Secondary metabolites of Hypericum richeri Vill. collected in Central Italy: chemotaxonomy and ethnomedicinal relevance

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ABSTRACT

The results of the phytochemical analysis of a Hypericum richeri Vill. accession collected from a population living in Appennines, Central Italy, are reported in this paper. The secondary metabolites pattern resulted mainly composed of flavonoids as both aglycones and in glycosidic form, such as quercetin (2), quercetin-3-O-methyl ether (3), 5,7,3',5'-tetrahydroxyflavanone (8), isorhamnetin glucoside (7), isoquercitrin (9), hyperoside (10), quercitrin (11) and myricetin-3-O-rutinoside (12). It was also observed the presence of the naphthodianthrones hypericin (5) and pseudohypericin (6) together with organic acids (benzoic acid (4)), a glycolipid (25)-1,2-di-O-((9Z,12Z,15Z)-octadeca-9,12,15-trienoyl)-3-O-β-D-galactopyranosyl glycerol (1) and the saccharides glucose (13), galactose (14) and sucrose (15). Among these constituents, compounds (1) and (3) have been identified in H. richeri Vill. for the first time during this study. The other difference in composition observed in the present study in respect to population from different regions is the absence of hyperforin and caffeoylquinic derivatives. The observed chemovariability, already reported in H. perforatum L., might be derived from the environmental characteristic of the collection site. The chemotaxonomic aspects, together with the pharmacologic relevance in traditional medicine of the isolated compounds were also discussed.

1. Introduction

Hypericum L. (Hypericaceae) is a large genus comprising about 450 species widespread in the temperate regions of the globe and well-represented in the Mediterranean area (Robson and Strid, 1986). Species belonging to this genus are easily recognizable by their yellow flowers. The only taxon of the genus generally used in medicine is H. perforatum L. which is employed in the treatment of mild to moderate depression and, externally, for the treatment of skin complaints such as blunts, wounds and burns (Fleming, 2000). However, several other Hypericum spp. are equally used in traditional medicines of several countries (Yazaki and Okada, 1994). For instance, H. hircinum L. is used in the Sardinian folk medicine for its antiseptic properties against chronic catarrhal affections, asthma and in burns treatment (Ballero et al., 1997; Atzei, 2003); in Lucanian traditional medicine, H. hircinum L. is used for the treatment of cough (Pieroni et al., 2004); H. androsaemum L. is another important species widely used in European traditional medicine. Its leaves are used for the diuretic properties in Portugal and also to treat ailments of liver, kidney and bladder (Valentão et al., 2002); in England, they are used to produce an ointment (mixed with lard) used to heal cuts and wounds (Phillips, 1977; Allen and Hatfield, 2004).

H. richeri Vill. (Fig. 1) (Martorati, 2005) is a species comprised in the Drosocarpium section of Hypericum genus and is a representative species of mountain environment of central south Europe (Kitanov, 2001). It is often used in the traditional medicine of Montenegro as a substitute for H. perforatum L. (Radanovic et al., 2006). Unfortunately, there are no references in literature about the ethnomedicinal uses of this species in the collection region. We have only some information from

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inhabitants about the use of the flowers of *H. richeri* Vill. with or without the contemporaneous presence of flowers of *H. perforatum* L. in the preparation of an ointment. This ointment is obtained by maceration of these plants organs in olive oil and is traditionally used to cure skin disorders such as wounds and burns. Recent studies on the phytochemistry of this species have been mainly conducted on the composition of the volatile fraction (Maffi et al., 2001; Ferretti et al., 2005; Maggi et al., 2010) from Italian and European accessions, mainly of Balkan origin (Smelcerovic et al., 2007). The analysis of the polar fraction revealed the presence of flavonoids, naphthodianthrones and phloroglucinols (Maffi et al., 2001; Smelcerovic and Spiteller, 2006). Also, the biological activities as antioxidant (Zdunič et al., 2011), antimicrobial (Maggi et al., 2010), anti-inflammatory and gastroprotective (Zdunič et al., 2010) agents have been explored in recent times.

