

Howard R. Davis  
Editor

# Hypericum



Botanical Sources, Medical  
Properties and Health Effects

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## **HYPERICUM**

### **BOTANICAL SOURCES, MEDICAL PROPERTIES AND HEALTH EFFECTS**

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**HYPERICUM**  
**BOTANICAL SOURCES, MEDICAL**  
**PROPERTIES AND HEALTH EFFECTS**

**HOWARD R. DAVIS**  
EDITOR



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*Chapter 2*

**POTENTIAL OF THE BALKAN FLORA AS A SOURCE  
OF PROSPECTIVE *HYPERICUM* GENOTYPES  
FOR THE CONVENTIONAL AND BIOTECHNOLOGICAL  
DELIVERY OF PHYTOPHARMACEUTICALS**

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**ABSTRACT**

Today plants still hold the leadership as the primary source of many bio-active molecules, irreplaceable for sustaining human health. One of the most widely applied medicinal plants is the St John's wort (*Hypericum perforatum* L., Hypericaceae) with its wide array of biological activities and relevance in treatment of ailments in the form of crude extracts, fractions and individual components. This chapter summarizes literature data on ethnobotanical practices, main active constituents, pharmacological activity and applications of *Hypericum* species throughout the world. Special attention is given to the available data on the phytochemical, pharmacological and biotechnological development of the Balkan *Hypericum* species based on literature survey as well as author's own experience.

**Keywords:** ethnobotanical practices, Balkan *Hypericum* species, polyphenolics, hypericins, plant cell tissue and organ culture

**INTRODUCTION**

Data for the medical application of *Hypericum perforatum* L. (Hypericaceae) use occur as early as the 1st century A.D. The species was known by Hippocrates, Pliny, and Dioscorides who included it in the Book III of *De materia medica* together with other related representatives of the genus (Reeds 2012). The plant is native to Europe, North Africa and

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Asia, but also naturalized to North America. The *Hypericum* genus comprises over 484 species grouped in 36 sections. Species are either naturally occurring or introduced to every continent on the world excluding Antarctica, their habitus varying from trees to herbaceous individuals (Crockett and Robson 2011).

The broad spectrum of activities of *H. perforatum* (St John's wort) is determined by the specific complexity of the *Herba Hyperici* extract and the potential additive effect, synergism or even possible antagonism between its different components. Research has led to elucidation of the most important biologically active substances in the plant – polyphenolic compounds, flavonoids, naphthodianthrone and phloroglucinols, terpenes (Nahrstedt and Butterweck 1997; Bruni and Sacchetti 2009; Nahrstedt and Butterweck 2010). These constituents were shown to possess antidepressive, antitumor, antiviral and antibiotic activity (Barnes et al. 2001; Saddiqe et al. 2010; Süntar et al. 2010). Research interest towards the plant has been incessant throughout the years.

Only a scarce number of *Hypericum* species have been thoroughly studied phytochemically and pharmacologically, thus providing the challenge of exploration and understanding the unstudied potential of new representatives of this multitudinous genus. In addition the tools of the plant cell tissue and organ culture techniques provide a convenient and flexible choice for preservation of valuable plant germplasm and conducting *ex situ* fundamental research on the molecular and biochemical mechanisms underlying secondary metabolite production in this genus (Danova 2014, and references cited within). These techniques also provide a sound alternative to conventional breeding practices for supplying raw material with controllable and constant active constituent content for the demands of agronomical practices. Although extensive research has been conducted on plant cell and tissue culture development of *Hypericum* species it is predominantly focused on *H. perforatum* or other closely related to it species in the genus (Danova 2014 and references cited within). This chapter provides overview of *Hypericum* ethnobotanical practices throughout the world and especially in the region of the Balkan Peninsula. Scientific evidence determining the pharmacological activities of the species has been reviewed. Achievements of plant biotechnology for the controllable yield of phytopharmaceuticals in this genus have been summarized with special attention given to Balkan *Hypericum* genotypic richness.

## 1. Contemporary Application of Medicinal Plants throughout the World

According to reports of the World Health Organization, a substantial part of the world population strongly relies on the resources of indigenous medicine, the traditional healers involving as a remedy plant extracts and/or their active constituents (WHO, 1993, 2002). Traditional medicine is the primary health care to meet the needs in countries of Africa, Asia and Latin America, the share reaching up to 80 percent of the population in Africa. In industrialized countries adaptations of traditional medicine are gaining increasing popularity, designated as “Complementary” or “Alternative” (WHO 2003).

In China the share of herbal preparations accounts 30-50% of total medical consumption. The share for the symptomatic malaria treatment of children with herbal remedies is up to 60% for Ghana, Mali, Nigeria and Zambia. For Europe, North America and other industrialized regions worldwide, over 50% of the population have used the treatment of complementary medicine, this share being as high as 90% for Germany. Throughout the

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world in both industrialized and developing regions up to 75% of the people living with HIV/AIDS have used the resources of complementary medicine (WHO 2003).

Nowadays, the popularity of whole plants and plant preparations for a range of therapeutic applications is constantly rising (Valiakos et al. 2015). Therefore, following the recommendations of WHO, ethnobotanical medicinal traditions are to be integrated with modern medical therapies in holistic strategies in order to effectively cope with increasing worldwide needs of curative resources.

## 2. Ethnobotanical Studies of *Hypericum* Species throughout the World

Traditional uses of *Hypericum* have been documented by the ancient Greek physicians Hippocrates (460-377 BC), Euryphon (288 BC) and Galen (130-200 AD) who used it for various curative indications as to treat burns, wounds, pain, sciatica and depression (Hobbs 1989; Hahn 1992; Bombardelli and Morazzoni, 1995; Blumenthal et al. 2000; Osbaldeston and Wood 2000; McClatchey et al. 2009). The Swiss physician Paracelsus applied it for psychological disorders and he called it an "almost universal medicine" (Wood 1997; Osbaldeston and Wood 2000). In his "Complete herbal", the English physician Culpepper described St John's wort as a plant "under the celestial sign Leo, and the dominion of the Sun". He recommended its use as diuretic and against pain and traumas and prescribed *Hypericum* ointment for wound healing.

In addition, the tincture of *Hypericum* blossoms in spirit or wine were advised "against melancholy and madness" (Culperer 1653, issue of 1954). Samuel Hahnemann introduced the plant into his *Materia medica* showing it to be specific for wounds to parts rich in nerves, injuries from sharp instruments etc., (Wood 1997). In the United States, first considered simply an invasive species by farmers and neglected by 19<sup>th</sup> century eclectic doctors, *H. perforatum* gradually gained more popularity with the settlement of the European immigrants (Wood 1997). So, its oily preparations were applied as cure for ulcers, diarrhea, hysteria and nervous conditions, including depression (Hobbs 1989). In the 20<sup>th</sup> century German physician Madaus prescribed it for conditions as neuralgia, neuroses, neurasthenias, hysteria and insomnia, as well as for wound healing (Hobbs, 1989). Throughout the centuries, *H. perforatum*, as well as other indigenous *Hypericum* species, have been applied in the ethnobotanical practices of many world cultures (Table 1). Investigation and utilization of the knowledge on *Hypericum* ancient resources has led world pharmaceutical industries to the development of the current date dietary supplements, homeopathic remedies and to the standardized extracts and prescription products recognized by official medicine. One of the topmost and commercially renowned species of the genus *Hypericum* is *H. perforatum*, commonly known as St. John's wort (Klemow et al. 2011).

On a world scale *H. perforatum* is considered to be among the top selling herbal preparations comprising a worldwide market value of about 600 million US dollars (Ernst 2003). Almost 13 % of European herbal products in 2004 were based on *H. perforatum* derived active constituents and in Germany they were valued at about 70 million Euro (Bäcker et al. 2006). In the United States it has been ranked as the top ten bestselling herbal dietary supplements with sales reaching ca. 8.2 million USD (ABC 2008).

Table 1. Ethnobotanical evidence of worldwide application of *Hypericum* species

Region*	<i>Hypericum</i> species	Preparation use	Application	Source
Brazil	<i>H. connatum</i> Lam. ('orelha-de-gato' (cat's ear))		Tonic and adstringent; Southern Brazil -treatment of oral lesions	Correa 1984; Mentz et al. 1997; Fritz et al. 2007
India	<i>H. panikum</i> Thumb. <i>H. hookertianum</i> Wight and Arnott	Leaves	Wound healing	Mukherjee and Suresh, 2000; Mukherjee et al., 2000
Konkan and Palani hills, (India)	<i>H. mysorensse</i> B.Heyne	Aerial parts	Spasmolytic, hypotensive and antibacterial activities	Gamble et al. 1984; Asolkar et al. 1992; Hariharapura et al. 2014
India	<i>H. hookertianum</i> Wight and Arnott	Aerial parts	Treatment of mental illness	Pongiya et al. 2007; Subakanmani and Umadevi 2012
Natural Park of "Serra de São Mamede" (Portugal)	<i>H. perforatum</i> L.	Flowering aerial parts - decoction	Liver ailments	Rodrigues et al. 2003
Alta Vall del Ter (Catalonia, Spain)	<i>H. perforatum</i> L.	Flowering aerial parts – lotion, liniment, decoction	Analgesic, anticatarrhal, antiechymotic, for burns, vulnerary, relaxing, antidepressant, improvement of memory, cerebral tonic	Rigat et al. 2007;
	<i>H. androsaemum</i> L.	Leaves – direct application, poultices or plasters	Vulnerary, wound healing,	Rigat et al. 2007;
Anadola (Turkey)	<i>H. perforatum</i> L.	Olive oil macerate of the flowering aerals, kept in the sun	Wound healing	Siıntar et al. 2010
Taurus mountains (Turkey)	<i>H. perforatum</i> L.	Decoction, infusions of flowering aerial parts	Uro-genital inflammations, diabetes mellitus, neuralgia, heart diseases, gastritis, hemorrhoids and peptic ulcers	Yesilada et al. 1993, 1995.

\* Listing of countries of origin in alphabetic order.

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**Table 2. Scientific evidence of ethnobotanic records for *Hypericum* species throughout the world**

Country of origin of the investigated species*	<i>Hypericum</i> species	Parts of the plant/chemical constituents	Pharmacological activity	Source
Brazil	<i>H. polyanthemum</i>	Three benzopyrans	Antitumor activity	Ferraz et al. 2005
	<i>H. connatum</i> Lam.	Crude methanolic extract, fractions (n-hexane, dichloromethane and methanol), Phloroglucinol derivative hyperbrasilol B, flavonoids amentoflavone, hyperoside, guajaverine and luteoforol from the aerial parts	Antiviral activity against herpes simplex viruses (HSV)	Fritz et al. 2007
India	<i>H. hookerianum</i> Wight and Arnott	Ethanolic extract of the aerial parts	Anxiolytic potential in stress induced swiss albino mice	Subakanmani and Umadevi 2012;
		Stem parts	Potent antitumor activity against DLA induced tumor in mice	Pulok et al. 2001
		Methanol extracts of the aerial parts, leaves, and stem	Selective cytotoxicity against tumor cells	Vijayan et al. 2003
		Methanol extract of stem parts	Antitumor (Ehrlich Ascites Carcinoma in Swiss Albino Mice)	Dongre et al. 2007
		Leaf and stem methanol extract	Wound healing	Mukherjee et al. 2001
		Leaf and stem methanol extract	Antibacterial	Mukherjee and Suresh 2000
		Ethanolic extract of aerial parts	Reversing haloperidol induced schizophrenia-like behaviors in Swiss albino mice	Pongiya et al. 2014
		Methanolic extract of the aerial parts	Antiviral effect towards HSV-1	Vijayan et al. 2004
		Methanolic extract of the aerial parts	Antiviral effect towards HSV-1	Vijayan et al. 2004
	<i>H. mysorens</i> B. Heyne			

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Table 2. (Continued)

Country of origin of the investigated species*	<i>Hypericum</i> species	Parts of the plant/chemical constituents	Pharmacological activity	Source
Endemic to Kazdagı Mount, Balıkesir, (Turkey)	<i>H. kazdaghenis</i> Gemici et Leblebici	Chloroform, acetone methanol extracts of air-dried leaves	Antibacterial activity	Dulger and Gonuz 2005
Turkey (Anatolia)	<i>H. perforatum</i> <i>H. scabrum</i>	Olive oil extract of flowering aerial parts	Wound healing ( <i>in vivo</i> excision and incision wound models); Anti-inflammatory ( <i>in vivo</i> inhibition of acetic acid-induced increase in capillary permeability. Activity established only for <i>H. perforatum</i> )	Stuntar et al. 2010
Taurus Mountains (Turkey)	<i>H. perforatum</i> L.	Ethanol extract of the flowering aerial parts.	Potent antiulcer activity (ethanol-induced gastric lesions in mice).	Yesilada and Gurbuz, 1998
		50 % ethanolic extract.	Wound healing (chicken embryonic fibroblasts).	Oznuik et al. 2007
Endemic to Canary Islands, Macaronesian Region	<i>H. grandifolium</i> Choisy	Aqueous, butanol and chloroform fractions obtained from the methanol extract of aerial parts	Potential antidepressant-like effects in the forced swimming test in mice	Sanchez-Mateo et al. 2009

\* Listing of countries of origin in alphabetic order.

