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**Title: Conservation and
utilisation of St. John's wort
(*Hypericum perforatum* L.) in
Herzegovina**

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Abstract

Collecting of medicinal and aromatic plants in Herzegovina has a very long tradition. *Hypericum perforatum* is reported to be collected from the wild in large quantities in Herzegovina. Whith this project it is aimed to investigate the presence of *Hypericum perforatum* in Herzegovina, as well as explore possibilities for *ex situ* conservation of this plant. Interviews with the collectors were conducted in order to explore situation on the terrain and investigate traditional knowledge related to that medicinal plant. In total 20 people were interviewed, 15 collectors and 5 herbalists. Since seed germination behavior is an integral part of *ex situ* conservation, germination rate of the seed of *Hypericum perforatum* was checked out. In the study one year old and new harvested seed obtained from *Hypericum perforatum* plants from the pasture near Stolac town were used.

According to interviewed collectors *Hypericum perforatum* is still not threaten in Herzegovina region, but sustainable harvesting has to be controlled. A potential risk for presence of *Hypericum perforatum* in study area is climate change; variations in richness are noticed from year to year. The erosion of traditional knowledge was noticed, since only 20% of interviewed herbalists obtained knowledge about medicinal and aromatic plants from ancestors. The main remedies prepared from *Hypericum perforatum* are herbal mixture tea, which is used against ulcus diseases, asthma and bronchitis and *Hypericum perforatum* oil, which is used for bruises, skin irritations, and varicose veins. Germination rate of new harvested seed is very low due to seed dormancy, while germination rate of one year old seed was satisfying from the aspect of *ex situ* conservation. Seed drying and cold storage significantly influenced on of the germination rate, but germination rate still was enough high for *ex situ* conservation. The light has a positive effect on seed germination of *Hypericum perforatum*.

Key words: *ex situ* conservation, germination rate, *Hypericum perforatum*, traditional knowledge

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Introduction

Aim of the study

With this project it is aimed to investigate the presence of *Hypericum perforatum* and threat of utilisation of *Hypericum perforatum* in Herzegovina, as well as explore possibilities for *ex situ* conservation of this plant. *Hypericum perforatum* is on the list of ten priority species, selected by the ECP/GR (European Cooperative Programme for Crop Genetic Resources Network) working group on medicinal and aromatic plants, as well as by the SEEDNet (South East Europe Development Network on Plant Genetic Resources) working group on medicinal and aromatic plants. Considering that it is reported that *Hypericum perforatum* is collected in larger quantities in Herzegovina, interviews with the collectors were conducted in order to explore presence of *Hypericum perforatum* on the terrain and collect traditional knowledge related to the use of that medicinal plant. It is intended to check germination rate of the seed of *Hypericum perforatum* which will serve as indicator of practicability of conserving this species *ex situ*. Seed germination behavior is an integral part of *ex situ* conservation, especially for developing standard viability monitoring protocols and to ensure sufficient populations size for germplasm regeneration (Bewley and Black 1994). Much is known about the optimum treatment of the seed of most of the major food crops, but there is a lack of that information regarding the wild species, including medicinal plants. The preservation of plant germplasm in seed banks, (or gene banks), is one of the more useful techniques of *ex situ* conservation of wild plant species.

Medicinal plants, use and exploitation

All cultures from ancient times to the present day have used plants as a source of medicines. The World Health Organization (WHO) estimates that some of 80% of the world's people depend on traditional medicine for their primary health care needs, of these 85% use plants or their extracts as the active substance (Sheldon et al., 1998).

This means that close to 3 billion people rely on plants for medicine. Medicinal plant species are still to a large extent harvested from the wild and relatively few are cultivated as crop plants (Kala, C.P.2000).

Thanks to the preventative and therapeutic importance and the additional benefits of their low cost, wide accessibility, and cultural significance in many traditional cultures, medicinal plants have always been a basic source for human health. Interest in and demand for traditional remedies is increasing worldwide, particularly in rapidly growing urban societies. Increased utilization of medicinal plants has increased pressure on a resource that is largely

harvested from exhausted wild populations in decrease wild habitats. Research on the conservation and sustainable use of medicinal plants and their habitats has fallen far behind the demand for this globally important resource. According to IUCN more than 20,000 species of plants are used medicinally somewhere on earth. Nearly half of these species are potentially threatened by over-harvest or loss of habitat. (IUCN) Relatively few medicinal and aromatic plant (MAP) species are cultivated. Excessive utilization of wild MAP from natural habitats and environmental degradation due to human activities are the main reasons why MAP is often considered endangered in many European regions.

The use of herbal preparations over millennia in the history of mankind, growing awareness of the role of medicinal plants in drug development and the modern "back to nature" trend, have increased global attention on medicinal and aromatic plants (MAP) research. (Maggioni L.1999).

The current and potential value of medicinal plants - their value to local community health, to regional markets, and to global health security and trade - is widely recognized as a reason to conserve medicinal plants. For most of the endangered medicinal plant species no conservation action has been taken. For example, there is very little material of them in gene banks. For most countries, there is not even a complete inventory of medicinal plants. Much of the knowledge on their use is held by traditional societies, whose very existence is now under threat (WHO, IUCN & WWF, 1993).

Medicinal plants in Bosnia and Herzegovina

A large share of the global medicinal plants trade, about 8 per cent, originates from the Balkans. The livelihoods of many people in rural areas largely depend on the wild collection of such plants. But over-harvesting has depleted wild populations of many medicinal plant species in areas where they were abundant only some 10 to 15 years ago. Most medicinal plants in the Bosnia and Herzegovina are collected from the wild by the local population (WWF/TRAFFIC, 2000).

The political changes over the past 15 years have offered both new opportunities and threats for utilization of medicinal plants. In Bosnia and Herzegovina medicinal and aromatic plants have the potential to contribute substantially to the national and local economy. In rural areas, the collection, use, processing, and trade is an important component of the livelihoods of marginal groups. The wild stock of many MAP species has declined during past decades; some species have become rare or endangered because of habitat loss or modification, overexploitation, soil erosion and other factors. Harvesting of medicinal and aromatic plants from the wild is an important economic factor in rural areas in Bosnia and Herzegovina (Kathe W. et al,

2003). During communism, wild herb collection was organized by the state, which bought the herbs through one central company and sold them, mostly to the domestic market. But now, hundreds of trading firms compete for a slice of the profits, and there are insufficient state funds to implement protective regulations.

No post-war studies on the effects of war on plant and animal species in Bosnia and Herzegovina are known because environmental monitoring is not yet well developed (REC 2000). The compilation of a national botanical Red List was started in 1990, continued during the war and is reported to have been published in 1997. The publication is believed to list 678 vascular plant species according to the old IUCN threat categories (REC 2000). However, this list was not available on request.

It is believed that in Bosnia and Herzegovina have over 700 species of medicinal and aromatic plants, from which is exploited around 200. (Borivoje Stepanovic et al, 2002.)

The use of medicinal and aromatic plants (MAPs) in Bosnia and Herzegovina for cosmetic, medicinal, coloring and aromatic purposes has a long tradition. MAPs are also used in herbal teas, food supplements, liquors, bitters, insecticides, fungicides, essential oil products, perfumes, flavoring liquids, varnishes and cleaning products.

Three of the top 15 best-selling herbs on world markets are wild-harvested in Herzegovina, including St. John's wort (*Hypericum perforatum* L., *Clusiaceae*), valerian (*Valeriana officinalis* L., *Valerianaceae*), and bilberry (*Vaccinium myrtillus* L., *Ericaceae*). Herzegovina's tradition of harvesting wild medicinal herbs dates back centuries, if not millennia. Herzegovina has huge potential thanks to its climate-Mediterranean as well as continental. In such a small area there is among the world's richest and well-developed herbal traditions. The quality of curing herbs from the region of Herzegovina is confirmed by its presence on European and USA market for over hundred years.

