UDC: 615.322:582.794.1
ID: 195742476
Original research paper

Acta Agriculturae Serbica, Vol. XVII, 33 (2012) 47-52

Antimicrobial activities of chloroform, ethyl acetate and petroleum ether extracts of plant species *Seseli rigidum W. K.*

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Abstract: This study was aimed at evaluating the antimicrobial activity and efficacy of the chloroform, ethyl acetate and petroleum ether extracts of the endemic plant species *Seseli rigidum* in inhibiting the development of selected fungi and bacteria. Antimicrobial activity was tested using broth dilution procedure for determination of minimum inhibitory concentration (MIC). The plant species extracts demonstrated important antimicrobial activity against 8 strains with MIC values from 16.62 to 62.50 μg/ml. The obtained results suggest that extract of the endemic species *Seseli rigidum* show antimicrobial activity.

Keywords: *Antimicrobial activity, Seseli rigidum*

Introduction

The use of traditional medicinal plants for primary health care and other purposes has progressively increased worldwide in recent years. Plants communicate with their environment by producing a diverse range of chemicals. These secondary metabolites are a common feature of specific plants and plant families. Many plant secondary metabolites have antimicrobial properties that

Received: 20. 05. 2012. / Accepted: 28. 09. 2012.

make plant extracts and products successful in the treatment of bacterial, fungal and viral infections (Gottschling *et all*, 2001), (Zhou and Duan 2005), (Iqbal *et all*., 2005). The different parts of plants (root, leaf, flower, fruit, stem, bark) are used to effectively treat a number of diseases. Their antioxidant and antimicrobial properties affect a range of physiological processes in the human body, thus providing protection against both free radicals and growth of undesirable microorganisms. The Boraginaceae family occurs worldwide, and it consists of about 100 genera with more than 2000 species (Josifović 1970,1977). Many members of the Boraginaceae family produce secondary metabolites such as alkaloids, naphthoquinones, polyphenols, phytosterols, terpenoids and fatty acids (Gottschling *et al*., 2001), (Zhou and Duan 2005). Polyphenols, including flavonoids and phenolic acids, produced by the family Boraginaceae, have a wide range of pharmaceutical activities, including antioxidant, anti-inflammatory, anti-viral and anti-bacterial activities (Iqbal *et al.*, 2005), (Wu,1990), (Šilić 1984).

Material and Methods

Chemicals used

All chemicals and reagents were of analytical grade and were purchashed from Sigma Chemical Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany).

Spectrophotometric measurements

Spectrophotometric measurements were performed using a UV-VIS spectrophotometer MA9523-SPEKOL 211 (ISKRA, Horjul, Slovenia).

Plant material

The test plant was collected at Ilijak Hill (Central Serbia) in May 2011. The species was identified and the voucher specimen was deposited at the Department of Botany, Faculty of Biology, University of Belgrade.

Preparation of the extracts

The air-dried aerial parts of the plant (50 g) were broken into small pieces by a cylindrical crusher, and extracted with chloroform, ethyl acetate and petroleum ether extracts using a Soxhlet apparatus. Extracts was filtered through a paper filter (Whatman, No.1) and concentrated to dry mass. The residues were stored in a dark glass bottle for further processing.

Test microorganisms

The antimicrobial activity of the plant extract was tested in vitro against the following bacteria; *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 13315, *Proteus mirabilis* ATCC 14153, *Bacillus subtilis* ATCC 6633, and fungi; *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. The fungi were reseeded on potato-glucose agar, on which they developed for 7 days at room temperature of 20 ºC under alternating day-night light conditions. They were reseeded on a new potato-glucose substrate, on which they developed for another 7 days. The reseeding procedure was performed four times, after which the pure cultures needed for determination were obtained. The identification of the test microorganisms was confirmed by the Laboratory of Mycology, Department of Microbiology, Institute Torlak, Belgrade, Serbia.

