

Full Length Research Paper

Antimicrobial and antioxidant activities of essential oil and crude extracts of *Hypericum tetrapterum* Fries (Hypericaceae)

Aleksandra Đorđević^{1*}, Andrija Šmelcerović², Dragan Veličković³, Vesna Stankov-Jovanović¹, Violeta Mitić¹, Danijela Kostić¹ and Radosav Palić¹

¹Department of Chemistry, Faculty of Science and Mathematics, University of Niš, 18000 Niš, Serbia.

²Department of Pharmacy, Faculty of Medicine, University of Niš, 18000 Niš, Serbia.

³College of Agriculture and Food Technology, 18400 Prokuplje, Serbia.

Accepted 21 June, 2010

***In vitro* antimicrobial activity of essential oil and crude extracts (petroleum ether, ethyl acetate and methanol extract) of *Hypericum tetrapterum* Fries (Hypericaceae) were investigated by disc diffusion and dilution methods. The studied samples were active against a panel of standardized bacteria and fungi. The maximum antimicrobial activity was shown by the essential oil and the petroleum ether extract. The antioxidant activity of the examined oil and extracts was determined by free radical scavenging assay and a phosphomolybdenum method. Both methods showed that the methanol crude extract of *H. tetrapterum* had the highest antioxidant capacity among all samples investigated. The results of the pharmacological tests were discussed with respect to up to now known constituents of essential oil and crude extracts of *H. tetrapterum*. A correlation between the total flavonoid content and the antioxidant activity was observed. To the best of our knowledge this is the first report about the antimicrobial and antioxidant activities of the essential oil, crude petroleum ether and ethyl acetate extracts of *H. tetrapterum*.**

Key words: *Hypericum tetrapterum* Fries, Hypericaceae, antimicrobial activity, antioxidant activity, essential oil, crude extracts.

INTRODUCTION

Plant species of the genus *Hypericum* (Hypericaceae) are well known for their use in traditional medicine due to their therapeutic efficacy. For homoeopathic preparations, European Pharmacopoeia recommends whole, fresh plant of St. John's wort (*Hypericum perforatum* L.) (European Pharmacopoeia, 2007). St. John's wort has also been widely used by folks as a tea. Due to the pharmaceutical activities of active compounds from *H. perforatum*, the number of studies on *Hypericum* species has increased rapidly over the years. Two detailed research studies were performed on the secondary metabolite contents of *Hypericum* species, collected from Serbia and former Yugoslav Republic of Macedonia, showing that methanol extracts of *H.*

tetrapterum contained hypericin, hyperforin, hyperoside and quercitrin (Smelcerovic and Spiteller, 2006a; Smelcerovic et al., 2006b). The chemical composition of *H. tetrapterum* essential oil, which originated from southeastern Serbia, was also reported (Smelcerovic et al., 2007). Our previous study revealed *in vitro* antimicrobial and antioxidant activity of nine *Hypericum* species from the Balkans. All investigated extracts possess a very broad spectrum of antimicrobial activity but, unexpectedly, the extract of the most recognized species of this genus, *H. perforatum*, was among the least active (Radulović et al., 2006). As part of our continuing study of the chemical composition (Smelcerovic and Spiteller, 2006a; Smelcerovic et al., 2006b; 2007; 2008; Verma et al., 2008;) and pharmacological activities (Radulović et al., 2006; Bonkanka et al., 2008; Spiteller et al., 2008) of *Hypericum* species, we now report in this paper the antimicrobial and antioxidant activities of essential oil and crude extracts

*Corresponding author. E-mail: sanjadj81@yahoo.com. Tel: +381 638079217. Fax: +381 18533014.

of *H. tetrapterum* Fries. In the previous reports, of the two earlier stated pharmacological activities of *H. tetrapterum*, only the methanol (Radulović et al., 2006) and methanol-acetone (Sagrattini et al., 2008) extracts were examined. In order to localize the range of polarity of the active constituents which are responsible for antimicrobial and antioxidant properties of *H. tetrapterum*, its essential oil as well as petroleum ether, ethyl acetate and methanol extracts were compared. The antimicrobial activity was investigated by disc diffusion and dilution methods while the antioxidant activity was determined using the free radical scavenging and phosphomolybdenum methods. An attempt to correlate the examined pharmacological effects with the chemical composition of the studied plant was also undertaken.