Description of study area

Geographical Situation of Herzegovina

Herzegovina is a historical and geographical region in the Dinaric Alps that comprises the southern part of present-day Bosnia and Herzegovina.

Herzegovina is situated between two regions: a mountainous region to the north and a coastal region to the south. The mountainous wall to the north holds back the circulation of atmospheric masses from the Mediterranean, creating favorable climatic conditions for various vegetal cultivations in the

area of Herzegovina. Herzegovina covers an area of 9,948 square kilometers. It is bordered to the east by Montenegro, to the west by Bosnia, to the south and southeast by southern Croatia and in one area forms part of the Adriatic coast. Borders between Bosnia and Herzegovina are unclear and often disputed. The geography of Herzegovina is mostly mountainous karsts with high mountain in the north Herzegovina. The Herzegovina terrain is mostly hilly, karsts except for the central valley of the river Neretva. Of the total land area of Herzegovina, 56.8% is given to agriculture (arable and pastoral lands), while 42.4% is taken up by the woodland, mostly low forests or shrub land. The remainder is 0.8%. The low forest has little production value, but is important from a biodiversity point of view as it shelters a number of endemic species.

Photo1. Herzegovina region



Climatic conditions

The nearby sea, relief, and height above sea level influence the climate of Herzegovina. Mediterranean climate, with its gentle winters and warm summers, is predominant. It is characterized by rainfall in the colder period of the year. During winter, the Adriatic Sea gives off the heat accumulated during

the summer period, easing winter atmospheric temperatures. In July and August, summer temperatures reach their maximum. Maximum precipitation takes place in the autumn, though rainfall can also be felt in the spring. However, the greatest quantities fall in November and December. Regarding wind, north and northeasterly winds are predominant. Herzegovina has another recurring wind which blows directly from the Adriatic Sea, most often during the autumn and spring. It is saturated with humidity and in the autumn brings rainfall in large quantities. On the basis of climatic evidence found in meteorological annals, it can be concluded that Herzegovina is influenced by Mediterranean, continental, and mountain climates. Herzegovina and the southern area have a modified Mediterranean climate with an average annual precipitation of 600 to 800 mm.

Hydrographical Conditions

Herzegovina is a rugged country in which limy layers originating in the Triassic, Jurassic, and Cretaceous ages extend for tens of meters. These layers spring up in spots, are sometimes crushed, or are in the form of large masses. Water follows these rifts, disappears from the surface for a while, creates receptacles underground, or pours underground and crops up later on the surface as a whole river. The copious autumn rains, which fall in the area of Herzegovina, create streams. These make the rivers swell and flood, and the Karst expanses become lakes. The area of southern Herzegovina is sparser regarding mountain springs.

Collecting tradition in Herzegovina

The local use of natural plant resources for medicinal purposes is an integral part of folk medicine in the whole region. Collecting of medicinal and aromatic plants in Herzegovina has a very long tradition; first documents are dated from the 13th century. It is estimated that altogether approximately 100.000 people are involved in collection of medicinal and aromatic plants (Group of authors, 2003).

It is estimated that in some habitats in Herzegovina there are 1300 species of plants with some medicinal characteristics. Some of them are of unique quality in the world, such as sage (*Salvia officinalis*), immortelle (*Helichrysum italicum*), heath (*Saturea montana*), juniper tree (*Juniperus communis*), autumn crocus (*Colchicum autumnale*), St. John's wort (*Hypericum perforatum*), hemp stalk (*Vitex agnus castus*), etc. (Kosovic, N. et al, 2000).

Traditionally, wild-harvesting of medicinal and aromatic plants predominates in this region. The history of MAP collection and use in Herzegovina is not well studied and documented. During the times of the former Socialist Republic of Yugoslavia, Bosnia-Herzegovina was mainly a provider of MAP raw material

(SEED HQ 2000). However, no reliable data are available on what quantities of the traded or exported material came from Bosnia-Herzegovina or from other parts of the former Yugoslavia.

Trade and marketability

According to Traffic International (1998), 90% of European MAP which are used commercially is collected in the wild. During the times of the former Socialist Republic of Yugoslavia, Bosnia-Herzegovina was mainly a provider of MAP raw material (Donnelly, R. et al., 2002). In Herzegovina exists a long tradition in collecting and usage of medicinal and aromatic plants. Collection activity in commercial purposes is passing mostly according to temporary market demands and a lot of enterprises in Herzegovina are involved in this activity (Gataric, Dj. et al., 1998).

The livelihoods of many rural people in Herzegovina largely depend on the wild collection of medicinal and aromatic plants. Over-harvesting has depleted wild populations of many medicinal plant species in areas where they were abundant only some 10 to 15 years ago. The war had a great impact on the wild-harvesting of medicinal and aromatic plants in Herzegovina. Consequences are an increase in deforestation and erosion, the further endangering of some plant species and a general loss of biodiversity. Some regions in Bosnia-Herzegovina are almost uninhabited today, and there are no or only little MAP collection activities in these regions. On those places there is a less risk of extinction of medicinal plants, but that has a negative impact on tradition of collecting of medicinal and aromatic plants in those areas. Yet, there is no survey to discover in which regions MAP wild-harvesting was most strongly affected by the consequences of the war. (Kathe, W. et al., 2003.) During the war, BiH's economy was totally destroyed, a large number of people were forced to flee from their homes, and the infrastructure of the country was almost fully laid to ruin.

In late 1990`s, the following MAP species were reported to be collected from the wild in larger quantities: Bearberry (*Arctostaphylos uva-ursi*), lime-tree (*Tilia spp.* (flowers)), nettle (*Urtica spp.* (herb)), St John's-wort (*Hypericum perforatum* (herb)), rose (*Rosa canina* (hips)), Yellow Gentian (*Gentiana lutea* (roots)), Marshmallow (*Althaea officinalis* (roots)), Hawthorn (*Crataegus monogyna*), Dandelion (*Taraxacum officinale*), Wormwood (*Artemisia absinthium*), Common Juniper (*Juniperus communis*), and Horse Chestnut (*Aesculus hippocastanum*) (SEED HQ 2000).

Local population, displaced persons and refugees are the inhabitants of the study area. Destruction and devastation of productive companies, general lack of financial resources and the political environment have caused a very low employment rate. The process of returning to normal is still slow. People

coming back to the villages and towns face a whole range of problems, but employment is the biggest one. A way to earn money is to deal with agriculture and collection of medicinal and aromatic plants and other NWF. At present, 5 large and a number of small companies are active in collecting MAPs in Herzegovina (Table 1.); they trade the collected material on both national and international markets. These companies mostly have their own contracted collectors, who regularly provide the raw material requested by the companies.

Table 1. Estimated number of collectors working for 5 of the most important companies trading MAPs in Herzegovina. (Kathe W et al, 2003.)

Name of company	Location	Region (collection)	Number of collectors
Ljekobilje	Trebinje	Herzegovina	500
Agroplod	Stolac	Herzegovina	180
ROING	Ljubuski	Herzegovina	30
Elmar	Trebinje	Herzegovina	100
Andjelic d.o.o.	Trebinje	Herzegovina	40

Hypericum perforatum

Plant description

Hypericum perforatum L. is a species which is plentiful of synonyms: *Hypericum officinarum* Crantz, *Hypericum vulgare* A. Froehl, *Hypericum officinale* L., *Hypericum veronense* Schrank, *Hypericum noeanum* Boiss, and within species are 3 subspecies: *Hypericum perforatum* L. var. *perforatum* L., *Hypericum perforatum* L. var. *angustifolium* DC., *Hypericum perforatum* L. var. *microphyllum* DC.