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### 3. Pharmacological Properties of *Hypericum* Species

The increasing needs of world population to cope with problems of widely piling “diseases of civilization”, pathogen resistance towards conventional treatment agents, as well as toxicity and side effects of synthetic drugs has urged researchers to look into the insights of scientific bases of ethnobotanical sources from all over the world. Incessant research on pharmacological properties of *Hypericum* species has been based on the evidence of its efficiency for various conditions like remedy against depressions, ulcers, dyspepsia, abdominal pains, burns, bacterial infections, migraine, headaches, and sciatica (German Commission E, 1998; WHO Monographs, 1999; ESCOP, 2003). Amongst the numerous applications of *Hypericum* bioactive preparations (Table 2) is the use in photodynamic therapy of cancer (PDT) of naphthodianthrone hypericin found in *Hyperici Herba* extract. This activity is based on the physical property of hypericin after photo-induction (by a particular type of light) to generate singlet oxygen which in its turn acts as a photosensitizer and attacks cancer cells upon topical application of the agent. Research has shown that the application of *H. perforatum* extract might cause fewer skin damage reactions, as compared to administration of hypericin alone (Schmitt et al. 2006). The authors of this study showed that two *H. perforatum* extracts (ethanol-chloroform, containing flavonoids and hypericin and a chloroform extract, where hypericin and flavonoids were not detected), when supplemented with hypericin exhibited 24% and 40% less phototoxicity than hypericin alone. Then, two *H. perforatum* constituents (chlorogenic acid pyropheophorbide), when supplemented with hypericin exhibited 24% and 40% less phototoxicity than hypericin alone. In addition, antioxidant, but also pro-oxidant activities of extracts of different parts of the plant at different stages have been established Gioti et al. (2009). Thus the question of possible antagonism and synergism between the different components of *Herba Hyperici* extract has brought to research indicating that the application of standardized extracts of the herb is advantageous to application of pure hypericin in PDT (Skalkos et al. 2006). In addition, the auto pro-oxidant effect of hypericins on the plant itself and subsequent oxidative stress alleviation by increasing phenolic and flavonoids production by the plant have been established in an *in vitro* culture model system of two hypericum species with different hypericins productivity (Danova et al. 2012).

### 4. Sourcing for *Hypericum* Genotypes with Higher Phytopharmaceuticals Productivity

Regarding the biosynthetic potential of the different representatives of the genus, in spite of a certain similarity of its main phytochemical constituents, remarkable intra- and interspecific differences of the quantitative and qualitative characteristics have been recorded (Umek et al. 1999; Kitanov 2001; Kusari et al. 2009), between populations of the same species from different locations (Buter et al. 1998; Kartnig et al. 1989) and within the individual developmental phases of the given individual (Tekelova et al. 2000). Literature surveys have shown that the evolutionary more developed *Hypericum* species possess an increased capacity of hypericins production as compared to the more primitive sections of the genus (Danova 2014 and references cited therein). Chemotaxonomic surveys have revealed that some *Hypericum* species, such as *H. boissieri*, *H. barbatum* and *H. rumeliacum*

(representatives of *Drosocarpium* section) may contain twofold to fourfold higher amounts of hypericins than *H. perforatum* (Kitanov 2001; Smelcerovic et al. 2006; Bruni and Sacchetti 2009). In addition, Smelcerovic et al. (2006) found a positive correlation between the secondary metabolite content in some *Hypericum* species and certain SSR and RAPD markers.

*Hypericum* (Hypericaceae; Stevens, 2007; APG III, 2009) is centred around the temperate regions of the Northern Hemisphere that underwent rapid radiation during the Pleistocene (Nürk et al. 2013). *H. perforatum* is a widespread Eurasian perennial plant species with remarkable variation in its morphology, ploidy and breeding system, which ranges from sex to apomixis (Koch et al. 2013). Recently Koch et al. (2013) have demonstrated that *H. perforatum* is not of hybrid origin, and for the first time recorded the occurrence of wild diploid populations of the species. It was shown that pseudogamous facultative apomictic reproduction is prevalent in the polyploids, whereas diploids are predominantly sexual, a phenomenon which also characterizes its sister species *H. maculatum*. Molecular markers characterized identical major gene pools, distinguishing *H. perforatum* from *H. maculatum* and two genetic groups in *H. perforatum*, the three gene pools being in close geographical contact. Extensive gene flow and hybridization throughout Europe within and between gene pools and species has been confirmed by the molecular data and morphometric analyses. The authors concluded that the geographical distribution of the different gene pools of *H. perforatum* demonstrates that Central Europe is divided, with a red gene pool primarily found in the north and a green gene pool found primarily in the south. High levels of gene flow between the two *H. perforatum* gene pools have been indicated. Further, the authors propose that the genetic and morphological distinctness is reflective of two taxa within a broadly defined *H. perforatum*. Although there is a broad interest in *H. perforatum* and its closest relatives, little is known regarding systematic relationships and evolutionary history, the distribution of genetic variation and intra- and interspecific introgression (Koch et al. 2013). Relationships between the 36 sections of the genus remain to still be speculative. In a recent work of (Meseguer et al. 2013) a complex complex pattern of morphological plasticity and inter-continental movement within the genus demonstrated by means of Bayesian reconstruction reconstruction of morphological character states and range evolution. It was hypothesized that ancestors of *Hypericum* were probably tropical shrubs that migrated from Africa to the Palearctic in the Early Tertiary, concurrent with the expansion of tropical climates in northern latitudes. Further climate cooling could have promoted adaptation to temperate conditions in some lineages, such as the development of the herbaceous habit or unspecialized corollas.

The diversity of Balkan *Hypericum* genotypes, ethnobotanical data on *Hypericum* species traditional applications, as well as scientific findings on *Hypericum* phytochemistry and biological activity have been summarized below.

## 5. Balkan *Hypericum* Traditions and Up-to-Date Research

The Balkan Peninsula encompasses territories to the south from central Europe into the Mediterranean Sea, the Balkan Peninsula, including Albania, Bosnia & Herzegovina, Bulgaria, Croatia, Greece, Macedonia, Montenegro, Romania, Serbia, Slovenia, and European Turkey. The Balkans are considered as one of the “hotspots” of European

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biodiversity being a glacial refuge for plant and animal species, and a crossroads for faunal and floral exchange with Central Europe on the one hand, and Asia Minor on the other, characterized by an outstanding diversity of cave and ancient lake environments, high terrestrial and riverine habitats (Griffits et al. 2004). Authors bring out the high level of endemism as one of the most striking elements of comparative patterns of biodiversity as compared with other parts of Europe. The use of *Herba Hyperici*, derived from the widely utilized *H. perforatum*, as well as from other *Hypericum* species indigenous for the region has been widely reported from numerous authors (Table 3). Together with *H. perforatum* which was prescribed by the ancient Greek healers, literature data on the application of also other species can be traced back to ancient Balkan phytotherapeutic history. Such example is *H. triquetrifolium*, which has been enlisted in medieval medical manuscript "Element Alpha" of Nikolaos Myrepsos', chapter "About the Antidotes" of the Byzantine physician at the court of John III Doukas Vatatzes at Nicaea (13th century) (Valiakos et al. 2015). Some practices of traditional application of the preparations by the Balkan people can also be traced in European written sources. Thus, a literature survey proved that the utilization of *Hyperici* oil for wound healing is not only known in the folk medicine of the Balkans and Turkey, but also practiced in all over the world. Thus, its production was described as "sunlight maceration method" in the supplement of German Pharmacopoeia [DAB 6] (Maisenbacher and Kovar, 1992). Based on chromatographical data hyperforin was considered the active principle determining this activity (Maisenbacher and Kovar, 1992). Motivated by the ethnobotanical evidence of utilization of *Hypericum* species other than the widely studied *H. perforatum*, a large number of works has been dedicated to the study of the therapeutic potential of Balkan representatives of the genus (Table 4).

## 6. Biotechnological Utilization of Balkan *Hypericum* Germplasm

Due to the high market demands for extractable raw material of *H. perforatum* with controlled quality of the secondary metabolite content, numerous programs have been developed for the selection, breeding and cultivation of improved cultivars of the species in Europe and North America (Gaudin et al. 2003; Azizi et al. 2011). In these, major effort has been put into the targeting of such genotypes, whose phenotypic expression would display desirable phenological, morphological, phytochemical and pathogenic-resistance characteristics; afterwards a secondary, equally important goal is the stabilization of the expression of these characteristics in the field cultivation environment (Azizi et al. 2011). But still to date, the problem is not satisfactorily solved with the existing commercially available to industry cultivars.

The phytochemical studies of *Hypericum* species indigenous to the Balkan region have provided evidence for the phytochemical potential of these accessions as novel sources of phytopharmaceuticals characteristic for the genus (Table 5). The tools of plant cell tissue and organ culture are a convenient approach for the conservation of valuable medicinal plants germplasm, supplementary of conventional methods for plant breeding. These tools are irreplaceable when the genotype of rare, threatened or endangered species are concerned.



Table 3. Ethnobotanic data on the application of *Hypericum* species in the region of the Balkan Peninsula

Balkan country	<i>Hypericum</i> species	Preparation use	Application	Source
Albania	<i>H. maculatum</i>	Decoction of the dried aerial parts. Oleolite	To treat digestive troubles and as anti-diarrhoeal (also used as a veterinary preparation, especially for sheep); treatment of stomach ache; as a tranquilizer; taken every morning as a diuretic; treatment of flu, sore throat, coughs and bronchitis; as an anthelmintic (used as a veterinary preparation for calves). The oil preparation – used for treatment of burns.	Pieroni et al. 2005
	<i>H. maculatum</i> L.	Aerial parts	Disorders of the digestive, respiratory, kidney, skin, menhal systems.	González-Tejero et al. 2008
Bosnia and Herzegovina	<i>H. montanum</i> L.	Aerial parts and blossoms	Uro-genital ailments, RT ailments, liver and gall disorders, gastro-intestinal ailments, blood system disorders, metabolism disorders, skin ailments, cardiovascular system disorders and musculoskeletal system disorders. Internal application: for cervical injuries, internal purification, gastrointestinal ailments, flatulence, pulmonary ailments, throat inflammations, bedwetting, renal stones and as roborantum for strengthening the corpus. External application: for ovarian inflammations, cervical wounds, arthritis, rheumatism, pain in the back, swollen legs, bruises, contusions, osteoporosis, burns, hemorrhoids, eye injuries and throat inflammations.	Sarić-Kundalić et al. 2010; Sarić-Kundalić et al. 2011
	<i>H. perforatum</i> L.	Aerial parts and blossoms	Respiratory tract ailments and skin ailments, uro-genital ailments, respiratory tract ailments, liver and gall disorders, gastro-intestinal ailments, blood system disorders, metabolism disorders, skin ailments, cardiovascular system disorders, and musculoskeletal system disorders, nervous system disorders, in nutrition.	Glück 1894; Sarić-Kundalić et al. 2010; Sarić-Kundalić et al. 2011
	<i>H. tetrapterum</i> Fr.		Uro-genital ailments, liver and gall disorders, blood system disorders, metabolism disorders, skin ailments, cardiovascular system disorders, musculoskeletal system disorders. Internal application: for cervical injuries, internal purification, gastrointestinal ailments, flatulence, pulmonary ailments, throat inflammations, bedwetting, renal stones and as roborantum for strengthening the corpus. External application: for ovarian inflammations, cervical wounds, arthritis, rheumatism, pain in the back, swollen legs, bruises, contusions, osteoporosis, burns, hemorrhoids, eye injuries and throat inflammations.	Glück 1894; Sarić-Kundalić et al. 2010; Sarić-Kundalić et al. 2011
Herzegovina	<i>H. perforatum</i> L.	Aerial parts	Tea (singly, or in combination with other herbs) against ulcus diseases, asthma and bronchitis, liver and kidney diseases, for appetite improvement and expectoration alleviation; Oil preparation (olive or sunflower oil and fresh flower placed in the sun or warm area for 6 weeks, the preparation then kept in cool and dark) - wounds and burns, for bruises and as anti-inflammatory, for sprains, skin irritations and laceration accompanied by severed nerve tissue, painful joints, varicose veins, muscle strain, arthritis, and rheumatism; Wine (fresh flowers covered with white wine, kept 30 days, then filtered) digestion improving and externally for bruises and ulcers.	Radun 2007