MATERIALS AND METHODS

Chemicals and reagents: Methanol, petroleum ether and ethyl acetate were purchased from Carlo Erba (Italy). Ammonium molybdate, sulfuric acid, sodium phosphate and α -tocopherol acetate were obtained from Merck (Darmstadt, Germany). Aluminum chloride hexahydrate and sodium acetate were provided by Reanal (Budapest, Hungary). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Karlsruhe, Germany) while 3,5-di-tert-butyl-4-hydroxytoluene (BHT) was purchased from Sigma (Taufkirchen, Germany). Rutin trihydrate and quercetin dihydrate were provided by Carl Roth GmbH (Karlsruhe, Germany). All chemicals and reagents were analytical grade purity.

Plant material and plant material extraction

The plant material (*H. tetrapterum* Fries) was collected at bloom stage on August 5th, 2004 on Rudina (surroundings of the town of Bosilegrad, southeast Serbia); a voucher specimen (No. 733) was deposited in the Herbarium Moesicum Doljevac (Serbia). The plant material was dried at room temperature and then milled. The dry plant material was then packed in paper bag and kept in a dark, dry and cool place. Aerial parts of the herb were used for the experiment. The essential oil was obtained by a Clevenger-type apparatus. The crude extracts were obtained by conventional maceration using 5 g of plant material and 50 ml of a solvent (petroleum ether, ethyl acetate and methanol).

Total flavonoid content

Total flavonoid content of crude extracts was determined using the slightly modified method of Ordonez et al. (2006). Total flavonoid content was calculated on the basis of the calibration curve of quercetin and expressed by mg quercetin equivalents/g of dry plant material. All spectrophotometric measures in this work were performed using a Perkin-Elmer Lambda 15 UV-VIS spectrophotometer and carried out in triplicate with average values considered.

Antimicrobial activity

The cultures of the following microorganisms were used: *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus*

ATCC 6538, *Sarcina lutea* ATCC 9341 and *Candida albicans* ATCC 10231 and were obtained from Oxoid (UK), as well as *Aspergillus niger* ATCC 16404, from the collection of microorganisms of the Biological laboratory of "Zdravlje-Actavis" Company, Pharmaceutical and Chemical Industry, Leskovac, Serbia. All the microorganisms were completely unsusceptible to the control discs imbued with appropriate solvents.

Diffusion method

A disc diffusion method was employed, according to National Committee for Clinical Laboratory Standards-NCCLS (NCCLS, 1997a), with slight modifications. Standard discs of gentamicin and nystatine (origin – Institute of Immunology and Virology "Torlak" (Serbia), 30 μ g of the active component, diameter 6 mm) were used individually as positive controls. Mean values of each test performed in triplicate were demonstrated.

Dilution method

A macro-dilution broth assay was used for the determination of minimal inhibitory (MIC), minimal bactericidal (MBC) and minimal fungicidal concentrations (MFC), recommended by NCCLS (NCCLS, 1997b; 1997c) (with slight modifications). Gentamicin and nystatine were used as positive controls.

Antioxidant assay

The antioxidant activity was determined using phosphomolybdenum and free radical scavenging methods. The phosphomolybdenum assay of the extracts and diluted essential oil (1:10 in ethanol, v/v) of *H. tetrapterum* were evaluated by slightly modified method described by Prieto et al. (1999). The free radical scavenging capacity of the same samples was determined by a modified DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Mimica-Dukić et al., 2008).

RESULTS AND DISCUSSION

The results of the antimicrobial activity of the essential oil and crude extracts of *H. tetrapterum* obtained by disc diffusion and dilution methods are given in Tables 1 and 2, respectively. The studied essential oil and crude extracts were active against all microorganisms tested. Both earlier stated methods indicate that the greatest activity was shown by the essential oil and the petroleum ether extract.

