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ANTIBACTERIAL ACTIVITY OF SOME PLANTS FROM FAMILY APIACEAE IN RELATION TO SELECTED PHYTOPATHOGENIC BACTERIA

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ABSTRACT. Water, ethanol and ethyl acetate extracts of 12 plants from the family Apiaceae were screened for antibacterial activities against selected phytopathogenic bacteria. Following plants were tested: *Aegopodium podagraria, Angelica silvestris, Chaerophyllum bulbosum, Daucus carota* subsp. *carota, Foeniculum vulgare, Heracleum sphondylium, Pastinaca sativa, Peucedanum cerevaria, Peucedanum oreoselinum, Pimpinella saxifraga, Sanicula europea, Torilis anthriscus.* The antibacterial activities were tested in relation to the following bacterial species*:* *Agrobacterium radiobacter* pv. *tumefaciens*, *Erwinia carotovora*, *Pseudomonas fluorescens* and *Pseudomonas glycinea*. Antibacterial activity was determined by filter disc diffusion method. All the aromatic plants investigated showed antimicrobial activities against selected phytopathogenic bacteria tested. 65.27% of all examined extracts showed antibacterial activities. The most active were the extracts of *Torilis anthriscus* especially in relation to *Pseudomonas glycinea. Aegopodium podagraria*, *Daucus carota*, *Heracleum sphondyilium* and *Pimpinella saxifraga* also showed significant antibacterial properties. The most sensitive bacteria were *Pseudomonas glycinea* followed by *Agrobacterium radiobacter* pv. *tumefaciens,* while the most resistant bacteria were *Erwinia carotovora.* Laboratory screening of plant extracts has given encouraging results indicating their potential uses in the control of selected phytopathogenic bacteria.

INTRODUCTION

Causes of many plant diseases are phytopathogenic bacteria which greatly determine the quality of plant production (Arsenijević, 1988; Čomić, 1999). Quick and effective management of plant diseases and microbial contamination is generally achieved by the use of synthetic pesticides (Agrios, 1997). The application of synthetic pesticides can develop the appearance of resistant strains and can cause environmental hazards - accumulation in food chain, high and acute toxicity, long degradation period and extension of their power to destroy both useful and harmful pests (Barnard *et al*., 1997). Therefore great attention is dedicated to producing of new antibacterial agents that are used in the control of pathogenic microorganisms without environmental effects. Different studies provide evidence that some

aromatic plants might indeed be potential valuable sources of natural pesticides (Balandrin *et al*., 1985). Plants produce antimicrobial secondary metabolites as response to pathogen attack or abiotic stress. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores (Cowan, 1999).

There are many reports of antimicrobial effect of plant essential oils and extracts in relation to medically significant bacteria and fungi (Deans and Ritchie, 1987; Larrondo *et al*., 1995; Drew and Myers, 1997), but there is less information of antibacterial activity against phytopathogenic bacteria (Nascimento *et al*., 2000).

Considering that there are not many data on biotic activity of the aromatic plants growing naturally in Serbia (Mirković *et al*., 1999; Mimica-Dukić *et al*., 2003; Veličković, 2003; Stanojković *et al*., 2004; Petrović *at al*., 2004), the objective of this study was to investigate the antibacterial properties of 12 plant species as well as to estimate whether some extracts of these plants could potentially be used in the control of four selected phytopathogenic bacteria.

MATERIALS AND METHODS

Plant material

The plant material was collected on mountains Rudnik and Ravna gora (Serbia) during summer 2005 and identified at Faculty of Science, University of Kragujevac. The following plant species were collected, identified and investigated: *Aegopodium podagraria* L., *Angelica silvestris* L., *Chaerophyllum bulbosum* L., *Daucus carota* L., *Foeniculum vulgare* Mill., *Heracleum sphondylium* L., *Pastinaca sativa* L., *Peucedanum cerevaria* L., *Peucedanum oreoselinum* Munch., *Pimpinella saxifraga* L., *Sanicula europea* L., *Torilis anthriscus* L.

Bacterial cultures

The following bacteria were used: *Agrobacterium radiobacter* pv. *tumefaciens* (PMFKg11), *Erwinia carotovora* (PMFKg031), *Pseudomonas fluorescens* (PMFKg028) and *Pseudomonas glycinea* (PMFKg040). All bacteria were obtained from the stock cultures of the Microbiology Laboratory of Faculty of Science, Kragujevac, Serbia.