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Balkan country	<i>Hypericum</i> species	Preparation use	Application	Source
Bulgaria	<i>H. perforatum</i> L.	Aerial parts; infusion, tea, oleic preparation	Gastric disorders (acidity, ulcer, gastritis, gall stones, kidney stones, against hemorrhages (hemorrhoids, menstruation), ulcerative colitis, treatment of heart disorders, neurasthenia.	Staneva et al. 1982
	<i>H. perforatum</i> L.	Aerial parts; infusion, tea, oleic preparation	Gout, tuberculosis (mild form), gynecologic disorders (vag. discharge), diarrhea, bed wetting in children, headache, migraine, hysteria.	Neshev and Landjev 1989
	<i>H. perforatum</i> L.	Aerial parts	Gastric and duodenal ulcer, regenerative, antiinflammatory agent in digestive tract diseases, epithelotonic	Ivanova 2005
	<i>H. perforatum</i> L.	Aerial parts – infusion, ethanolic tincture, oleic preparation	Anti-inflammatory, diuretic, spasmolytic, analgesic, as tonic, antimicrobial, mood regulation, against insomnia and fear conditions.	Kunchev 2005
Greece	<i>H. empetrifolium</i>	Aerial parts	External use as a wash to speed wound-healing, heal scalds, and treat outbreaks of herpes	Vokou et al. 1993
Montenegro	<i>H. perforatum</i> L.	Blossoms – red colored oil preparation	First - degree burns, bruises and other skin conditions. Utilized as “tanning oil” to create a dark tan (contra-indicated in light skin individuals).	Foster 2006
	<i>H. maculatum</i> Crantz, <i>H. perforatum</i> L.,	Aerial parts	Anxiety, depressive moods, gastritis. Externally, inflammation of the skin, blunt injuries, wounds, burns.	Menković et al. 2011
Serbia	<i>H. annulatum</i> Moris (syn. <i>H. atomarium</i> Boiss. subsp. <i>degenii</i> (Bomm.) Hayek and <i>H. degenii</i> Bomm.)	n.a.	treatment of gastric and liver disorders	Đorđević et al. 2013
	<i>H. perforatum</i>	Aerial parts, blossoms	An astringent, calming properties. Internally for moderate depression, insomnia and gastrointestinal ailments (stomach ulcer, liver and bile ailments, jaundice) (tea). Externally for haemorrhoids (oil); creams and infused oils are applied to burns, wounds, cuts, muscular pain, sciatica, neuralgia (flowering tops fresh or dried put into olive oil and left in the sun for 30–40 days to obtain a red oil). For newborn infant’s gastric spasms (by putting oil on the baby’s abdomen and by dressing warmly).	Jarić et al. 2007
	<i>H. perforatum</i> L.	Infusion and oil extract of the aerial parts	Internally: moderate depression and gastrointestinal ailments; externally: skin complaints, wounds and burns, hemorrhoids.	Šavikin et al. 2013
	<i>H. perforatum</i>	Infusion, oil of the aerial parts	Most popular amongst 45 other medicinal plants. Immune system strengthening, peptic ulcers, stimulant. Externally, skin disorders, purulent wounds and wound healing.	Zlatković et al. 2014

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Table 3. (Continued)

Balkan country	<i>Hypericum</i> species	Preparation use	Application	Source
Romania, Bukovina	<i>H. perforatum</i> L. and <i>H. maculatum</i> Crantz	Aerial parts	Teas for stomach problems, medicinal tea for cows, relaxing teas, blessed in bouquets on the Day of Assumption, present in Apocrypha	Kolodziejaka-Degórska 2012
Romania, Transilvania		Aerial parts	Aerial parts for wound, bleeding, diarrhoea, gastric ulcer, kidney and heart diseases as tea. The tea was poured into the nostril of horses against diarrhea. Considered a blessed plant. It was believed that blood of Jesus dropped on the leaf causing spots. aerial parts for diarrhea.	Grynaeus and Szabó 2002; Bremner and Chey 2010; Saito et al. 2010; Papp et al. 2014
Turkey	<i>H. empetrifolium</i>	Aerial parts	Treatment of kidney stones and gastric ulcers	Tuzlacy 2006
Turkey (Kukludere Province)	<i>H. perforatum</i> L.	Decoction of the aerial parts	Kidney stones, urinary diseases, diabetes, antihypertensive, cold, stomach ache, enteritis, eczema, antifungal, cardiac diseases, arteriosclerosis.	Kültür 2007

\* Listing of countries of origin in alphabetic order.

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**Table 4. Research on the biological activity of extracts and individual compounds derived from Balkan *Hypericum* species**

Balkan country*	<i>Hypericum</i> species	Parts of the plant/chemical constituents	Pharmacological activity	Source
Bulgaria	<i>H. annulatum</i> Moris	Aerial parts - 5 benzophenones and a xanthone .	Cytoprotective effects.	Momekov et al. 2006
		Aerial parts – five benzophenones and a xanthone	Protective effect against carbon tetrachloride toxicity in isolated rat hepatocytes	Mitcheva et al. 2006
		Annulatamarin.	Growth-inhibitory activity in vitro against human chronic myeloid leukemia.	Nedialkov et al. 2007b
		Aerial parts – hyperatomarin.	Cytotoxic effect on different human tumor cell lines.	Momekov et al. 2008
	<i>H. perforatum</i>	Aerial parts - (1) ethanol; (2) chloroform; and (3) the residue from the chloroform extraction, extracted with ethanol; pure compounds isolated from the plant (chlorogenic acid and pyropheophorbide).	Reduction in hypericin-induced phototoxicity.	Schmitt et al. 2006
Greece	<i>H. empetrifolium</i>	Phloroglucinol derivatives, isolated from the petrol ether extract of the aerial.	Two of the acylphloroglucinols substituted with monoterpenoids displayed high anti-angiogenic activity. One of them was also effective in a cell migration assay with HMEC-1 cells.	Schmidt et al. 2012
	<i>H. perforatum</i>	Methanolic extract	Anti-inflammatory and analgesic activity.	Trovato et al. 2001
		Methanolic extract and polar methanolic extract of the aerial parts.	Photosensitizer in photodynamic therapy for the leukemic cell line HL-60 and cord blood (CB) hemopoietic progenitors	Kapsokalyvas et al. 2005
		Aerial parts boiled in methanol for 30 min. Extract defatted with n-hexane and polar methanolic fraction (PMF) obtained.	Comparison of photosynthesizing capacity (photoexcitation caused) of pure hypericin (as reference) and obtained PMF.	Skalkos et al. 2006
		Polar methanolic fraction (PMF) of the extract of the aerial parts, as described above.	Phototoxic activity against urinary bladder carcinoma using T24 (high grade metastatic cancer), and RT4 (primary low grade papillary transitional cell carcinoma) human bladder cancer cells.	Stavropoulos et al. 2006
		Hyperforin and semi-synthetic analogues (acetate hyperforin, deoxycoumumolone, mono-acetyl deoxycoumumolone, di-acetyl deoxycoumumolone, tri-acetyl deoxycoumumolone	Larvicidal activity against <i>Culex pipiens</i> (mosquitoes transmitters of filariasis, West Nile virus, St. Louis encephalitis, Japanese encephalitis, Venezuelan equine encephalitis, Western equine encephalitis).	Mitsopoulou et al. 2014

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Table 4. (Continued)

Balkan country*	<i>Hypericum</i> species	Parts of the plant/chemical constituents	Pharmacological activity	Source
Serbia	<i>H. annulatum</i> Moris	Hyperatomarin – from the aerial parts.	Antibacterial activity.	Šavikin-Fodulović et al. 2003
	<i>H. barbatum</i> Jacq., <i>H. hirsutum</i> L., <i>H. limarioides</i> Bosse, <i>H. maculatum</i> Crantz, <i>H. olympicum</i> L., <i>H. perforatum</i> L., <i>H. richeri</i> Vill., <i>H. rumeliacum</i> Boiss., <i>H. tetrapterum</i> Fries	Crude methanol extracts of the aerial parts	Antimicrobial and antioxidant activity.	Radulović et al. 2007
	<i>H. alpinum</i> <i>H. barbatum</i> <i>H. rumeliacum</i> , <i>H. hirsutum</i> , <i>H. maculatum</i> , <i>H. perforatum</i>	Essential oils of the aerial parts	Antimicrobial activity. <i>H. barbatum</i> essential oil was proved to be the most active.	Saroghi et al. 2007
	<i>H. annulatum</i> Moris and <i>H. elegans</i> Stephan ex Willd.	Essential oils of the aerial parts	Antimicrobial (antibacterial, antifungal) activity. <i>H. annulatum</i> : better antibacterial than antifungal activity, more effective against <i>P. aeruginosa</i> and <i>E. coli</i> ; <i>H. elegans</i> - no significant difference between antibacterial and antifungal activity	Bordević et al. 2013a
	<i>Hypericum rochelii</i> Griseb. & Schenk, <i>H. umbellatum</i> A. Kern.	Essential oils of the aerial parts	Moderate antimicrobial effects against five bacterial and two fungal strains	Bordević et al. 2013b
	<i>H. maculatum</i> ssp. <i>immaculatum</i> , <i>H. olympicum</i> , <i>H. richeri</i> ssp. <i>grisebachii</i> , <i>H. barbatum</i> ).	Phenolic acids, flavonoids, hyperforin, hypericin isolated from the aerial parts	Notable antioxidant potential, prominent inhibition of acetylcholinesterase	Božin et al 2013
Romania	<i>H. maculatum</i> Crantz, <i>H. perforatum</i> L.	Ethanol (70 %) extraction at room temperature	Antiproliferative effects on Ehrlich ascitic carcinoma in white NMRI male mice.	Prodan et al. 2009
	<i>H. maculatum</i> Crantz, <i>H. perforatum</i> L.	Ethanol (70 %) extraction at room temperature	Antidepressant effect (forced swimming test in Swiss Mice).	Manalache et al. 2010

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Balkan country*	<i>Hypericum</i> species	Parts of the plant/chemical constituents	Pharmacological activity	Source
	<i>H. maculatum</i> Crantz, <i>H. perforatum</i> L.	1:10 alcoholic tincture of the aerial parts. For <i>H. perforatum</i> (4.421% rutoside and 0.444% total hypericin); <i>H. maculatum</i> (4.957% rutoside and 0.543% total hypericin)	Administration on mice intoxicated with alcohol. Most effective (hepatoprotective and immunostimulating), <i>H. perforatum</i> extract (0.15% hypericin) and <i>H. maculatum</i> extract (0.15% hypericin). <i>H. perforatum</i> extract with 0.3% hypericin/kg corp increased liver morphological changes induced by alcohol.	Roman 2011
	<i>H. maculatum</i> Crantz, <i>H. perforatum</i> L.	1:10 alcoholic tincture of the aerial parts. For <i>H. perforatum</i> (4.421% rutoside and 0.444% total hypericin); <i>H. maculatum</i> (4.957% rutoside and 0.543% total hypericin)	Administration on rats intoxicated with alcohol. <i>Hypericum perforatum</i> and <i>Hypericum maculatum</i> had sedative effects, animals manifesting somnolence.	Roman et al 2013

\* Listing of countries of origin in alphabetic order.