The activity of the essential oil and extracts on Gram-negative bacteria, Gram-positive bacteria, and fungal organisms was similar. The diameters of growth inhibition zones ranged from 7.5 mm (5 μ l of essential oil against *C. albicans*) to 17.5 mm (10 μ l of petroleum ether extract against *C. albicans*) (Table 1). The values of examined samples at dose of 10 μ l per disc were comparable with inhibition zone diameters of standard antibiotics (30 μ g of the active component per disc) (Table 1). The MIC values for the all test cultures were 2.5 μ l/ml (Table 2). The MBC and MFC values ranged from 2.5 μ l/ml to 80 μ l/ml for the *H. tetrapterum* essential oil and extracts while standard antibiotics exhibited values from 16.0 to 25.0 μ g/ml (Table 2).

Table 1. Diameters of growth inhibition zones (in mm; including the diameter of the disc, 6 mm) caused by the action of essential oil and crude extracts of *H. tetrapterum*.

Sample/Microorganism		<i>S. enteritidis</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. lutea</i>	<i>C. albicans</i>	<i>A. niger</i>
Essential oil (μl)	5	9.4	11.4	12.0	12.7	8.2	7.5	8.6
	10	15.0	15.2	15.8	15.5	8.4	10.5	13.5
Petroleum ether extract (μl)	5	11.0	13.2	12.7	11.7	9.6	13.8	13.5
	10	13.8	13.8	15.1	14.3	10.8	17.5	15.6
Ethyl acetate extract μl	5	11.4	11.2	11.3	11.4	7.8	14.1	12.8
	10	11.8	11.3	11.5	12.0	8.4	15.5	12.9
Methanol extract (μl)	5	9.5	10.8	12.0	11.8	8.6	8.8	8.4
	10	12.4	13.0	13.0	13.4	9.2	12.3	11.2
Gentamicin (μg)	30	14.0	15.0	15.0	17.0	16.0	n.t.	n.t.
Nystatine (μg)	30	n.t.	n.t.	n.t.	n.t.	n.t.	18.0	17.0

n.t. - not tested.

Table 2. MIC and MBC/MFC (μl/ml) values of essential oil and crude extracts of *H. tetrapterum*

Sample/Microorganism		<i>S. enteritidis</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. lutea</i>	<i>C. albicans</i>	<i>A. niger</i>
Essential oil	MIC	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	MBC/MFC	40.0	40.0	40.0	40.0	50.0	40.0	40.0
Petroleum ether extract	MIC	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	MBC/MFC	50.0	50.0	30.0	20.0	10.0	5.0	5.0
Ethyl acetate extract	MIC	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	MBC/MFC	50.0	50.0	30.0	60.0	50.0	30.0	50.0
Methanol extract	MIC	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	MBC/MFC	5.0	30.0	40.0	80.0	50.0	30.0	20.0
Gentamicin (μg/ml)	MIC	8.0	10.0	8.0	8.0	8.0	n.t.	n.t.
	MBC	16.0	16.0	25.0	16.0	16.0	n.t.	n.t.
Nystatin (μg/ml)	MIC	n.t.	n.t.	n.t.	n.t.	n.t.	8.0	8.0
	MFC	n.t.	n.t.	n.t.	n.t.	n.t.	16.0	16.0

n.t. - not tested.

The showed antimicrobial activity of the essential oil and extracts of *H. tetrapterum* was related to their chemical composition (Smelcerovic and Spiteller, 2006a; Smelcerovic et al., 2006b; 2007). The *H. tetrapterum* oil is a complex mixture of non-terpenes, monoterpenes and sesquiterpenes (Smelcerovic et al., 2007), and it contains some terpenes with known antibacterial (linalool, α -terpineol and terpinen-4-ol) (Kotan et al., 2007; Hinou et al., 1989) and antifungal (anethol) (Pauli and Knobloch, 1987) activity. The

methanol extracts of *H. tetrapterum* contained hyperforin (phloroglucinol derivative) and flavonoids (Smelcerovic and Spiteller, 2006a; Smelcerovic et al., 2006b). Hypericin (Avato et al., 2004) and phloroglucinol derivatives (Avato et al., 2004; Rocha et al., 1995) are reported to be responsible for the antimicrobial activity against various bacteria and fungi in some *Hypericum* species. Hyperforin, a phloroglucin derivative, exhibited an excellent effect against methicillin-resistant strains of *S. aureus* with a MIC

Table 3. Total antioxidant capacity (μmol of α -tocopherol acetate per g of dry plant material) and DPPH-RSC antioxidant activity of *H. tetrapterum* essential oil and crude extracts.

