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REVIEW PAPER

MUTUALISTIC AND ENDOPHYTIC MICROORGANISMS OF *ARTEMISIA ANNUA*: DESCRIPTION, ROLE AND USE

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Abstract: *Artemisia annua* is an important medical plant that produces artemisinin used for its antimalarial, antibacterial and antifungal effects in modern medicine. The high demand and low artemisinin content in plants (0.01-2 %) has led to studies about alternative methods to increase yield. Biofertilizers (beneficial microbes and/or biological products that colonize roots, improve plant nutrition and growth) have been reported affecting secondary metabolism and the production of active ingredients of herbs. The purpose of this paper is to draw attention to the current status of the research on mutualistic and endophytic microorganism of *A. annua* that have the potential to increase the quality and quantity of the crude drugs, derived from the herb. Scientific papers in this field focus on the effects on inoculation with different microorganisms (arbuscular micorrhizal fungi, endophytic bacteria and fungi) and the isolation of endophytes from *A. annua*. Bioinoculants can affect biomass, artemisinin and essential oil concentration, disease resistance, nutrient status, phosphatase activity, foliar glandular trichome density, leaf chlorophyll content, guaiacol peroxidase enzyme concentration, stomatal conductance and transpiration rate, and plant growth parameters (total weight, leaf yield, height, seed yield). The endophytes isolated from the plant are potential artemisinin content and plant stress resistance enhancers.

Keywords: *Artemisia annua*, artemisinin, mutualism, arbuscular mycorrhizal fungi, endophytic bacteria, endophytic fungi, plant stress resistance.

1. Introduction

The *Artemisia annua*, also known as sweet wormwood (Chinese: qing hao), is a plant belonging to the Asteraceae family native to temperate Asia, especially in China where it has been used for several centuries as therapy for cerebral fever (Chaudhary et al., 2008; Fairhust and Wellems, 2015). It was naturalized in many countries including Argentina, Australia, Bulgaria, France, Hungary, Italy, Romania, Spain and the USA (Das, 2012; Bilia et al., 2014).

A. annua has become the focus of hundreds of papers since the early 1970s, after the discovery of its active component artemisinin (Wang et al., 2011; Calderón et al., 2013; Naeem et al., 2014). This complex structure, from the family of sesquiterpene trioxane lactone (Martínez et al., 2014) provides a wide spectrum of action against many diseases. Although artemisinin is no longer used as a drug itself, its derivates are highly effective anti-malarials, which are produced by chemical alteration of artemisinin (Hommel, 2008). According to WHO (2017), an estimated 216 million cases of malaria occurred worldwide with most cases being in the African region (90%). Parasite, predominantly *Plasmodium falciparum* resistance to artemisinin has been reported in five countries of the Greater Mekong subregion, therefore exploring all possible modes of action of artemisinin in order to develop new generation antimalarial drugs has become of great importance (Fairhust and Dondorp, 2016).

Artemisinins (artemisinin and its chemical derivates) have cytotoxic and inhibitory effect on various cancers, inflammatory diseases, viral (e.g. *Human cytomegalovirus*), protozoal (e.g. *Toxoplasma gondii*), helminthic (*Schistosom* sp., *Fasciola hepatica*) and fungal (e.g. *Cryptococcus neoformans*) infections (Ho et al., 2013). Hence, the interest in the isolation of artemisinin and the production of *A. annua* has increased worldwide (Sadiq et al., 2014). Unfortunately, its demand to production ratio is low. Artemisinin extraction from *A. annua* is highly influenced by the low artemisinin percentage in plants, which usually ranges from 0.01 to 2% dry weight (Liu et al., 2006; Keshavarzi et al., 2012), and the herb's dependence of temperature, humidity and soil types (White, 2008). Chemical synthesis is uneconomical, non-cost-effective and low yielding (Pandey and Pandey-Rai, 2015), however microbial genetic engineering is a potential alternative (Hommel, 2008). Progress has been made towards breaking the cost/yield barrier while it is yet unproven as commercially viable syntheses of artemisinin (Kung et al., 2018). Currently biosynthetic processes have proven to be the most efficient synthetic methods to produce artemisinin (Tang et al., 2018). Therefore, it is likely that *A. annua* will continue to be the main source of artemisinin (Hommel, 2008).

Various efforts have been carried out to increase the antimalarial compounds. These attempts can be divided into two categories that focus on improving the efficiency of artemisinin extraction (Briars and Paniwnyk, 2014) and the increment of antimalarial compounds in *A. annua* (Namuli et al., 2018). In order to increase the artemisinin concentration fertilizers (chemical, biological, organic and vermicompost), plant growth regulators (hormones), variation of growth conditions (light, water, macro and micronutrients) and the use of high-yielding clones or strains have been tried (Namuli et al., 2018). One of the issues encountered in medicinal plants cultivation is the unstable quality of the product. Biofertilizers, however, have been reported affecting secondary metabolism and the production of active ingredients of herbs (Zeng et al., 2013).

Microbial inoculants or biofertilizers are beneficial microbes and/or biological products that colonize roots, improve plant nutrition, growth, development and resistance to abiotic stresses (Monfil and Casas-Flores, 2014), thus they play an important role in sustaining productivity (Malhi et al., 2013). Bioinoculants can fix atmospheric nitrogen or enhance the solubility of soil nutrients which leads to their potential to increase the yield of crops (Namuli et al., 2018). However, their efficiency varies with nutrient type, source, soil type, climatic conditions, and species compatibility in the respective environment (Malhi et al., 2013; Berruti et al., 2016).

