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Antibacterial effects of *Origanum onites* against phytopathogenic bacteria: Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria

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ABSTRACT

The aim of this study was to determine the antibacterial activity of the essential oil, the extracts and pure metabolites of *Origanum onites* L. against plant pathogenic bacteria and potential use of the extracts against the diseases caused by some phytopathogenic bacteria in vitro and in vivo conditions. The essential oil and the extracts of *O. onites*, and its pure compounds isolated from the acetone extract were individually tested against a total of 14 phytopathogenic bacterial strains. The essential oil and hexane extract contained mainly carvacrol (70.50% and 80.50%), *p*-cymene (13.97% and 0.96%), thymol (2.19% and 7.53%, respectively). The oil showed potent antibacterial effect against all tested phytopathogenic bacteria, with inhibition zones of 22–40 mm. The hexane, chloroform and acetone extracts also inhibited the growth of the tested plant pathogenic bacteria exhibiting 12–24 mm inhibition zones, except *Xanthomonas campestris* pv. *zinniae*. MIC values for the oil and effective extracts were determined between 7.81 and 31.25 μl/ml and 40 and 100 mg/ml, respectively. However, methanol extract did not show any antibacterial effects against the pathogenic bacteria. Seven compounds were isolated from acetone extract by column and thin layer chromatography and their chemical structures were characterized by UV, IR, ^{1}H-NMR, ^{13}C-NMR and 1D and 2D NMR spectroscopic methods as caryophyllene oxide, carvacrol, *n*-tetracosanol, β-sitosterol, betulinic acid, ursolic acid, naringenin and aromadendrin. Among these compounds, carvacrol inhibited the growth of all the bacteria tested, whereas aromadendrin and naringenin inhibited only a few bacterial species. In the Petri plate assays, disease severity on the tomato and lettuce was reduced by the extracts as compared to control, whereas the high concentrations of the extracts showed a negative effect on seed germinations of the tomato and lettuce. The effects of the extracts on the germination, disease severity and seedling growth of tomato coated with *Clavibacter michiganensis* ssp. *michiganensis*, *Xanthomonos axonopodies* pv. *vesicatoria* and lettuce coated with *Xanthomonas campestris* pv. *vitians* were also studied on pots. The results showed that the extract applications significantly reduced the disease symptoms and they did not affect the germination and seedling growth of tomato and lettuce as compared to control. Furthermore, some applications increased the germination of tomato and lettuce. The current results showed that the extracts can be used in inhibiting some bacterial plant disease.

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1. Introduction

Plant diseases caused by plant pathogenic bacteria are one of the major problems of crop loss (Kotan et al., 2005, 2010). Avoiding or mitigating crop losses due to the plant diseases is an important

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consideration in plant production. Rapid and effective control of the plant disease is generally achieved by use of synthetic pesticides and antibiotics. However, these chemicals are associated with undesirable effects on the environment due to their slow biodegradation in the environment and some toxic residues in the products for mammalian health (Barnard et al., 1997; Isman, 2000). The risk of developing resistance by microorganisms and the high cost–benefit ratio are also other disadvantages of synthetic pesticide uses (Brent and Hollomon, 1998; Roy and Dureja, 1998).

Bacterial diseases caused *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al., *Xanthomonas axonopodis* pv. (syn. *campestris*) *vesicatoria* (Doidge) Dye and *Xanthomonas campestris* pv. *vitians* (Brown) Dyes have devastated various host plants, leading to considerable losses in productivity and quality of harvests. They are seed-borne diseases and characterized by necrotic lesions on leaves, steams, and/or fruits. In warm and rainy weather, bacterial spot may cause severe defoliation of the plants that results in reduced yield, and diseased fruits may not be suitable for fresh-market sale.

Management strategies of the phytopathogenic bacterial pathogens include the use of disease-free seed and seedlings, resistant cultivars, antibiotics and copper sprays. However, these strategies are not always effective, especially when environmental conditions are optimal for disease or inoculums levels are high. Spraying with the antibiotics and copper compounds have never been satisfactory. Furthermore, antibiotics are forbidden in many countries because of their general toxicity and exert a negative impact on both yield and the environment. Only the United States and a few other countries allow the use of oxytetracycline and streptomycin for the control of the bacterial diseases on important crops (McManus et al., 2002).

Seed-borne diseases can be spread with seed trade and control of them using commercial disease management methods is extremely difficult (Bradbury, 1986). Therefore, the use of healthy seeds is the most important manner for controlling the above diseases. Of course this matter is an important problem in organic agriculture. New awareness to reduce the usage of the chemical pesticides by developing alternative strategies or technologies in order to improve plant disease resistance and control of pathogens are being promoted. Therefore, there has been a growing interest in research concerning the alternative pesticides and antimicrobial active compounds, including the plant extracts and essential oils of aromatic plants (Bajpai et al., 2010, 2011; Pradhanang et al., 2003; Kotan et al., 2010). Among the aromatic plant species, the genus *Origanum* L. has received great attention because of the fact that essential oil and extracts of members of this genus showed a very strong antimicrobial activity against various species of bacteria and fungi (Mosch et al., 1990; Lambert et al., 2001; Baydar et al., 2004; Arfa et al., 2006; Cavalcanti et al., 2006; Vasinauskiene et al., 2006; Sarac and Ugur, 2008; Wogiatzi et al., 2009; Copur et al., 2010; Bajpai et al., 2011; Arslan et al., 2012).