The phytochemical studies of medicinal plants are of primary importance in several scientific fields, ranging from the studies of chemical composition of plants of ethnomedicinal relevance to the study of their biological activity. The phytochemical analysis is an important tool to identify the active components of a plant species and is an irreplaceable method of investigation to reveal the presence of not previously described compounds and, lastly, it may reveal the possible presence of poisonous or harmful constituents (Venditti et al., 2016a, 2016b, 2016c; Camilo et al., 2017; Mohammadhosseini, 2017; Mohammadhosseini et al., 2017; Venditti et al., 2017a, 2017b; Frezza et al., 2018). The nature of secondary metabolites observable in a plant species could also give information at the ecology level and/or about the physiology of the studied entity, e.g. revealing chemical evidence of hemiparasitic behavior (Venditti et al., 2017c) or the adaption to a specific environment (Venditti et al., 2016d). In continuation of our work on the analysis of the secondary metabolites and bioactivities of *Hypericum* spp. from Italy (Esposito et al., 2013; Toniolo et al., 2014; Mandrone et al., 2015; Caprioli et al., 2016), we report here the results of the phytochemical analysis conducted on a sample of *H. richeri* Vill. collected in the Appennines area of Central Italy. The aim of the study was to deepen the knowledge about the phytochemical pattern of this species and to highlight an eventual different secondary metabolites pattern in respect to the other studied populations since the tendency to the chemovariability has been already observed in related species such as *H. perforatum* L. (Toniolo et al., 2014) collected in different areas. The phenomenon of chemovariability has been already recognized in several botanical entities, such those living in restricted areas and/or in isolated populations and, obviously, in endemic species (Venditti et al., 2015a; Bianco et al., 2016; Venditti et al., 2017b).

2. Experimental

2.1. Plant materials

Plant materials consisting of fresh flowering aerial parts (60.0 g) were collected in Central Italy during July 2015 from the population living on the slopes of “Monte Agnello” (GPS: N 41.84′25″; E 13.33′64″) (Fig. 2) in the locality called “Campocatino”, Latium region, at about 1900 m a.s.l.. The botanical identification was performed by one of us (A.V.) using available literature (Pignatti, 1982; Aeschimann et al., 2004; Bartolucci et al., 2018). A representative sample of the studied accession (about 10 g) has been preserved in the laboratory under the code HR07072015 for any further reference.

2.2. Chemicals

The following chemicals were employed during the study: ethanol 96%, distilled water, methanol, chloroform as pure solvents or in mixture at several concentrations to be used as eluting systems for the chromatographic separation; silica gel (40-63 μm particle size) as stationary phase; deuterated solvents such as CDCl₃ and CD₃OD to solubilize the samples for NMR spectroscopy analysis; methanol of HPLC grade was instead used to solubilize the samples for mass spectrometry analysis.

All the solvents having RPE purity grade if not differently specified, were purchased from Sigma Aldrich as well as the deuterated solvents and the methanol of HPLC grade, whereas silica gel was purchased from Fluka Analytical.

Fig. 1. Flowers of *H. richeri* Vill.

Fig. 2. Satellite view of Central Italy and expansion of the collection area.
2.3. Instruments

NMR spectra were recorded on a Varian Mercury 300 MHz (now Agilent Technologies) and/or on a Bruker Avance III 400 MHz instruments. Bidimensional experiments were performed on the Bruker Avance III 400 MHz instrument, operating at 9.4 T at 298 K. The chemical shifts were expressed from TMS (δ = 0 ppm) as internal reference standard for spectra in CDCl₃, while the internal solvent signal of CD₂HOD (m5, δ2 = 3.31 ppm; m7 δ2 = 49.00 ppm) was the reference for spectra in CD₂OD.

MS spectra were performed on a Q-TOF MICRO spectrometer (Micromass; now Waters, Manchester, UK) equipped with an ESI source operating in the negative and/or positive ion mode. The flow rate of sample infusion was 20 μL/min with 100 acquisitions per spectrum. Data were analyzed by using the Masslynx software developed by Waters.

2.4. Extraction and isolation

The fresh plant materials, represented by flowering aerial parts (50.0 g), were macerated in ethanol 96% (3x200 mL) for 48 h at room temperature. After filtration, the three consecutive organic extracts were gathered and the ethanol was removed at reduced pressure (45 °C) in a rotary evaporator. During the concentration of the extract, the pH value was checked on litmus paper and resulted to be 6.5 pH units. This operation is necessary to avoid the possible production of artifact due to extreme acidic or alkaline conditions which may lead to the hydrolysis of esters and glycosides. Once the ethanol was removed, an aqueous suspension was obtained which was frozen to -20 °C and later lyophilized at the same temperature to preserve eventual temperature-sensitive components. From this procedure, 5.9 g of dry crude ethanolic extract was obtained.