The aim of this article is to discuss the current status of the research on relationship between mutualistic or endophytic microorganisms and *A. annua* by reviewing a total of 37 papers in order to draw attention to, and arouse more interest in this research field. The publications were collected from two main research directions: *A. annua* inoculation with different microorganisms (arbuscular micorrhizal fungi, endophytic bacteria and fungi) and their effect on plant grow and development; isolated endophytic microorganism from *A. annua* and their relationship with their host plant. Plants grown in poor-quality soil, under stressful conditions (arid conditions, low nutrient soil) or in intensely farmed land where topsoil is tripped-away can benefit from these organisms. They can positively influence mineral uptake (N, P, K, Ca, Mg, Zn, Cu, S), chlorophyll content in leaves, essential oil concentration, optimal growth of herbs, increase salt toxicity and water deficiency resistance in hosts and reduce susceptibility to root rot pathogens. The Global North becomes more suitable for plant growth due to global warming, while there is a decrease in agriculturally suitable land in the Global South and the Mediterranean area. Limits of land extensions and the increasing demand for agricultural products and medical plants raised attention to the usage of mutualistic and endophytic microorganisms (Zabel et al, 2017). Therefore, they could help meet the demand for artemisinin by increasing the artemisinin content, survival rate of herbs and enabling the production of plants in less favorable conditions, where malaria is present.

2. The effect of arbuscular mycorrhizal fungi (AMF) on *Artemisia annua* plants

Mycorrhiza is a mutualistic relationship between fungi and the roots of a plant, which are infected by the fungus. The rest of the fungal mycelium continues to grow in the soil and shares the absorbed nutrients and water with the plant host, while the fungus is provided with photosynthetic sugars by the host (Moore et al., 2011). Mycorrhizal networks have a significant effect on plant communities by leading to plant-to-plant transfers of nutrients or signals, thus increasing stress resistance (Heijden and Horton, 2009).

Mycorrhizas are present in more than 80% of angiosperms and gymnosperms (Bonfante and Anca, 2009; Barman et al., 2016). They have been classified into two types depending on the location of the fungal hyphae: ectotrophic (outside the root) and endotrophic (inside the root) (Bonfante, 2001). Endomycorrhizas can be divided in three groups: arbuscular (AM), ericoid and orchidaceous endomycorrhizas (Moore et al., 2011).

AMF, members of Glomeromycota, are not host specific, but depend on their host plant to complete the fungal life cycle (Balestrini et al., 2015; Berruti et al., 2016). AM colonies are usually formed by intracellular and intercellular hyphae, and intracellular arbuscules, while in soil AMF are distributed in large extraradical mycelium (Giovannetti and Sbrana, 1998; Moore et al., 2011). A wide range of soil microorganisms (nonbacterial microorganisms, mycorrhiza helper bacteria and plant growth promoting rhizobacteria) maintain a relationship with AMF (Miransari, 2011).

The effects of AMF on secondary metabolites of different host plants have been researched since the mid-twentieth century (Santander et al., 2017). AMF enhance the absorption of different soil nutrients including phosphorus, zinc, silicium, copper, magnesium, potassium, calcium, iron and nitrogen, thus alleviate salt stress (Evelin et al., 2009) and increase K^+/Na^+ ratio, growth and productivity of plants (Jeffries et al., 2003; Zeng et al., 2013; Garg and Bhandari, 2016). AM symbiosis can promote accumulation of terpenes, alkaloids, phenolic compounds and other substances in medicinal plants (Zeng et al., 2013). AMF hyphal length can reduce soil loss by preventing soil erosion (Mardhiah et al., 2016). Water transport in host plants can be influenced by AMF with hormonal changes, effective water absorption, direct water uptake through mycelium and osmotic regulation

(Santander et al., 2017). The aforementioned effects vary depending on the host plant species, soil type, nutrient status of the soil and the AMF species (Kim et al., 2017).

A. annua has been treated previously by AMF species from the genera *Acaulospora*, *Glomus* and *Rhizophagus*. Changes have been reported regarding biomass, plant growth, total leaf yield, artemisinin and essential oil content, nutrient status, phosphatase activity, foliar glandular trichome density, leaf chlorophyll content, GPOX enzyme concentration, stomatal conductance and transpiration rate (**Table 1**).

3. The effect of endophytic fungi on *Artemisia annua* plants

Endophytes are microorganisms including bacteria, archaea, fungi, and protists that inhabit the plant endosphere for at least parts of their life cycle where they can form different relationships (mutualistic, symbiotic, commensalistic, trophobiotic) (Ryan et al., 2008; Hardoim et al., 2015; Kandel et al., 2017; Singh et al., 2017). They can be classified into two groups: prokaryotic and eukaryotic endophytes (Hardoim et al., 2015). Another classification distinguishes endophytes that are unable to develop external structures and those that do generate external structures (nodules of nitrogen fixing bacteria) (Lacava and Azevedo, 2014).

Endophytic fungi-host interactions can be mutualistic, antagonistic and neutral. Their distribution is influenced by ecological and environmental conditions (temperature, humidity and levels of soil nutrition) (Jia et al., 2016). Endophyte-host association can be used to enhance the production of useful metabolites (Suryanarayanan et al., 2009). Three types of endophytic fungus-host relationship have been described: some endophytic fungi produce hormones to affect plant growth, some promote the accumulation of secondary metabolites such as medical components or drugs (Jia et al., 2016), while others produce antimicrobial and antifungal substances (Torres and White, 2012). Chemicals produced by endophytic fungi can improve the resistance and yield of crops (Cavaglieri et al., 2004) and also can be toxic for insects (Hartley and Gange, 2009).

Associations between *A. annua* and endophytic fungi have been evaluated after treatment with species from the genera *Colletotrichum*, *Penicillium* and *Piriformospora*. Artemisinin concentration, growth of biomass, chlorophyll content, seed yield, secondary metabolites, adaptation to biotic and abiotic stresses have been influenced by the treatment (**Table 2**).

4. The effects of endophytic bacteria on *Artemisia annua* plants

The large variety of endophytic bacteria and the adaptation to different environments (tropic, temperate, aquatic, Antarctic, rainforests, xerophytic, deserts, mangrove swamps and also coastal forests, geothermal soils etc.) lead to a wide pharmaceutical and agricultural application (Singh et al., 2017).

