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Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi

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ABSTRACT

The essential oils of eighteen Egyptian plants, namely, *Artemisia judaica*, *A. monosperma*, *Callistemon viminals*, *Citrus aurantifolia*, *C. lemon*, *C. paradisi*, *C. sinensis*, *Cupressus macrocarpa*, *C. sempervirens*, *Myrtus communis*, *Origanum vulgare*, *Pelargonium graveolens*, *Rosmarinus officinalis*, *Syzygium cumini*, *Schinus molle*, *S. terebinthifolius*, *Thuja occidentalis* and *Vitex agnus-castus*, were isolated by hydrodistillation. The chemical composition of the isolated oils was identified by gas chromatograph/mass spectrometer (GC/MS). The major constituents of the isolated oils were limonene (40.19%, 56.30%, 74.29% and 89.23% in *C. aurantifolia*, *C. lemon*, *Citrus paradise* and *C. sinensis*, respectively), α-pinene (37.88%, 35.49%, 26.16% and 17.26% in *C. sempervirens*, *T. occidentalis*, *M. communis* and *S. cumini*, respectively), 1,8-cineole (71.77% and 19.60% in *C. viminals* and *R. officinalis*), pulegone (77.45% in *O. vulgare*), β-thujone (49.83% in *A. judaica*), capillene (36.86% in *A. monosperma*), sabinene (14.93% in *S. terebinthifolius*), α-phellandrene (29.87% in *S. molle*), 4-terpeneol (20.29% in *C. macrocarpa*), *trans*-caryophyllene (15.19% in *V. agnus-castus*) and β-citronellol (35.92 in *P. graveolens*). The isolated oils were tested for their antimicrobial activity against the most economic plant pathogenic bacteria of *Agrobacterium tumefaciens* and *Erwinia carotovora* var. *carotovora*, and fungi of *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum* and *Fusarium solani*. The isolated oils showed variable degree of antibacteabril activity against *A. tumefaciens* and *E. carotovora* var. *carotovora*. Based on minimum inhibitory concentration (MIC) values, the oils were more effective against *E. carotovora* var. *carotovora* than *A. tumefaciens*. The oil of *T. occidentalis* revealed the highest antibacterial activity among the tested oils showing the lowest MIC values of 400 and 350 mg/L, on *A. tumefaciens* and *E. carotovora* var. *carotovora*, respectively. In mycelial growth inhibition assay, most of the essential oils showed pronounced effect and the oil of *A. monosperma* was the most potent inhibitor with EC_{50} = 54, 111, 106 and 148 mg/L against *A. alternata*, *B. cinerea*, *F. oxysporum* and *F. solani*, respectively. On the other hand, the oils caused strong reduction in spore germination of fungi compared with control. The oils of *A. judaica* and *A. monosperma* caused the highest spore germination inhibition of *F. oxysporum* and their EC_{50} values were 69 and 62 mg/L, respectively. Among the tested fungi, *F. oxysporum* was the most susceptible fungus to all of the tested oil except the oil of *S. molle*. The relationship between the antimicrobial activity and the chemical composition of the isolated oils was disclosed. The findings of the present study suggest that the isolated oils have a potential to be used as antimicrobial agents.

1. Introduction

Essential oils (EOs) are aromatic oily liquids obtained from various plant parts. They are often responsible for a plant's distinctive scent or taste. As such, essential oils play a prominent role as flavoring agents in the food industry and as fragrances for the perfume industry (Bruneton, 1999). An estimated 3000 EOs are known, of which about 300 are commercially important. EOs are a complex mixture of compounds, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides). Some volatile compounds include phenylpropenes and specific sulphur- or nitrogen-containing substances. Generally, essential oil composition is a balance of various compounds, although in many species one constituent may prevail over all others (Cowan, 1999).

The growing interest in the substitution of synthetic antimicrobial agents by natural ones has fostered research on vegetable sources and the screening of plant materials in order to identify new compounds. It has been known since ancient times that spices and their essential oils have varying degrees of antimicrobial activity (Wan et al., 1998; Lachowicz et al., 1998). Recently, numerous studies have been reported on the antifungal activity of essential

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oils against human pathogenic, food spoilage and plant pathogenic fungi was described (Sokovic and Van Griensven, 2006; Souza et al., 2007; Gundidza et al., 2009; Mahboubi and Bidgoli, 2010; Mazari et al., 2010). The antibacterial properties of essential oils on human and animal pathogens, food poisoning and spoilage, and plant pathogenic bacteria were also reported (Burt, 2004; Delamare et al., 2007; Mevy et al., 2007).

The development of microbial resistance to presently available antimicrobial chemicals has necessitated the search for new antimicrobial agents. Therefore, the present study aimed to describe the chemical analyses and antimicrobial activities of essential oils of eighteen Egyptian plants, *Artemisia judaica*, *A. monosperma*, *Callistemon viminals*, *Citrus aurantifolia*, *C. lemon*, *C. paradisi*, *C. sinensis*, *Cupressus macrocarpa*, *Cupressus sempervirens*, *Myrtus communis*, *Origanum vulgare*, *Pelargonium graveolens*, *Rosmarinus officinalis*, *Syzygium cumini*, *Schinus molle*, *S. terebinthifolius*, *Thuja occidentalis* and *Vitex agnus-castus*. The chemical compositions of the essential oils were analyzed by gas chromatography/mass spectrophotometric (GC–MS) analysis. The antimicrobial activities of the essential oils were studied against plant pathogenic bacteria of crown gall *Agrobacterium tumefaciens* and soft mold disease *Erwinia carotovora* var. *carotovora* and fungi *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum* and *Fusarium solani*.