A portion of 3.0 g of the crude extract was subjected to a first chromatographic separation on silica gel (100.0 g) and followed by elution with a solution of chloroform/methanol (8:2 v/v). The polarity of the mobile phase was then gradually raised to 7:3 and 6:4 (v/v). From this separation step, 45 fractions were obtained which was frozen to -20 ºC and later lyophilized to a first chromatographic separation on silica gel (9.0 g) and followed by elution with a solution of chloroform/methanol (9:1 v/v) which was gradually raised to 85:15, 75:25 and 6:4 (v/v). From this second separation step, hypericin (5) and pseudohypericin (6) in mixture (quantity not estimated) in Fr(1–18)B (1.2 mg); isorhamnetin glucoside (7) (Dourou et al., 2017) and 5,7,3′,5′-tetrahydroxyflavanone (8) (Esposito et al., 2013) in mixture (3:1) from the assembly of fractions Fr(3–6)B (15.3 mg); isoquercetin (9) (Estork et al., 2014; Maggi et al., 2015; Flores-Bocanegra et al., 2015; Caprioli et al., 2016), hyperoside (10) (Estork et al., 2014; Flores-Bocanegra et al., 2015) and quercitrin (11) (Flores-Bocanegra et al., 2015) in mixture (3:2:1) from the assembly of fractions Fr(9–12)B (9.8 mg); myricetin-3-O-rutinoside (12) (Ahmed et al., 2011; Venditti et al., 2016a) and saccharides (quantity not estimated) from the assembly of fractions Fr(15–18)B (6.7 mg) were identified. The unequivocal identification of substances in mixture has been conducted on the basis of chemical shift values, multiplicity and the hetero- and homonuclear correlations observed in the 2D-NMR spectra, following a method already applied by our research group also to describe not previously identified compounds when in mixture (Sciubba et al., 2014; Venditti et al., 2016b; Venditti and Ukwueze, 2017; Frezza et al., 2018; Venditti et al., 2018b).

2.5. NMR data of isolated compounds

(2S)-1,2-di-O-[(9Z,12Z,15Z)-octadeca-9,12,15-trienoyl]-3-O-β-D-galactopyranosyl glycerol (1):
1H-NMR (300 MHz, CD2OD) δ: 5.46-5.24 (13H, m, overlapped olefinic protons and H2-glycerol), 4.44 (1H, bd, J = 12.0, 3.0 Hz, H-1′′′(Gal)), 4.23 (1H, d, J = 7.2 Hz, H-1′′′(Gal)), 4.22 (1H, dd, J = 12.0, 6.7 Hz, H-1′′′(Gal)), 3.99 (1H, dd, J = 10.9, 5.4 Hz, H-6″(Gal)), 3.83 (1H, part. overl., H-6″′(Gal)), 3.78 - 3.68 (m′, 3.55 - 3.43 (m′), 2.81 (8H, br t, J = 5.6 Hz, H-11′′, H-11′′), H-14′′, H-14′′′), 2.31 (4H, ddd, J = 11.9, 7.9, 4.3 Hz, H-2′′′, H-2′′), 2.15-2.02 (8H, m, H-8′, H-8′′, H-8′′′, H-17′′), 1.37 - 1.25 (8H, m, H-4′′, H-4′′′, H-7′′, H-7′′′), 0.98 (6H, br t, J = 7.5 Hz, H-18′′′, H-18′′′). *overlapped signals of galactose and glycerol.

13C-NMR (75 MHz, CDCl3) δ: 173.9 (C-1′), 173.6 (C-1′′′), 132.1 (C-9′, C-9′′), 130.3 (C-10′, C-10′′), 128.5 (C-12′, C-12′′), 128.4 (C-13′, C-13′′), 127.9 (C-15′, C-15′′), 127.3 (C-16′, C-16′′), 104.1 (C-1″′′), 77.1 (C-5″′′), 74.7 (C-3″′′), 73.6 (C-2″′′), 71.7 (C-2′′′), 70.4 (C-4″′′), 69.5 (C-1), 68.4 (C-3), 62.9 (C-6′′), 34.4 (C-2′′′), 34.3 (C-2″′), 33.8 (C3), 32.1 (C-3′), 29.8 (C-4′), 29.7 (C-4″), 29.6 (C-7′′), 29.5 (C-7′′′), 29.3 (C-8′), 29.2 (C-8′′), 27.3 (C-11″′, C-11″), 25.8 (C-6′), 25.7 (C-6″), 25.0 (C-14′), 24.9 (C-14″), 22.8 (C-17), 20.7 (C-17′′), 14.4 (C-18′′), 14.2 (C-18′′′).
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5.18 (1H, d, J = 10.8, 3.1 Hz, H-1′′), 6.89 (1H, d, J = 8.6 Hz, H-5′′), 6.37 (1H, br s, H-8′), 6.19 (1H, br s, H-6), 5.25 (1H, d, J = 7.3 Hz, H-1′′′), other sugar signals overlapped.