Extraction

The water, ethanol and ethyl-acetate extracts were made by boiling dry, macerated plant (30g for each solvent) at the water-bath at the temperature of 80ºC for 1 hour. The filtration and evaporation were done after 24 hours. The ethanol and ethyl-acetate solutions were evaporated in vacuum at 40ºC, while the evaporation of the water solution was done at the water bath. The solutions were evaporated until the dry extracts were obtained. The suitable solvents (water, ethanol, and ethyl-acetate) in the amount of 50ml were added into the obtained extracts. Related to the mass of dry matter (mg), the concentrations of the extracts for antibacterial investigation (ml) were calculated.

Evaluation of antibacterial activity

Evaluation of the antimicrobial activity of water, ethanol and ethyl acetate extracts was conducted according to the disk diffusion method (NCCLS 1999). The extracts were tested at concentration of 15mg per disc. Discs of Chloramphenicol (concentration 30μg/disc, Torlak, Beograd), Streptomycin (concentration 30μg/disc, Torlak, Beograd) and

Tetracyclin (concentration 30μg/disc, Torlak, Beograd) were used as standard to confirm that all the microorganisms tested were inhibited by the antibiotic. The antibacterial activity was interpreted from the size of the diameter of zone inhibition (mm) including disk diameter of 10mm. Each test was performed in triplicate and results analyzed for statistical significance.

RESULTS

The results of the investigation are presented in Table 1.

All the aromatic plant species investigated showed antimicrobial activities against selected phytopathogenic bacteria tested with the exception *Sanicula europea* which was inactive against *Erwinia carotovora* and *Pseudomonas fluorescens.* The antibacterial activities differed significantly depending on taxonomic characteristics of the plant species as well as biological characteristics of the tested bacteria.

65.27% of all examined extracts showed antibacterial activities but their values were usually lower than standard values. In relation to the value of the most active antibiotic, the values of the plant extracts' activities were as follows: 39.69-100.00% for *Agrobacterium radiobacter* pv *tumefaciens,* 30.35-64,70% for *Erwinia carotowora*, 28.66-66.66% for *Pseudomonas fluorescens* and 28.66-72.22% for *Pseudomonas glycinea.*

The most active plant was *Torilis anthriscus* especially in relation to *Pseudomonas glycinea.* In relation to tetracycline activity, ethanol and ethyl acetate extracts showed similar or same activities while the water activity was higher than standard (100.00%). Species *Aegopodium podagraria, Daucus carota*, *Heracleum sphondylium* and *Pimpinella saxifraga* also showed significant antibacterial properties. The extracts of these plants inhibited all the tested bacteria. The extracts of other plant species also showed antibacterial activities but their intensity was small, which indicates that they are not significant in the control of selected phytophatogenic bacteria. Of the plants used in the investigation, the least active were *Sanicula europea, Pastinaca sativa* and *Chaerophyllum bulbosum*.

DISCUSSION

The acquired results confirm and supplement present findings about antibacterial characteristics of aromatic plants from the Apiacea family. This is particularly important if we consider the variety of these characteristics in relation to the geographic origin of the plants. The results related to plants of whose antibacterial activity we do not have data (*Torilis anthriscus, Aegopodium podagraria*) or it is little researched; also stand out for its importance. Different studies provide evidence that there is a relationship between the plant chemical structures and their antimicrobial activity (Iwu *et al*., 1999; Cowan, 1999).

The shown antibacterial activity of *Torilis anthriscus* in relation to the selected species of the phytopathogenic bacteria contributes to better knowing of its biotic characteristics. The juice of the root is used in the treatment of indigestion. The seed *Torilis anthriscus* L. is anthelmintic, antifungal, antiviral, expectorant. It is used in Korea in the treatment of amnesia, prurit, acidosis and scabies. Fruits have spasmolitical effect in spasms of small intestine caused by histamin and barium chloride (Alefirov, 1998) Sesquiterpene torilin, isolated from the fruit, has anti-angiogenic activity as well as anti-tumour

characteristics (Kim *et al*., 2000). The methanolic extract of the fruits showed a potent inhibition against 5alpha-redeuctase activity in vitro (Park *et al*., 2003). *Torilis anthriscus* shows certain bioactive characteristics such as: antifungal, antiviral, antihelmintic, antitrichomonas sex hormone-like activities (Hsu *et al*., 1986). No evidence has been found on antibacterial activity.

This plant contains a higher number of biotically active components. The root contains polyacetil compounds. Leaves contain 7-glucosid luteolin, while fruits contain etheral oils and vegetable oils (Alefirov, 1998). The main components of the essential oil were germacrene-D (57.9-71.8% inthe oil), alpha-humulene (2.4-13.2%), bicyclogermacrene (1.9-5.4%), beta-caryophyllene (1.5-4.6%), and delta-cadinene (1.0-1.9%) (Fujita, 1990). Essential oil contains –cadinene and torilin. Fatty oils contain petroselin, linolein as well as torirolide, oxytorilolide, (-)- Germacrene-D, germacra-4(15), trans-5, 10(14)-trien-1-ol.