Folks names - bljuzga, bljuzgavac, bogorodicna ruka, vražiji beg, gorac, gorčan, gospin cvet, gospino zelje, greotaljka, žuta metlica, žuti kantarion, žućenica, zasekliva trava, zaseknica, zvekac, zvončac, ivanova trava, ivanjčica, izdatljivica, kantarion, krvavac, marina ručica, pljuskavica, smicaljka, tantur, trava od izdati, trava od poseka (dr Jovan Tucakov, 1971.)



The name *Hypericum* derives from the Greek name for the plant "hyperikon." The word roots are *hyper* (meaning over) and *eikon* (meaning image). The common name St. John's wort derives from Anglo-Saxon tradition, when the plant was considered to drive away evil spirits and ward off the devil's temptations.

The plant was named by early Christians in honor of John the Baptist. They believed that burning the plant in bonfires would drive away evil spirits, purify the air and promote healthy crop growth. The herb was traditionally gathered on mid-summer's eve, June 23. This date was later christianized as the eve of the feast day of St. John the Baptist. This folk custom gave the plant its popular name. The Anglo-Saxon word wort means medicinal herb (Hahn, G. 1992).

Hypericum perforatum is native to Europe, occurring throughout that continent, except the extreme North. The center of distribution is around the Mediterranean in southern Europe. *Hypericum* is a large genus with about 300 species. *Hypericum perforatum* occurs in a range of habitats and plant communities, including forest, woodland, rangeland, and prairie communities. In forested areas, *Hypericum perforatum* is commonly associated with disturbances such as roads, logging, grazing, and fire. The plant is not particular about soil conditions and grows in any average garden soil. However, it prefers good drainage, full sun, moist conditions, and a rather lean soil, which reduces competition from taller plants. Rocky or gravelly soil in calcareous grasslands is quite acceptable.

Hypericum perforatum L. is an herbaceous perennial belonging to the *Hypericaceae* family. It is upright plant, about 30 – 100 cm high. Stems in the upper part are branchy, the branches are alternating. The stems bear two characteristic longitudinal ridges that distinguish the plant from other species. It has simple, opposite leaves, which are narrow, elliptic and oval, sessile and well dotted with glandular dots with essential oil. Leaves are 1.5-3 cm long and 1.5 to 5 mm wide. Branched clusters of golden yellow flowers with tiny black spots are produced from June to August. Flowers are numerous (generally 25-100 per stem), about 2 cm in diameter. The fruit is a sticky, many-seeded, 3-celled capsule, 5 to 10 mm long. There are around 50 seeds per seed capsule. The average seed number per plant is 26,000 to 34,000. Seeds are about 1 mm long, peaked, black or dark brown. Seed is ripening in August and September. Weight of 1000 seeds is 0, 12 - 0, 14 g. (Tisdale, E. et al., 1959)
Common *Hypericum perforatum* reproduces by seed and short runners. Root system spreads horizontally and forms new buds.

Chemistry and Pharmacology

Hypericum perforatum herb contains 6.5–15% catechin-type tannins and condensed-type proanthocyanidins (catechin, epicatechin, leucocyanidin); 2–5% flavonoids, mostly hyperoside (0.5–2%), rutin (0.3–1.6%), quercitrin (0.3%), isoquercitrin (0.3%), quercetin, and kaempferol; biflavonoids (approximately 0.26% biapigenin); phloroglucinol derivatives (up to 4% hyperforin); phenolic acids (caffeic, chlorogenic, ferulic); 0.05–1.0% volatile oils, mainly higher *n*-alkanes; 0.05–0.15% naphthodianthrones (hypericin and pseudohypericin); sterols (*b*-sitosterol); vitamins C and A; xanthenes (up to 10 ppm); and choline (Bruneton, 1995; ESCOP, 1997; Leung and Foster, 1996; Newall et al., 1996; Wichtl and Bisset, 1994).

Medicinal benefits

Hypericum perforatum has been used medicinally for at least 2000 years. Initially it was promoted by physicians as a tranquilizer, and was thought to protect the user from ghosts or witches' spells. Many of *Hypericum perforatum* therapeutic applications (except antiviral use), including its uses as a vulnerary, diuretic, and treatment for neuralgic conditions, stem from traditional Greek medicine, originally documented by ancient Greek medical herbalists Hippocrates (ca. 460–377 B.C.E.), Theophrastus (ca. 372–287 B.C.E.), Dioscorides (first century C.E.), and Galen (ca. 130–200 C.E.) (Bombardelli and Morazzoni, 1995) The first century Greek physicians Galen and Dioscorides recommended it as a diuretic, wound healing herb, and a treatment for menstrual disorders. In the sixteenth century Paracelsus, who ushered in the era of mineral medicines, used St. John's wort externally for treating wounds and for allaying the pain of contusions. The aerial flowering parts of *Hypericum perforatum* have been used in traditional European medicine for centuries to treat neuralgia, anxiety, neurosis, and depression (Rasmussen, 1998). The traditional way to take *Hypericum perforatum* was as herbal tea, an aqueous extract whose single dose corresponded to 2–3 g of dried crude drug (Schulz et al., 1997).

Clinical studies have shown *Hypericum perforatum* is an effective antidepressant, sedative and anti-anxiety treatment. Oil-based *Hypericum* preparations have an anti-inflammatory effect. In Europe, *Hypericum perforatum* has been approved for use by physicians in treating anxiety, depression, and inflammation of the skin, blunt injuries, wounds and burns.

Over the centuries it has been used for many other purposes: to treat colds, chest congestion, menstrual cramps, headaches, diarrhea, fever, snake bit, asthma, tuberculosis, skin problems, wounds, bruises, burns, and infections. The plant is known to contain flavonoids, which may have value in treating immunity problems, naphthodianthrones, mainly hypericin and

pseudohypericin, which have antiviral and antidepressant qualities, essential oils and tannins.

Today *Hypericum perforatum* is widely used in Europe to treat mild to moderate depressive states (restlessness, anxiety and irritability), particularly in Germany, and its use is growing in North America. More recently it has been used in aromatherapy, and is still used in treating ulcers, bowel inflammation, wounds and bruises. It is currently under investigation for the treatment of HIV conditions (Porter B.2006).

Material and methods

Interviews

An ethnobotanical survey was conducted through Herzegovina (Trebinje, Ljubinje, Stolac, Mostar) during period August - September in 2006. Unstructured and semi structured questionnaire surveys were conducted with selected collectors and herbalists. (Appendices). The questionnaires were in their base the same for both groups of interviewees, slightly developed for each group. Collectors are the persons who are mostly just collecting medicinal plants in order to sell it, while herbalists are the persons who besides collecting are preparing remedies as well. Information was collected and recorded on the status/abundance of *Hypericum perforatum* in the study area, collecting techniques, the usable plant part, gathering, reasons for medicinal plants collecting, the preparation method for medicine, application and the dosage, and cultivation practices of *Hypericum perforatum*.

Interviews and discussions were conducted in Serbian language. The time given for interview with one collector was about 2 hours. In order to establish confidence interviews were conducted in a place where the collectors were most comfortable. Mostly the interviews were taken place on the field, in respondents working environment. In total 20 people were interviewed, 15 collectors and 5 herbalists. Two groups of people were interviewed in order to obtain information about the presence of *Hypericum perforatum* on the terrain, as well as to obtain information regarding usage and remedies preparation methods. Persons for interview were selected with the help of key informants. Key informants are persons who have been dealing with trade and purchase of medicinal plants for a long period and at the moment they are representatives of one of the enterprises which are buying medicinal plants from the collectors. In each town one key informant was present during the interview, but he didn't interfere into discussion. It was tried to cover the most important areas for medicinal plant collection. Five collectors were from Mostar, five from

Trebinje and five from Ljubinje and Stolac. Two herbalists were from Trebinje, two from Mostar and one from Ljubinje. Collectors were mostly asked questions regarding collections techniques and abundance of *Hypericum perforatum*, while herbalists were asked information regarding preparing of remedies and traditional knowledge related to *Hypericum perforatum*.