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**Table 5. Research on the phytochemical characterization on wild collected *Hypericum* species indigenous to the Balkan flora**

Balkan country	<i>Hypericum</i> species	Parts of the plant/ extraction procedure	Phytochemical data	Source
Bulgaria	Evaluation of 36 <i>Hypericum</i> ssp. belonging to 17 sections	Aerial parts	Mangiferin – in 26 species; Isomangiferin – 33 species. highest amounts of mangiferin were observed in <i>H. rochelii</i> Griseb. et Schenk, <i>H. perforatum</i> L., <i>H. aucheri</i> Jaub. et Spach and <i>H. montanum</i> L.	Kitanov and Nedialkov 1998
	<i>H. annulatum</i> Moris subsp. <i>annulatum</i> ( <i>H. atomarum</i> subsp. <i>degenii</i> )	Total hydrolysis of purified ethanol extract	Genistein, xanthohypericoside	Kitanov and Akhtardziev, 1979; Kitanov and Nedialkov 2000
		Aerial parts	Hypericin, pseudohypericin	Kitanov, 2001
		Methanol extract of the aerials	hypericophenonoside and annulatophenone	Kitanov and Nedialkov 2001
		Methanolic and chloroform extracts	Annulatophenonoside, acetylanulatophenonoside and a chromone	Nedialkov and Kitanov, 2002
		Aerial parts	Annulatomanin, physcion and $\beta$ -sitosterol	Nedialkov et al, 2007b
	<i>H. perforatum</i>	Aqueous infusion of the aerials.	Total tannins, total flavonoids, and antioxidant capacity	Ivanova et al. 2005
	<i>H. umbellatum</i>	Methanol extract of the flowering aerial parts	2 arabinofuranosides, avicularin, quercitrin, isoquercitrin), hyperoside, xanthone (norathyniol), kaempferol, I-3,II-8-biapigenin, quercetin, myricetin	Nedialkov et al. 2007a
	<i>H. aucheri</i> Jaub. et Spach, <i>H. barbatum</i> Jacq, <i>H. cerastoides</i> (Spaugh) N., <i>H. elegans</i> Stephan ex Willd., <i>H. linarioides</i> Bosse, <i>H. maculatum</i> Crantz, <i>H. montbretii</i> Spach, <i>H. olympicum</i> L., <i>H. perforatum</i> L., <i>H. richeri</i> Vill., <i>H. rumeliacum</i> Boiss., <i>H. tetrapterum</i> Fries, <i>H. umbellatum</i> A. Kerner	Methanolic extract of the aerial parts	Total flavonoid and tannins content, radical scavenging and antioxidant activities. The highest level of tannins was found in <i>H. perforatum</i> and the lowest – in <i>H. elegans</i> . Highest levels of flavonoids - <i>H. cerastoides</i> , lowest <i>H. olympicum</i> . Highest DPPH and ABTS quenching - <i>H. cerastoides</i> . Highest total antioxidant activity - <i>H. maculatum</i> and <i>H. olympicum</i> .	Zheleva-Dimitrova et al. 2010

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Balkan country	<i>Hypericum</i> species	Parts of the plant/ extraction procedure	Phytochemical data	Source
Croatia	<i>Hyperici herba</i>	Aqueous infusion of the aerial parts	Total phenolic content antioxidant capacity	Katalinic et al. 2006
Greece	<i>H. perforatum</i>	Soxhlet extraction of the aerial parts with methanol. LC–MS, LC–UV–SPE–NMR. On-line DPPH and ABTS radical scavenging screening.	chlorogenic acid, hyperoside, taxifolin 3- $\alpha$ -l-rhamnopyranoside, quercitrin, quercetin	Exarchou et al. 2006
		Extraction by methanol, ethanol, EtOH–water and water. Comparative analysis of floral buds, blossoms, shoots, branches	Hyperforin, adhyperforin, hypericin, pseudohypericin, phenolic content, antioxidant activity; pro-oxidant activity of extracts established	Gioti et al. 2009
		Methanolic extract of the aerial parts	Total phenolics, epicatechin, catechin, quercetin, kaempferol, genistein, chrysin, naringenin, chlorogenic acid, protocatechuic acid, caffeic acid, vanillic acid, ferulic acid, gallic acid, p-coumaric acid, p-OH-benzoic acid, resveratrol, syringic acid, homovanillic acid, phloretic acid, cinnamic acid, O-coumaric acid, p-OH-phenylacetic acid, malvidin 3-O-glucoside, catechin, hyperoside, isoquercitrin, astilbin, quercetin 3-O-xyloside, quercitrin, quercetin 7-O-glucoside, quercetin aglycon. Significant antioxidant activity of the extract established.	Kalogeropoulos et al. 2010
	<i>H. empetrifolium</i> Willd., <i>H. rumeliacum</i> Boiss. ssp. <i>apollinis</i> Robson & Strid, <i>H. perforatum</i> L., <i>H. triquetrifolium</i> Turra and <i>H. perforatum</i> L.	Essential oil of the aerials	Comparison based on 98 terpenoid compounds (occurred in concentration over 1%) out of 132 terpenoids present in one or more oils	Petrakis et al. 2005
	<i>H. empetrifolium</i>	Petrol ether extract of the aerial parts	Five acylphloroglucinols substituted with monoterpenoids (empetrifelin A–D and empetrikajafarin), three monocyclic acylphloroglucinols, one monocyclic acylphloroglucinol	Schmidt et al. 2012
	<i>H. perforatum</i>	Polar methanolic fraction of the extract of aerial parts	Total hypericins, total chlorophylls, chlorophyll a and chlorophyll b, hypericin, pseudohypericin	Skalkos et al. 2006
		Ultrasonic methanolic extraction in dark conditions.	hypericin, pseudohypericin, protohypericin, protopseudohypericin, hyperforin, adhyperforin, quercetin, quercitrin, isoquercitrin.	Tatsis et al. 2007

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Table 5. (Continued)

Balkan country	<i>Hypericum</i> species	Parts of the plant/ extraction procedure	Phytochemical data	Source
		Separation and identification by LC/DAD/SPE/NMR and LC/UV/(ESI)MS techniques	hyperoside, astilbin, miquelianin, B, B8-biapigenin, chlorogenic acid, 3-O-coumaroylquinic acid	
		Ultrasonic methanol extraction of the aerial parts. Method development for the direct identification and quantification by one-dimensional <sup>1</sup> H NMR spectroscopy	Hypericin and pseudohypericin	Tatsis et al. 2008
Romania	<i>H. perforatum</i> L., <i>H. maculatum</i> Crantz, with two subspecies, <i>H. tetrapterum</i> Fries, <i>H. hirsutum</i> L.	Methanolic extracts of the aerial parts.	Spectrophotometric assay of total flavonoids and hypericins. <i>H. maculatum</i> had highest content of total hypericins and flavonoids.	Gitea et al. 2010
Serbia	<i>H. annulatum</i> Moris subsp. <i>annulatum</i> ( <i>H. atomarium</i> subsp. <i>degenii</i> )	Aerial parts	Hyperatomarin	Šavikin-Fodulović et al. 2003
	<i>H. annulatum</i> Moris subsp. <i>annulatum</i> ( <i>H. atomarium</i> subsp. <i>degenii</i> )	Aerial parts	Essential oil composition	Guđić et al. 2004; Niketić and Tomović 2008
	<i>H. barbatum</i> , <i>H. hirsutum</i> , <i>H. linarioides</i> , <i>H. maculatum</i> , <i>H. olympicum</i> , <i>H. perforatum</i> , <i>H. richeri</i> , <i>H. rumeliacum</i> , <i>H. tetrapterum</i>	Methanol extracts of the aerial parts	hyperoside, quercitrin, hyperforin and hypericin (hypericin in <i>H. barbatum</i> was 3.9 times higher than that in <i>H. perforatum</i> ).	Smelcerović and Spiteller 2006
	<i>H. barbatum</i> Jacq., <i>H. hirsutum</i> L., <i>H. linarioides</i> Bosse, <i>H. maculatum</i> Crantz, <i>H. rumeliacum</i> Boiss., <i>H. tetrapterum</i> Fries	Accelerated solvent extraction with methanol	Hyperoside, quercitrin, pseudohypericin, hyperforin, hypericin. Highest content of hypericin and pseudohypericin: in <i>H. barbatum</i> , highest hyperforin and quercitrin in <i>H. tetrapterum</i> , highest hyperoside in <i>H. maculatum</i> .	Smelcerovic et al. 2006
	Six <i>Hypericum</i> species	essential oils of the aerial parts	Essential oil composition. Main components: <i>H. alpinum</i> : β-pinene, γ-terpinene, caryophyllene; <i>H. barbatum</i> : α-pinene, β-pinene, limonene, caryophyllene, caryophyllene oxide; <i>H. rumeliacum</i> : α-pinene, β-pinene, limonene.	Saroglou et al. 2007

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Balkan country	<i>Hypericum</i> species	Parts of the plant/ extraction procedure	Phytochemical data	Source
			<i>H. hirsutum</i> : nonane, undecane, caryophyllene, caryophyllene oxide, <i>H. maculatum</i> : spathulenol, globulol; <i>H. perforatum</i> : $\alpha$ -pinene, $\beta$ -farnesene, germacrene D;	
	<i>H. barbatum</i> , <i>H. hirsutum</i> , <i>H. linarioides</i> , <i>H. maculatum</i> , <i>H. olympicum</i> , <i>H. perforatum</i> , <i>H. richeri</i> , <i>H. rumeliacum</i> , <i>H. tetrapterum</i>	Essential oils of the aerial parts	High content of non-terpene compounds and a low content of monoterpenes. <i>H. barbatum</i> , <i>H. richeri</i> and <i>H. rumeliacum</i> (section <i>Drosocarpum</i> ) – similarity and high content of fatty acids. <i>H. hirsutum</i> and <i>H. linarioides</i> (section <i>Taeniocarpum</i> ) – high n-nonane. <i>H. maculatum</i> , <i>H. perforatum</i> and <i>H. tetrapterum</i> (section <i>Hypericum</i> ) – similar contents of non-terpenes and sesquiterpenes. <i>H. olympicum</i> differed from others by higher terpene content.	Smelcerovic et al. 2007
	<i>H. maculatum</i> ssp. <i>immaculatum</i> , <i>H. olympicum</i> , <i>H. richeri</i> ssp. <i>grisebachii</i> , <i>H. barbatum</i>	Maceration with 70% of ethanol of the aerial parts at full blossoming stage	chlorogenic acid, caffeic acid, rutin, quercitrin, hyperforin, hypericin	Božin et al 2013
	<i>Hypericum rochelii</i> Griseb. & Schenk, <i>H. umbellatum</i> A. Kern.	Essential oils of the aerial parts - hydrodistillation by Clevenger - type apparatus	<i>H. rochelii</i> – 79 components: n-nonane, $\beta$ -pinene, germacrene D, n-undecane, and $\alpha$ -pinene (5.8%) as main constituents. <i>H. umbellatum</i> – 126 components: germacrene D, ( <i>E</i> )-nerolidol, n-nonane, ( <i>E</i> )-caryophyllene and caryophyllene oxide as the most abundant components.	Đorđević et al. 2013b
Northern Turkey	<i>H. perforatum</i> L.	Covering aerial parts with 96% ethanol, then shaken for 1 h and left for 72 h in dark at room temperature.	Hypericin, chlorogenic acid, rutin, hyperoside, apigenin-7-O-glucoside, quercitrin and quercetin	Çirak et al. 2007
Northern Turkey	<i>H. perforatum</i> and <i>H. origanifolium</i>	Stem, leaf and reproductive tissues -	Pseudohypericin and hyperforin – increased during ontogenesis. Full opened flowers produced the highest pseudohypericin and hyperforin in <i>H. origanifolium</i> . Highest pseudohypericin was in full opened flowers in <i>H. perforatum</i> , while floral buds of this species produced the highest amount of hyperforin.	Çirak et al. 2008
Turkey (Kayışdağı, Istanbul)	<i>H. calycinum</i> L.	80% methanolic extraction at 45°C of the aerial parts.	Butyl chlorogenate, chlorogenic acid, quercetin, quercitrin, hyperoside, isoquercitrin, miquelianin, rutin, I3, IIS-biapigenin, (+)-catechin and (-)-epicatechin were isolated. Compounds showed strong DPPH and moderate NO scavenging activities. (+)-catechin and (-)-epicatechin were most active.	Kırmızıbekmez et al. 2009

\* Listing of countries of origin in alphabetic order.