2. Materials and methods

2.1. Plant materials

Various parts of eighteen plant species: *A. judaica* L. (aerial parts), *Artemisia monosperma* Del. (leaves), *C. viminals* (Sol.ex Gaertn.) G. Don (leaves), *C. aurantifolia* (Christm.) Swingle (fruit peels), *Citrus lemon* (L.) Burm.f. (fruit peels), *Citrus paradisi* Macfad (fruit peels), *C. sinensis* (L.) Osbeck (fruit peels), *C. macrocarpa* Hartw. ex Gordon (leaves), *C. sempervirens* L. (leaves), *M. communis* L. (leaves), *O. vulgare* L. (aerial parts), *P. graveolens* L'Her (leaves) *R. officinalis* L. (leaves), *S. cumini* L. Skeels (leaves), *S. molle* L. (leaves), *Schinus terebinthifolius* Raddi (leaves), *T. occidentalis* L. (leaves) and *V. agnus-castus* L. (leaves) were collected during the flowering stage from different locations of Alexandria, Behira and Matrouh Governorates, Egypt, in August, 2010 to April, 2011. The plant materials were identified by Prof. FathAllah Zaitoon of Plant Pathology Department, Faculty of Agriculture, Alexandria University. Voucher specimens have been deposited in Department of Pesticides Chemistry, Faculty of Agriculture, Alexandria University.

2.2. Isolation of essential oils

The aerial plant parts were partially dried at room temperature ($26 \pm 1\,^{\circ}C$) for five days and the fruit peels were used fresh. Essential oils were extracted by hydrodistillation in a Clevenger-type apparatus for 3 h. The oils were dried over anhydrous sodium sulfate, and stored at 4 °C until used for GC–MS analysis and biological activity tests.

2.3. Analysis of essential oils

Essential oils were diluted in diethyl ether and 0.5 μL was injected into the gas chromatography (Hewlwett Packard 5890)/mass spectrometry (Hewlwett Packard 5989B) (GC–MS) apparatus. The GC column was a 30 m (0.25 mm i.d., film thickness 0.25 μm) HP-5MS (5% diphenyl) dimethylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 240 °C; column temperature, isothermal at 70 °C and held for 2 min, then programmed to 280 °C at 6 °C/min and held at this temperature for 2 min; ion source temperature, 200 °C; detector temperature, 300 °C. Helium was used as the carrier gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 400 amu for 5 s. The oil components were identified by comparison of their retention indices and mass spectra with the NIST Mass Spectral Library.

2.4. Test microorganisms and growth conditions

Two phytopathogenic bacterial strains, *A. tumefaciens* and *Erwinia carotovora* var. *carotovora*, and four plant pathogenic fungal strains, *A. alternata*, *B. cinerea*, *F. oxysporum* and *F. solani*, were obtained from Microbiology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Egypt. The bacterial strains were maintained on Nutrient Agar (NA) medium at 37 °C. and the fungi were maintained on Potatoes Dextrose Agar (PDA) medium at 25 °C.

The bacterial strains were cultivated in NA medium (Oxoid Ltd., UK) at 37 °C and fungi were cultured on PDA medium at 25 °C. Working bacterial cultures were prepared by inoculating a loopful of each test bacterium in 5 mL of Nutrient broth (NB) medium and were incubated at 37 °C for 24 h. For the test, final inoculum concentrations of 10^8 CFU/mL were used. Fungal spore suspensions were collected from the surface of fungal colonies by gentle scraping with a sterile glass rod and suspended in 10 ml Potato Dextrose Broth (PDB). The suspension was filtered through three layers of cheesecloth to remove mycelia fragments. This suspension was mixed vigorously by vortexing for 15–20 min. The suspensions were diluted with sterile water to an absorbance of 0.25 at 425 nm which contains about 1.0×10^6 conidia/mL.

2.5. The antibacterial assay

The *in vitro* antibacterial activity of the essential oils was assayed using NA dilution method (EUCAST, 2000) against *A. tumefaciens* and *E. carotovora* var. *carotovora*. Preliminary screening tests were performed at concentrations ranging from 100 to 1000 mg/L. For determination of a minimum inhibitory concentration (MIC), essential oils were dissolved in dimethyl sulfoxide (DMSO) and added to NA medium immediately before it was poured into the Petri dishes at a temperature of 40–45 °C. Parallel controls were maintained with distilled water and DMSO mixed with NA medium. After solidifications, 6 μL of bacterial cultures (approximately 10^8 CFU/mL) was spotted (three spots per each plate) using 2 μL standard loop on the surface of agar. The inoculum spots were allowed to dry before inverting the plates for incubation at 37 °C for 24 h. The MIC was determined as lowest concentration of the essential oils showing no visible bacterial growth in the agar plates.

2.6. The antifungal assay

2.6.1. Mycelial growth inhibition assay

The inhibitory effect of the essential oils on the mycelial growth of *A. alternata*, *B. cinerea*, *F. oxysporum* and *F. solani* was tested using a radial growth inhibition technique (El-Ghaouth et al., 1992). The oils were dissolved in dimethyl sulfoxide (DMSO) and added to PDA medium immediately before it was poured into the Petri dishes at a temperature of 40–45 °C. The compounds were tested at concentrations ranged from 10 to 1000 mg/L and each concentration was tested in triplicate. Parallel controls were maintained with distilled water and DMSO mixed with PDA medium. The discs of mycelial culture (0.5 cm diameter) of fungi, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the center of the Petri dishes. The plates were incubated in the dark at 25 °C. The mycelial growth diameter was measured when the fungal growth in the