A preference ranking technique was used to rank medicinal plants according to their degree of scarcity. Preference ranking is one of the simplest analytical tools which involve asking people to think of 5 – 7 items and then arrange those items according to a given criterion (Martin 1995). In this case herbalists were asked to rank top 5 medicinal plants in Herzegovina; their preference was related to collection and marketability.

Seed material

In order to investigate possibilities for *ex situ* conservation of *Hypericum perforatum*, the seed was taken from the same locality two years successive, the seed from 2005 year and 2006 year. The location where seed had been taken was pasture near Stolac town. The seed from 2006 was received directly from collectors during visits for interview session and seed from 2005 was received from the company “Ljekobilje” in Trebinje. The seed from 2005 had been cleaned and stored on dark, dry place at room temperature in paper bags and was accompanied with information about locality where it had been taken. Seed from 2006 was directly taken and received from the collectors from the same location as seed stored in Ljekobilje. Longitude and latitude of location were recorded using GPS. Seed samples from 2006 had been cleaned manually, kept in paper bags and stored in a dry dark location at room temperature.

Germination tests

In the study one year old seed and new harvested seed obtained from *Hypericum perforatum* plants from the pasture near Stolac town were used. In order to test out potential for *ex situ* conservation, germination tests were conducted during the research. The germination tests were made under controlled conditions to find out how many seeds will germinate and produce normal seedlings which could develop into normal reproductively mature plants. Seeds from 2005 and 2006 years were used.

The material was divided into three groups:

1. fresh seed (from 2006) and seed stored in room temperature (from 2005)
2. seed dried to 5% moisture content (from 2005 and 2006)
3. seed stored at -18C for 60 days (from 2005 and 2006)

All germination tests were conducted according to the following protocol: seeds were placed in Petri dishes on double filter paper wetted with distilled water. The Petri-dishes were maintained in germinators under alternate temperatures of 20°/30°C (8 hours temperature was 30°C and 16 hours temperature was 20°C) and continuous white fluorescent light or darkness. Two Petri dishes (2x100 seeds) were used in each treatment. The germinated seed were counted every 7 days. Protrusion of the radicle was the criterion used for a germinated seed. One germination test took 21 days, which means 3 countings were done during one germination test.

The germination of the first group gave us the answer about germination rate of seeds from 2005 and 2006. Different conditions during germination tests was tried to clarify the best conditions for seed germination. Two different light regimes were tested on the first germination group of material, as well as cold pretreatment. 200 (2x100) seeds were placed in germinator under constant light conditions and 200(2x100) seeds were germinated without any light. In the dark treatment Petri dishes were wrapped in aluminium foil. Also, intention was to check out how chilling period can increase germination rate. 200(2x100) seeds which passed chilling period for 5 days in fridge were placed under light conditions in germinator, and 200(2x100) seeds which passed chilling period for 5 days in fridge were placed in germinator without light. In total 16 Petri dishes of the first group were germinated under alternate temperatures of 20°/30°C.

The second group of material was germinated after seed drying until moisture content approximately 5%. The silica gel is used for seed drying. Equal amount of silica gel and seed were placed for a 7 days in air tight jars. In the second germinated group 200 seed (2x100) for each year were germinated with constant presence of light and under alternate temperatures of 20°/30°C. In total 4 Petri dishes were employed in the second germinated group. The third group of seed material was germinated after cold storage. Dried seed was stored on -18C for 60 days. The germination was done under light condition. 200 seeds (2x100) for each year were germinated under alternate temperatures of 20°/30°C. In total 4 Petri dishes were employed for germination of the third group of material.

Data analysis

Data obtained from the interviews was analyzed qualitatively. Responses from the open ended questions were grouped into classes that expressed similar ideas, while percentages based on responses were calculated from the close ended questions.

Seed germination data were analyzed by performing analyses of variance with a one-way ANOVA. Differences among different treatments were considered significant only for $P < 0,05$. Chi - square tests were employed as *ad hoc* tests in order to prove that germination rate of *Hypericum perforatum* seed is the biggest under light conditions. In case of this test each seed was treated individually. Mini tab statistic program has used for data analyses.

Results and discussion

Information from interviews

General information

During the study 10 male and 5 female collectors were interview and 2 female herbalists and 3 male herbalists. Mostly they had medium level of education, 70% of the collectors have finished secondary school, till 30% of them had finished only primary school or they are illiterate. 4 interviewed herbalists had finished secondary school. 40% of respondents stated that they have been dealing with medicinal plants for more than 10 years, 30 % of respondents have been in that sector between 5 and 10 years and 30% less than 5 years. Tradition of medicinal plants collection is still rich in Herzegovina, mostly the collectors have been involved in medicinal plant sector more than 10 years, but also it was noticed that after the war the number of collectors has been enlarged. All interviewed collectors and herbalists declared that they are collecting *Hypericum perforatum*. According to herbalists preference ranking made in terms of collection and trade, *Hypericum perforatum* is placed among the top 5 medicinal plants.

Abundance of *Hypericum perforatum* in Herzegovina

According to interviewed collectors *Hypericum perforatum* is still not threaten in Herzegovina region, but sustainable collection have to be controlled. 60% of interviewed collectors responded that still there is no need to be worried about accessibility of *Hypericum perforatum*, but 40% of interviewed collectors told that the situation has changed during last few years, mainly because of climate change. 46.7% of interviewed collectors declared that richness of *Hypericum perforatum* in Herzegovina varies from year to year, while 26.7% of interviewed persons think that there is decrease and equal number of interviewed persons thinks there are no changes regarding richness of this plant. (Fig. 1) Accessibility of *Hypericum perforatum* can vary from year to year depending on climate conditions. Too much precipitation can have negative influence on abundance of *Hypericum perforatum*. Changes regarding material quality are not noticed. Quality of collected material is constant, the buyers are satisfied. All

interview herbalists responded that they did not notice any changes in the quality of raw material. During the collecting season one collector is able to collect between 0, 5 and 1, 5 tone of material, but that mainly depend on buyers demand and also, as is mentioned above it can vary from year to year depending on the situation on the field. 70% of collectors are following rules for sustainable harvesting and during collection they are leaving some plants for regeneration. Educated collectors are following rules of sustainable collection and they are leaving approximately 30% of flowers on the field in order to let the plants regenerate. Those collectors are getting instruction from buyers how to collect or they attended programme for education of collectors (73%). In the last few years the buyers started with education of their collectors. Those programs are mainly funded by international organizations such as GTZ (German Agency for Technical Cooperation), USAID LAMP (United States Agency for International Development), SEED (Southeast Europe Enterprise Development), and IMO (Institute for Marketecology) etc.