Bacterial endophytes have shown to control plant pathogens, insects and nematodes (Mercado-Blanco and Lugtenberg, 2014) in order to reduce biotic and abiotic stresses for their host (Liarzi et al., 2016), they promote plant growth and increase plant yield by inhibiting growth promoting hormones, fixation of nitrogen, water uptake and phosphate solubilization (Taghavi et al., 2009; Griffin, 2014; Singh et al., 2017; Etminani and Harighi, 2018). Some endophytes are resistant to heavy metals or antimicrobials, and can degrade organic compounds (Sheng et al., 2008; Bian et al., 2011; Rajkumar et al., 2012), which makes them a possible option for phytoremediation (Ryan et al., 2008). Endophytes were successful in decreasing drought, heat and salt stress in host plants (Kandel et al., 2017).

Table 1. The effect of AM fungi on *Artemisia annua* plants

AMF species	The effect of the treatment	References
Acaulospora tuberculata	-addition of mycorrhiza did not affect leaf yield, total biomass yield -inoculation enhanced the number of branches and the leaf artemisinin content (0.29%) -manure and mycorrhiza improved plant height, total dry weight, the number of branches	Rahman et al., 2014
Glomus aggregatum	-the treatment increased leaf yield and P content, total weight and height of the plants, and phosphatase activity in soil -no significant differences were found in leaf artemisinin content between treated (0.65%) and control plants (0.61%)	Awasthi et al., 2011
Glomus macrocarpum	-inoculation significantly increased the dry weight of shoot, production of herbage, nutrient status (P, Zn, Fe) of shoot, concentration of essential oil and artemisinin in leaves compared to control plants -inoculation resulted in highest artemisinin concentration -Mn concentration significantly decreased -Cu concentration was not influenced by the treatment	Chaudhary et al., 2007
	-the treatment significantly increased concentration of artemisinin -mycorrhizal plants had higher foliar glandular trichome density, accumulated more phosphorus in their shoots compared to control plants -Cu concentration was not influenced	Kapoor et al., 2007
Glomus mosseae	-inoculated plants had higher artemisinin content and biological yield -the addition of 40-80 mg/kg phosphorus fertilizer to the mycorrhizal colonization has increased mycorrhizal colonization and enhanced plant growth rate	Tan et al., 2013
	-the treatment enhanced leaf yield and total dry weight of the plants -significant increase in phosphatase activity and phosphorus concentration was observed -co-inoculation with *Bacillus subtilis* was effective in improving the height by 57.78%, total plant weight by 62.64%, leaf yield of plants by 66.6%, phosphatase activity and the artemisinin content (0.77 %) compared to control (0.61 %) or other tested bio-inoculants	Awasthi et al., 2011
	-mycorrhizal treatment improved the nitrogen, phosphorus and potassium uptake -mycorrhizal colonization led to changes in volatile components and increased the volatile oil content by 45.0% -stem, branch and leaf biomass was enhanced by 32.8%, 15.2% and 19.6% -inoculation increased the leaf chlorophyll content, net photosynthetic rate, stomatal conductance, transpiration rate, stem diameter and aboveground biomass	Huang et al., 2011
Glomus spp. (*G. mosseae*, *G. intraradices*, *G. viscosum*)	-AM inoculation increased shoot elongation, total shoot length, the foliar mineral concentration of Mg -the emission of limonene and artemisia ketone was stimulated by the treatment -the total terpene content and emission was not affected by AM inoculation with or without bacteria -co-inoculation with bacteria led to higher stem dry weight and leaf mass per area compared to other treatments (AM only, P-supplemented non-mycorrhizal plants and control)	Rapparini et al., 2008

	-Na and K concentrations were lower in AM treatments compared to control plants -no differences in the rate of photosynthesis were observed	
Glomus versiforme	-mycorrhizal treatment improved the uptake of nitrogen, phosphorus and potassium -stem, branch and leaf biomass was enhanced by 26.5%, 10.1% and 14.9% -mycorrhizal colonization increased the leaf chlorophyll content, net photosynthetic rate, stomatal conductance, transpiration rate, stem diameter and aboveground biomass -the treatment changed the volatile oil components and increased volatile oil content in leaf by 25.0%	Huang et al., 2011
Rhizophagus fasciculatus (*Syn. Glomus fasciculatum*)	-AMF treatment positively affected the uptake of plant nutrients and the density of glandular trichomes -a significant increase in biomass production and accumulation of artemisinin was observed	Giri, 2017
	-the treatment was not effective alone in enhancing growth and yield of plants -co-inoculation with *Stenotrophomonas* spp. improved its efficiency in increasing total dry weight and height -significant increase in phosphatase activity was observed -no significant differences were found in leaf artemisinin content between treated (0.68%) and control plants (0.61%) -combination with *Bacillus subtilis* led to significant increase in artemisinin content with 24.6%	Awasthi et al., 2011
	-inoculation significantly increased the production of herbage, dry weight of shoot, nutrient status (P, Zn, Fe) of shoot, concentration of essential oil and artemisinin in leaves compared to control plants -Mn concentration significantly decreased -an increase in essential oil concentration of 66% was observed compared to non-inoculated plants -Cu concentration was not influenced by the treatment	Chaudhary et al., 2007
	-the treatment significantly increased concentration of artemisinin -mycorrhizal plants had higher foliar glandular trichome density, accumulated more phosphorus in their shoots compared to control plants -inoculation in P-enriched soil significantly increased biomass, Fe and Zn concentrations in shoots, glandular trichome density, artemisinin concentration in leaves -Cu concentration was not influenced	Kapoor et al., 2007
Rhizophagus intraradices (*Syn. R. irregularis, Glomus intraradices*)	-the treatment significantly improved guaiacol peroxidase enzyme concentration, fresh and dry biomass of leaves -the number of trichomes and artemisinin concentration was significantly increased by 40.7% and 17% compared to control	Domokos et al., 2018
	-inoculation increased root biomass, had significantly greater inter-nodal length, more flavonoid content compared to control plants -the treatment did not significantly affect artemisinin content, the glandular trichome density and leaf biomass	Fortin and Melchert, 2015
	-inoculation resulted in increased endogenous jasmonic acid levels -the glandular trichome density and artemisinin concentration was enhanced	Mandal et al., 2015
	-the treatment was effective in enhancing leaf yield, total dry weight, significantly improved K uptake and phosphatase activity -no significant differences were found in leaf artemisinin content between treated (0.69%) and control plants (0.61%)	Awasthi et al., 2011