**Table 6. Research on plant cell tissue and organ culture of *Hypericum* species characteristic for the Balkans**

Country of origin of the plant	<i>Hypericum</i> species	Type of <i>in vitro</i> culture	Experimental design in the work	Source
Bulgaria	<i>H. rumeliacum</i> Boiss.	Shoot culture	Initiation of shoot cultures from <i>ex situ</i> sterilized stem explants. Assay of phenolic and flavonoid levels.	Danova et al. 2007a
	<i>H. tetrapterum</i> Fries and <i>H. rumeliacum</i> Boiss.	Sterile explants	Study of morphogenic response of leaf, stem, nodule and root explants towards plant growth regulators. Double stage culture for biomass production <i>in vitro</i> .	Danova et al. 2007b
	<i>H. rumeliacum</i> Boiss.	Shoot culture	Cryopreservation of shoot apical meristems. Study of levels of phenolic and flavonoid compounds, malondialdehyde and hydrogen peroxide levels, and histological characteristic of photosynthetic apparatus of regenerated plantlets <i>in vitro</i> as compared with non-frozen control.	Danova et al. 2009
	<i>Hypericum rumeliacum</i> , <i>H. tetrapterum</i> Fries, <i>H. calycinum</i> L.	Shoot cultures	Growth parameters, polyphenolic content, phenolics accumulation, total soluble sugars content, malondialdehyde and hydrogen peroxide levels in a model system of abscisic acid pre-culture treatments (as pre-conditioning step prior to liquid nitrogen storage).	Danova 2010
	<i>H. rumeliacum</i> Boiss.	Shoots, leaf, stem, nodule, root explants.	Light microscopy study of morphogenetic structures formed as a result of plant growth regulators treatment. Phenolic and flavonoid levels, double stage culture for biomass formation and retaining of biosynthetic potential <i>in vitro</i> .	Danova et al. 2010
	<i>H. rumeliacum</i> Boiss., <i>H. tetrapterum</i> Fries, <i>H. calycinum</i> L.	Shoot cultures	Modification of vitamin supplementation. Study of polyphenolic content, hypericin, pseudohypericin, malondialdehyde and hydrogen peroxide, morphometric parameters, dark glands density.	Danova et al. 2012a
	<i>H. rumeliacum</i> Boiss.,	Shoot cultures	Regenerants after cryo-storage in liquid nitrogen. Polyphenolics, hypericin and pseudohypericin, activity of enzymes responsible for the antioxidant defense of the plant.	Danova et al. 2012b
	<i>H. rumeliacum</i> Boiss., <i>H. richeri</i> Vill., <i>H. tetrapterum</i> Fries	Shoot cultures	Slow growth stock cultures. Nitrogen oxide scavenging activity.	Mehandzhiyski et al. 2013
	<i>H. calycinum</i> L.	Shoot cultures	Modification of plant growth regulators content. Polyphenolics content and antioxidant enzymatic activity study.	Trenea et al. 2014
	<i>H. rumeliacum</i> Boiss.	Shoot cultures	Karyotype characterization.	Mártonfiová et al. 2014

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Country of origin of the plant	<i>Hypericum species</i>	Type of <i>in vitro</i> culture	Experimental design in the work	Source
Macedonia	<i>Hypericum perforatum L.</i>	Callus, shoots and regenerated plantlets <i>in vitro</i> .	Hypericin, pseudohypericin	Gadzovska et al. 2005
	<i>Hypericum perforatum L.</i>	Suspension cultures	Jasmonic acid elicitation and stimulation of increased production of phenylpropanoids and naphthodianthrones production.	Gadzovska et al. 2007
	<i>Hypericum perforatum L.</i>	Suspension cultures	Elicitation of phenylpropanoids (phenolic compounds, flavanols, flavonols and anthocyanins) and naphthodianthrones (hypericins) with mycelia extract from the fungus <i>Aspergillus flavus</i> .	Gadzovska-Simic et al. 2012
	<i>Hypericum perforatum L.</i>	Hairy roots	Quinic acid, 3-caffeoylquinic acid, 3-p-coumaroylquinic acid, 3-feruloylquinic acid, rosmarinic acid, quercetin 6-C-glucoside, kaempferol 3-O-rhamnoside, isorhamnetin O-hexoside, kaempferol hexoside, rutin, kaempferol 3-O-rutinoside, catechin, (epi)catechin, proanthocyanidin dimer, quercetin, kaempferol	Tusevski and Gadzovska 2013
	<i>Hypericum perforatum L.</i>	Hairy root cultures	Quinic acid, flavonol glycosides, flavonol aglycons, xanthones	Tusevski et al. 2013a
	<i>Hypericum perforatum L.</i>	Hairy root cultures	Comparative study of the phytochemical profile of dark-grown and photoperiod-exposed cultures. Phenolic acids, flavonols, flavan-3-ols (quinic acid, kaempferol, and seven identified xanthones stimulated by light); 3-p-coumaroylquinic acid and 3-feruloylquinic acid – <i>de novo</i> synthesized in light.	Tusevski et al. 2013b
	<i>Hypericum perforatum L.</i>	Shoot cultures	Polysaccharide elicitors (chitin, pectin, and dextran) – stimulation of phenylpropanoids (phenolics and flavonoids) and naphthodianthrones (hypericin and pseudohypericin). Study of nonenzymatic antioxidant properties, peroxidase activity, phenylalanine ammonia lyase and chalcone-flavanone isomerase.	Gadzovska Simic et al. 2014
	<i>Hypericum perforatum L.</i>	Suspensions	Evaluation of growth, antioxidant activity, phenolic compounds production, xanthone profile after elicitation with <i>Agrobacterium tumefaciens</i> and <i>Agrobacterium rhizogenes</i> .	Tusevski et al. 2014a

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Table 6. (Continued)

Country of origin of the plant	<i>Hypericum</i> species	Type of <i>in vitro</i> culture	Experimental design in the work	Source
	<i>Hypericum perforatum</i> L.	Hairy roots	Regeneration of plantlets <i>in vitro</i> from hairy roots. Determination of phenolic acids, flavonols, flavan-3-ols, naphthodianthrones, phloroglucinols, and xanthones.	Tusevski et al. 2014b
Serbia	<i>Hypericum perforatum</i> L.	Hairy roots	Effect of sucrose on growth and biomass production	Vinterhalter et al. 2006
	<i>Hypericum perforatum</i> L.	Hairy roots	Effect of sucrose on shoot regeneration potential	Vinterhalter et al. 2015
Romania	<i>H. maculatum</i> Crantz	Micropropagation	Efficient <i>in vitro</i> propagation protocol resulting in an average of 97 – 100% acclimatized plantlets. Study the effect of plant growth regulators.	Băcilă et al. 2010
	<i>H. hirsutum</i> , <i>H. maculatum</i> Crantz	Shoot cultures	Effects of plant growth regulators and elicitors (jasmonic acid and salicylic acid) on production of hypericin, pseudohypericin and hyperforin	Coste et al. 2011
	<i>H. richeri</i> ssp. <i>transilvanicum</i> , <i>H. umbellatum</i>	Shoot cultures	Cryopreservation protocol, study of effects of type of explant and cytokinin type on <i>in vitro</i> plant regeneration.	Coste et al. 2012

\* Listing of countries of origin in alphabetic order.

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The biotechnological development of *in vitro* accessions also allows for the development of protocols for the constant and reproducible delivery of certain components of the phytochemical spectrum of the species. After the pioneering works on plant cell tissue and organ culture of *H. perforatum*, more than twenty years ago (Zdunek and Alfermann 1992; Čellarova et al. 1992), a huge number of research effort has been dedicated to biotechnological development of *Hypericum* species. However, with only few exceptions, research has been focused predominantly on *H. perforatum* or also other closely related species (Danova 2014 and references cited within). The recent decade has also marked an active development of research on the biotechnological experiment on *Hypericum* species indigenous to the Balkan Peninsula (Table 6). It has been shown that both differentiated shoots, as well as genetically transformed hairy root cultures of *Hypericum* species possess a high biosynthetic potential regarding secondary metabolites characteristic for the genus.

Development of callus and suspensions, as well as hairy roots of *H. perforatum* by Gadzovska-Simich et al. (Table 6) has led to *in vitro* system development for the production of xanthenes, which are otherwise not present in *H. perforatum*. Bulgarian flora provides the richness of wild accessions of 22 *Hypericum* species (Table 7). Of them 5 are Balkan and one Bulgarian endemic species (the latter, however, extinct).

**Table 7. Species of the *Hypericum* genus characteristic for the Bulgarian flora (Yordanov and Kojuharov 1970; Robson 1977; Kitanov 2001; Anchev et al. 2009)**

Species	Section	Endemic status	Threatened status
<i>H. calycinum</i> L.	III. <i>Ascyreia</i> Choisy		EN
<i>H. androsaemum</i> L.	V. <i>Androsaemum</i> (Duhamel) Godron		EN
<i>H. tetrapterum</i> Fries	IX. <i>Hypericum</i>		
<i>H. maculatum</i> Crantz	IX. <i>Hypericum</i>		
<i>H. perforatum</i> L.	IX. <i>Hypericum</i>		
<i>H. elegans</i> Stephan	IX. <i>Hypericum</i>		
<i>H. olympicum</i> L.	X. <i>Olympia</i> (Spach) Nyman		
<i>H. cerastoides</i> Spach	XI. <i>Campylopus</i> Boiss	Balkan	
<i>H. montbretii</i> Spach	XIII. <i>Drosocarpium</i> Spach		
<i>H. setiferum</i> Stef.	XIII. <i>Drosocarpium</i> Spach	Bulgarian	EX
<i>H. umbellatum</i> Kern.	XIII. <i>Drosocarpium</i> Spach	Balkan	NT
<i>H. richerii</i> Vill.	XIII. <i>Drosocarpium</i> Spach		
<i>H. rochelii</i> Griseb. et Shenk	XIII. <i>Drosocarpium</i> Spach		
<i>H. boissieri</i> Petr.	XIII. <i>Drosocarpium</i> Spach	Balkan	VU
<i>H. barbatum</i> Jacq.	XIII. <i>Drosocarpium</i> Spach		
<i>H. rumeliacum</i> Boiss	XIII. <i>Drosocarpium</i> Spach	Balkan	LC
<i>H. aucheri</i> Jaub. et Spach	XIV. <i>Oligostema</i> (Boiss.) Stef.		
<i>H. thasium</i> Griseb	XV. <i>Thasia</i> Boiss.	Balkan	VU
<i>H. hyssopifolium</i> Chaix	XVII. <i>Hirtella</i> Stef.		
<i>H. hirsutum</i> L.	XVIII. <i>Taeniocarpium</i> Jaub. et Spach		
<i>H. linarioides</i> Bosse	XVIII. <i>Taeniocarpium</i> Jaub. et Spach		NT
<i>H. annulatum</i> Moris	XXVII. <i>Adenosepalum</i> Spach		NT

Abbreviations: Protection status EN – endangered, EX – extinct, NT – near threatened; LC – least concerned; VU – vulnerable.

Table 8. Obtained conditions for long-term maintenance of stock shoots of some *Hypericum* species characteristic for the Balkan flora

<i>Hypericum</i> species	Medium formulation	Sub-culture period
<i>H. richeri</i> Vill.	The basic macro- microsalts and vitamins of MS medium +	3 months
<i>H. rumeliacum</i> Boiss.	<ul style="list-style-type: none"> <li>– For stimulated hypericin production (the basic MS macro- microsalts, Gamborg vitamins + glycine supplementation after the MS formula), 30 g/l sucrose, 6.5 g/l agar</li> <li>– For stimulated polyphenolics production the basic MS formula, 30 g/l sucrose, 6.5 g/l agar</li> </ul>	3 months for both formulations
<i>H. calycinum</i> L.	The basic MS formula, 30 g/l sucrose, 6.5 g/l agar	7 months
<i>H. tetrapterum</i> Fries	The basic MS formula, 30 g/l sucrose, 6.5 g/l agar	7 months
<i>H. perforatum</i> L.	The basic MS formula, 30 g/l sucrose, 6.5 g/l agar	7 months

As a part of broader program for germplasm conservation as well as for biotechnological development purposes we have started the establishment of a slow growth culture of some of these species (Table 8). Research has led to the optimization of culture conditions for the double stage culture of *H. rumeliacum* Boiss for the biomass formation, as well as retaining its biosynthetic potential of polyphenolic compounds (References in Table 6). The latter species showed to have an indigenously high hypericins production which was optimized up to levels exceeding the ones from the samples of the wild habitats, as well as of other reported *Hypericum* species *in vitro* without the conducting of elicitation experiments (Danova et al. 2012a).

In a comparative study of different *in vitro* culture accessions in our Lab, *H. rumeliacum* and *H. richeri* extracts were shown to exhibit nitrogen oxide radical scavenging activity exceeding the one of *H. tetrapterum*, as well as the other species. The obtained values were also higher than the ones obtained for the referent Vitamin C in this work (Mehandzhiyski et al. 2013). The hypericin non-producing *H. calycinum* was shown to be a promising producer of polyphenolic compounds *in vitro* (Treneva et al. 2014).

Further research is in progress for the scale-up of the obtained processes in bioreactor conditions, as well as to assess the pharmacological potential of extracts, fractions or pure compounds obtained from *Hypericum* genotypes indigenous to the Balkan region.