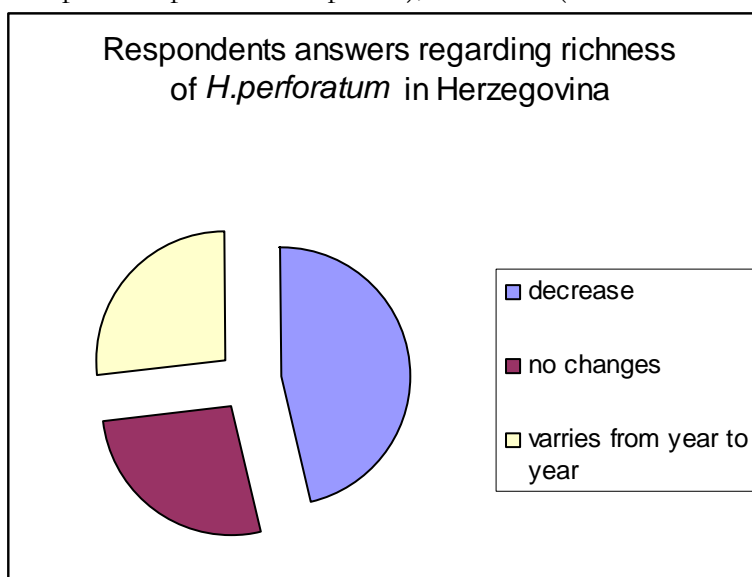


Fig.1. Changes in richness of *Hypericum perforatum* in Herzegovina according to respondents

Collecting techniques

Organization of collecting

Collection of *Hypericum perforatum* is seasonal work; only during the vegetation period collectors harvest the herbs. In Herzegovina region there are two harvest periods of *Hypericum perforatum* per year. The first yield is taken at the end of June – beginning of July, and the second in August. But according to collectors, it is noted that sometimes, depending on year and climate conditions it is possible to have longer vegetation period and 3 harvests per year.

Gathering and usable plant part

According to the respondents, the recommended harvest stage is at the stage of 50% flower buds and 50% open flowers, which is generally around the last week of June or the first week of July in Herzegovina. Plant parts harvested by the collectors and herbalists are the flower (*Hiperici flos*) and upper green parts of the plant with flowers (*Hiperici herba*). Collection is done in the morning, by cutting with sickle or scissors and put in the baskets to avoid the damaging of bright dots on the leaves where the essential oil is placed. Collection, very often, is combined with other activities, such as herding or land cultivation. Apart from simple air-drying, there is no further processing carried out by the collectors. The herbs are stored at home until dry.

Reasons for medicinal plants collecting

The main reason for collecting of medicinal plants according to collectors is to gain income. Collectors sell the collected raw material to processors, traders, herbalists and to anybody who is ready to pay or offer the best price. Collectors either take the herbs to buyers or buyers come to the collectors, depending on the distance to the processing unit and on the quantities collected. Approximately 65% of interviewed collectors said that there is a number of different buyers they could sell collected herbs to, and approximately 35% have only one or two buyers, either because of lack of market information or because of established relationship with a buyer. Approximately 70% of interviewed collectors said that they are organized in terms of collection and material selling, while 30% are not. Collectors organized by buyers usually receive training, seem to respect more the sustainable collection of herbs and produce a better quality. 11 interviewed collectors (69%) are collecting medicinal plants only for selling and for 60% of them, money obtained on that way are their only source of income. The rest of interviewed herbalists are collecting medicinal herbs for their own usage and for selling as well. For 80% of the herbalists, preparing and selling of remedies are the only source of income. Only 1 herbalist declared that he has additional source of income. They stressed that collecting of medicinal plants is economically a very perspective work, even very hard labor. The price which they can get from the buyers for 1kg of *Hypericum perforatum* vary from year to year and for 2006 it was 0.80 euros per kilogram.

Interviewed herbalists are doing collection by themselves and also they are buying material from the collectors. Air drying, cleaning and cutting of raw material and preparing remedies are processes which are carried out by herbalists.

Preparation method of remedies and application and dosage

Traditional knowledge

Data obtained from interviews indicated that there is considerable knowledge and use of *Hypericum perforatum* remedies in Herzegovina. Most of the people interviewed had themselves taken herbal remedies and their parents and grandparents knew how to prepare them. Unfortunately, the younger generation tended not to use medicinal plants and preferred conventional forms of medicine instead.

Only 1 herbalist declared that he obtained knowledge about MAP from ancestors i.e. he is keeping knowledge which is accumulated from generation to generation. The rest of them are mainly self-educated about MAP. This implicate that erosion of traditional knowledge is obvious. Collectors of medicinal plants in Herzegovina region are also mostly self-educated (47%), 27% of them obtained knowledge from ancestors and 27% of collectors obtained knowledge through education.

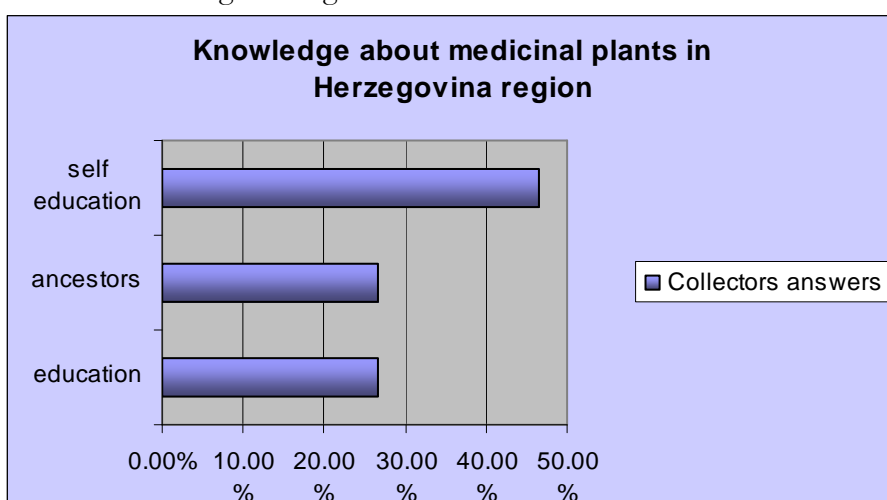


Fig.2. Sources of collector's knowledge obtained regarding medicinal plants in Herzegovina region

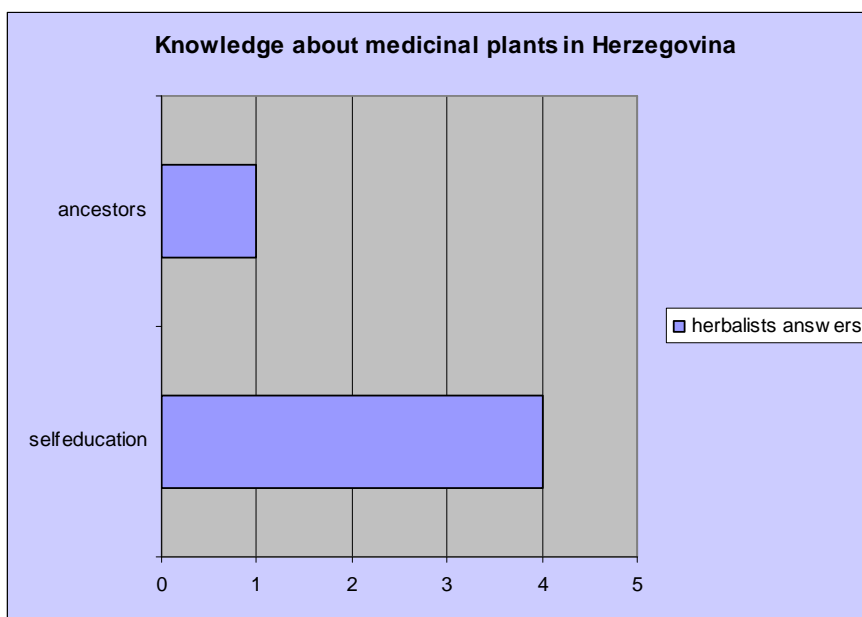


Fig. 3. Sources of herbalist's knowledge obtained regarding medicinal plants in Herzegovina region

All collectors emphasized that they are harvesting the flower of *Hypericum perforatum* (Hiperici flos) and upper green parts of the plant with flower (Hiperici herba). Plant should be dried in cold, dark place with air circulation. *Hypericum perforatum* tee and *Hypericum perforatum* oil are the remedies which they are preparing and which are the most useful. Also, some of them (30% of interviewed) are preparing *Hypericum perforatum* wine, tincture and balm. Thanks to the herbalists some methods for preparing the most used remedies from *Hypericum perforatum* are recorded. (Appendix III)

Cultivation practices in Herzegovina

Interviewed collectors emphasized that they are considering cultivation of *Hypericum perforatum*. Since demand for raw material of *Hypericum perforatum* is increasing, they think cultivation is a good option from the aspect of providing raw material as well as from the aspect of conservation.

