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Antimicrobial potential of essential oil from *Pastinaca sativa* L.

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***Abstract*:**
Matejić, J., Džamić, A., Mihajlov-Krstev, T., Ranđelović, V., Krivošej, Z., Marin, P.: *Antimicrobial potential of essential oil from Pastinaca sativa L. Biologica Nyssana, 5 (1), Septmeber 2014: 31-35.*

The aim of the current investigation was to evaluate the antimicrobial effect of *Pastinaca sativa* L. (Apiaceae) essential oil. The aerial parts of plants were collected at Kopaonik Mountain (Serbia) and the essential oil has been isolated by hydrodistillation from this plant material. Essential oil was dominated by (Z)-β-ocimene (10.8%), hexyl butanoate (10.4%), (E)-β-farnesene (6.1%) and lavandulyl acetate (5.2%). The antimicrobial activity of the essential oil was investigated using a micro-well dilution assay against the most common human gastrointestinal pathogenic microbial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* and yeast *Candida albicans*. The results showed that minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations of essential oil ranged from 0.72 μg/ml (for the most sensitive *B. cereus*) to above 92.5 mg/ml for *S. enteritidis* and *L. monocytogenes*. This finding suggests that *P. sativa* may be considered as a natural source of antimicrobial agents.

Key words: *antimicrobial potential, essential oil, Pastinaca sativa* L.

***Apstract*:**
Matejić, J., Džamić, A., Mihajlov-Krstev, T., Ranđelović, V., Krivošej, Z., Marin, P.: Antimikrobni potencijal etarskog ulja vreste *Pastinaca sativa L. Biologica Nyssana, 5 (1), Septmeber 2014: 31-35.*

Cilj ovog rada bio je utvrđivanje antimikrobne aktivnosti etarskog ulja vrste *Pastinaca sativa* L. (Apiaceae). Nadzemni deo biljke sakupljen je na Kopaoniku (Srbija). Etarsko ulje nadzemnog dela izolovano je metodom hidrodestilacije. Kao glavne komponente etarskog ulja bile su: (Z)-β-ocimen (10.8%), heksil butanoat (10.4%), (E)-β-farnezen (6.1%) i lavandulil acetat (5.2%). Antimikrobna aktivnost etarskog ulja ispitivana je korišćenjem mikrodilucione metode na patogene gastrointestinalnog trakta kao što su: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* i *Candida albicans*. Rezultati su pokazali da su se minimalne inhibitorne koncentracije (MIC) kao i minimalne fungicidne koncentracije kretale u opsegu od 0.72 μg/ml (za najosetljiviju bakterijsku vrstu *B.*

cereus) do 92.5 mg/ml za *S. enteritidis* i *L. monocytogenes*. Na osnovu dobijenih rezultata slobodno se može reći da *P. sativa* može služiti kao prirodni izvor antimikrobnih agenasa.

Ključne reči: *antimikrobni potencijal, etarsko ulje, Pastinaca sativa* L.

Introduction

Essential oils are complex mixtures of compounds, synthesized in plant tissues as secondary metabolites. The role of essential oil in plant is multiple: defense against pathogens and herbivores, attraction of pollinators, inhibition of other plant's seed germination, formation of protective layer which reduces transpiration and formation of specific microclimate. Due to their lipophylic character, essential oils can interact with microbial membranes and achieve significant antimicrobial effect (Stojanović-Radić, 2012). In Serbian flora, family Apiaceae consists of 138 species. From the genus *Pastinaca*, two species are found in Serbia - *P. sativa* L. and *P. hirsuta* Panč. (Nikolić, 1973).

Pastinaca sativa L. is biennial, more or less pubescent plant native to Europe except the Arctic. Stem is hollow or solid, angled or terete. Basal leaves are usually simply pinnate. Fruit is broadly elliptical and vittae on the commissural face not reaching the ends of the fruit (Tutin, 1968).

The seed of *P. sativa* species contains essential oils. The dominant constituents in this oil are myristicin (64.20%), *β*-ocimene (29.10%) and *β*-farnesene (24.50%). For this reason *P. sativa* is used in food industry in Serbia (Jančić et al., 1995). In the oil obtained from crushed seeds of *P. sativa* subsp. *urens* from Turkey, 18 components were characterized representing 95% of the oil with octyl butyrate (79.5%) and octyl hexanoate (5.3%) as the major constituents (Kurkcuoglu et al., 2006).

