SHORT REPORT

A C G publications

Rec. Nat. Prod. 5:4 (2011) 319-323

records of natural products

Chemical Composition and Antimicrobial Activity of *Echinophora spinosa* L. (Apiaceae) Essential Oil

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(Received July 28, 2010; Revised March 21, 2011; Accepted March 24, 2011)

Abstract: The present study was undertaken to investigate the chemical composition and effectiveness of the essential oil isolated from *Echinophora spinosa* on different bacterial and fungal species. Chemical analysis (GC/MS) showed that δ^3-carene (60,86 %), α-phellandrene (7,12%), p-cymene (6,22 %), myrcene (4,82 %) and β-phellandrene (2,73 %) were dominant components in this oil. Essential oil tested showed good antimicrobial activity. Antimicrobial potential of this oil was higher than potential of commercial antimicrobial drugs tested, streptomycin, bifonozole and ketoconazole.

Keywords: *Echinophora spinosa*; essential oil composition; δ^3-carene; α-phellandrene; p-cymene; myrcene; β-phellandrene; antifungal activity; antibacterial activity.

1. Plant Source

Echinophora spinosa L. (Apiaceae) is a perennial plant, distributed only in Mediterranean region mostly at maritime sands [1]. The underground rhizome has a wide growth, the erect stem is full of branches. Leaves end with spines. It flowers form June to September. In terms of its flora, Montenegro represent very interesting country. In this area of the world, wild edible plants have been used as a source of food and medicaments from ancient times onward [2]. The plants genera *Echinophora* species are also used in folk medicine to heal wounds and to treat gastric ulcers due to its antifungal, carminative, and digestive properties [3]. Plant material was collected from Buljarice Cost, Herceg Novi in Montenegro, on August 2009. Voucher specimens (ESP0407) have been deposited at the Herbarium of the Department of Plant Physiology, Institute for Biological Research, University of Belgrade, Serbia.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 04 /15/2011 EISSN: 1307-6167

2. Previous Studies

Earlier, it was one reported that the oil of aerial parts of *E. spinosa* contained α-phellandrene (36.8%) whereas the root oil contained predominantly terpinolene (77.2%) [4]. There is no any literature data concerning on antimicrobial activity of *E. spinosa* oil.

3. Present Study

The air-dried plants of *E. spinosa* were submitted for 3 h to water-distillation using Clevenger apparatus. The obtained essential oils were stored at +4 °C until tested and analyzed. Qualitative and quantitative analyses of the oils were performed using GC and GC/MS.

GC and GC/MS: The GC analysis of the oil was carried out on a GC HP-5890 II apparatus, equipped with split-splitless injector, attached to HP-5 column (25 m x 0.32 mm, 0.52 µm film thickness) and fitted to FID. Carrier gas flow rate (H_2) was 1 mL/min, split ratio 1:30, injector temperature was 250°C, detector temperature 300°C, while column temperature was linearly programmed from 40°-240°C (at rate of 4°/min). The same analytical conditions were employed for GC/MS analysis, where HP G 1800C Series II GCD system equipped with HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-400. The components of the oil were identified by comparison of their mass spectra to those from Adams [5], Wiley, NIST/NBS libraries. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1., DTRA/NIST, 2002). Results obtained were correlated with retention indices with data available in common literature [5]. For quantification purpose, area percent data obtained by FID were used.

*Antimicrobial Activity***:** For the bioassays we used eight bacteria: *Pseudomons aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Micrococcus flavus* (ATCC 9341), *Bacillus cereus* (clinical isolate), *Listeria monocytogenes* (NCTC 7973), *Staphylococcus aureus* (ATCC 25923) *Salmonella typhimurium* (ATCC 13311) and *Enterobacter faecalis cloacae* (human isolate). and seven fungi: *Aspergillus flavus* (ATCC 9643), *A. niger* (ATCC 6275), *A. versicolor* (ATCC 11730), *A. ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Trichoderma viride* (IAM 5061) insted; The micromycetes were maintained on malt agar (MA), bacteria on Mueller-Hinton agar (MH) and cultures were stored at +4°C and subcultured once a month [6].

The modified microdilution technique was used [7, 8]. The bacterial inocula applied contained approximately 1.0 x 10^5 cells in a final volume of 100 µL per well. The fungal spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10^5 in a final volume of 100 µL per well. Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique using 96-well microtitre plates. The microplates were incubated for 48 h at 37 °C (for bacteria) and 72 h at 28° C (for fungi). The lowest concentrations without visible growth were defined as concentrations which completely inhibited bacterial and/or fungal growth (MICs). The minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFCs) were determined by serial subcultivation. The lowest concentration with no visible growth was defined as the MBC/MFCs, indicating ≥ 99.5% killing of the original inoculum. Streptomycin, Bifonazole and Ketoconazole were used as a positive control.

GC and GC-MS analyses showed a total of 42 compounds in the essential oil of *E. spinosa* (Table 1). The major compound is δ^3-carene (60,86%), following by α-phellandrene (7,12%), p-cymene (6,22 %), myrcene (4,82%), β-phellandrene (2,73%), α-pinene (2,55%), β-phellandren-8-ol

(2,04%), trans,trans-2,6-dimethyl-1,3,5,7-octatetraene, (1,78%), terpinolene (1,59%) and myristicin (1,04%).

Table 1. Chemical composition of *Echinophora spinosa* essential oil.