67% of interviewed collectors stated that they are planning cultivation of *Hypericum perforatum*. But the main obstacle for their plans is lack of appropriate soil. The soil in Herzegovina is mostly rocky and shallow, so there is deficiency of good soil even for agriculture and food. Cultivation of MAPs in BiH is still a small-scale business; an area of 200-300 hectares of agricultural land is believed to be used to grow MAPs in BiH, mainly in Republika Srpska (SEED HQ 2000).

Cultivation of *Hypericum perforatum* in Herzegovina region has not been done up till now. Some experimental attempts have been done in the region of central Bosnia and results were promising. Company Ljekobilje from Trebinje run one experiment regarding cultivation of *Hypericum perforatum* in Bosnia and Herzegovina. Cultivation is commercially attractive to companies because they have greater control over quality and supply. Experimental fields are located in the surroundings of Sokolac. According to their reports, cultivation attempts were very successful. (key inf.,pers.comm.) But the quality of raw material from that location is a question for the further investigation. It is well known that characteristic climate conditions in Herzegovina are the reason why medicinal plants from that region are of high quality. Geographical location of cultivation is important. The most vigorous *Hypericum perforatum* plant colonies in the wild only grow naturally from the coast inland to about 200 miles from the sea coast, and not in the interior of the continents (Huffman, M., 2004)

Conservation measures

GPS information

Seed from 2005 and 2006 is received from the same location, which was recorded using GPS. Latitude is 43°04'59.7", longitude 17°56'4.5" and altitude 87m.

Germination tests

Generally germination rate of fresh seed of *Hypericum perforatum* is very low due to seed dormancy. The dormancy is caused by chemical inhibitor exudates from the capsule and absence of light has a negative effect on germination. (Perez-Garcia, F. et al.2005) During the germination experiments *Hypericum perforatum* seed was germinated under different conditions in order to break seed dormancy, but new harvested seed, seed from 2006 showed very low germination rate no matter which treatment was employed. The highest germination rate was noticed in seed germinated under light condition (8 %) and lowest was for seed germinated in dark (4 %). That was the reason why the germinations of the second and third group of material were made only in light condition. During the germination of the second group of material (after seed drying) and the third group of material (after cold storage) of the seeds from 2006 increasing of germination rates was noticed (14 % after seed drying and 20, 5 % after cold storage).

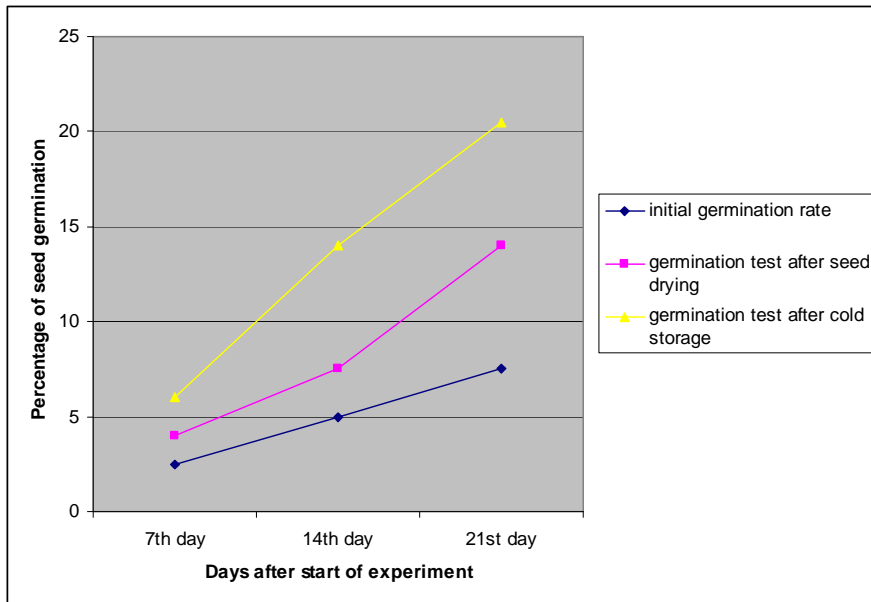


Fig.4. Germination percentages for *Hypericum perforatum* seed harvested in 2006

The situation with the seeds from 2005 year was different. Germination rate after the first germination test was the highest for seed germinated under light condition (87.5 %) and the lowest was for seed germinated without light (68 %). Seed drying and cold storage in this case have decreased germination rates. Germination rate after seed drying was 75.5 % and after cold storage were 70.5 %.

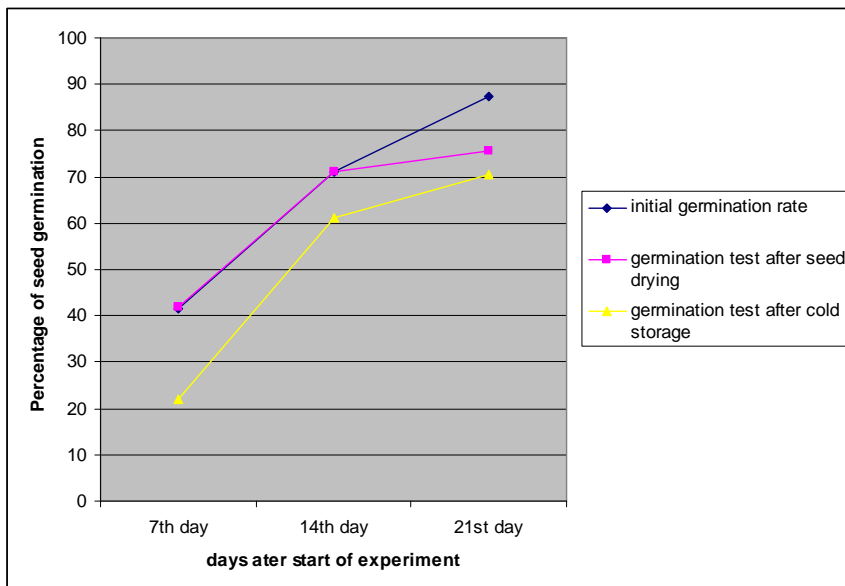


Fig.5. Germination percentages for *Hypericum perforatum* seed harvested in 2005

Table3. Germination rates of *Hypericum perforatum* seed

Year of harvesting	Germination of the first group of material	Germination of the second group of material (after seed drying)	Germination of the third group of material (after cold storage)
2005	87.5 %	75.5 %	70.5 %
2006	8 %	14 %	20.5 %

Considering the results mentioned above, it can be noticed that germination rate of new harvested *Hypericum perforatum* seed is increasing with time. These results are in accordance with literature statement, it is stressed that the best way to break dormancy in *Hypericum perforatum* seed to let time pass. Seeds of *Hypericum perforatum* required 4-6 months following harvesting before they became germinable and 12 months for them to reach maximum germination capacity. (Campbell M.H., 1985). Theory of the necessity of an after ripening period for germination could be explained by drying and wearing away of the capsule exudates over time.

Seed collected in 2005 shows quite satisfied germination rate from the aspect of ex situ conservation. Germination experiments show that light is an important factor for seed germination and light has a positive effect on seed germination.