13C-NMR (100 MHz, CD3OD) δ: 179.50 (C-4'), 165.98 (C-3'), 159.30 (C-5), 159.83 (C-3), 153.78 (C-13), 123.18 (C-11), 122.95 (C-12), 117.79 (C-2'), 116.06 (C-5'), 105.62 (C-10), 104.38 (C-1′′), 99.87 (C-6), 94.78 (C-7′), 78.71 (C-5′), 78.37 (C-3′), 75.72 (C-2′′), 70.03 (C-4′′), 62.57 (C-6′).

ESI-MS: m/z 465.16 [M+H+]; m/z 488.39 [M+Na+].

Hyperoside (10): 1H NMR (400 MHz, CD3OD) δ: 7.83 (1H, br s, H-2′), 7.56 (1H br d, J = 8.9 Hz, H-6′), 6.89 (1H, d, J = 8.9 Hz, H-5′), 6.37 (1H, br s, H-8), 6.19 (1H, br s, H-6), 5.18 (1H, d, J = 7.8 Hz, H-1′′′).

13C-NMR (100 MHz, CD3OD) δ: 179.96 (C4), 166.00 (C7), 163.02 (C-5), 159.01 (C-9), 158.79 (C-2), 149.92 (C4′), 146.83 (C-3′), 135.63 (C-3), 123.18 (C-6′), 122.87 (C-1′), 117.57 (C-2′), 116.94 (C-5′), 105.62 (C-10), 105.45 (C-1′′), 99.80 (C-6), 94.76 (C-8), 77.17 (C-5′′), 75.10 (C-3′), 73.18 (C-2′′), 70.03 (C-4′′), 61.95 (C-6′).

ESI-MS: m/z 465.16 [M+H+]; m/z 488.39 [M+Na+].

Quercitrin (11): 1H NMR (400 MHz, CD3OD) δ 7.39 - 7.21 (2H, m, overlapped, H-2′′,H-6′), 6.85 (1H, d, J = 9.0 Hz, H-5′), 6.37 (1H, br s, H-8′), 6.19 (1H, br s, H-6), 5.34 (1H, br s, H-1′′), 0.96 (3H, d, J = 6.0 Hz, H-3′′).

13C-NMR (100 MHz, CD3OD) δ: 179.47 (C-4), 165.82 (C-7), 162.97 (C-2′), 158.53 (C-5), 158.43 (C-9), 149.77 (C-4′), 145.78 (C-3'), 134.90 (C-3), 122.95 (C-1), 122.87 (C-6), 116.94 (C-2′′), 115.99 (C-5′'), 104.38 (C-1′′), 103.54 (C-1′′′), 99.80 (C-6), 94.76 (C-8), 72.14 (C-4′′), 72.02 (C-3′), 71.90 (C-2′′′), 71.23 (C-5′′), 17.65 (C-6′′).

ESI-MS: m/z 471.36 [M+Na+]; m/z 487.39 [M+K+].

Myricetin-3-O-rutinoside (12): 1H NMR (400 MHz, CD3OD) δ: 7.12 (2H, br s, H-2′,H-6′), 6.37 (1H, br s, H-8′), 6.19 (1H, br s, H-6), 5.16 (1H, d, J = 7.8 Hz, H-1′′′ (Glc)), 4.33 (1H, br s, H-1′′′ (rutinoside), 0.93 (3H, d, J = 5.9 Hz, H-6′′′ (rutinoside)).

