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Antibacterial activity of *Thymus* phenols by direct bioautography

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ABSTRACT Planar chromatography is a powerful technique for separating certain classes of compound of biological interest. In higher plants there are many substances which have antimicrobial effect. Bioautography is a method to localize antibacterial activity on a chromatogram. Thymol and carvacrol have antibacterial effect against *Erwinia amylovora* and *Erwinia carotovora*. Rosmarinic and caffeic acids were detected in some Thymus taxa but have no antibacterial activity with direct bioautography. **Acta Biol Szeged 46(3-4):145-146 (2002)**

KEY WORDS

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Direct bioautography is a method to localize antibacterial activity on a chromatogram (Botz et al. 2001). Rosmarinic and caffeic acids were detected from some Thymus taxa (*Thymus vulgaris, Thymus serpyllum, Thymus citriodorus and Thymus citriodorus "Archers Gold"*) by TLC. Thymol, carvacrol and chlorogenic acid were tested with bioautography. The essential oils of thyme as well as two of their main constituents, thymol and carvacrol (de Bouchberg et al. 1976) have been found to antagonize the propagation of several bacteria. When added to microbiological media, more spices and essential oils were more effective against bacteria (Shelef et al. 1984). *Erwinia* strains were chosen as test bacteria. The aim of this study was to investigate the antibacterial activity of some common phenoloids.

Materials and Methods

Bioactive compounds: Thymol, carvacrol (Sigma), chlorogenic acid (Fluka), rosmarinic acid (Roth), caffeic acid (Serva)

Reagent: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma)

Antibiotica: Streptomycin sulphate (Biogal),gentamycin (Biochemie)

Bacteria: *Erwinia amylovora, Erwinia carotovora subsp. carotovora, Erwinia carotovora subsp. atroseptica.* Origin of these bacterium isolata: Health and Soil Conservation Service of Baranya County, Bacteriology Laboratory.

Culture media: cultures were grown in 100 mL nutrient broth Mueller-Hinton (Oxoid) (Bouillon, pH 7.3) at 26-28 ˚C for 24 h, in shaker incubator at a speed of 60 rpm. Bacterial suspension was diluted with fresh nutrient broth to furnish optical density 0,4 at 600 nm; this is equivalent to approximately 4 x 10^7 cfu mL^{-1}.

Direct bioautography: 10 x 10 cm silica gel 60 F_{254} sheets (Merck, Darmstadt, Germany) were used. Samples: 1-2 µL methanol extract of plant material, caffeic acid, rosmarinic acid, 10 µL thymol and carvacrol, 10 µL streptomycin sulphate and gentamycin. The concentration of the tests was 1 mg/mL. Mobile phase: benzol-ethyl-acetate-acetic acid (4:5:2). Chamber: Camag twin through chamber with chamber saturation. Detection: light source: deuterium lamp (UV) at 327 nm. Densitometry: Camag TLC Scanner II (Mutlenz, Switzerland) equipped with CATS software. Scanning condition: monochromator bandwidth: 30 nm.

TLC adsorbents were preconditioned by heating at 120˚C for 3 h. Without this procedure adsorbent layers became partly detached when soaked. 5-10 µL samples were applied to 10 x 10 cm TLC silica gel 60 F_{254} type plates with 5 µL capillaries. Plates were dipped in approximately 50 mL of the culture medium containing the test organism for 10 s and the layers were dried under air flow for 2 min. Layers were stored in a water-vapor chamber at 26-28 ˚C for 17 h. Layers were dipped in an aqueous solution of 0,8 g L^{-1} MTT for 15 s. Layers were incubated at 28 ˚C for 24 h. Layers were then dipped in 70% ethanol for 10 s.

Results and Discussion

The applied thymol and carvacrol had antibacterial activity by direct bioautography. Gentamycin showed stronger antibacterial activity against *Erwinia* strains than plant extracts. The sensitivity of detection of the tests on TLC plates was assessed by comparing the areas of zones of inhibition. The zones of inhibition of gentamycin were 13 mm in diameter in case of *Erwinia amylovora*, 12 mm against *Erwinia carotovora subsp. atroseptica* and 11 mm against *Erwinia carotovora subsp. carotovora*. Thymol against *Erwinia amylovora* produced zones of inhibition of 7 mm in diameter. Against *Erwinia carotovora subsp. atroseptica* the zones of inhibition were 9 mm in diameter. In case of *Erwinia carotovora subsp. carotovora* zones of inhibition of 10 mm in diameter were detected. Carvacrol produced zones of inhibition of 8 mm in diameter against *Erwinia* strains. All the strains of *Erwinia* reduced MTT in 24 h. The rosmarinic, caffeic and chlorogenic acids did not showe inhibition on TLC plate against *Erwinia* strains by direct bioautography.

Optimized direct bioautography is useful both for the analytical determination of the main compounds and for characterization of their antibacterial effects.

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