The genus *Origanum* (oregano) is an important genus of the Lamiaceae family and is represented in Turkey by about 23 species and 32 taxa (Baser, 2002; Skoula and Harborne, 2002; Gurbuz et al., 2011). *Origanum* species known “kekik” in Anatolia are aromatic and are used as condiment or herbal tea (Baser et al., 1993; Baser, 2002; Chan et al., 2010; Bagci et al., 2013). *Origanum onites* L. is the dominant species in the northwest of Turkey (Coskun et al., 2008). Recently, this spice plant has drawn more attention of consumers due to its antibacterial (Mosch et al., 1990, 1996; Baydar et al., 2004), antifungal (Reddy et al., 1998; Daferera et al., 2003; Dervis and Arslan, 2010), insecticidal (Isman, 2000; Aslan et al., 2005; Kordali et al., 2008; Ayvaz et al., 2010), acaricidal (Coskun et al., 2008) and antioxidative effects on human healthy (Kulisic et al., 2004; Bakkali et al., 2008). These activities have been mainly attributed to carvacrol, the major constituent of these oils. To our knowledge, there is a lack of information on the antibacterial property of the extracts and/or essential oil obtained from *O. onites* against plant pathogenic bacteria under in vivo assays. Therefore, this study was undertaken to assess the in vitro and in vivo antibacterial efficacy of the essential oil, *n*-hexane, chloroform, acetone and methanol extracts of the aerial parts of *O. onites* against plant pathogenic bacteria on the petri plate and pot assays.

2. Materials and methods

2.1. General experimental procedures

NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer, operating at 400 MHz and 100 MHz for ^1H and ^{13}C, respectively, using chloroform-d ($CDCl_3$) and dimethyl sulfoxide-d_6 (DMSO-d_6). Chemical shifts were expressed in δ (ppm) downfield from TMS as an internal standard and coupling constants reported in Hz. The IR spectra were determined on a FT-IR PerkinElmer Model 1600 spectrophotometer. Column chromatography (CC) was carried out using silica gel 60 (70–230 and 200–400 mesh), thin layer chromatography (TLC) and preparative TLC on silica gel 60 precoated plates, F-254 (Merck) (Cakir et al., 2003; Kotan et al., 2010). The spots on TLC were visualized by UV_{254}, UV_{366} and spraying with 1% vanillin-H_2SO_4 followed by heating (105 °C). Melting points were determined with a Thermo Scientific 9200 apparatus.

2.2. Plant material

Plant material was provided from the aerial parts of *O. onites* grown under field conditions at Hatay, Turkey in July 2009. The plant was collected at the flowering stage and dried in shade.

2.3. Extraction procedures

To obtain the essential oil, the dried plant samples (500 g) were powdered and subjected to hydro-distillation using a Clevenger-type apparatus for 4 h. The oil was extracted with $CHCl_3$ and then were dried over anhydrous sodium sulfate (Na_2SO_4) and stored under N_2 atmosphere at 20 °C in a sealed vial until use. The yield of the oil was 3.67%.

Furthermore, to obtain plant extracts, the dried plant samples were powdered in a blender and then samples of 50 g extracted individually with *n*-hexane, chloroform, acetone and methanol at room temperature. After filtration, the organic solvents were evaporated under reduced pressure and temperature. For the methanol extract of the plant sample, the concentrated methanol extract was individually dissolved in distilled water (60 °C) and then filtered. Thus, chlorophyll was removed from the solution. Then, this solution was lyophilized in a Labconco 117 freeze-dryer at 5 m-Hg and −50 °C. The extract yields (w/w) of hexane, chloroform, acetone and methanol were 3.92, 7.04, 5.62 and 8.04, respectively.

2.4. Isolation procedures of pure compounds

To isolate compounds in the acetone extract responsible for antibacterial activity, the powdered plant sample (1125 g) was extracted with acetone (3L × 5). After filtration, acetone was evaporated under reduced pressure and temperature and a brown crude extract (72.64 g) was obtained.

The concentrated extract (35 g) was fractioned on silica gel CC (350 g, 200–400 mesh) using $CHCl_3$-hexane (8:2, 9:1 and 1:0) and $CHCl_3$-ethyl acetate (8:2, 7:3, 6:4, 1:1, 4:6, 2:8 and 0:1) as eluents. The fractions (50 ml each) were compared by TLC (silica gel) using $CHCl_3$-hexane (8:2) and $CHCl_3$-ethyl acetate (8:2, 6:4, 4:6, 2:8 and

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