13C-NMR (100 MHz, CD3OD) δ: 179.52 (C-4), 165.98 (C-7), 159.30 (C-5), 158.53 (C-9), 149.82 (C-2′,C-3′), 145.78 (C-3′), 135.77 (C-3), 135.63 (C-6′), 122.95 (C-1), 109.53 (C-2′′′,C-6′′′), 105.45 (C-10), 104.38 (C-1′′′), 103.54 (C-1′′′′), 99.80 (C-6′), 94.70 (C-8′), 78.71 (C-5′), 78.36 (C-3′), 75.10 (C-2′′′), 72.14 (C-4′′′), 72.02 (C-3′′′), 71.90 (C-2′′′′), 71.23 (C-5′′′′), 70.03 (C-4′′′′), 66.57 (C-6′′′), 16.75 (C-6′′′′).

ESI-MS: m/z 625.24 [M+H+]; m/z 649.36 [M+Na+]; m/z 665.19 [M+K+].

Glucose (13): NMR and MS data fully in accordance with those reported in literature (Sciubba et al., 2014; Venditti et al., 2017f, 2017g; 2018a).

ESI-MS: m/z 203.06 [M+Na+].

Galactose (14): NMR and MS data fully in accordance with those reported in literature (Sciubba et al., 2014).

ESI-MS: m/z 203.06 [M+Na+].

Sucrose (15): NMR and MS data fully in accordance with those reported in literature (Sciubba et al., 2017f, 2017g; 2018a).

ESI-MS: m/z 365.14 [M+Na+].

3. Results and Discussion

3.1. Phytochemical analysis of H. richeri Vill.

The phytochemical analysis of H. richeri Vill. collected from Appennines mountain in Central Italy revealed the presence of fifteen compounds (Fig. 3), namely, (25)-1,2-di-O-[(9Z,12Z,15Z)-octadeca-9,12,15-trienoyl]-3-O-β-D-galactopyranosyl glycerol (1) (glycolipid), quercetin (2), quercetin-3-O-methyl ether (3) and 5,7,3′,5′-tetrahydroxyflavanone (8) (flavonoids aglycones), benzoic acid (4) (organic acid), hypericin (5) and pseudohypericin (6) (naphthodianthrones), isorhamnetin glucoside (7), isoquercitrin (9), hyperoside (10), quercitrin (11) and myricetin-3-O-rutinoside (12) (glycosidic flavonoids), glucose (13), galactose (14) and sucrose (15) (saccharides). Therefore, from this specific accession it was recognized a phytochemical pattern
perfectly in accordance with those observed in other entities of the genus and, therefore, with the current botanical classification of the species.

The flavonoid components resulted the most represented class of natural products with compounds in both aglycone and glycosidic form (mono- and diglycosides). Among these, the flavonol (3-hydroxyflavone) derivatives resulted the major components. In this context, it is worth noting the presence of compound (8) recently identified in H. hircinum L., (Esposito et al., 2013) and reported for the first time in this study as a component of H. richeri Vill.. The presence of (8) may have a chemosystematic relevance since it represents a chemical trait of proximity between these two species. Moreover, it resulted to be one of the active components as inhibitor of both HIV-1 reverse transcriptase-associated DNA polymerase and ribonuclease H activities (Esposito et al., 2013). It showed also a high radical scavenging activity and exerted an inhibitory action toward the collagenase (Mandrone et al., 2015) with a non-competitive mechanism.

For what concerns the biological activities, all the isolated flavonoids are well-known to possess a pronounced antioxidant and radical scavenging action, but to the date, other useful pharmacological potentialities are also known for these phytochemicals. For instance, isorhamnetin glucoside (7) and quercetin derivaties have been revealed as potential inhibitors of acetylcholinesterase (Olennikov et al., 2017); isoquercitrin (9) and other quercetin and myricetin related flavonoids showed a significative concentration-dependent inhibition of cell growth in bladder cancer cells with IC_{50} values ranging from 8 to 92 μM (Prasain et al., 2016); hyperoside (10) exerted protective effects of on CCl_4-induced chronic liver fibrosis in mice by increasing the activity of both the antioxidant and phase II detoxifying enzymes and through the activation of Nrf2 pathway (Zou et al., 2017).

The flavonoids play an important role in the traditional medicines as tranquilizers for their high sedative and antispasmodic properties (Venditti et al., 2014; 2015b; 2017i). In recent studies, it has been shown that several flavonoids have a selective affinity for the benzodiazepine receptors with a pharmacological profile consistent with a partial agonistic mechanism of action (Medina et al., 1989, 1997). This property has been observed in several flavonoid derivatives, both natural and semisynthetic.