Sample	Total antioxidant capacity	DPPH-RSC (%)
Methanol extract	5588.0 \pm 22	86.6
Ethyl acetate extract	1371.3 \pm 13	63.5
Petroleum ether extract	27.0 \pm 2	1.3
Essential oil	107.2 \pm 9	4.0
BHT (30 mg/ml)	n.t.	95.6

n.t. - not tested.

value of 1.0 $\mu\text{g/ml}$ (Schempp et al., 1999).

The antioxidant capacity of the examined samples, determined by using phosphomolybdenum method, ranged from 27.0 to 5588.0 $\mu\text{mol/g}$ (expressed as equivalents of α -tocopherol acetate per g of dry plant material) (Table 3). The highest antioxidant capacity was produced by the methanol extract. The value of the activity for the methanol extract was similar with those reported by Radulović et al. (2006). The three recent investigations of the antioxidant activity of *H. perforatum* and *H. triquetrifolium* extracts (Conforti et al., 2002; Silva et al., 2005; Zou et al., 2004) have already concluded that the activity is related to the flavonoid derivatives. Zou et al. (2004) indicated that flavonoid-rich extract of *H. perforatum* might be proposed as a dietary supplement or drug for the treatment of various coronary heart diseases (Zou et al., 2004), based on its antioxidant activity. In order to confirm this relationship among different *H. tetrapterum* extracts, total flavonoid content was determined. The total flavonoid content for methanol, ethyl acetate and petroleum ether extracts amounted 23.0 \pm 0.8, 4.4 \pm 0.4 and 0.9 \pm 0.1 mg quercetin per g of dry plant material, respectively. A significant correlation ($r^2=0.9926$) between the total flavonoid content and the antioxidant activity was observed.

The results of the free radical scavenging antioxidant assay (DPPH-RSC) are given in Table 3. According to the results obtained by both methods, the antioxidant activity decreases in the following order: methanol extract > ethyl acetate extract > essential oil > petroleum ether extract. The results suggest that polar compounds, such as flavonoids, are responsible for antioxidant effects observed in this species.

Conclusion

The main conclusion from the earlier stated data is that the studied *H. tetrapterum* essential oil shows excellent antimicrobial activity, and it may be used against bacterial and fungal infections in traditional and modern medicine. Contrary to the antimicrobial activity, the highest antioxidant ability was expressed by methanol extract. To the best of our knowledge, this is the first report about the antimicrobial and antioxidant activities

of the essential oil as well as the ethyl acetate and petroleum ether extracts of *H. tetrapterum*.

ACKNOWLEDGEMENTS

This work was funded by the Ministry of Science and Technological Development of Serbia (Project 142054 B). The authors wish to thank Prof. Dr. N. Ranđelović, Faculty of Occupation Safety, Niš, Serbia, for the taxonomic identification of the plant material.