Minimum inhibitory concentration (MIC)

The minimal inhibitory concentrations (MIC) of the extract and cirsimarin against tested bacteria were determined based on a microdilution method in 96 multi-well microtiter plates (Satyajit *et al*., 2007). All tests were performed in Muller–Hinton broth (MHB) with the exception of the yeast when Sabouraud dextrose broth was used. A volume of 100 L stock solutions of oil (in methanol, 200 µL/mL) and cirsimarin (in 10 % DMSO, 2 mg/ml) was pipette into the first row of the plate. To all other wells 50 µL of Mueller Hinton or Sabouraud dextrose broth (supplemented with Tween 80 at a final concentration of 0.5% (v/v) for analysis of oil) was added. A volume of 50 µL from 1st test wells was pipetted into the 2nd well of each microtiter line, and then 50 µL of scalar dilution was transferred from the 2nd to the 12th well. To each well 10 µL of resazurin indicator solution (prepared by dissolving a 270-mg tablet in 40 mL of sterile distilled water) and 30 µL of nutrient broth were added. Finally, 10 µL of bacterial suspension (10^6 CFU/mL) and yeast spore suspension (3×10^4 CFU /mL) was added to each well. For each strain, the growth conditions and the sterility of the medium were checked. Standard antibiotic amracin was used to control the sensitivity of the tested bacteria, whereas ketokonazol was used as control against the tested yeast. Plates were wrapped loosely with cling film to ensure that bacteria did not become dehydrated and prepared in triplicate, and then they were placed in an incubator at 37 °C for 24 h for the bacteria and at 28 °C for 48 h for the yeast. Color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value. The average of 3 values was calculated and that was the MIC for the tested compounds and standard drug.

Results and Discussion

The results of the antimicrobial activity obtained by the dilution method are reported in Table 1. Minimum inhibitory concentrations were determined for 8 selected indicator strains. The results presented in Table 1 revealed antimicrobial activity of extracts of *Seseli rigidum* within the concentration range from 15.62 μg/ml to 62.50 μg/ml. Amracin and ketokonazol were used as a standard antimycotics. The results of present investigation clearly indicate that the antibacterial and antifungal activity vary with the species extracts the plants. The highest antimicrobial activity on the studied strains showed chloroform extract of plant species *Seseli rigidum*, which is the subject of further investigation. In conclusion, this is the first study focused on the biological activities of *Seseli rigidu.*

Tab. 1. Minimum inhibitory concentrations (MIC) of the chloroform, ethyl acetate and petroleum ether extracts *Seseli rigidum*

	MIC *μg/ml*				
Microbial strains	chloroform extract	ethyl acetate extract	petrol ether extract	Amracin	Ketokonazol
Staphylococcus aureus ATCC 25923	15.62	15.62	15.62	0.97	/
Klebsiella pneumoniae ATCC 13883	15.62	31.25	31.25	0.49	/
Escherichia coli ATCC 25922	31.25	62.50	62.50	0.97	/
Proteus vulgaris ATCC 13315	15.62	15.62	31.25	0.49	/
Proteus mirabilis ATCC 14153	15.62	31.25	62.50	0.49	/
Bacillus subtilis ATCC 6633	15.62	15.62	31.25	0.24	/
Candida albicans ATCC 10231	15.62	31.25	31.25	/	1.95
Aspergillus niger ATCC 16404	15.62	15.62	31.25	/	0.97

Conclusion

Antimicrobial properties of essential oils and various extracts of many plants are of great interest in both fundamental science and food industry, since their potential use as natural additives has emerged from a growing tendency to

replace synthetic antioxidants by natural ones. The present study confirmed the antimicrobial activities of Serbian *Seseli rigidum* extracts. The obtained results suggest that extracts of the endemic species *Seseli rigidum* show antimicrobial activity.

Acknowledgements: This work is part of the Project III 046009 and Project 172016, Funded by Ministry of Education and Science of Serbia.

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ANTIMIKROBNA AKTIVNOST HLOROFORMNOG, ETILACETATNOG I PETROLETARSKOG EKSTRAKTA ENDEMIČNE BILJNE VRSTE *SESELI RIGIDUM W. K.*

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Rezime

Rad je koncipiran sa ciljem da ispita antimikrobnu aktivnost tj. da odredi efikasnost hloroformskog, etil-acetatnog i petroletarskog ekstrakta endemicne biljne vrste *Seseli rigidum* u inhibiciji razvoja odabranih gljiva i bakterija. Antimikrobna aktivnost je testirana korišćenjem razblazenja, postupka za utvrđivanje minimalne inhibitorne koncentracije (MIC). Ekstrakti ove biljne vrste su testirani na 8 sojeva i pokazuju antimikrobnu aktivnost u opsegu koncentracija od 16.62 do 62.50 μg/mL. Dobijeni rezultati ukazuju da ekstrakti endemicne vrste *Seseli rigidum* pokazuju dobru antimikrobnu aktivnost.