Table 2. The effect of endophytic fungi on *Artemisia annua* plants

Endophytic fungi	**The effect of the treatment**	**Reference**
Colletotrichum gloeosporioides	-the treatment promoted the artemisinin production by 51.63% in *A. annua* hairy root cultures	Wang et al., 2006
Colletotrichum sp.	-when using elicitor extract from the fungus the artemisinin content increased in hairy roots by 64.29 % compared to control -peroxidase activity of hairy roots and Ca^{2+} accumulation in cortical cells was influenced	Wang et al., 2002
	-the elicitor extract from the fungus led to an increase in artemisinin content in hairy roots of *A. annua* (44% increase over the control)	Wang et al., 2001
Penicillium oxalicum	-inoculation of 30-day-old rooting plantlets with fungus enhanced the artemisinin concentration by 43.5% over control -the endophyte had positive effect over the growth of *in vitro* propagated *A. annua* plantlets and induced oxidative stress through the production of reactive oxygen species	Zheng et al., 2016
Piriformospora indica	-inoculation promoted the growth of on *in vitro* grown plantlets and increased the carotenoid, total soluble sugar, total soluble protein and flavonoids content -the artemisinin concentration was enhanced by 1.7-fold compared to control	Arora et al., 2017
	-the treatment significantly increased the plant biomass, total chlorophyll content in leaves, phosphorus and nitrogen content by 65.95% and 13.27% -the endophyte led to an increment in artemisinin content -dual inoculation with *Azotobacter chroococcum* enhanced plant height, total biomass and total leaf yield per plant by 63.51%, 52.61% and 79.70% compared to control	Arora et al., 2016
	-root length, shoot length, stem size, root thickness, dry weight and fresh weight of *in vitro* raised plantlets increased due to the treatment -the chlorophyll and carotenoid content was also positively affected	Baishya et al., 2015
	-the treatment led to a 1.57 times increase in artemisinin production and promoted growth in hairy roots in liquid cultures	Ahlawat et al., 2014
	-the treatment significantly increased the average shoot number and biomass of *A. annua* cultures -the artemisinin content was enhanced by 60% compared to control	Sharma and Agrawal, 2013

Table 3. The effect of endophytic bacteria on *Artemisia annua* plants

Endophytic bacteria	The effect of the treatment	References
Agrobacterium rhizogenes	-the infected *A. annua* seedling tissues produced neoplastic roots characterized by high growth rate, genetic stability and producing higher levels of secondary metabolites	Soleimani et al., 2012
Azospirillum sp.	-the treatment increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
Azotobacter chroococcum	-an increase in total soluble sugar, total soluble protein and flavonoids content and carotenoid accumulation was observed -the treatment promoted plantlet growth -the artemisinin content was increased by 1.3-fold compared to control	Arora et al., 2017
	-the treatment increased plant biomass, total chlorophyll content by 51.97%, phosphorus content by 31.90%, nitrogen content by 29.20% and artemisinin content by 70% -dual inoculation with *Piriformospora indica* enhanced plant height, total biomass and total leaf yield per plant by 63.51%, 52.61% and 79.70% compared to control	Arora et al., 2016
Azotobacter sp.	-the treatment increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
Bacillus sp.	-inoculation increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
	- AM fungi in association with different bacteria increased stem dry weight and leaf mass per area -the total terpene content and emission, and the rate of photosynthesis was not affected	Rapparini et al., 2008
Bacillus subtilis	-inoculation improved height, leaf yield and total dry weight of the plants -significantly increased the mycorrhizal colonization of *G. mossae* by 50.32 % and phosphorus concentration in plants -co-inoculation with *G. mossae* was highly effective by improving the height by 57.78%, total plant weight by 62.64%, leaf yield of plants by 66.60%, phosphatase activity, and the artemisinin content by 26.2 % compared to control -co-inoculation with *G. fasciculatum* increased the artemisinin content by 24.6%	Awasthi et al., 2011
Pseudomonas fluorescens	-AM fungi in association with different bacteria increased stem dry weight and leaf mass per area -the total terpene content and emission, and the rate of photosynthesis was not affected	Rapparini et al., 2008
Pseudomonas sp.	-the treatment increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
Radiobacter spp.	-AM fungi in association with different bacteria increased stem dry weight and leaf mass per area -the total terpene content and emission, and the rate of photosynthesis was not affected	Rapparini et al., 2008

Stenotrophomonas spp.	-inoculation improved the height, leaf yield and total weight of the plants -the leaf artemisinin content was 0.73%, compared to 0.61% in control -the treatment improved the population of *G. fasciculatum* -co-inoculation with *G. mosseae* increased phosphorus concentration	Awasthi et al., 2011
Streptomyces spp.	-AM fungi in association with different bacteria increased stem dry weight and leaf mass per area -the total terpene content and emission, and the rate of photosynthesis was not affected	Rapparini et al., 2008

