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Ethnopharmacological uses of *Sempervivum tectorum* L. in southern Serbia: Scientific confirmation for the use against *otitis* linked bacteria

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ABSTRACT

Ethnopharmacological relevance: *Sempervivum tectorum* L. (Crassulaceae), known as houseleek, is used in traditional medicine in the treatment of ear inflammation. It can be spread as a pack on wounds, sores, burns, and abscesses and also on painful areas attacked by gout as a refrigerant and astringent. Drinking tea prepared from leaves of *S. tectorum* is recommended for ulcer treatment. The present study was designed to investigate ethopharmacological use of *S. tectorum* in the southern Serbia and to further scientifically justify and confirm effectiveness of the leaf juice used in ethnomedicine for ear inflammation, against *otitis* linked bacteria.

Material and methods: Ethnopharmacological survey on the use of *S. tectorum* in southern Serbia was performed using semi structured questionnaires via a face-to-face interview. Chemical composition of the leaf juice regarding phenolic compounds and organic acids was analyzed. Antimicrobial activity was tested on bacteria isolated from ear swabs of the patients suffering from the ear pain (*otitis*). Anti-quorum-sensing activities of the juice were further investigated on *Pseudomonas aeruginosa*.

Results: Ethnopharmacological survey revealed the use of *S. tectorum* in southern Serbia for the treatment of ear pain, warts, cancer, stomachache, ulcer and high blood sugar level with the highest fidelity level (FL) for the ear pain. The phenolic composition of the *S. tectorum* leaf juice consisted of flavonol glycosides, with kaempferol-3-*O*-rhamnosyl-glucoside-7-*O*-rhamnoside as the majority compound. Organic acids composition revealed malic acid as the most dominant one. Antimicrobial and anti-quorum-sensing activities of the juice showed to be promising.

Conclusion: Ethnopharmacological use of *S. tectorum* juice for treating ear pain is justified, since the juice possessed antimicrobial activity towards clinical isolates of bacteria linked to *otitis*.

1. Introduction

Traditional medicines are continuously increasing in their status, and show that there are some features, which are unique to them, contributing to both efficacy and safety (Nanjan, 2010). In recent years, special attention has been given to alternative natural bio-remedies to cure different diseases (Savikin et al., 2013) because of their less or no side effects, high efficacy and low cost.

Sempervivum tectorum L. (Crassulaceae), known as houseleek, is an evergreen plant with perennial root, crowned with imbricated fleshy leaves, which are smooth on both sides and ciliated at the margin, with the stem rising from the center of the tuft of leaves and terminated with a cymose corymb flowers (Muselin et al., 2014). Its use in the treatment of ear inflammation in Serbian folk medicine has been reported (Savikin et al., 2013), having also an antinociceptive activity (Alberti et al., 2012). Fresh juice from squeezed leaves of *S. tectorum* is used as a folk medicine almost exclusively for external purposes. It can be spread as a pack on wounds, sores, burns, and abscesses and also on painful areas attacked by gout as a refrigerant and astringent. Drinking tea prepared from leaves of *S. tectorum* is recommended for ulcer treatment (Bremness, 1996).

Otitis is a general term used to describe inflammation or infection of the ears. *Otitis* is classified as *otitis interna*, *otitis media* and *otitis externa*, depending on it affects inner ear, middle ear and outer ear and canals, respectively (NIDOCD, 2006). *Otitis media* is linked to inflammation of the middle ear, which often

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begins when infections that cause sore throats, colds, or other respiratory or breathing problems spread to the middle ear (Berman et al., 1997). Acute *otitis media* is a bacterial infection of the mucosal line ear (Culpepper and Froom, 1997). Chronic suppurative *otitis media* is the condition when the ear drum has been perforated. In an acute attack of *otitis media* the infection remains patent and becomes chronic upon secondary invaders: *Staphylococcus aureus* and *P. aeruginosa* (Tierney et al., 2005). *Otitis externa* may be caused by bacteria and fungi, particularly *P. aeruginosa*, *S. aureus*, *Candida albicans* and some *Aspergillus* species (Karma et al., 1978).

Quorum sensing (QS) is an intercellular signaling system in which bacteria communicate and regulate gene expression by releasing small compounds called autoinducers in the environment. Due to its role in various regulatory processes it can serve as an important target. Knowledge about quorum sensing is resulting in identification of new targets for therapeutics against *P. aeruginosa* infection (Petrović et al., 2014; Glamočlija et al., 2015).

The aim of this study was first to investigate folk use of *S. tectorum* in Vranje area, southern Serbia. After the data about the use in folk medicine were obtained, chemical characterization of the squeezed *S. tectorum* juice was analyzed by HPLC–DAD–ESI/MS. Since the fidelity level revealed the most frequent use of *S. tectorum* for ear inflammation, bacteria were isolated from several patients suffering with *otitis*. Antimicrobial activity of *S. tectorum* juice was tested against the isolated bacteria. Furthermore, influence of the juice was tested against certain quorum-sensing-regulated functions in *Pseudomonas aeruginosa* clinical isolate.

2. Material and methods

2.1. Ethnopharmacological investigation

2.1.1. Study area

Vranje is administrative, health care, educational and cultural center of Pcinja district, located in southern Serbia. The City of Vranje covers the space of 860 km^2. Vranje is the economic, political and cultural center of Pčinja District consisting of Bosilegrad, Bujanovac, Vladičin Han, Preševo, Surdulica, Trgovište and Vranje municipalities. The city is located in the southwest of Vranje valley, on the left bank of the South Morava River (Fig. 1). All the participants interviewed were living in the municipality of Vranje or surrounding villages.

2.1.2. Ethnopharmacological survey

The survey was performed using semi structured questionnaires via a face-to-face interview and circulating these questionnaires among cross section of people above 20 years of age; 212 filled up reports were collected visiting the Vranje area in southern Serbia. A questionary in Serbian language was prepared about the use of *S. tectorum* L. (Crassulaceae) by the local people in Vranje area. All the respondents were aware of the present investigation and have signed the informed consent. The survey was conducted during two months. The survey covered different age groups of both the sexes, whose gender, age, educational background, professional status and knowledge on the use of *S. tectorum* were also documented. Each participant was interviewed separately to generate data on diseases, regarding the treatment through medicinal plant *S. tectorum*. The record of questionnaires used included the following information: (a) the local name,

Fig. 1. Study area-location of the city of Vranje on the map of Serbia and Europe.