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## REFERENCES

- American Botanical Council (ABC) (2008) Herbal supplement sales experience slight increase in 2008.
- Anchev M, Apostolova I, Assyov B, Bancheva S, Denchev CM, Dimitrov D, Dimitrova D, Evstatieva L, Genova E, Georgiev V, Goranova V, Gushev Ch, Ignatova P, Ivanova D, Meshinev T, Peev D, Petrova A, Petrova AS, Sopotlieva D, Stanev St, Stoeva M, Stoyanov St, Tashev A, Tosheva A, Tsoneva S, Tzonev R, Vitkova A, Vladimirov V (2009) *Red List of Bulgarian vascular plants*. Petrova A & Vladimirov V (eds), *Phytologia Balcanica* 15 (1): 63 – 94.
- APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.
- Asolkar LV, Kakkar KK, Chakre OJ (1992) Second Supplement of Glossary of Indian Medicinal Plants with Active Principles; Publication and Information Directorate, CSIR: New Delhi, India, Volume 1, pp. 138–139.
- Azizi M, Ghani A, Ebadi T, Crockett S (2011) The *ex situ* comparison of two improved St. John's wort (*Hypericum perforatum*) cultivars with an Iranian wild population. *Acta Hort.* 925: 163–170.
- Băcilă I, Coste A, Halmagyi A, Deliu C (2010) Micropropagation of *Hypericum maculatum* Cranz an important medicinal plant. *Romanian Biotechnological Letters*. 15: 86–91.
- Bäcker W, Bart H, Bischoff F, Grabley S, Goedecke R, Johannsbauer W, Jordan V, Stockfleth R, Strube J, Wiesmet V (2006) *Phytoextrakte – Produkte und Prozesse*.
- Barnes J, Anderson LA, Phillipson JD (2001) St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. *Journal of Pharmacy and Pharmacology*. 53: 583–600.
- Blumenthal M, Goldberg A, Brinckmann J (2000) The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines. American Botanical Council. Austin, TX: Integrative Medicine Communications;
- Bombardelli E, Morazzoni P. *Hypericum perforatum* (1995) *Fitoterapia* 66: 43–68.
- Božin B, Kladar N, Grujić N, Anačkov G, Samojlik I, Gavarić N, Srđenović Čonić B (2013) Impact of origin and biological source on chemical composition, anticholinesterase and antioxidant properties of some St. John's wort species (*Hypericum* spp., Hypericaceae) from the Central Balkans. *Molecules*. 18: 11733–11750.
- Brenner DM, Chey WD (2010) St. John's Wort for the treatment of irritable bowel syndrome: reminders of the Hippocratic Oath. *Gastroenterology* 139: 1788–1790.
- Bruni R, Sacchetti G (2009) Factors Affecting Polyphenol Biosynthesis in Wild and Field Grown St. John's Wort (*Hypericum perforatum* L. Hypericaceae/Guttiferae). *Molecules*: 14: 682–725
- Buter B, Orlacchio C, Soldati A, Berger K (1998) Significance of genetic and environmental aspects in the field cultivation of *Hypericum perforatum*. *Planta Med.* 64: 431–437.
- Čellárová, E., Kimáková, K. and Brutovská, R. (1992). Multiple shoot formation and phenotypic changes of R0 regenerants in *Hypericum perforatum* L. *Acta Biotechnol.*, 12: 445–452.



- Çirak C, Radušienė J, Karabük B(S), Janulis V (2007) Variation of bioactive substances and morphological traits in *Hypericum perforatum* populations from Northern Turkey. *Biochemical Systematics and Ecology* 35: 403-409.
- Çirak C, Radušienė J, Çamas N (2008) Pseudohypericin and hyperforin in two Turkish *Hypericum* species: Variation among plant parts and phenological stages. *Biochemical Systematics and Ecology* 36: 377-382.
- Correa, M.P., 1984. Dicionário das Plantas Uteis do Brasil e das Exóticas Cultivadas. Instituto Brasileiro de Desenvolvimento Florestal, Rio de Janeiro.
- Coste A, Vlase L, Halmagyi A, Deliu C, Coldea G (2011) Effects of plant growth regulators and elicitors on production of secondary metabolites in shoot cultures of *Hypericum hirsutum* and *Hypericum maculatum*. *Plant Cell Tiss Organ Cult* 106: 279-288.
- Coste A, Halmagyi A, Butiuc-Keul AL, Deliu C, Coldea G, Hurdu B (2012) *In vitro* propagation and cryopreservation of Romanian endemic and rare *Hypericum* species. *Plant Cell Tiss Organ Cult* 110: 213-226.
- Crockett SL, Robson NKB (2011) Taxonomy and chemotaxonomy of the genus *Hypericum*. *Medicinal and Aromatic Plant Science and Biotechnology* 5 (Special issue 1), pp 1-13, Global Science Books
- Culperer N (edition of 1954) Culpeper's complete herbal:consisting of a comprehensive description of nearly all herbs with their medicinal properties and ibections for compounding the medicines extracted from them. Library of the Ontario College of Pharmacy.
- Danova K, Markovska Y, Dimitrov D, Kapchina-Toteva V (2007a) *In vitro* culture initiation and phenol and flavonoid determination of some medicinal plants, endemic to the Balkan flora, *Proceedings book of International Scientific Conference Stara Zagora*, June 7-8, 2007, vol. 1 "Plant breeding", pp 222 - 229.
- Danova K, Damianova P, Kapchina-Toteva V (2007b) Utilization of the methods of *in vitro* propagation for resource purposes in medicinal plants breeding. *In vitro cultivation of some Hypericum species. Journal of mountain agriculture* 10(6): 1074-1089.
- Danova K, Urbanová M, Skyba M, Čelárová E, Stefanova M, Koleva D, Kapchina-Toteva V (2009) Impact of cryopreservation on biochemical parameters of *in vitro* cultured *Hypericum rumeliacum* Boiss. *Proceedings of International Symposium "New Research in Biotechnology"* (19-20 Nov, Bucharest), pp 78-85.
- Danova K (2010) Production of polyphenolic compounds in shoot cultures of *Hypericum* species characteristic for The Balkan Flora. *Botanica Serbica*, 34(1): 29-36.
- Danova K (2014) Biotechnological utilization of the indigenous biosynthetic capacity of medicinal and aromatic plants. Experience in the genera *Hypericum*, *Pulsatilla* and essential oil bearing *Artemisia alba* characteristic for the Balkan region; Book chapter for Series Recent Progress in Medicinal Plants; Vol.39: *Biotechnology and Genetic Engineering II*, 355-392 pp., Series ISBN: 0-9656038-5-7, Vol ISBN: 1-933699-99-X, Studium Press LLC, USA.
- Danova K, Čelárová E, Macková A, Daxnerová Z, Kapchina-Toteva V (2010) *In vitro* culture of *Hypericum rumeliacum* Boiss. and production of phenolics and flavonoids. *In Vitro Cellular and Developmental Biology – Plant* 46: 422-429.
- Danova K, Nikolova-Damianova B, Denev R, Dimitrov D (2012a) Influence of vitamins on polyphenolic content, morphological development, and stress response in shoot cultures of *Hypericum* spp. *Plant Cell Tiss Organ Cult*, 110:383-393.

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- Danova K, Nikolova-Damianova B, Denev R, Markovska Y (2012b) Impact of pre-culture on short- and long-term *in vitro* recovery of the biosynthetic potential and enzymatic and non-enzymatic antioxidant defense of *Hypericum rumeliacum* Boiss. after cryostorage. *Plant Growth Regulation*, 68:447–457.
- Dongre SH, Badami S, Natesan S, Chandrashekhara R (2007) Antitumor activity of the methanol extract of *Hypericum hookerianum* stem against Ehrlich ascites carcinoma in Swiss albino mice. *J Pharmacol Sci*. 103: 354–359.
- Đorđević AS, Lazarević JS, Mitić VD, Palić RM, Stojanović GS (2013a) Antimicrobial activity of *Hypericum annulatum* Moris and *Hypericum elegans* Stephan ex Willd. essential oils from Serbia. *Chemical Industry & Chemical Engineering Quarterly* 19: 7–11
- Đorđević A, Lazarević J, Šmelcerović A, Stojanović G (2013b) The case of *Hypericum rochelii* Griseb. & Schenk and *Hypericum umbellatum* A. Kern. essential oils: Chemical composition and antimicrobial activity. *Journal of Pharmaceutical and Biomedical Analysis* 77 (2013) 145– 148.
- Dulger B, Gonuz A (2005) Antibacterial activity of the endemic *Hypericum kazdagensis*. *Fitoterapia* 76 (2005) 237 – 239.
- Ernst E (2003) *Hypericum. The Genus Hypericum*. Taylor & Francis, Inc., London.
- ESCOP Monographs, 2003. The European Scientific Cooperative on Phytotherapy, 2nd edition. Exeter, United Kingdom.
- Exarchou V, Fiamingos YC, van Beek TA, Nanos C, Vervoort J (2006) Hyphenated chromatographic techniques for the rapid screening and identification of antioxidants in methanolic extracts of pharmaceutically used plants. *Journal of Chromatography A*, 1112 (2006) 293–302.
- Ferraz ABF, Grivicich I, von Poser GL, Faria DH, Kayser GB, Schwartzmann G, Henriques AT, da Rocha AB (2005) Antitumor activity of three benzopyrans isolated from *Hypericum polyanthemum*. *Fitoterapia* 76: 210-215.
- Foster S (2006) Medicinal plants of Montenegro. *HerbalGram*. 72: 48-54.
- Fritz D, Venturi CR, Cargnin S, Schripsema J, Roehe PM, Montanha JA, von Poser GL (2007) Herpes virus inhibitory substances from *Hypericum connatum* Lam., a plant used in southern Brazil to treat oral lesions. *Journal of Ethnopharmacology* 113: 517–520
- Gadzovska S, Maury S, Ounnar S, Righezza M, Kascakova S, Refregiers M, Spasenoski M, Joseph C, Hagège D (2005) Identification and quantification of hypericin and pseudohypericin in different *Hypericum perforatum* L. *in vitro* cultures. *Plant Physiology and Biochemistry* 43: 591–601
- Gadzovska S, Maury S, Delaunay A, Spasenoski M, Joseph C, Hagège D (2007) Jasmonic acid elicitation of *Hypericum perforatum* L. cell suspensions and effects on the production of phenylpropanoids and naphthodianthrones. *Plant Cell Tiss Organ Cult*. 89: 1–13.
- Gadzovska-Simic S, Tusevski O , Antevski S, Atanasova-Pancevska N, Petreska J, Stefova M, Kungulovski D, Spasenoski M (2012) Secondary metabolite production in *Hypericum perforatum* L. cell suspensions upon elicitation with fungal mycelia from *Aspergillus flavus*. *Arch. Biol. Sci., Belgrade*, 64: 113-121.
- Gadzovska Simic S, Tusevski O, S Maury, Delaunay A, Joseph C, Hagège D (2014) Effects of polysaccharide elicitors on secondary metabolite production and antioxidant response

- in *Hypericum perforatum* L. shoot cultures. Hindawi Publishing Corporation's Scientific World Journal Volume 2014, Article ID 609649, 10 pages.
- Gamble JS (1984) *Flora of the Presidency of Madras*; Bishen Singh & Mahendra Pal Singh: Dehradun, India, Volume 1, pp. 69–71. 21.
- Gaudin M, Simonnet X, Debrunner N (2003) *Colletotrichum gloeosporioides* as the cause of St. John's wort (*Hypericum perforatum*) dieback in Switzerland and breeding for a tolerant variety. In: *Hypericum: The Genus Hypericum*. (Ernst E editor) Taylor and Francis, New York, USA, pp. 23–42.
- German Commission E Monographs, 1998. American Botanical Council. Boston, MA.
- Gioti EM, Fiamingos YC, Skalkos DC, Stalikas CD (2009) Antioxidant activity and bioactive components of the aerial parts of *Hypericum perforatum* L. from Epirus, Greece. *Food Chemistry* 117: 398–404.
- Gitea D, Şipoş M, Mircea T, Paşca B (2010) The analysis of alcoholic extracts of *Hypericum* species by UV/VIS spectrophotometry. *Analele Universităţii din Oradea - Fascicula Biologie*, XVII: 111–115.
- Gluck L (1894) Skizzen aus der Volksmedizin und dem medicinischen Aberglauben in Bosnien und der Hercegovina. In: *Wissenschaftliche Mitteilungen aus Bosnien und der Hercegovina*, pp. 392–454, 2.
- González-Tejero MR, Casares-Porcel M, Sánchez-Rojas CP, Ramiro-Gutiérrez JM, Molero-Mesa J, Pieroni A, Giusti ME, Censori E, de Pasquale C, Della A, Paraskeva-Hadjichambi D, Hadjichambis A, Houmani Z, El-Demerdash M, El-Zayatf M, Hmamouchi M, ElJohrig S (2008) Medicinal plants in the Mediterranean area: Synthesis of the results of the project Rubia. *Journal of Ethnopharmacology* 116: 341–357.
- Griffiths HI, Kryštufek B, Reed JM (2004) *Balkan Biodiversity: Pattern and Process in the European Hotspot*, Kluwer Academic Publishers.
- Grynaeus T, Szabó LGy (2002) A bukovinai hadikfalvi székelyek növényei. *Gyógyszerészet* 46, 251–259, 327–336, 394–399, 588–600.
- Gudžić B, Dorđević S, Nedeljković J, Šmelcerović A (2004) Essential oil composition of *Hypericum atomarium* Boiss. *Chemische Industrie* 58: 413–415.
- Hahn G (1992) *Hypericum perforatum* (St. John's wort) - a medicinal herb used in antiquity and still of interest today. *Journal of Naturopathic Medicine* 3:94–96.
- Hariharapura RC, Srinivasan R, Ashok G, Dongre SH, Jagani HV, Vijayan P (2014) Investigation of the antioxidant and hepatoprotective potential of *Hypericum mysorens*. *Antioxidants*. 3: 526–543.
- HerbalGram*. 2008; 82:58–61.
- Hobbs C (1989) St. John's wort: A literature review. *Herbalgram* 18: 24–33.
- Ivanova D, Gerova D, Chervenkov T, Yankova T (2005) Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology*. 96: 145–150.
- Jarić S, Popović Z, Macukanović-Jocić M, LDjurdjević L, Mijatović M, Karadžić B, Mitrović M, Pavlovic P (2007) An ethnobotanical study on the usage of wild medicinal herbs from Kopaonik Mountain (Central Serbia). *Journal of Ethnopharmacology* 111: 160–175.
- Kartnig T, Gruber A, Sauer H (1989) Comparative phytochemical investigations of *Hypericum* species. *Planta Med.* 55: 215.
- Kalogeropoulos N, Yannakopoulou K, Gioxari A, Chiou A, Makris DP (2010) Polyphenol characterization and encapsulation in  $\beta$ -cyclodextrin of a flavonoid-rich *Hypericum perforatum* (St John's wort) extract. *LWT - Food Science and Technology* 43: 882–889.