Table 4. Isolated fungi from *Artemisia annua* and their importance

Isolated fungi	**The effect of the fungi**	**Reference**
Acremonium persicum	-the elicitor extract prepared from the fungi increased the potted plants biomass and artemisinin content	Hussain et al., 2017
Aspergillus spp.	-the 11 endophytic extracts had different inhibitory effects on microbial pathogens -*Aspergillus* spp. exhibited the strongest activity against *Escherichia coli*, *Staphylococcus aureus* and *Trichophyton rubrum*	Zhang et al., 2012
Aspergillus terreus	-the fungi was isolated from *A. annua*, but no effect was studied	Zhang et al., 2010
Cephalosporium sp.	-*Cephalosporium* sp. extract had the strongest antimicrobial activity against *Magnaporthe grisea*	Zhang et al., 2012
Cladosporium sp.	-the pure cultures of the fungi had the greatest antibacterial (against *Staphylococcus aureus*, *Streptococcus mutans*, *Salmonella typhi*, *Bacillus subtilis*) and antifungal activity (*Malassezia furfur, Candida albicans*) out of the seven endophytic fungi obtained	Purwantini et al., 2015
Cochliobolus lunatus	-the elicitor extract prepared from the fungi increased the plant biomass and artemisinin content	Hussain et al., 2017
Colletotrichum gloeosporioides	-an increase in the plant biomass and artemisinin content was observed	Hussain et al., 2017
Colletotrichum sp.	-the fungi can produce antimicrobial and plant growth regulatory metabolites -the extract has antibacterial (against *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea* and *Pseudomonas* sp.) and antifungal activity (against *Candida albicans*, *Aspergillus niger*, *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia cerealis*, *Helminthosporium sativum, Phytophthora capisici*)	Lu et al., 2000
Curvularia pallescens	-the elicitor extract prepared from the fungi increased the potted plants biomass and artemisinin content and induced maximum growth (amongst the tested endophytic fungi)	Hussain et al., 2017
Leptosphaeria sp.	-leptosphaerone, a new bioactive and/or chemically new compound that may contain great medicinal or agricultural potential, was isolated from the studied endophyte of *A. annua*	Liu et al., 2002
Mucor sp.	-*Mucor* sp. extract showed the most pronounced effect on *Rhizoctonia cerealis*	Zhang et al., 2012

Studies have been conducted on the relationship between *A. annua* and prokaryotic endophytes from the phylum Firmicutes (genus *Bacillus*), Actinobacteria (genera *Stenotrophomonas* and *Streptomyces*) and Proteobacteria (genera *Agrobacterium*, *Azopirillum*, *Azotobacter*, *Pseudomonas* and *Radiobacter*). The majority of these studied bacterial endophytes are nitrogen-fixing (NF) bacteria. Plants are unable to directly assimilate molecular nitrogen, which is a limiting nutrient in most environments. However, biological nitrogen fixation developed only by prokaryotic cells enables this process (Franche et al., 2009).

The treatment lead to increased protein, chlorophyll and artemisinin content, and enhanced phosphatase activity and plant growth parameters (total weight, leaf yield, height) (**Table 3**).

5. Isolated endophytes from *Artemisia annua* and their importance

Prokaryotic endophytes isolated from *A. annua* belong to the genera *Nocardia*, *Nonomuraea*, *Pseudonocardia*, *Rhodococcus* and *Streptomyces*: *Nocardia artemisiae* sp. nov. (Zhao et al., 2011a), *Nonomuraea endophytica* sp. nov. (Li et al., 2011), *Pseudonocardia bannaensis* sp. nov. (Zhao et al., 2011b), *Pseudonocardia* sp. (Li et al., 2012), *Pseudonocardia xishanensis* sp. nov. (Zhao et al., 2012a), *Rhodococcus artemisiae* sp. nov. (Zhao et al., 2012b) and *Streptomyces endophyticus* (Li et al., 2013). Their impact on *A. annua* has not been the main goal of these studies. However, the results suggest that certain endophytic actinobacteria can stimulate plant defense responses thus affecting the artemisinin content.

The isolated endophytic fungi are from the genera *Acremonium*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Cochliobolus*, *Colletotrichum*, *Curvularia*, *Leptosphaeria* and *Mucor*. The antimicrobial and antifungal activities of the fungal endophytes have been studied (**Table 4**).

Conclusions

The shortage of wild medicinal plant resources and the increasing demand for herbal material increased the interest in alternative methods to enhance productivity. In this review, it has been pointed out that overall the inoculation with mutualistic and endophytic microorganisms leads to positive outcomes (enhancing plant growth, biomass, artemisinin and essential oil concentration, disease resistance), which makes them a potential alternative to existing yield increasing methods.

Some of the AM fungi and endophytes obviously favor the plant growth and development, influencing the quality and quantity of the crude drugs derived from *A. annua*. Co-inoculation of AM fungi and bacterial endophytes had the most impact on host plants, followed by single inoculation with AM fungi.

No publications about cultivation of *Artemisia annua* in open field conditions by inoculation with mutualistic or endophytic microorganisms were found. In the future the initiation of competition experiments with AMF and other soil inhabiting microorganisms would be of high interest.

AMF inoculation increased guaiacol peroxidase enzyme (GPOX) activity in *A. annua* plants. Further studies would be necessary to elucidate the mechanism of tolerance to water deficiency in case of inoculated *A. annua* plants.

