Journal of Arid Environments 120 (2015) 26–32

Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Screening inland halophytes from the central Balkan for their antioxidant activity in relation to total phenolic compounds and flavonoids: Are there any prospective medicinal plants?

Milan S. Stanković [a], Milica Petrović [b], Dejan Godjevac [c], Zora Dajić Stevanović [b, *]

[a] Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia
[b] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Republic of Serbia
[c] Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Republic of Serbia

ARTICLE INFO

Article history:
Received 25 June 2013
Received in revised form
15 January 2015
Accepted 10 April 2015
Available online 15 April 2015

Keywords:
Halophytes
Semiarid arid habitats
Salinity
Secondary metabolites
Biological activity

ABSTRACT

This paper presents new data for secondary metabolites and biological activity of the insufficiently studied European inland halophytes as remarkable indicators of Balkan saline habitats. Phenolic content, flavonoid concentrations and antioxidant activity were analyzed in fifteen terrestrial salt tolerant plants collected from eight saline habitats situated in two distinct semiarid regions of Serbia. Total phenols ranged from 31.86 to 212.71 mg of GA/g of extract (gallic acid equivalent – milligrams of gallic acid per gram of extract). Concentration of flavonoids ranged from 41.21 to 146.06 mg of RU/g of extract (rutin equivalent – milligrams of rutin per gram of extract). Antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl radical reagent; obtained values was ranged from 1785.81 to 17.55 μg/ml. A significant relation was noticed between the concentration of phenols and antioxidant activity. Parallel to the analysis of researched halophytes, *Camellia sinensis* and *Ginkgo biloba* were analyzed for comparison. The best antioxidant properties were determined for *Statice gmelinii*, *Artemisia santonicum* and *Mentha pulegium*. The results indicate a potential of studied halophytic species as candidates for natural sources of secondary metabolites with high biological activity, whereas their bioactive compounds might have a significant role in adaptations to salt and drought stress.

1. Introduction

Salinity and sodicity are common phenomena for arid and semiarid regions of the world. In arid and semiarid regions annual rainfall is insufficient to leach out salts from the upper layers of the soil because of high rates of evaporation and loss of water through the vegetation canopy. Halophytic flora and vegetation are remarkable indicators of the content and composition of salts in the soil giving the main characteristics to saline habitats of the semiarid regions (Dajic Stevanovic et al., 2008). Plants of saline habitats have developed special adaptations of tolerance to salinity stress, as having been faced with osmotic (similar to plants under drought conditions) and toxic effects in conditions of increased salinity. Various adaptive mechanisms of the salt tolerance include: ion compartmentation, osmotic adjustment and succulence, ion transport and uptake regulation, salt exclusion and excretion, regulation of Na/K selectivity, maintenance of redox and energetic status, as well as a range of biochemical and signaling pathways under complex genetic control (e.g. Hasegawa et al., 2000; Dajic, 2006; etc.). There are many similar responses of plants exposed to salt and drought stress conditions. However, some halophytes can tolerate salt stress but not drought, and some xerophytes can tolerate drought but not salt stress (Kefu et al., 2003). In the case of halophytes occurring in arid and semiarid regions, adaptive mechanisms of response to combined stresses (salinity and drought), have evolved in a more complex way.

Oxidative stress is known as an additional phenomenon of stress impact on plants. This secondary effect emerges as a consequence of hyperosmolarity caused by the imposing of plants to drought or salt stress conditions, resulting in the appearance of the reactive oxygen molecules, such as hydrogen peroxide, hydroxyl radicals and superoxide anions (Xiong et al., 2002). The scavenging of reactive oxygen species (ROS) in plants exposed to salt and

* Corresponding author.
E-mail addresses: mstankovic@kg.ac.rs (M.S. Stanković), mpetrovic.azs@gmail.com (M. Petrović), dgodjev@chem.bg.ac.rs (D. Godjevac), dajic@agrif.bg.ac.rs (Z.D. Stevanović).

http://dx.doi.org/10.1016/j.jaridenv.2015.04.008
0140-1963/

drought stress is associated with both activity of the enzymes involved in antioxidant processes of the cell, particularly superoxide dismutase, glutathione peroxidase and catalase (Sekmen et al., 2012) and the presence of osmoprotectant compounds, such as mannitol, betaine and proline (Xiong et al., 2002). The harmful influence of ROS on cell macromolecules may be also alleviated by the activity of non-enzymatic antioxidant compounds such as ascorbic acid, glutathione, thioredoxin, carotenoids, vitamins and phenolics (Xiong and Zhu, 2002; Ksouri et al., 2007).

Biosynthesis and accumulation of polyphenols and other secondary metabolites in plants is considered as evolutionary response of biochemical pathways under unfavorable environmental influences, i.e. biotic/abiotic constraints), including increased salinity (e.g. Navarro et al., 2006; Meot-Duros et al., 2008) and drought (e.g. Selmar and Kleinwächter, 2013).

Since halophytes have been evaluated as valid sources of polyphenols (Benhammou et al., 2009) and other secondary metabolites of medicinal properties in adaptive responses to stress conditions (e.g. Ksouri et al., 2007), and because of a rapid increase in demand for natural bioactive substances on the world market, there is a need for further phytochemical and pharmacological characterization of halophytes. Potential of some halophytic species for antioxidant activity (e.g. Meot-Duros et al., 2008; Lee et al., 2011) and additional demonstration of antimicrobial (e.g. Trabelsi et al., 2010), antiviral, anticancer and other biological effects (Queslati et al., 2012) have been recently reported.