A whole plant is used for strengthening the appetite, better digestion, and as a diuretic. The fruit is bitter aromatics that increases milk yield (Tucakov, 1986). The root is rich in starch and sugar and is used as food (parsnip), animal fodder, and for wine making. The sap is liable to cause skin irritation by sensitizing skin to UV radiation (Zehui & Watson, 2005). The root is delicious baked, and it can be used in soups, added to cakes, pies and puddings (Facciola, 1990). Leaves and young shoots can be usedcooked with other greens as a vegetable or added to soups (Launert, 1981; Facciola, 1990). The seed is used as a condiment (Launert, 1981). It is similar in taste to dill (Facciola, 1990). Native parts of plants have been applied in feeding as a spice, pharmaceutical industry and folk medicine (Nikolić, 1973).

Material and methods

Plant material and isolation of the essential oils

Aerial parts of *P. sativa* were collected in July 2003 from Kopaonik Mountain. A voucher specimen (PSa 60316), has been deposited at the Herbarium of the Institute of Botany and Botanical Garden “Jevremovac”, Faculty of Biology, University of Belgrade (BEOU). The oil from the dried aerial parts (210 g) was separately obtained by hydro distillation for 3 h using a Clevenger-type apparatus. Yield of the essential oil from the aerial parts was 0.1%, ρ=925.5 g/cm^3**.**

Antimicrobial activity

Microbial strains

The antimicrobial activity of all tested samples was evaluated using laboratory control strains obtained from the American Type Culture Collection: Gram (-) bacteria - *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enteritidis* ATCC 13076; Gram (+) bacteria: *Bacillus cereus* ATCC 10876, *Listeria monocytogenes* ATCC 15313, *Staphylococcus aureus* ATCC 25923 and yeast *Candida albicans* ATCC 10231.

Micro-well Dilution Assay

The inocula of the microbial strains were prepared from the overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity (corresponding to 10^7-10^8 CFU/ml, depending on genera - consensus standard by the NCCLS (2003)). Serial doubling dilutions of the tested samples (tested oil in 5% DMSO) were prepared in a 96/well microtiter plate over the range of 0.05–100 μl/ml (for oil) in inoculated Mueller-Hinton broth. The final volume was 100 μl and the final microbial concentration was 10^6 CFU/ml in each well. The plate was incubated for 24 h at 37 °C. All experiments were performed in triplicate. Two controls were included - medium with 30% ethanol (negative control) and medium with Streptomycin, Chloramphenicol and Nystatin (positive control). Microbial growth was determined by adding 20 μl of 0.5% triphenyl tetrazolium chloride (TTC) aqueous solution (Sartoratto et al., 2004).

Minimal inhibitory concentration (MIC) was defined as the lowest concentration of the samples inhibiting visible growth (red colored pellet on the bottom of the wells after the addition of TTC). To determine MBC/MFC, the broth was taken from each well without visible growth and inoculated in Mueller-Hinton agar (MHA) for 24 h at 37°C. Minimal bactericidal/fungicidal concentration (MBC/MFC) was defined as the lowest samples concentration killing 99.9% of bacterial/fungal cells.

Results and Discussion

Chemical analysis of *P. sativa* essential oil was already tested and the main components were: (Z)-*β*-ocimene (10.8%), hexyl butanoate (10.4%), (E)-*β*-farnesene (6.1%) and lavandulyl acetate (5.2%), and *P. hirsuta* was dominated by butyl butyrate (2.5%). In both species, hexyl butanoate is dominant (10.4 and 55.4%, resp.) (Kapetanos et al., 2008). Yield of the essential oil for 13 components in this essential oil varied in the range of 5-1% (germacrene D, (+)-*γ*-terpinene, *ar*-curcumene, *α*-zingiberene, *β*-sesquiterpene, (+)-(E)-*β*-caryophyllene, geranyl acetone, myrcene, *β*-bourbonene, nonanyl hexanoate, nonanal, (+)-*β*-pinene, ethyl 2-phenylbutanoate (Kapetanos et al., 2008). In Kubeczka & Stahl (1977) study, oils from the wild parsnip *P. sativa* were characterized by the presence of octyl acetate. The composition of essential oils in the roots of *P. sativa* changes during the course of plant development: at the seedling stage (0-18 days), the primary oil ducts between the pericycle cells contain mainly the sesquiterpene hydrocarbon trans-*β*-farnesene (34.9%) with smaller amounts of myristicin (14.4%) and terpinolene (3.1%); as the roots thicken during the secondary stage (23-30 days) the sesquiterpene content decreases, whereas the myristicin and terpinolene contents increase; and finally, at the adult stage (38-160 days) when secondary oil ducts develop, the percentage of sesquiterpene falls to 1.5%, while that of myristicin and perpinolene increase to 62.6 and 25.5%, respectively (Stahl-Biskup & Wichtmann, 1991).