Endophytes isolated from the plant could be used in medicine and in agriculture for their antimicrobial effect. These endophytes are potential artemisinin content and plant stress resistance enhancer.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Ahlawat S, Saxena P, Alam P, Wajid S, Abdin M Z (2014) Modulation of artemisinin biosynthesis by elicitors, inhibitor, and precursor in hairy root cultures of Artemisia annua L. Journal of Plant Interactions, 9:1,811-824, doi: 10.1080/17429145.2014.949885
2. Arora M, Saxena P, Abdin M Z, Varma A (2017) Interaction between Piriformospora indica and Azotobacter chroococcum governs better plant physiological and biochemical parameters in Artemisia annua L. plants grown under in vitro conditions. Symbiosis, Volume 75, Issue 2, pp 103–112. doi: 10.1007/s13199-017-0519-y
3. Arora M, Saxena P, Choudhary D K, Abdin M Z, Varma A (2016) Dual symbiosis between Piriformospora indica and Azotobacter chroococcum enhances the artemisinin content in Artemisia annua L. World J Microbiol Biotechnol (2016) 32:19. doi: 10.1007/s11274-015-1972-5
4. Awasthi A L, Bharti N, Nair P, Singh R, Shukla K A, Gupta M M, et al., (2011). Synergistic effect of Glomus mosseae and nitrogen fixing Bacillus subtilis strain Daz26 on artemisinin content in Artemisia annua. Appl. Soil Ecol. 49:125–130. doi: 10.1016/j.apsoil.2011.06.005
5. Baishya D, Deka P, Kalita M C (2015) In vitro co-cultivation of Piriformospora indica filtrate for improve biomass productivity in Artemisia annua (L.). Symbiosis, Volume 66, Issue 1, pp 37–46. doi: 10.1007/s13199-015-0331-5
6. Balestrini R, Lumini E, Borriello R, Bianciotto C (2015) Plant-soil biota interactions. Soil Microbiology, Ecology and Biochemistry, ed Paul E A, London, Academic Press, Elsevier, pp 311-338. doi: 10.1016/b978-0-12-415955-6.00011-6
7. Barman J, Samanta B, Saha B, Datta S (2016) Mycorrhiza: the oldest association between plant and fungi. Siraj Datta, Dec 14, 2016
8. Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front. Microbiol., 6:1559. doi: 10.3389/fmicb.2015.01559
9. Bian G, Zhang Y, Qin S, Xing K, Xie H, Jiang J (2011) Isolation and biodiversity of heavy metal tolerant endophytic bacteria from halotolerant plant species located in coastal shoal of Nantong. Wei Sheng Wu Xue Bao, 51(11):1538-47.
10. Bilia A R, Santomauro F, Sacco C, Bergonzi M C, Donato R (2014) Essential oil of Artemisia annua L.: an extraordinary component with numerous antimicrobial properties. Complementary and Alternative Medicine 2014(1):159819. doi: 10.1155/2014/159819
11. Bonfante P (2001) At the interface between mycorrhizal fungi and plants: the structural organization of cell wall, plasma membrane and cytoskeleton. In: Hock B. (eds) Fungal Associations. The Mycota (A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research), vol 9. Springer, Berlin, Heidelberg, pp 45-61. doi: 10.1007/978-3-662-07334-6_4
12. Bonfante P, Anca I A (2009) Plants, mycorrhizal fungi, and bacteria: a network

of interactions. Annual Review of Microbiology, 63:363-383. doi: 10.1146/annurev.micro.091208.073504

13. Briars R, Paniwnyk L (2014) Potential methods to improve the efficiency of artemisinin extraction from Artemisia annua. In: Aftab T., Ferreira J., Khan M., Naeem M. (eds) Artemisia annua - Pharmacology and Biotechnology. Springer, Berlin, Heidelberg, pp 124–137. doi: 10.1007/978-3-642-41027-7_8
14. Calderón F, Wilson D M, Gamo J (2013) Antimalarial drug discovery: Recent progress and future directions. Progress in Medicinal Chemistry 52:97–151. doi: 10.1016/B978-0-444-62652-3.00003-X
15. Cavaglieri L., Passone A., Etcheverry M. (2004). Correlation between screening procedures to select root endophytes for biological control of Fusarium verticillioides in Zea mays L. Biol. Control. 31, 259–267. doi: 10.1016/j.biocontrol.2004.07.006
16. Chaudhary L V, Kapoor R, Bhatnagar K A (2008) Effectiveness of two arbuscular mycorrhizal fungi on concentrations of essential oil and artemisinin in three accessions of Artemisia annua L. Appl. Soil Ecol. 40, 174–181. doi: 10.1016/j.apsoil.2008.04.003
17. Das S (2012) Artemisia annua (qinghao): a pharmacological review. International journal of pharmaceutical sciences and research: 4573–4577. doi: 10.13040/IJPSR.0975-8232.3(12).4573-77
18. Domokos E, Jakab-Farkas L, Darkó B, Bíró-Janka B, Mara Gy, Albert Cs, Balog A (2018) Increase in Artemisia annua plant biomass content and guaiacol peroxidase activity using the arbuscular mycorrhizal fungus Rhizophagus irregularis. Front Plant Sci. 2018; 9: 478. doi: 10.3389/fpls.2018.00478
19. Etminani F, Harighi B (2018) Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. Plant Pathol. J. 34(3):208-217. doi: 10.5423/PPJ.OA.07.2017.0158
20. Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Annals of Botany, 104: 1263–1280. doi:10.1093/aob/mcp251
21. Fairhust R M, Dondorp A M (2016) Artemisinin-Resistant Plasmodium falciparum Malaria. Microbiol Spectr. 4(3). doi: 10.1128/microbiolspec.EI10-0013-2016.
22. Fairhust R M, Wellems T E (2015) Malaria (Plasmodium species). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition):3070-3090. doi: 10.1016/B978-1-4557-4801-3.00276-9
23. Fortin S, Melchert V (2015) Effect of mycorrhizae on Artemisia annua. Worcester Polytechnic Institute.
24. Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59. doi: 10.1007/s11104-008-9833-8
25. Garg N, Bhangari P (2016) Silicon nutrition and mycorrhizal inoculations improve growth, nutrient status, K+ /Na+ ratio and yield of Cicer arietinum L. genotypes under salinity stress. Plant Growth Regulation 78(3):371-387. doi: 10.1007/s10725-015-0099-x
26. Giovannetti M, Sbrana C (1998) Meeting a non-host: the behaviour of AM fungi. Mycorrhiza 8:123-130. doi: 10.1007/s005720050224
27. Giri B (2017) Mycorrhizal fungus Rhizophagus fasciculatus promotes artemisinin accumulation in Artemisia annua. In Paper Presented at the Tropentag,

Future Agriculture: Socio-Ecological Transitions and Bio-Cultural Shifts, Bonn.