All results analyzed with one way ANOVA test shows significant difference in germination rate of *Hypericum perforatum* seed germinated under different conditions. For the seed from 2005 P value is 0.04, and for the seeds from 2006 P value is 0, 33. In both cases P value is <0.05 which means that differences in germination rate under different conditions are significant. (Table 4)

Table 4 ANOVA test/ influence of different treatments on germination rate for seeds from 2005 and 2006

year/seed	F	P
2005	7.59	0.04
2006	8.44	0.033

Chi square test shows that the best germination rate is achieved with seed germinated under light conditions. Chi square test the results (Chi-Square = 19.680, DF = 1, P-Value = 0.000) indicate very significant difference in number of germinated seeds under light and dark conditions. Also, comparing number of germinated seeds in darkness and after chilling period, Chi square test shows that chilling period also improves germination rate significantly. (Chi-Square = 4.226, DF = 1, P-Value = 0.040) According to the results, light was the most significant factor affecting germination. (Table 5)

Table 5 Chi-Square test/influence of different treatments on germination rate of seed from 2005

Treatment	Chi-Square	DF	P
light vs. dark	19.68	1	0.00
dark vs. chilling	4.226	1	0.04

ANOVA test shows that there is significant difference between germination rate of *Hypericum perforatum* seed from 2005 in different conditions after seed drying and cold storage. (P=0.013) Since P- value is 0.013, it can be concluded that differences in germination rate of *Hypericum perforatum* seed after seed drying and seed storage are significant. (Table 6)

Table 6 ANOVA test/ influence of seed drying and cold storage on germination rate of seed from 2005

treatment	F	P
seed drying vs. cold storage	26.17	0.013

Seed drying and seed storage affected seed germination rate of one year old seed. Decreasing in germination rate is significant after seed drying and seed storage. But, still percentage of germinated seeds after drying (75.5%) and cold storage (70.5%) was acceptable for *ex situ* conservation.

Conclusion

The quality of curing herbs from the region of Herzegovina is confirmed by its presence on the European and American market for over hundred years. *Hypericum perforatum* has been used in folk medicine in Herzegovina since the ancient time, especially against slashes, burns, hemorrhoids, for wounds scaring and as antiseptic. Today, in modern medicine *Hypericum perforatum* is best known and most widely used for the treatment of depression. According to interviews carried out, St John's wort is currently not threatened, although it is massharvested in Herzegovina region. A growth in interest in a particular medicinal plant accompanied by media interest, such as has occurred in the case of St John's wort can result in a major and sudden rise in demand; sufficient quantities of good quality supplies may not be available. Other factors which can have influence on *Hypericum perforatum* availability are environmental factors, since variations in abundance are noticed from year to year. Traditional knowledge regarding *Hypericum perforatum* in Herzegovina is considerable, but erosion is noticeable since the number of herbalists which knowledge is accumulated from generation to generation is decreasing.

Based on experiences from this study and taking into consideration seed behavior, *ex situ* conservation is possible method for protection of this plant. But also taking into consideration present situation in medicinal sector in Bosnia and Herzegovina some practical advices can be recommended for conservation, such as:

- Cultivation as the best way to provide plant material needed for medicine, although this requires further research.
- further education of collectors and raising public awareness about sustainable harvesting methods
- The Government should regulate the collection of medicinal plants. Ideal system would be permit system for commercial collection of any plant and determination of maximum quantities of annual wild collection.

Although the aim of this project was to conserve *Hypericum perforatum*, the experiences gained can easily be adapted to conservations of any medicinal plant.

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References

- Bombardelli, E. and P. Morazzoni. 1995. *Hypericum perforatum*. *Fitoterapia*. 66(1):43–68.
- Bruneton, J. 1995. *Pharmacognosy, Phytochemistry, Medicinal Plants*. Paris: Lavoisier Publishing.
- Campbell, M.H.1984., Germination, emergence and seedling growth of *Hypericum perforatum* L., *Weed Research* 1985., Volume 25. 259-266
- Dragić, B. 1998, Lekovito bilje u preventivi i lečenju, Izdavacko preduzeće "Dobrotvor"- Beograd
- Donnelly R, Helberg U. Balkans Herbal Development Initiative—Phase 1 – Draft Report-Economic&Activity Mapping in Bosnia and Herzegovina. Southeastern Europe Enterprise Development and The Corporate Citizenship Facility; 2002
- Mabey R. et al., 1988, The New Age Herbalist, London,
- ESCOP. 1997. "Hyperici Herba." *Monographs on the Medicinal Uses of Plant Drugs*. Exeter, U.K.: European Scientific Cooperative on Phytotherapy
- Gataric, Dj., Radanovic, D., Cvikic,Z. Durman, P. Experience in cultivation and production economy of medicinal and aromatic plants in Banjaluka region, Republic of Srpska, Lek.Sirov Vol. XLVII No.18 PP 33-40 Belgrade 1988.
- Group of authors, Medicinal Plants, Manual for Collectors Based on Principles of Organic Production, Bosnia and Herzegovina, 2003
- Hamilton, A., 2003. Medicinal plants and conservation: issues and approaches, International Plants Conservation Unit, WWF-UK
- Hahn, G. *Hypericum perforatum* (St. John's Wort) - A Medicinal Herbs Used in Antiquity and Still of Interest Today. *The Journal of Naturopathic Medicine*, 1992, 3(1):94-96
- Huffman, M., 2004. Challenges and Opportunities for Herb Farmers: The Case of St. John's Wort, *HerbalGram* 61:38-43
- Kala, C.P.2000. Status and conservation of rare and endangered medicinal plants in the Indian Trans-Himalaya, *Biological Conservation*, Volume 93, Number 3, pp. 371-379(9)
- Kathe W., Honnef S. & Heym A., Medicinal and Aromatic Plants in Albania, Bosnia-Herzegovina, Bulgaria, Croatia and Romania, WWF Deutschland / TRAFFIC Europe Germany, 2003
- Kosović, N,Dunjić,Lj. Some indicators of the status and possibilities to improve the collection, purchase and processing of medicinal and aromatic plants in the region of Herzegovina- Neretva canton in the Federation of Bosnia and Herzegovina,
- Maggioni, L.,1999, Report of a Network Coordinating Group on Minor Crops, IPGRI

- Martin, G.J. 1995. *Ethnobotany: A Conservation Manual*.- Chapman & Hall, London
- Porter B., *St. John's Wort Production in Saskatchewan*, Saskatchewan Agriculture and Food, 2006
- Rasmussen, P. 1998. *St. John's wort—A review of its use in depression. Australian J Med Herbalism* 10(1):8–13.
- Regionalni centar za okoliš za Centralnu i Istočnu Evropu (REC); *Izvyještaj za Bosnu i Hercegovinu u okviru strateške okolišne analize za Albaniju, Bosnu i Hercegovinu, Kosovo i Makedoniju*; REC office for Bosnia and Herzegovina, Sarajevo and Banjaluka, Bosnia and Herzegovina
- Rhonda Janke, 2004. *Farming a Few Acres of Herbs: St. John's Wort*, Kansas State University
- Sheldon, J. W., M. J. Balick, and S. A. Laird. 1997. Medicinal plants: can utilization and conservation coexist? *Advances in Economic Botany* 12:1–104
- Schulz, V., Hbner, W.D., Ploch, M., 1977. Clinical trials with phytopharmacologic-al agents. *Phytomed* 4(4):379–387
- SEED HQ (2000). *The Balkan Herb Sector.- A descriptive review*. 15 pp., Sarajevo.
- Tisdale, E. W.; Hironaka, M.; Pringle, W. L. 1959. Observations on the autecology of *Hypericum perforatum*. *Ecology*. 40(1): 54-62
- Tucakov, J. 1971, *Lecenje biljem*, Kultura, Beograd
- Wichtl, M. and N.G. Bisset (eds.). 1994. *Herbal Drugs and Phytopharmaceuticals*. Stuttgart: Medpharm Scientific Publishers.
- WHO, IUCN & WWF. *Guidelines on the Conservation of Medicinal Plants*, 1993. The International Union for Conservation of Nature and Natural Resources (IUCN), Gland, Switzerland,
- WWF Deutschland/TRAFFIC Europe-Germany, 2000, "Medizin und Artenschutz", Hannover