The acquired results, in contrast to present data, show significant antibacterial characteristics of *Daucus carota* growing naturally on Ravna Gora Mauntain (Serbia). Osclik *et al*. (2004) did, by examining antimicrobe characteristics of four plants that contain furanocoumarins, establish sligh antibacterial activity of ethanol extract of *D. carota* in relation to *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus*. By examining effect of spice plants on the bacteria *Listeria monocytogenes, Yersinia enterolitica, Pseudomonas aeruginosa* and *Lactobacillus plantarum* (Elgayyar *et al*., 2001) they established that *Angelica* sp., *Daucus* sp. and *Foeniculum* sp. do not have more important antibacterial characteristics. Out of 21 examined plants of Scotland, methanol seed extracts of Scotland, methanol seed extracts of 11 plants, including *Daucus carota*, showed certain antimicrobial properties. *D. carota* showed antibacteriak activity in relation to only one bacterial species (Kumarasamy *et al*., 2002). The differences in intensity of antibacterial characteristics of *Daucus carota* found in this research and the mentioned data are most probably a result of different origin of the plants (cultivated and wild ones) and different content of biotically active components which is to be confirmed in further research. Apart from that, the synthesis of biotically active components is directly linked to climate or geological factors.

The methanol seed extract of *Daucus carota* that is components of luteolin and 4-0-glucoside showed antibacterial effect on *Staphylococcus aureus*, *Escherichia coli*, *Cirobacter freundii* and *Lactobacillus plantarum* (Kumarasamy *et al*., 2005). Essential seed oils of *Daucus carota* subsp. *carota* and cultivated carrots were monitored as antibacterial substances to four species of bacteria and two species of fungi. The oils obtained from cultivated carrot seed were more effective against all tested organisms (Staniszewska, 2005). Before this, six components that contained sesquiterpens had been isolated from root of cultivated *Daucus carota* subsp. *carota* plants. The six bacteria (*Staphylococcus aureus, Streptomyces scabies, Bacillus subtilis, B. cereus, Pseudomonas aeruginosa* and *Escherichia coli*) were treated with this mixture. The components showed slight antimicrobial activity (Ahmed *et al*., 2005).

Antibacterial characheristics of plants from *Heracleum* genus have relatively slightly been researched. Methanol, ethylacetate extracts of plant species *Heracleum crenatifolium, H. Platytaenium, H. sphondylum* showed more important medical use and, partly, antibacterial, effect on six pathogenic bacteria (Ergene *et al*., 2004). 16 species of bacteria and one species of yeast were treated with essential oil of crushed seed of *Heracleum sphondylium* subsp. *ternatum*. The essential oil showed stronger inhibition against the plant pathogenic microorganisms *Pseudomonas syringae* pv. *syringae*, *Xanthomonas campestris* pv. *phaseoli* and *Xanthomonas campestris.* (Iscan *et al*., 2002). Antimicrobial mixture

(sesquiterpenes) of *Pimpinella* species, which grows in Turkey, showed antimicrobe activity to *Mycobacterium intracellulare* (Tabanca *et al*., 2003).

Essential oil of *Foeniculum vulgare* slightly inhibits *E. coli* (Stefanini *et al*., 2001). Essential oils are extracted from fruits of *Coriandrum sativum* and *Foeniculum vulgare* var *vulgare* and an in vitro experiment to activity of *Escherichia coli* and *Bacillus megatherium* was carried out (Cantore *et al*., 2004). Essential oils extracted from seeds of seven plants species, including *Foeniculum vulgare* were monitored for antibacterial activity of eight pathogenic bacteria which cause infections in humans. Apart from etheric oil of *Carum capticum*, the other etheric oils had a slight antibacterial effect (Singh *et al*, 2002).

Angelica extracts were analyzed for potential antimicrobial activity against various bacteria *(Bacillus subtilis, Escherichia coli, Micrococcus luteus, M. flavus, Pseudomonas aeruginosa, Staphylococcus epidermidis, S. aureus* and *S. faecalis*). Chloroform extract *Angelica* showed that it had the most important radical which showed the highest antibacterial activity (Kujundžić *et al*., 2002).

Water, ethanol and ethyl acetate extracts of *Torilis anthriscus* as well as *Daucus carota*, *Aegopodium podararia, Heracleum sphondylium* and *Pimpinella saxifraga* have significant antibacterial activities so potentially they might be used as biological pesticides in the control of some phithopathogenic bacteria although further researches are also needed.

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