28. Griffin M R (2014) Biocontrol and bioremediation: two areas of endophytic research which hold great promise. Advances in endophytic research. Verma V C, Gange A C, Springer India, 2014, pp 257-282. doi: 10.1007/978-81-322-1575-2_14
29. Hardoim P R, van Overbeek L S, Berg G, Pirttilä A M, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev. 79(3):293-320. doi: 10.1128/MMBR.00050-14
30. Hartley S E, Gange A C (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu. Rev. Entomol. 54, 323-342. doi: 10.1146/annurev.ento.54.110807.090614
31. Heijden M G A, Horton T R (2009) Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. Journal of Ecology. 97, 1139–1150. doi: 10.1111/j.1365-2745.2009.01570.x
32. Ho W E, Peha H Y, Chan T K, Wong F (2013) Artemisinins: Pharmacological actions beyond anti-malarial. Pharmacology & Therapeutics 142:126–139. doi: 10.1016/j.pharmthera.2013.12.001
33. Hommel M (2008) The future of artemisinins: natural, synthetic or recombinant? Journal of Biology 2008, 7:38. doi: 10.1186/jbiol101
34. Huang J H, Tan J F, Jie H K, Zeng R S (2011) Effects of inoculating arbuscular mycorrhizal fungi on Artemisia annua growth and its officinal components. Yingyong Shengtai Xuebao, 2011, Vol. 22 Issue 6, p1443-1449.
35. Hussain M A, Mahajan V, Rather I A, Awasthi P, Chouhan R, Dutt P, Sharma Y P, Bedi Y S, Gandhi S G (2017) Isolation and identification of growth promoting endophytic fungi from Artemisia annua L. and its effects on artemisinin content. Trends Phytochem. Res. 1(4): 207-214.
36. Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea J M (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils, 37:1–16. doi: 10.1007/s00374-002-0546-5
37. Jia M, Chen L, Xin H L, Zheng C J, Rahman K, Han T, Qin L P (2016) A Friendly Relationship between Endophytic Fungi and Medicinal Plants: A Systematic Review. Front Microbiol. 2016; 7: 906. doi: 10.3389/fmicb.2016.00906
38. Kandel S L, Firrincieli A, Joubert P M, Okubara P A, Lestion N D, McGeorge K M, Doty S L (2017) An in vitro study of bio-control and plant growth promotion potential of Salicaceae endophytes. Front Microbiol, 13;8:386. doi: 10.3389/fmicb.2017.00386
39. Kapoor R, Chaudhary V, Bhatnagar AK (2007) Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in Artemisia annua L. Mycorrhiza 17:581–587. doi: 10.1007/s00572-007-0135-4
40. Keshavarzi M H B, Mousavi-Nik S M, Abdin M Z (2012). The effect of biological and chemical fertilizers on chlorophyll and artemisinin content in Artemisia annua L. In The 1st international and the 4th national congress on recycling of organic waste in agriculture. pp. 1–8. doi:
41. Keshavarzi M H B, Nik A M M (2011) The effect of biological and chemical fertilizers on protein content in Artemisia annua L. Leaves. Annals of Biological Research, 2011, 2 (5) :596-601

42. Kim S J, Eo J K, Lee E H, Park H, Eom A E (2017) Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. Mycobiology, 45(1): 20–24. doi: 10.5941/MYCO.2017.45.1.20
43. Kung S H, Lund S, Murarka A, McPhee D, Paddon C J (2018) Approaches and Recent Developments for the Commercial Production of Semi-synthetic Artemisinin. Front Plant Sci. 31;9:87. doi: 10.3389/fpls.2018.00087
44. Lacava P T, Azevedo J L (2014) Biological control of insect-pest and diseases by endophytes. Advances in endophytic research. Verma V C, Gange A C, Springer India, 2014, pp 231-256. doi: 10.1007/978-81-322-1575-2_13
45. Li J, Zhao G Z, Huang H Y, Zhu W Y, Lee J C, Zu L H, Kim C J, Li W J (2011) Nonomuraea endophytica sp. nov., an endophytic actinomycete isolated from Artemisia annua L. International Journal of Systematic and Evolutionary Microbiology, 61, 757–761. doi: 10.1099/ijs.0.022558-0
46. Li J, Zhao G Z, Zhu W Y, Huang H Y, Xu L H, Zhang S, Li W J (2013) Streptomyces endophyticus sp. nov., an endophytic actinomycete isolated from Artemisia annua L. International Journal of Systematic and Evolutionary Microbiology 63, 224–229. doi: 10.1099/ijs.0.035725-0
47. Li J, Zhao G-Z, Varma A, Qin S, Xiong Z, et al., (2012) An Endophytic Pseudonocardia Species Induces the Production of Artemisinin in Artemisia annua. PLoS ONE 7(12): e51410. doi:10.1371/journal.pone.0051410
48. Liarzi O, Bucki P, Miyara S B, Ezra D (2016) Bioactive volatiles from an endophytic *Daldinia cf. concentrica* isolate affect the viability of the plant parasitic nematode *Meloidogyne javanica*. PLoS ONE 11(12): e0168437. doi:10.1371/journal.pone.0168437
49. Liu C, Zhao Y, Wang Y (2006) Artemisinin: current state and perspectives for biotechnological production of an antimalarial drug. Appl Microbiol Biotechnol, 72:11–20. doi: 10.1007/s00253-006-0452-0
50. Liu J Y, Liu C H, Zou W X, Tian X, Tan R X (2002) Leptosphaerone, a metabolite with a novel skeleton from Leptosphaeria sp. IV403, an endophytic fungus in Artemisia annua. Helvetica, Volume 85, Issue9, pp 2664-2667. doi: 10.1002/1522-2675(200209)85:9<2664::AID-HLCA2664>3.0.CO;2-R
51. Lu H, Zou W X, Meng J C, Hu J, Tan R X (2000) New bioactive metabolites produced by Colletotrichum sp., an endophytic fungus in Artemisia annua. Plant Science 151:67–73. doi: 10.1016/S0168-9452(99)00199-5
52. Malhi S S, Sahota T S, Gill K S (2013) Potential of Management Practices and Amendments for Preventing Nutrient Deficiencies in Field Crops under Organic Cropping Systems. Agricultural Sustainability pp 77–101. doi: 10.1016/B978-0-12-404560-6.00005-8
53. Mandal S, Upadhyay S, Wajid S, Ram M, Jain D C, Singh V P, Abdin M Z, Kapoor R (2015) Arbuscular mycorrhiza increase artemisinin accumulation in Artemisia annua by higher expression of key biosynthesis genes via enhanced jasmonic acid levels. Mycorrhiza. 25(5):345-57. doi: 10.1007/s00572-014-0614-3
54. Mardhiah U, Caruso T, Gurnell A, Rillig M (2016) Arbuscular mycorrhizal fungal hyphae reduce soil erosion by surface water flow in a greenhouse experiment. Appl Soil Ecol 99:137–140. doi: 10.1016/j.apsoil.2015.11.027
55. Martínez M J A, Olmo L M B, Ticona L A, Benito P B (2014) Pharmacological Potentials of Artemisinin and Related