In order to evaluate antioxidant potential (in relation to total phenol and flavonoid content) of the insufficiently studied European inland halophytes, fifteen terrestrial salt tolerant plants of the central Balkan area (territory of Serbia) were selected. Plants were collected from different saline habitats that occur in the form of mosaic plots frequently distributed within two distinct semiarid regions: one in the north (corresponding to the southern part of the Pannonian plain) and the other in the south of Serbia, and which are exposed to strong sub-Mediterranean influences, that of mainly dry and warm summer periods. Influences of ancient boreal periods when primary solonchaks were formed and the current semiarid climate, are both essential for current floristic composition of saline habitats in Serbia, explaining the significant prevalence of xerophytes (mostly of a Pontic, continental and Mediterranean floristic element) over mesophytes (Knezević et al., 2002).

To the best of our knowledge, these results represent the first report on secondary metabolites content and biological activity for halophytes of European inland saline habitats, additionally representing first time information regarding most of the species researched here, including three Pannonian endemics (*Artemisia santonicum, Camphorosma annua* and *Aster tripolium* subsp. *pannonicus*). Stress tolerant species were studied to assess their possible utilization as new medicinal plants.

2. Material and methods

2.1. Plant material

Total of 15 indigenous, mostly obligate halophytes from Serbian inland saline habitats was collected at their full flowering time during 2011 and 2012. The researched halophytes belong to six plant families and include: *Artemisia santonicum* L., *Achillea collina* Becker ex Rchb., *Aster tripolium* L. subsp. *pannonicus* (Jacq.) Soó, *Chamomilla recutita* (L.) Rauschert (Asteraceae), *Atriplex littoralis* L., *A. tatarica* L., *Camphorosma annua* Pall., *C. monspeliaca* L., *Salicornia europaea* L., *Suaeda maritima* (L.) Dumort (Chenopodiaceae), *Hordeum hystrix* Roth, *Puccinellia limosa* (Schur) Holmb. (Poaceae), *Lepidium ruderale* L., (Brassicaceae), *Mentha pulegium* L. (Lamiaceae) and *Statice gmelinii* Willd (Plumbaginaceae).

Plants were collected from natural populations occurring on different types of salt affected soils, such as solonetz and solonchak, differing in composition and content of total sodium salts. Saline habitats of central Balkan, i.e. within the territory of Serbia are distributed mostly in the northern part of the country, known as Vojvodina region, as well as in the south of the country, but presented in the form of a few small and disjunctive areas. Plant material was collected from eight sites two of which are situated in the south of Serbia (Fig. 1 and Table 1). Climatic data for 30 year period (1981–2010) obtained from the Republic Hydrometeorological Service of Serbia (http://www.hidmet.gov.rs/), show the clear evidence of semi-aridity for both regions of the collecting sites (sites assigned 1–6 in the north and sites 7 and 8 in the south of Serbia) in terms of annual precipitation of 586 mm and 550 mm and number of hot days (with temperatures higher than 25 °C) of 29 and 35 days, respectively; for both regions annual evaporation exceeds 856 mm. Such observations on semi-aridity have been recently confirmed by analyses using special climatic indexes (Hrnjak et al., 2014).

Taxonomy of plants followed Flora of Serbia (1970–1980), while Flora Europaea (1968–1980) was used as a complementary taxonomical source. The voucher specimens were confirmed and deposited at the Herbarium of the Faculty of Agriculture, University of Belgrade.

The aboveground parts (shoots) were collected as bulk samples, containing a sufficient number of representative individuals for the purpose of future phytochemical analyses. Plant material was air-dried in the dark, at ambient temperature. Air-dried material was milled in a grinder and stored in tightly sealed dark containers until the analysis.

2.2. Chemicals

Organic solvents and sodium hydrogen carbonate were purchased from „Zorka pharma“ Šabac, Serbia. Gallic acid, rutin hydrate, chlorogenic acid and 2,2-dyphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. Folin-Ciocalteu phenol reagent, 3-tert-butyl-4-hydroxyanisole (BHA) and aluminium chloride hexahydrate ($AlCl_3 \times 6H_2O$) were purchased from Fluka Chemie AG, Buchs, Switzerland. All other solvents and chemicals were of analytical grade. The samples of Green tea

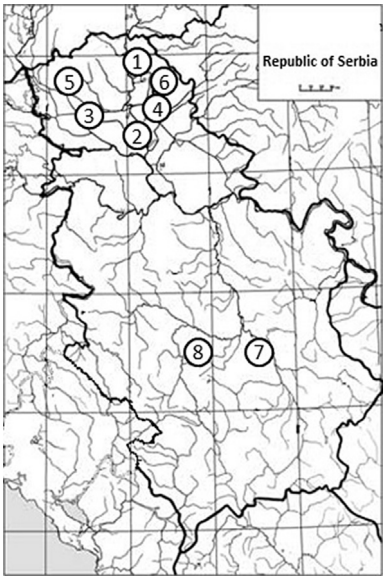

Fig. 1. Distribution of saline habitats of the investigated halophytes on the territory of Serbia (indication of sites as in Table 1).