REFERENCES

- Avato P, Raffo F, Guglielmi G, Vitali C, Rosato A (2004). Extracts from St John's wort and their antimicrobial activity. *Phytother. Res.*, 18: 230-232.
- Bonkanka CX, Smelcerovic A, Zuehlke S, Rabanal RM, Spittler M, Sánchez-Mateo CC (2008). HPLC-MS analysis and anti-oedematogenic activity of *Hypericum grandifolium* Choisy (Hypericaceae). *Planta Med.*, 74: 719-725.
- Conforti F, Statti GA, Tundis R, Menichini F, Houghton P (2002). Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part. *Fitoterapia*, 73: 479-483.
- European pharmacopoeia (2007). Directorate for the Quality of Medicines and HealthCare of the Council of Europe (EDQM) Strasbourg, 6th edition, vol. 1, pp. 1080-1081.
- Hinou JB, Harvala CE, Hinou EB (1989). Antimicrobial activity screening of 32 common constituents of essential oils. *Pharmazie* 44: 302-303.
- Kotan R, Kordali S, Cakir A (2007). Screening of antibacterial activities of twenty-one oxygenated monoterpenes. *Z. Naturforsch. C* 62: 507-513.
- Mimica-Dukić N, Simin N, Cvejić J, Jovin E, Orčić D, Božin B (2008). Phenolic compounds in field horsetail (*Equisetum arvense* L.) as natural antioxidants. *Molecules*, 13: 1455-1464.
- NCCLS (National Committee for Clinical Laboratory Standards) (1997b). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th edition, Wayne, Pa. M7-A4.
- NCCLS (National Committee for Clinical Laboratory Standards) (1997a). Performance standards for antimicrobial disc susceptibility testing, 6th International Supplement, Wayne Pa. M2-A6.
- NCCLS (National Committee for Clinical Laboratory Standards) (1997c). Reference method for broth dilution antifungal susceptibility testing for yeasts, Wayne, Pa. M27-A.
- Ordóñez AAL, Gomez JD, Vattuone MA, Isla MI (2006). Antioxidant activities of *Sechium edule* (Jacq.) swart extracts. *Food Chem.*, 97: 452-458.
- Pauli A, Knobloch K (1987). Inhibitory effects of essential oil components on growth of food-contaminating fungi. *Z. Lebensm. Unters. F. A*, 185:10-13.
- Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.*, 269: 337-341.

- Radulović N, Stankov-Jovanović V, Stojanović G, Šmelcerović A, Spiteller M, Asakawa Y (2006). Screening of *in vitro* antimicrobial and antioxidant activity of nine *Hypericum* species from the Balkans. *Food Chem.*, 103: 15-21.
- Rocha L, Marston A, Potterat O, Kaplan MAC, Stoeckli-Evans H, Hostettmann K (1995). Antibacterial phloroglucinols and flavonoids from *Hypericum brasiliense*. *Phytochemistry*, 40: 1447-1452.
- Sagratini G, Ricciutielli M, Vittori S, Öztürk N, Öztürk Y, Maggi F (2008). Phytochemical and antioxidant analyses of eight *Hypericum* taxa from Central Italy. *Fitoterapia*, 79: 210-213.
- Schempp CM, Pelz K, Wittmer A, Schöpf E, Simon JC (1999). Antibacterial activity of hyperforin from St. John's wort, against multiresistant *Staphylococcus aureus* and gram-positive bacteria. *Lancet*, 353: 2129.
- Silva BA, Ferreres F, Malva JO, Dias ACP (2005). Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chem.*, 90: 157-167.
- Smelcerovic A, Spiteller M (2006a). Phytochemical analysis of nine *Hypericum* L. species from Serbia and the F.Y.R. Macedonia. *Pharmazie*, 61: 251-252.
- Smelcerovic A, Spiteller M, Ligon AP, Smelcerovic Z, Raabe N (2007). Essential oil composition of *Hypericum* L. species from southeastern Serbia and their chemotaxonomy. *Biochem. Syst. Ecol.*, 35: 99-113.
- Smelcerovic A, Verma V, Spiteller M, Ahmad SM, Puri SC and Qazi GN (2006b). Phytochemical analysis and genetic characterisation of six *Hypericum* species from Serbia. *Phytochemistry*, 67: 171-177.
- Smelcerovic A, Zuehlke S, Spiteller M, Raabe N, Özen T (2008). Phenolic constituents of 17 *Hypericum* species from Turkey. *Biochem. Syst. Ecol.*, 36: 316-319.
- Spiteller M, Özen T, Smelcerovic A, Zuehlke S, Mimica-Dukic N (2008). Phenolic constituents and the *in vitro* antioxidant activity of the flowers of *Hypericum venustum*. *Fitoterapia*, 79: 191-193.
- Verma V, Smelcerovic A, Zuehlke S, Hussain MA, Ahmad SM, Ziebach T, Qazi G, Spiteller M (2008). Phenolic constituents and genetic profile of *Hypericum perforatum* L. from India. *Biochem. Syst. Ecol.*, 36: 201-206.
- Zou Y, Lu Y, Wei D (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. *J. Agric. Food Chem.*, 52: 5032-5039