The high presence of flavonoids in Hypericum spp. may substantiate its uses as anxiolytic and sedative agents in traditional medicine from a phytochemical basis. In this context, it is noteworthy the huge presence of compounds of this class of natural products in the studied accession of H. richeri Vill., which further confirmed the previous findings that this plant species, having a similar pharmacological potential, may be used for the same purposes of H. perforatum L., (Stojanovic et al., 2013). This finding also substantiate on phytochemical basis the substitution for H. perforatum L., in traditional medicine, as reported by Radanovic and collaborators (Radanovic et al., 2006) as well as the inhabitants of the region near the collection site of the studied sample.

To the best of our knowledge, quercetin-3-O-methyl ether (3) has never been reported before as a constituent of H. richeri Vill.. This flavonoid was previously recognized in Achillea nobilis L. (Asteraceae family) (Krenn et al., 2003) and more recently in Rhamnus disperma Ehrenb. ex Boiss. roots (Rhamnaceae family) (Ragab, 2015). The same is for the glycolipid (1) which, instead, has recently been identified in Agathis robusta (C.Moore ex F.Muell.) F.M.Bailey (Venditti et al., 2018a).

The naphtodianthrones, hypericin (5) and pseudohypericin (6), have been already observed in H. richeri Vill. collected in different North Appennines regions (Maffi et al., 2001) and the studied accession

Fig. 3. Molecular structures of isolated compounds.
from Central Italy revealed the presence of a comparable amount of naphthodianthrones with those reported in other studies. On the contrary, the hyperforin was not recognized in the studied accession, as well as the caffeoylquinic derivatives such as chlorogenic acid and neo-chlorogenic acid already observed in *H. richeri* Vill. (Zdunić et al., 2010, 2017) and in several other species of this genus (Esposito et al., 2013; Mandrone et al., 2015; Caprioli et al., 2016). The absence of hyperforin and caffeoylquinic derivatives represents one of the main difference of the studied accession in respect to samples collected from different areas. Anyway, a certain grade of chemovariability, for what concerns the naphtodianthrones, chloroglycerins and caffeoylquinic derivatives contents, has also been already observed in *H. perforatum* L., collected from different geographic areas as well as for its subspecific taxa (Toniolo et al., 2014). For what concerns the absence of hyperforin, it could be also due to spontaneous degradation. In fact, it is known that this is an unstable compound (Wolfender et al., 2003) which may form several oxidized products before its degradation. The other possible causes of variability might be derived from the different phenological stages of plants and, obviously, from the different environmental conditions. In respect to this last aspect, the altitude of the collection place (about 1900 m a.s.l.) and the good exposure (South-East/South) to the sunlight might have a primary role in the development of the observed secondary metabolites pattern with a prevalence of flavonoids which have, among the other actions in plants, a protective role against UV light. Anyway, further studies are necessary to confirm these hypotheses.

4. Concluding remarks

The phytochemical analysis of *H. richeri* Vill. from Central Italy revealed in general a secondary metabolites pattern in accordance with those observed in previous studies on accessions from other areas (North Appennines and Balkan), but it also showed some differences in respect to the absence of hyperforin and caffeoylquinic acids and the occurrence of new components for the species. In fact, compounds (1) and (3) have been identified for the first time during the present study in *H. richeri* Vill.. The observed chemovariability of the studied sample could likely be due to the environmental factors but further studies are necessary to confirm such hypothesis. The recognized phytochemical pattern resulted therefore in accordance with the current classification of the species also from the chemosystematic standpoint and may substantiate, from a chemical base, the ethnomedicinal use of this species as well as the possible substitution with *H. perforatum* L., in the preparation of traditional remedy as it already happens in several countries, such as Balkans and central Italy, where *Hypericum* spp. are used in traditional medicine. Further studies should be done to verify the influence of environmental factors, e.g. the level of exposure to sun of plants, toward the expression of the secondary metabolites and how they could determine the differences in composition. Furthermore, being the species *H. richeri* Vill. exchangeable with *H. perforatum* L. for ethnomedicinal applications, it would be desirable that in the future some biotechnological and chemical studies will be conducted also on this species and its phytoconstituents as has already been done on *H. perforatum* L. and other *Hypericum* spp (Franklin et al., 2016; Ornano et al., 2018).

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