven temperature program started at 50°C and was kept there for 3 minutes before ramping up to 220°C at a rate of 3°C/min. As a carrier gas, helium (99.9% purity) was employed (1.0 ml/min). Hexane is used to dilute the essential oil (2 ml cyclohexane, 5 ml essential oil). The Xcalibur program (Wiley 9) was used to analyse mass spectra to determine each compound's structure (Adams, 2001).

Isolation of test microorganisms

The bacterial disease agents *Pseudomonas syringae* pv. *phaseolicola* and *Pectobacterium carotovorum* subsp. *carotovorum* were used for the antibacterial activity of essential oils used in this study. Each of the bacterial disease agents was obtained from their respective host plants (bean and potato) that have been growing in the region. The pure bacterial cultures were maintained and evaluated for antibacterial tests on King B (KB) medium.

Antibacterial susceptibility testing of the essential oils

The paper disc diffusion assay was used to determine the antibacterial activity of the essential oils. The overnight cultures of bacterial suspensions (200 µl at the 10^8 cells/mL concentrations) were used to inoculate on KB plates. Six-mm-diameter sterile filter paper discs were coated with 5 µl of the essential oil and put in the centre of the agar. To avoid unwanted evaporation or interaction, the lids of each individual petri dish were replaced immediately, sealed with parafilm, and incubated at 25°C for 48 hours. Additionally, water-amended discs were used as a control. The diameter of the inhibitory zones, which included the paper discs, was then measured to evaluate the antibacterial activity of each essential oil against bacterial disease agents. Studies were carried out in triplicate and three times. All computations were done using the SPSS statistical program, and the significance was assessed using Duncan's Multiple Range Test ($P<0.05$).

RESULTS AND DISCUSSION

GC-MS analysis was used to determine the chemical components of essential oils from *H. italicum, I. graveolens, C. creticus, E. purpurea* and *H. perforatum*. Following GC-MS analysis, 50 different components were identified in *H. italicum*, 42 components in *I. graveolens*, 48 components in *C. creticus*, 40 components in *E. purpurea* and 44 components in *H. perforatum* essential oil, respectively. According to GC-MS analysis, neryl acetate was the most abundant volatile compound, comprising 27.17% of total volatiles of *H. italicum* essential oil, followed by α-pinene (12.3%) and β-selinene (8.14%), respectively. In the case of *I. graveolens*, fenchyl (bornyl) acetate was the most abundant volatile compound, comprising 50.33% of total volatiles of *I. graveolens* essential oil, followed by borneol (36.95%) and caryophyllene oxide (4.27%). For *C. creticus,* α-pinene (19.4%) was the major component which was followed by veridiflorol (9.96%) and bornyl acetate (8.58%), respectively. The major component of *E. purpurea* essential oil was caryophyllene oxide (16.69%) which was followed by α-pinene (16.54%) and spathulenol (9.91%), respectively. In the case of *H. perforatum*, α-pinene was the most abundant volatile compound, comprising 32.15% of total volatile essential oil, followed by isoaromadendrene (12.07%) and decane 2-methyl- (10.29%), respectively (Table 1).

The main compounds determined in the essential oils of the plants used in this study were largely similar to the compounds previously determined in the essential oils of the *I. graveolens* (Djenane et al., 2012; Sellem et al., 2020), *H. italicum* (Dzamic et al., 2019; Mollova et al., 2020), *H. perforatum* (Moleriu et al., 2017), *C. creticus* (Demetzos et al., 1997), *E. purpurea* (Diraz et al. 2012).

The antibacterial activities of *H. italicum, I. graveolens*, *C. creticus*, *E. purpurea* and *H. perforatum* essential oils were estimated by using the paper disc diffusion technique and the response of bacterial disease agents to the essential oil was presented in Table 2. *H. italicum* essential oil had the highest antibacterial activity against *P. syringae* pv. *phaseolicola* corresponding to 19.33 mm in the zones of inhibition over the control (sterile water). This essential oil was followed by *H. perforatum* (12.33 mm) and *C. creticus* (10.33 mm) respectively (Table 2). No antibacterial activity was, however, displayed by *E. purpurea* essential oil against bacterial disease agent *P. syringae* pv. *phaseolicola*.

Table 1. Major chemical compounds determined in the essential oils used in this study

Essential oil	No. of compound identified	Major compounds[a] identified
Helichrysum italicum	50	neryl acetate (27.17%), α-pinene (12.3%), β-selinene (8.14%),
Inula graveolens	42	fenchyl (bornyl) acetate (50.33%), borneol (36.95%), caryophyllene oxide (4.27%)
Cistus creticus	48	α-pinene (19.4%), veridiflorol (9.96%), bornyl acetate (8.58%)
Echinacea purpurea	40	caryophyllene oxide (16.69%), α-pinene (16.54%), spathulenol (9.91%)
Hypericum perforatum	44	α-pinene (32.15%), isoaromadendrene (12.07%), decane 2-methyl- (10.29%)

[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

H. perforatum essential oil had the highest inhibitory activity against *P. carotovorum* subsp. *carotovorum* corresponding to 14.33 mm in the zones of inhibition over the control (sterile water). This essential oil was followed by *C. creticus* (10.67 mm) and *E. purpurea* (9.67 mm) essential oils, respectively (Table 2). *I. graveolens* essential oil did not display antibacterial activity against bacterial disease agent *P. carotovorum* subsp. *carotovorum*.

Table 2. Antibacterial activities of essential oils against bacterial disease agents

Essential oils	Bacterial species	
	Pseudomonas syringae* pv. *phaseolicola	***Pectobacterium carotovorum* subsp. *carotovorum***
	Inhibition zone (mm)	
Helichrysum italicum	19.33e*	7.33b
Inula graveolens	8.67b	0.00a
Cistus creticus	10.33c	10.67d
Echinacea purpurea	0.00a	9.67c
Hypericum perforatum	12.33d	14.33f
Antibiotic (Streptomycin sulphate)	19.00e	13.33e
Control (DMSO)	0.00a	0.00a

*Diameter of inhibition zone including disc diameter of 6 mm. Means in the column followed by different letters are significantly different according to Duncan Multiple Range Test ($P<0.05$).

Essential oils (EOs) and their major constituents offer a novel source of antimicrobial agents against a broad spectrum of plant pathogenic bacterial, fungal, and viral disease agents. Although antimicrobial activities of essential oils from various medicinal and aromatic plants were previously investigated against certain plant pathogenic bacterial disease agents such as *P. syringae* pv. *phaseolicola* and *P. carotovorum* pv. *carotovorum* (Horvath et al., 2010; Elshafie et al., 2016; Cadena et al., 2018; Bouchekouk et al., 2019; Della Pepa et al. 2019; Elshafie et al., 2019; Elshafie et al., 2020; Camele et al., 2021), to the best of our knowledge there is no record available on the antibacterial activities of essential oils of *H. italicum*, *I. graveolens*, *C. creticus*, *E. purpurea* and *H. perforatum* against plant pathogenic bacterial disease agents used in this study.

CONCLUSIONS

The findings of this study conclusively demonstrated that *P. syringae* pv. *phaseolicola* and *P. carotovorum* subsp. *carotovorum* are both susceptible to the antibacterial effects of essential oils derived from the leaves of local plants. Although the exact mechanism by which essential oils inhibit bacteria is unknown. The presence of essential oil components may damage the cell membrane of the bacteria and alter its permeability have been reported in previous studies (Sivropoulou et al., 1995). The essential oils of these plants contained a high concentration of phenolic chemicals which are thought to be the cause of their strong antibacterial effects.

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