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- Kapsokalyvas D, Dimitriou H, Skalkos D, Konstantoudakis G, Filippidis G, Stiakaki E, Papazoglou Th, Kalmanti M (2005) Does *Hypericum perforatum* L. extract show any specificity as photosensitizer for HL-60 leukemic cells and cord blood hemopoietic progenitors during photodynamic therapy? *Journal of Photochemistry and Photobiology B: Biology* 80: 208–216.
- Katalinic V, Milos M, Kulisic T, Jukic M (2006) Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry* 94: 550–557.
- Kırmızıbekmez H, Bassarello C, Piacente S, Celep E, Ataya İ, Mercanoğlu G, Yeşilada E (2009) Phenolic compounds from *Hypericum calycinum* and their antioxidant activity. *Nat Prod Commun.* 4: 531 – 534.
- Kitanov GM (2001) *Hypericin* and pseudohypericin in some *Hypericum* species. *Biochem. Syst. Ecol.* 29: 171–178.
- Kitanov G, Akhtardzhiev Ch (1979) Isolierung von Gentisein aus *Hypericum degeneri* Bornm. *Pharmazie* 34, 447–448.
- Kitanov GM, Nedialkov PT (1998) Mangiferin and isomangiferin in some *Hypericum* species. *Biochemical Systematics and Ecology*. 26: 647–653
- Kitanov GM, Nedialkov PT (2000) Xanthohypericoside, a new xanthone-O-glucoside from *Hypericum annulatum*. *Pharmazie* 55,397–398.
- Kitanov GM, Nedialkov PT (2001) Benzophenone O-glucoside, a biogenic precursor of 1,3,7-trioxygenated xanthones in *Hypericum annulatum*. *Phytochemistry*. 57: 1237–1243.
- Klemow KM, Bartlow A, Crawford J, Kocher N, Shah J, Ritsick M (2011) Chapter 11 Medical Attributes of St. John's Wort (*Hypericum perforatum*) In: Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Benzie IFF, Wachtel-Galor S, (editors). Boca Raton (FL): CRC Press;
- Koch MA, Scheriau C, Betzin A, Hohmann N, Sharbel TF (2013) Evolution of cryptic gene pools in *Hypericum perforatum*: the influence of reproductive system and gene flow. *Annals of Botany* 111: 1083–1094.
- Kołodziejska-Degórska I (2012) Mental herbals – a context-sensitive way of looking at local ethnobotanical knowledge: examples from Bukovina (Romania). *Trames*, 16: 287–301,
- Kültür S (2007) Medicinal plants used in Kırklareli Province (Turkey). *Journal of Ethnopharmacology* 111: 341–364.
- Kunchev Ts (2005) *Identification and collection of herbs*. Colchida, Sofia (in Bulgarian)
- Kusari S, Zühlke S, Borsch T, Spiteller M (2009) Positive correlations between hypericin and putative precursors detected in the quantitative secondary metabolite spectrum of *Hypericum*. *Phytochemistry* 70: 1222–1232.
- Maisenbacher P, Kovar KA (1992) Analysis and stability of *Hyperici oleum*. *Planta Medica* 58: 351–354.
- Manalachioaie R, Sevastre1 B, Prodan I, Toiu AM, Benedec D, Oniga I, Marcus I, C Deliu (2010) Comparative evaluation of antidepressant effects of two *Hypericum* species (*H. perforatum* L. and *H. maculatum* Cranz) in Swiss Mice. Bulletin UASVM, Veterinary Medicine 67: 115-119.
- Mártonfióvá L, Danova K, Toteva-Kapchina V, Čellárová E (2014) *Karyotype analysis of Hypericum rumeliacum* Boiss., Thaiszia - J. Bot., Košice, 24 (2): 143-150.
- McClatchey WC, Mahady GB, Bennett BC, Shiels L, Savo V (2009) Ethnobotany as a Pharmacological Research Tool and Recent Developments in CNS-active Natural Products from Ethnobotanical Sources. *Pharmacol Ther.* 123: 239–254.



- Mehandzhyski A, Batovska D, Dimitrov D, Evstatieva, L, Danova K (2013) Nitric Oxide-Scavenging Activity Of In Vitro Cultured Balkan Medicinal And Aromatic Plants. *Bulgarian Journal of Agricultural Science*. 19: 31–34.
- Menković N, Šavikin K, Tasić S, Zdunić G, Štesević D, Milosavljević S, Vincek D (2011) Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains (Montenegro). *Journal of Ethnopharmacology* 133: 97–107.
- Mentz LA, Lutzemberger LC, Schenkel EP (1997) Da flora medicinal do Rio Grande do Sul: notas sobre a obra de D'Avila (1910). *Cadernos de Farmácia* 15: 25–47.
- Meseguer AS, Aldasoro JJ, Sanmartín I (2013) Bayesian inference of phylogeny, morphology and range evolution reveals a complex evolutionary history in St. John's wort (*Hypericum*). *Molecular Phylogenetics and Evolution*. 67 (2013) 379–403.
- Mitcheva M, Kondeva M, Vitcheva V, Nedialkov P, Kitanov G (2006) Effect of benzophenones from *Hypericum annulatum* on carbontetrachloride-induced toxicity in freshly isolated rat hepatocytes. *Redox Rep.* 11: 3–8.
- Mitsopoulou KP, Vidali VP, Koliopoulos G, Couladouros EA, Michaelakis A (2014) Hyperforin and deoxycoumulinone as a larvicidal agent against *Culex pipiens* (Diptera: Culicidae). *Chemosphere* 100 (2014) 124–129.
- Momekov G, Nedialkov PT, Kitanov GM, Zheleva-Dimitrova D, Tzanova T, Girreser U, Karaivanova M (2006) Cytoprotective effects of 5 benzophenones and a xanthone from *Hypericum annulatum* in models of epi rubicin-induced cytotoxicity: SAR analysis and mechanistic investigations. *Med.Chem.* 2: 377–384.
- Momekov G, Ferdinandov D, Dimitrova DZ, Nedialkov P, Girreser U, Kitanov G (2008) Cytotoxic effects of hyperatomarin, a prenylated phloroglucinol from *Hypericum annulatum* Moris subsp. *annulatum*, in a panel of malignant cell lines. *Phytomedicine* 15: 1010–1015.
- Mukherjee PK, Saritha GS, Suresh B (2001) Antibacterial spectrum of *Hypericum hookerianum*. *Fitoterapia*. 72: 558–560.
- Nahrstedt, A., Butterweck, V., 1997. Biologically active and other chemical constituents of the herb of *Hypericum perforatum* L. *Pharmacopsychiatry* 30, 129–134.
- Nahrstedt A, Butterweck V (2010) Lessons learned from herbal medicinal products: the example of St. John's wort. *J. Nat. Prod.* 73: 1015–1021.
- Nedialkov PT, Kitanov GM (2002) Two benzophenone O- arabinosides and a chromone from *Hypericum annulatum*. *Phytochemistry* 59: 867–871.
- Nedialkov PT, Kitanov GM, Zheleva-Dimitrova D, Girreser U (2007a) Flavonoids and a xanthone from *Hypericum umbellatum* (Guttiferae). *Biochemical Systematics and Ecology*. 35: 118–120.
- Nedialkov P, Zheleva-Dimitrova D, Girreser U, Kitanov GM (2007b) A new isocoumarin from *Hypericum annulatum*. *Nat. Prod. Res. A* 21: 1056–1060
- Neshev G, Landgeev I (1989) *Reference book of herbs*. Dr P. Beron, Sofia, pp 69–70 (in Bulgarian).
- Niketić M, Tomović G (2008) Survey of some rare and endangered plants in Serbia with new chorological data In: *Bulletin of the Natural History Museum of Belgrade*, vol 1, ISSN 1820-9521.
- Nürk NM, Madriñán S, Carine MA, Chase MW, Blattner FR (2013) Molecular phylogenetics and morphological evolution of St. John's wort (*Hypericum*). *Molecular Phylogenetics and Evolution*. 66: 1–16.

Complimentary Contributor Copy

- Osbaldeston TA, Wood RPA (2000) *De Materia Medica* (English translation). Johannesburg, South Africa: IBIDIS Press, Dioscorides
- Öztürk N, Korkmaz S, Öztürk Y (2007) Wound-healing activity of St. John's Wort (*Hypericum perforatum* L.) on chicken embryonic fibroblasts. *Journal of Ethnopharmacology* 111: 33–39.
- Pappa N, Birkás-Frendl K, Bencsik T, Stranczinger S, Czégényi D (2014) Survey of traditional beliefs in the Hungarian Csángó and Székely ethnomedicine in Transylvania, Romania. *Rev Bras Farmacogn* 24: 141–152.
- Petrakis PV, Couladis M, Roussis V (2005) A method for detecting the biosystematics significance of the essential oil composition: The case of five Hellenic *Hypericum* L. species. *Biochemical Systematics and Ecology* 33: 873–898.
- Pieroni A, Dibra B, Grishaj G, Grishaj I, Maçai SG (2005) Traditional phytotherapy of the Albanians of Lepushe, Northern Albanian Alps. *Fitoterapia* 76: 379–399.
- Pongiya UD, Kandanath BM, Rao YR, Khan MR (2014) Protective effect of *Hypericum hookerianum* in reversing haloperidol induced schizophrenia-like behaviors in Swiss albino mice. *Asian Journal of Biomedical and Pharmaceutical Sciences* 32: 14–23.
- Prodan I, Sevastre B, Toiu AM, Benedec D, Oniga I, Deliu C, Marcus I (2009) Antitumour Activity of *Hypericum perforatum* and *Hypericum maculatum* in Ehrlich Ascitic Carcinoma. *Bulletin UASVM, Veterinary Medicine* 66: 176–181.
- Pulok K, Mukherjee GS, Saritha B, Suresh B (2001) Antibacterial spectrum of *Hypericum hookerianum*. *Fitoterapia*; 72:558–560.
- Radulović N, Stankov-Jovanović V, Stojanović G, Šmelcerović A, Spiteller M, Asakawa Y (2007) Screening of in vitro antimicrobial and antioxidant activity of nine *Hypericum* species from the Balkans. *Food Chemistry* 103 (2007) 15–21.
- Radun M (2007) Conservation and utilisation of St. John's wort (*Hypericum perforatum* L.) in Herzegovina. Master theses (Thörn E, supervisor) No. 47, *International Master Programme at the Swedish Biodiversity Centre*, Uppsala, ISSN: 1653-834X.
- Redžić SS (2006) Wild edible plants and their traditional use in the human nutrition in Bosnia-Herzegovina. *Ecology of Food and Nutrition* 45: 189–232.
- Redžić SS (2007) The Ecological Aspect of Ethnobotany and Ethnopharmacology of Population in Bosnia and Herzegovina. *Collegium Antropologicum* 31: 869–890.
- Reeds K (2012) Saint John's wort (*Hypericum perforatum* L.) in the age of Paracelsus and the Great Herbals: Assessing the Historical Claims for a Traditional Remedy. In: *Herbs and Healers from the Ancient Mediterranean through the Medieval West: Essays in Honor of John M. Riddle*. Anne Van Arsdall, Timothy Graham (Eds) Ashgate Publishing, Ltd.,
- Rigat M, M. A. Bonet, S. Garcia, T. Garnatje, and J. Vallès (2007) "Studies on pharmaceutical ethnobotany in the high river Ter valley (Pyrenees, Catalonia, Iberian Peninsula)," *Journal of Ethnopharmacology*, vol. 113, no. 2, pp. 267–277.
- Robson NKB (1977) Studies in the genus *Hypericum* L. (Guttiferae). 1. Infrageneric classification. *Bull. Brit. Mus. (Nat. Hist.), Bot.* 5: 325.
- Rodrigues JC, Ascensão L, Bonet MÀ, Vallès J (2003) An ethnobotanical study of medicinal and aromatic plants in the Natural Park of "Serra de São Mamede" (Portugal). *Journal of Ethnopharmacology* 89: 199–209.
- Roman I, Cristescu M, Puică C (2011) Effects of *Hypericum perforatum* and *Hypericum maculatum* extracts administration on some morphological and biochemical parameters