Appendix 1 Questionnaire for collectors

Location:	Code:
Circle one: Female	Male
Year of birth:	
Level of education:	
Occupation:	

1. For how long you have been collecting medicinal plants?

- > 10 years
- 5 – 10 years
- < 5 years

2. Why are you collecting medicinal plants?

- for own purposes
- for selling
- other_____

3. Is the money obtained from selling herbs important for your livelihood?
 70 – 100% of income, 40 – 70% of income, less than 40%

4. How much (or what proportion) is collected for the different uses?

5. Is it collecting of MAP economically perspective job?

- yes
- no

6. How did you gain knowledge about medicinal plants?

- from family member/ relatives
- from book/magazine (selfeducation)
- Education

7. Are you collecting *Hypericum perforatum*?

- yes,
- no (If yes continue with questions)

8. Which part of the plant are you collecting?

- leaves (herba)
- flowers (flos)
- roots
- seeds
- all

9. On what stage of plant development are you collecting?
- before flowering
 - during flowering
 - after flowering
10. On what time of the day are you collecting?
- early in the morning
 - midday
 - afternoon
 - anytime
11. How much of *H. perforatum* are you collecting yearly / per season?
- < 0.5 t
 - 0.5 – 1 t
 - > 1t
12. During collection do you leave some species to regenerate or you collect everything you find?
13. Do you get instructions from buyers how to collect?
14. Is it collection of medicinal plant well organized in Herzegovina?
- yes no
15. Have you ever attend programme for education of collectors?
- yes, no
- If yes, who organized it?
16. Are you selling collected material only to one buyer or to several buyers?
- one buyer
 several buyers
17. How do you process raw material? clean, dry, cut, other.
- clean
 - dry
 - cut
 - other
18. Did you notice any changes in richness of *H. perforatum*?
- yes-decrease
 - yes-increase
 - yearly variations

- no change
19. Do you consider that *H. perforatum* is rarer now? yes, no
20. If yes, what is the reason?
- overharvesting
 - grazing
 - environmental changes
 - other_____
21. Did you notice any changes in collected material of *H. perforatum*?
 yes, no (If yes explain which one_____)
22. Do you have in plan to cultivate *H. perforatum*?

Appendix 2 Questionnaire for herbalists

Location: Code: Circle one: Female Male Year of birth: Level of education: Occupation:
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1. For how long you have been collecting medicinal plants?
 - > 10 years
 - 5 – 10 years
 - < 5 years

2. Why are you collecting medicinal plants?
 - for own purposes
 - for selling
 - other _____

3. Is the money obtained from selling herbs and remedies important for your livelihood?
 - 70 – 100% of income, 40 – 70% of income, less than 40%

4. Is it dealing with MAP economically perspective job?
 - yes no

5. How did you gain knowledge about medicinal plants?
 - from family member/ relatives
 - From book/magazine (selfeducation)
 - Education

6. Are you collecting *Hypericum perforatum*?
 - yes, no (If yes continue with questions)

7. Which part of the plant are you collecting?
 - leaves (herba)
 - flowers (flos)
 - roots
 - seeds
 - all

8. On what stage of plant development are you collecting?
- before flowering
 - during flowering
 - after flowering
9. On what time of the day are you collecting?
- early in the morning
 - midday
 - afternoon
 - anytime
10. During collection do you leave some species to regenerate or you collect everything you find?
11. How do you process raw material? clean, dry, cut, other.
- clean
 - dry
 - cut
 - other
12. Did you notice any changes in richness of *H. perforatum*?
- yes-decrease
 - yes-increase
 - yearly variations
 - no change
13. Do you consider that *H. perforatum* is rarer now? yes, no
14. If yes, what is the reason?
- overharvesting
 - grazing
 - environmental changes
 - other_____
15. Did you notice any changes in collected material of *H. perforatum*?
- yes, no (If yes explain which one_____)
16. Do you have in plan to cultivate *H. perforatum*?
17. For what purposes are you using *H. perforatum*? Against which diseases?
18. How are you preparing remedies?
19. Which are the top 5 medicinal plants in Herzegovina?

Appendix 3 Methods for preparing the most used remedies from *Hypericum perforatum* in Herzegovina

Hypericum perforatum tea

According to the herbalists, *Hypericum perforatum* tea is used against ulcer diseases, asthma and bronchitis. *Hypericum herba* alone or in combination with other drugs can be used as an additional medicine for curing of liver and kidney diseases, for appetite improvement and expectoration alleviation. The herbalists are preparing tea on the following way: In 2 dl of water 2 spoons of *Hypericum perforatum* flower have to be putted, then covered, stirred, filtrated, added honey and drink three times per day and directly before sleeping. Treatment is lasting 4 - 5 weeks. Improvement is expecting after 14 days. With combination with honey all good *Hypericum perforatum* ingredients are emphasized.

Very popular is Herzegovina native tea which is the mixture of the most quality medical herbs (Yarrow, St. John's Wort, Sage, Germander and Thyme).

Hypericum perforatum oil

Hypericum perforatum oil is preparing from virgin olive oil and fresh flower according to the herbalists. Alternatively, sunflower oil can be used for preparing *Hypericum perforatum* oil. For a very long time it is used for curing wounds and burns, especially for curing the old, purulent wounds raised from hard burns and other injuries which are hard for curing. The herbalists state that *Hypericum perforatum* oil is used for bruises, is anti-inflammatory, and is often used by herbalists to help speed healing of wounds and sores. *Hypericum perforatum* oil helps to reduce inflammation. Externally it is applied to bruises, sprains, burns, skin irritations, or any laceration accompanied by severed nerve tissue. According to the herbalists it is simple to make *Hypericum perforatum* oil. The herb should be harvested just as the plant comes into bloom. About one cupful of the fresh flowers has to be taken, a sufficient quantity of olive oil added just to cover the flowers. The fresh herb should be finely cut or crushed, covered with the oil, then placed in the sun or warm area for 6 weeks until the herb imparts its qualities to the oil. Once the allotted time has passed, the herb should be pressed, strained from the oil, and then stored in a dark, closed container in a cool place. The yellow flowers will turn the oil a deep blood-red color. The herbalist declared that medicinal oil will maintain its potency for two years or more. This oil makes a good rub for painful joints, varicose veins,

muscle strain, arthritis, and rheumatism. Used in a compress it can help to heal wounds and inflammation, and relieve the pain of deep bruising.

Hypericum perforatum wine

In the first century, the Roman naturalist Pliny, prescribed *Hypericum perforatum* steeped in wine for the bites of poisons snakes. In the 17 century, Nicholas Culpeper called *Hypericum perforatum* " A singular wound herb, boiled in wine and drank, it health inward hurts and bruises, it opens obstructions, dissolves swellings and closes up the lips of wounds" *Hypericum perforatum* wine in Herzegovina is used for digestion improving and externally for bruises and ulcers.

For internal usage, 40 g of the fresh flowers have to be covered with 1l of white wine, and then left for 30 days with occasionally shaking. After the allotted time has passed the wine should be filtrated and sweeten with honey. Recommended dosage is one little glass before meal.

For external usage against bruises and ulcers, 250 g of the fresh flowers have to be mixed with 1/2l of olive oil and 250 ml of white wine and stored 4 days. After that period, mixture has to be boiled 2-3 hours. After boiling, solution has to be filtrated and stored in a bottle.