Biological activities of phenolic compounds and ethanolic extract of *Halacysa sendtneri* (Boiss) Dörfler

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**Abstract:** The objective of this study was to evaluate the efficacy of the ethanolic extract of endemic plant *Halacysa sendtneri* in inhibiting the growing of the test fungi and bacteria as well as to determine its genotoxic potential and toxicity using the Allium anaphase-telophase assay. Minimum inhibitory concentrations (MIC) were determined for 15 indicator strains of pathogens, representing both bacteria and fungi. The highest susceptibility to the ethanolic extract of *H. sendtneri* was exhibited by *Pseudomonas glycinea* (FSB4) (MIC = 0.09 mg/ml) among the bacteria, and by *Phialophora fastigiata* (FSB81), (MIC = 1.95 mg/ml) among the fungi. The composition of *H. sendtneri* extracts was also determined using HPLC analysis. Rosmarinic acid was found to be the dominant phenolic compound. The *Allium* anaphase-telophase genotoxicity assay revealed that the ethanolic extract of *H. sendtneri* at concentrations of 31.5 mg/l and below does not produce toxic or genotoxic effects. This is the first report of chemical constituents, genotoxic and antimicrobial activities of the endemic species, *H. sendtneri*.

**Keywords:** Antimicrobial activity • Genotoxicity • *Halacysa sendtneri* • Phenolic compounds

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1. Introduction

The use of traditional medicinal plants for primary health care and other purposes has progressively increased worldwide in recent years. Much attention has been given to various plant secondary metabolites that are a common feature of specific plants and plant families. Many plant secondary metabolites and essential oils have antimicrobial properties that make plant extracts and products successful in the treatment of bacterial, fungal and viral infections [1-3]. The Boraginaceae family occurs worldwide, and it consists of about 100 genera with more than 2000 species, (one of the species is *Halacysa sendtneri* [4]. Many members of the Boraginaceae family produce secondary metabolites such as alkaloids, naphthaquinones, polyphenols, phytosterols and terpenoids [5,6]. Polyphenols, including flavonoids and phenolic acids, produced by the family Boraginaceae, have a wide range of pharmaceutical activities, including anti-inflammatory, anti-viral and anti-bacterial activities [7-9].

*Halacysa sendtneri* is a member of the monotypic genus *Halacysa* of the family Boraginaceae, its range
being limited to parts of the habitat in the central Balkans. The species inhabits open serpentine rocky landscapes at altitudes ranging from 190 to 1500 m [10]. In Serbia, it is found along the serpentine of certain mountains, as well as in some gorges. H. sendtneri is considered a Tertiary relict [11]. So far, antimicrobial properties have not been indicated for this endemic species. It is qualified as a vulnerable species (V) in the European Red List (marked as +) [12]. Apart from being highly important in terms of world plant gene pool preservation, endemic plants can also contribute substantially to studies on antimicrobial activity [13]. In order to minimize the risk of using new natural antioxidants as preservers or for other purposes (as drugs or food supplements) it is necessary to have data on their potential toxicity and genotoxicity. For evaluation of genotoxicity, a significant number of assays using different biological systems (plants, fruits, vegetables etc.) have been developed. Plant bioassays, characterized by generally higher sensitivity in comparison to other systems, proved useful in different situations and for different end points [14]. Among them, the Allium (A. cepa) test has been used to assess a great number of chemical compounds [15], wastewater [16], rivers and lakes [17,18], drinking water [19] and melted snow [20]. The advantages of this assay include high sensitivity, similarity in chromosome organization to that in humans, good correlation to other test systems and the possibility of studying the effects under a wide range of conditions [21]. So far, only one study reported on the activities of H. sendtneri. This research is based on studies of antioxidant activity H. sendtneri extracts [22].

No previous studies on the biological activity or chemical constituents of Halacsya sendtneri have been reported in the literature. The objective of the study was to determine the antimicrobial, antioxidant, and genotoxic activities of Halacsya sendtneri growing in Serbia. We also identified the major phenolic constituents of H. sendtneri using HPLC.

2. Experimental Procedures

2.1 Chemicals used

Standards for HPLC (chlorogenic, caffeic, ferulic, rosmarinic, protocatechuic, gallic and p-coumaric acid, myricetin, quercetin, resveratrol, rutin, epigallocatechin, catechin, apigenin) analysis were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA). Acetonitrile gradient grade (J. T. Baker, Philipsburg, NJ, USA), phosphoric acid p.a. grade and 18 M deionized water (Millipore, Bedford, MA) were used. Ethanol was of analytical grade (Aldrich Chemical Co., Steinheim, Germany).

2.2 Plant material

*Halacsya sendtneri* (Boiss) Dörfler. was collected at Ilijak Hill, Central Serbia (214 m above sea, coordinates: 43°52’02”N, 20°31’28”E) from May to June 2008. The species was identified and the voucher specimen was deposited at the Department of Botany, Faculty of Biology, University of Belgrade (16336 BEOU, Lakušić Dmitar).

2.3 Preparation of extracts

The air-dried parts of the above-ground of plant (90 g) were broken into small pieces by a cylindrical crushe, and extracted (60°C, 5 h and, 1:5, g/ml (drug:solvent ratio)) with ethanol (99.8%) using a Soxhlet apparatus. The ethanolic extract was filtered through filter paper (Whatman, No.1) and concentrated to dry mass (6.51 g). The residues were stored in a dark glass bottles for further processing.

2.4 Test microorganisms

The antimicrobial activity of the plant extract was tested *in vitro* against the following Gram-positive bacteria: *Staphylococcus aureus* (American Type Culture Collection (ATCC) 12600), *Micrococcus lysodeikticus* (ATCC 4698) and *Bacillus mycoides* (ATCC 6462); and the following Gram-negative bacteria: *Klebsiella pneumoniae* (ATCC 6462), *Pseudomonas glycinea* (Faculty of Biological Sciences, Serbia (FSB) 40) and *Escherichia coli* (ATCC 11775) and fungi *Candida albicans* (ATCC 10259), *Fusarium oxysporum* (FSB91), *Penicillium canescens* (FSB24), *Aspergillus glaucus* (FSB32), *Alternaria alternata* (FSB51), *Penicillium verrucosum* (FSB21), *Aspergillus niger* (FSB31), *Trichoderma viride* (FSB11) and *Phialophora fastigiata* (FSB81). Pure cultures were generated by subculturing four times on the same media for seven days. All test microorganisms used are from the Institute for Immunobiology and Virology, Torlak, Belgrade, Serbia. Identification of the test microorganisms was confirmed by the Laboratory of Mycology, Department of Biology, Faculty of Science, University of Kragujevac, Serbia.

2.5 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of the extracts were determined using a microdilution method in 96 multi-well microtiter plates [23]. All tests were performed in Müller–Hinton broth (MHB), with the exception of yeast, in which case Sabouraud dextrose broth was used. A volume of 100 µl stock solutions of extract (in methanol, 200 µl/ml) was pipetted into the first row of the plate. 50 µl of Müller–Hinton or Sabouraud dextrose broth (supplemented with Tween 80 at a final concentration of 0.5% v/v) for extract analysis) was added to the other wells. 50 µl from the first test well