In the oil of *P. hirsuta,* twenty-nine compounds were identified in root oil and thirteen compounds in fruit oil (92.8% and 90.8% of the total oils, respectively) using GC-FID and GC-MS. The principal components of the root oil were apiole (56.0%) and myristicin (21.0%), followed by *β*-bisabolene (7.2%). The main compounds in the fruits oil were hexyl hexanoate (59.8%) and hexyl butanoate (21.4%) (Petrović et al., 2008).

Our results of antimicrobial activity obtained for the examined essential oil are presented in **Table 2**. We were using the antibiotics Streptomycin, Chloramphenicol and Nystatin for comparison.

The essential oil of *P. sativa* showed the greatest activity against *B. cereus* strains (MIC=MBC=0.72 mg/ml). Bacterial strains *S. enteritidis* and *L. monocytogenes* showed the lowest susceptibility to the tested essential oil. Terpenes, in general, possess a wide spectrum of biological activity through which they appear to play a role in plant defense mechanisms (Fraga, 2001, 2003). In addition, it was possible that components present in lower amounts in the oil might be involved in some type of synergism with the other active compounds (Marino et al., 2001). Due to their bioactivity, the great number of terpenes has been evaluated for antibacterial or antifungal activity. Components of essential oil such as *p*-cymene, *α*-pinene, *β*-pinene, limonene, *α*-terpinene, *α*–terpinolene, caryophyllene oxide, camphene, 1,8-cineole and linalool have been reported for their antimicrobial activity (Viljoen, 2003; Knobloch, 1989; Sökmen, 2004). The main components of *P. sativa* essential oil were not up to now evaluated for their antimicrobial activity.

Brković et al. (2006) tested antimicrobial activity of water, ethanol and ethyl acetate extracts from *P. sativa* and 11 other plants from the family of Apiaceae. Comparison with the other species showed that *P. sativa* had the least antimicrobial activity.

The root oil of *P. hirsuta* Pančić was active against all tested microorganisms and exhibited the best activity against *B. subtilis* (MIC 6.25 µg/ml), *S. aureus, M. luteus* and *C. albicans* ATCC 10259 (MIC 12.5 µg/ml). The oil from the fruits of *P. hirsuta* was active only against *E. coli* and *C. albicans* (the most active against *C. albicans* ATCC 10259: MIC 12.5 µg/ml) (Petrović et al., 2008). When comparing the results, we can conclude that the results of antimicrobial activity points to higher potential of *P. hirsuta* than the herein tested oil. The reason for this can be different chemical composition of the root and the fruits as well as different solvent in which the oil is dissolved during the antimicrobial testing.

Table 2. Antimicrobial activity of *Pastinaca sativa* essential oil against pathogenic microbial strains using micro-well dilution assay

Pathogenic microbial strains	***P. sativa* essential oil (MIC/MBC(MFC) in mg/ml)**	**Antibiotic (MIC/MBC(MFC) in µg/ml)**
Gram (-) bacteria		**Streptomycin**
E. coli ATCC 25922	23.1/92.5	16.0/16.0
P. aeruginosa ATCC 9027	46.2/46.2	8.0/8.0
S. enteritidis ATCC 13076	92.5/92.5	4.0/4.0
Gram (+) bacteria		**Chloramphenicol**
B. cereus ATCC 10876	0.72/0.72	4.0/16.0
L. monocytogenes ATCC 15313	92.5/92.5	8.0/16.0
S. aureus ATCC 25923	46.2/46.2	1.0/8.0
Yeast		**Nystatin**
C. albicans ATCC 10231	46.2/46.2	16.0/16.0

Conclusion

The essential oil from *Pastinaca sativa* inhibited the growth of tested microorganisms. These microorganisms cause food spoilage and are recognized as human and animal pathogens. Thus, the oil from this species deserves further investigation in search for natural food preservatives.

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