- in rat liver intoxicated with alcohol. *Studia Universitatis "Vasile Goldiș", Seria Științele Vieții*. 21: 361-370.
- Roman I, Puică C, Cristescu M (2013) Effects of *Hypericum perforatum* and *Hypericum maculatum* extracts on the rats' brain intoxicated with alcohol. *Studia Universitatis "Vasile Goldiș", Seria Științele Vieții*. 23: 189-193.
- Saddiqe Z, Naeem I, Maimoona A (2010) A review of the antibacterial activity of *Hypericum perforatum* L. *Journal of Ethnopharmacology*. 131: 511-521.
- Saito YA, Rey E, Almazar-Elder AE, Harmsen WS, Zinsmeister AR, Locke GR, Talley NJ (2010) A randomized, double-blind, placebo-controlled trial of St John's wort for treating irritable bowel syndrome. *Am. J. Gastroenterol.* 105: 170-177.
- Sanchez-Mateo CC, Bonkanka CX, Rabanal RM (2009) *Hypericum grandifolium* Choisy: A species native to Macaronesian Region with antidepressant effect. *Journal of Ethnopharmacology* 121: 297-303.
- Sarić-Kundalić B, Dobes C, Klatte-Asselmeyer V, Saukel J (2011) Ethnobotanical survey of traditionally used plants in human therapy of east, north and north-east Bosnia and Herzegovina. *Journal of Ethnopharmacology* 133: 1051-1076.
- Sarić-Kundalić B, Dobes C, Klatte-Asselmeyer V, Saukel J (2010) Ethnobotanical study on medicinal use of wild and cultivated plants in middle, south and west Bosnia and Herzegovina. *Journal of Ethnopharmacology* 131: 33-55.
- Saroglou V, Marin PD, Rancic A, Veljic M, Skaltsa H (2007) Composition and antimicrobial activity of the essential oil of six *Hypericum* species from Serbia. *Biochemical Systematics and Ecology* 35: 146-152.
- Šavikin-Fodulović K, Aljancić I, Vajs V, Menković N, Macura S, Gojčić G, Milosavljević S (2003) Hyperatomarin, an antibacterial prenylated phloroglucinol from *Hypericum atomarium* ssp. *degenii*. *Journal of Natural Products* 66: 1236-1238.
- Šavikin K, Zdunić G, Menković N, Živković J, Čujić N, Tereščenko M, Bigović D (2013) Ethnobotanical study on traditional use of medicinal plants in South-Western Serbia, Zlatibor district. *Journal of Ethnopharmacology* 146: 803-810.
- Schmidt S, Jürgenliemk G, Skaltsa H, Heilmann J (2012) Phloroglucinol derivatives from *Hypericum empetrifolium* with antiproliferative activity on endothelial cells. *Phytochemistry* 77 (2012) 218-225.
- Schmitt LA, Liu Y, Murphy PA, Petrich JW, Dixon PM, Birt DF (2006) Reduction in hypericin-induced phototoxicity by *Hypericum perforatum* extracts and pure compounds. *Journal of Photochemistry and Photobiology B: Biology* 85: 118-130.
- Skalkos D, Gioti E, Stalikas CD, Meyer H, Papazoglou ThG, Filippidis G (2006) Photophysical properties of *Hypericum perforatum* L. extracts – Novel photosensitizers for PDT. *Journal of Photochemistry and Photobiology B: Biology* 82 (2006) 146-151.
- Smelcerovic A, Spiteller M (2006) Phytochemical analysis of nine *Hypericum* L. species from Serbia and the F.Y.R. Macedonia. *Pharmazie* 61: 251-252.
- Smelcerovic A, Verma V, Spiteller M, Ahmad SM, Puri SC, Qazi GN (2006) Phytochemical analysis and genetic characterization of six *Hypericum* species from Serbia. *Phytochemistry* 67: 171-177.
- Smelcerović A, Spiteller M, Ligon AP, Smelcerović Z, Raabe N (2007) Essential oil composition of *Hypericum* L. species from Southeastern Serbia and their chemotaxonomy. *Biochemical Systematics and Ecology* 35: 99-113.



- Staneva D, Panova D, Raynova L, Assenov A (1982) Herbs at each home. Medicina I fizkultura, Sofia (in Bulgarian).
- Stavropoulos NE, Kim A, Nseyo UU, Tsimaris I, Chung TD, Miller TA, Redlak M, Nseyo UO, Skalkos D (2006) *Hypericum perforatum* L. extract – Novel photosensitizer against human bladder cancer cells. *Journal of Photochemistry and Photobiology B: Biology* 84: 64–69.
- Stevens PF (2007) *Hypericaceae. The families and genera of vascular plants*. In: (Kubitzki K. ed.). Vol. XI. Heidelberg: Springer Verlag, 194–201.
- Subakanmani S, Umadevi P. Physicochemical characterization and phytochemical analysis of aerial parts of *Hypericum hookerianum*. *Journal of Pharmacy Research* 2012; 5(3):1387-1391.
- Süntar IP, Akkol EK, Yilmazer D, Baykal T, Kirmizibekmez H, Alper M, Yeşilada E (2010) Investigations on the *in vivo* wound healing potential of *Hypericum perforatum* L. *Journal of Ethnopharmacology* 127: 468–477.
- Tatsis EC, Boeren S, Exarchou V, Troganis AN, Vervoort J, Gerothanassis IP (2007) Identification of the major constituents of *Hypericum perforatum* by LC/SPE/NMR and/or LC/MS. *Phytochemistry* 68 (2007) 383–393.
- Tatsis EC, Exarchou V, Troganis AN, Gerothanassis IP (2008) <sup>1</sup>H NMR determination of hypericin and pseudohypericin in complex natural mixtures by the use of strongly deshielded OH groups. *Analytica chimica acta* 607: 219–226.
- Tekelova D, Repcak M, Zemkova E, Toth J (2000) Quantitative changes of dianthrones, hyperforin and flavonoids content in the flower ontogenesis of *Hypericum perforatum*. *Planta Med.* 66: 778–780.
- Trenea G, Markovska Y, Wolfram E, Danova K (2014) Effect of plant growth regulators on growth patterns and enzymatic antioxidant activities in *Hypericum calycinum* shoot cultures. *Bulg J Agr Sci* (2014) 20: 46-50.
- Trovato A, Raneri E, Kouladis M, Tzakou O, Taviano MF, Galati EM (2001) Anti-inflammatory and analgesic activity of *Hypericum empetrifolium* Willd. *Guttiferae. Farmaco* 56: 455–457.
- Tusevski O, Gadzovska S (2013) Phenolic acids and flavonoids in *Hypericum perforatum* L. hairy roots. *Int J Pharm Bio Sci* 4: 737 – 748.
- Tusevski O, Stanoeva JP, Stefova M, Kungulovski D, Atanasova-Pancevska N, Sekulovski N, Panov S, Gadzovska-Simic S (2013a) Hairy roots of *Hypericum perforatum* L.: a promising system for xanthone production. *Centr. Eur. J. Biol.* 8: 1013-1022.
- Tusevski O, Stanoeva JP, Stefova M, Gadzovska Simic S (2013b) Phenolic Profile of dark-grown and photoperiod-exposed *Hypericum perforatum* L. hairy root cultures. Hindawi Publishing Corporation. *The Scientific World Journal*. Volume 2013, Article ID 602752, 9 pages.
- Tusevski O, Stanoeva JP, Stefova M, Simic SG (2014a) Agrobacterium enhances xanthone production in *Hypericum perforatum* cell suspensions. *Plant Growth Regulation*. DOI 10.1007/s10725-014-9989-6.
- Tusevski O, Stanoeva JP, Stefova M, Pavokovic D, Gadzovska Simic S (2014b) Identification and quantification of phenolic compounds in *Hypericum perforatum* L. transgenic shoots. *Acta Physiologiae Plantarum*, 36: 2555-2569. DOI: 10.1007/s11738-014-1627-4.

- Tuzlacy E (2006) *Türkiye'nin Bitkisel Halk Yılları* (Herbal folk medicines) Alfa Basımyaym Daıyım Pırketi, İstanbul.
- Umek A, Kreft S, Karting T, Heydel B (1999) Quantitative phytochemical analyses of six *Hypericum* species growing in Slovenia. *Planta Med.* 65: 388–390.
- Valiakos E, Marselos M, Sakellariadis N, Constantinidis Th, Skaltsa H (2015) Ethnopharmacological approach to the herbal medicines of the “Antidotes” in Nikolaos Myrepsos' *Dynameron*. *Journal of Ethnopharmacology* 163: 68–82
- Vijayan P, Vinodkumar S, Badami S, Mukherjee PK, Dhanaraj SA, Suresh B (2003) Selective *in vitro* cytotoxicity of *Hypericum hookerianum* towards cancer cell lines. *Oriental Pharm Expt Med.* 3:141–146.
- Vijayan P, Raghu C, Ashok G, Dhanaraj SA, Suresh B (2004) Antiviral activity of medicinal plants of Nilgiris. *Indian J Med Res* 120: 24–29.
- Vinterhalter B, Ninković S, Cingel A, Vinterhalter D (2006) Shoot and root culture of *Hypericum perforatum* L. transformed with *Agrobacterium rhizogenes* A4M70GUS. *Biologia Plantarum*, 50: 767–770.
- Vinterhalter B, Zdravković-Korać S, Mitić N, Bohanec B, Vinterhalter D, Savić J (2015) Effect of sucrose on shoot regeneration in *Agrobacterium* transformed *Hypericum perforatum* L. roots. *Acta Physiologiae Plantarum* January 2015: 37:37.
- Vokou D, Katrati K, Kokkini S (1993) Ethnobotanical survey of Zagori (Epirus, Greece), a renowned centre of folk medicine in the past. *J. Ethnopharmacol.* 39: 187–196.
- WHO (1993) Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram* 28: 13–14.
- WHO (1999) World Health Organization Monographs on Selected Medicinal Plants, vol. 2, Geneva.
- WHO (2002) *Traditional Medicine Strategy* 2002–2005.
- WHO (2003) Fact sheet N°134, <http://www.who.int/mediacentre/factsheets/2003/fs134>.
- Wood M (1997) *The Book of Herbal Wisdom: Using Plants as Medicine*. North Atlantic Books.
- Yesilada E, Gurbuz I (1998) Evaluation of the anti-ulcerogenic effect of the flowering herbs of *Hypericum perforatum*. *Journal of Faculty of Pharmacy of Gazi University* 15: 25–31.
- Yesilada E, Honda G, Sezik E, Tabata M, Goto K, Ikeshiro Y (1993) Traditional medicine in Turkey. IV. Folk medicine in the Mediterranean subdivision. *Journal of Ethnopharmacology* 39: 31–38.
- Yesilada E, Honda G, Sezik E, Tabata M, Fujita T, Tanaka T, Takeda Y, Takaishi Y (1995) Traditional medicine in Turkey. V. Folk medicine in the inner Taurus Mountains. *Journal of Ethnopharmacology* 46: 133–152.
- Yordanov D, Kojuharov St (1970) *Guttiferae Family: Flora of the PR of Bulgaria*, vol IV, Bulgarian Academy of Sciences, Sofia, p 227 (in Bulgarian)
- Zdunek K, Alfermann AW (1992) Introduction of shoot organ cultures of *Hypericum perforatum* and formation of hypericin derivatives. *Planta Med.*, 58: 621–2.
- Zheleva-Dimitrova D, Nedialkov P, Kitanov G (2010) Radical scavenging and antioxidant activities of methanolic extracts from *Hypericum* species growing in Bulgaria. *Pharmacogn Mag.* 6: 74–78.
- Zlatković BK, Bogosavljević SS, Radivojević AR, Pavlović MA (2014) Traditional use of the native medicinal plant resource of Mt. Rtanj (Eastern Serbia): Ethnobotanical evaluation and comparison. *Journal of Ethnopharmacology*. 151: 704–713.

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