RI	Compound	(%)	RI	Compound	(%)
925	*α*-thujene	0.17	1134	cis-p-metha-2,8-dien-1-ol	0.2
930	*α*-pinene	2.55	1152	eucarvone	0.13
968	Verbenene	0.42	1160	*β*-phellandren-8-ol	2.04
971	Sabinene	0.06	1167	p-metha-1,5-dien-8-ol	0.9
973	*β*-pinene	0.13	1183	m-cymen-8-ol	0.79
991	Myrcene	482	1187	p-cymen-8-ol	0.27
999	δ^2-carene	0,1	1191	*α*-terpienol	0.11
1003	*α*-phellandrene	7,.2	1194	cis-4-caranone	0.3
1010	δ^3-carene	60.86	1208	verbenone	0.8
1015	*α*-terpinene	0.37	1222	car-2-en-4-one	0.39
1023	p-cymene	6.22	1254	car-3-en-2-one	0.34
1027	*β*-phellandrene	2.73	1286	dihydroedulan II	0.08
1038	cis-*β*-ocimene	0.19	1291	dihydroedulan I	0.11
1048	trans-*β*-ocimene	0,.44	1387	trans-*β*-damascenone	0.17
1057	*γ*-terpinene	0.1	1390	*β*-elemene	0.14
1084	p-metha-2,4(8)-diene	0.1	1479	*γ*-muurolene	0.08
1086	Terpinolene	1.59	1493	*α*-selinene	0.82
1101	Perillene	0.25	1522	Myristicin	1.04
1107	Hotrienol	0.17	1640	*δ*-cadinol	0.28
1121	trans-sabinol	0.53	1654	*α*-cadinol	0.2
1129	trans,trans-2,6-dimethyl-1,3,5,7-octatetraene	1.78	1839	Neophytadiene	0.12

The major compounds of *E. spinosa* oil, δ^3-carene (60,86%) was found to be the main constituent only in oil of *E. lamondiana* (48.1%), [9]. The composition of the oil of some *Echinophora* species is described in the literature [3, 9-13].

Antibacterial effect of *E. spinosa* oil in microdilution test was the most prominent against *Escherichia coli* and *Pseudomonas aeruginosa* (Table 2). The most resistant bacterial species was *Staphylococcus aureus*. In our research it can be seen that *E. spinosa* oil showed better antibacterial activity against gram-negative bacterial species. Minimum inhibitory and fungicidal concentrations of oil are presented in the Table 3. The most resistant fungal species were *Penicillium ochrocloron* and *P. funicoloum* while *Trichoderma viride* was the most sensitive. The essential oil tested showed higher antifungal potency than tested commercial drugs bifonazole and ketoconazole only against *T. viride*.

It is also imporatant to notice that this is the first record of antimicrobial activity of *E. spinosa* essential oil. There is no any literature data that essential oils from other *Echinophora* species are tested for antimicrobial activity. Only ethanol extarcts of *E. platyloba* are tested for antimicrobial activity agisnt *Candida albican,* dermatomycetes and bacteria (*Staphylococcus aureus, S. epidermidis* and *Streptocioccus pyogenes*). This 5% ethanol extarct showed weekly antibacterial but potent antifungal activity [14-16]. Methanol extract of *E. platyloba* showed good antibacterial activity against agaisnt *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while there is non indicated considerable growth prevention for *Aspergillus flavus, A. niger* and *Candidia albicans* [17].

Numerous antimicrobial agents have been formulated and evaluated for use in the management of bacterial and fungal diseases, but the high toxicity of these agents makes it necessary to find new one. The oil used in this study reveal that it could be very good alternatives to chemicals in the treatment of microbial diseases because of its potent activities and their reduced toxicity.

Table 2. Antibacterial activity of *E. spinosa* essential oil (mg/mL) and streptomycin (mg/mL).

Bacteria	Oil MIC MBC	Streptomycin MIC MBC
Staphylococcus aureus	2.5	0.05
	10	0.1
Bacillus cereus	0.5	0.5
	1	0.5
Micrococcus flavus	1	0.1
	5	0.1
Listeria monocytogenes	1	0.05
	5	0.1
Pseudomonas aeruginosa	0.25	0.05
	0.5	0.1
Enterobacter cloacae	0.25	0.05
	0.5	0.1
Salmonella typhimurium	0.1	0.1
	2.5	0.2
Escherichia coli	0.0625	0.2
	0.5	0.4

Table 3. Antifungal activity of *E. spinosa* essential oil (mg/mL) and bifonazole, ketoconazol (mg/mL).

Fungi	Oil MIC MFC	Bifonazole MIC MFC	Ketoconazol MIC MFC
Aspergillus ochraceus	1	0.1	0.025
	1	0.1	0.1
Aspergillus versicolor	0.5	0.1	0.1
	1	0.1	0.25
Aspergillus niger	1	0.1	0.025
	5	0.1	0.025
Aspergillus flavus	0.5	0.1	0.025
	1	0.1	0.05
Trichoderma viride	0.0625	0.15	0.05
	0.125	0.25	0.1
Penicillium ochrochloron	2.5	0.15	0.01
	10	0.2	0.025
Penicillimu funiculosum	2.5	0.15	0.025
	10	0.2	0.05

Acknowledgments

We are grateful to the Ministry of Science and Technological Development of Republic of Serbia for financial support, Pr. No 173032 and 173024

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