Sesquiterpene Lactones: Recent Advances and Trends, In: Aftab T., Ferreira J., Khan M., Naeem M. (eds) Artemisia annua - Pharmacology and Biotechnology. Springer, Berlin, Heidelberg, pp 75-93. doi: 10.1007/978-3-642-41027-7_5

56. Mercado-Blanco J, Lugtenberg B J J (2014) Biotechnological applications of bacterial endophytes. Current Biotechnology, 3(1):60-75. doi: 10.2174/22115501113026660038
57. Miransari M (2011) Interactions between arbuscular mycorrhizal fungi and soil bacteria. Appl Microbiol Biotechnol, 89:917–930. doi:10.1007/s00253-010-3004-6
58. Monfil V O, Casas-Flores S (2014) Molecular mechanisms of biocontrol in Trichoderma spp. and their applications in agriculture. Biotechnology and Biology of Trichoderma, pp 429–453. doi: 10.1016/B978-0-444-59576-8.00032-1
59. Moore D, Robson G, Trinci A (2011) 21th century guidebook of fungi. Cambridge University Press, United States of America, New York
60. Naeem M, Idrees M, Singh M, Kahn M M A, Moinuddin (2014) Artemisia annua: a miraculous herb to cure malaria. In: Aftab T., Ferreira J., Khan M., Naeem M. (eds) Artemisia annua - Pharmacology and Biotechnology. Springer, Berlin, Heidelberg, pp 27–49. doi: 10.1007/978-3-642-41027-7_3
61. Namuli A, Bazira J, Casim T U, Engeu P O (2018) A review of various efforts to increase artemisinin and other antimalarial compounds in Artemisia Annua L plant. Cogent Biology 4: 1513312. doi: 10.1080/23312025.2018.1513312
62. Pandley N, Pandley-Ray S (2015) Updates on artemisinin: an insight to mode of actions and strategies for enhanced global production. Protoplasma, 253(1):15-30. doi: 10.1007/s00709-015-0805-6
63. Purwantini I, Wahyono, Mustofa, Asmah R (2015) Isolation of endophytic fungi from Artemisia annua, L and identification of their antimicrobial compound using bioautography method. Int J Pharm Pharm Sci, Vol 7, Issue 12, 95-99.
64. Rahman W, Widyatmoko D, Lelono A A (2014) The effect of NPK fertilizer, manure, vesicular arbuscular mycorrhiza (VAM) on the growth, biomass and artemisinin content of Artemisia annua L. Jurnal Biologi Indonesia 10(2): 285-296. doi: 10.14203/jbi.v10i2.2107
65. Rajkumar M, Sandhya S, Prasad M N, Feritas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv., 30(6):1562-74. doi: 10.1016/j.biotechadv.2012.04.011
66. Rapparini F, Llusia J, Penuelas J (2008) Effect of arbuscular mycorrhizal (AM) colonization on terpene emission and content of Artemisia annua L. Plant Biol 10:108–122. doi: 10.1055/s-2007-964963
67. Ryan R P, Germaine K, Franks A, Ryan D J, Dowling D N (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiology Letters, volume 278, issue 1, pp 1-9. doi: 10.1111/j.1574-6968.2007.00918.x
68. Sadiq A, Hayat M O, Ashraf M (2014) Ethnopharmacology of Artemisia annua L.: A Review. In: Aftab T., Ferreira J., Khan M., Naeem M. (eds) Artemisia annua - Pharmacology and Biotechnology. Springer, Berlin, Heidelberg, pp 9–25. doi: 10.1007/978-3-642-41027-7_2
69. Santander C, Aroca R, Ruiz-Lozano J M, Olave J, Cartes P, Borie F, Cornejo P (2017) Arbuscular mycorrhiza effects on plant performance under osmotic stress.

Mycorrhiza, 27(7):639-657. doi: 10.1007/s00572-017-0784-x

70. Sharma G, Agrawal V (2013) Marked enhancement in the artemisinin content and biomass productivity in Artemisia annua L. shoots co-cultivated with Piriformospora indica. World J Microbiol Biotechnol. 29:1133–1138. doi: 10.1007/s11274-013-1263-y
71. Sheng X F, Xia J J, Jiang C Y, He L Y, Qian M (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. Environ. Pollut., 156:1164–1170. doi: 10.1016/j.envpol.2008.04.007
72. Singh D G, Kumar A, Lata S, Johri R M (2017) Effect of arbuscular mycorrhizal fungi on plant growth, essential oil and Artemisinin content of Artemisia annua L. International Journal of Pharmacology and Biological Sciences, 11(1): 33-44.
73. Soleimani T, Keyhanfar M, Piri K, Hasanloo T (2012) Morphological evaluation of hairy roots induced in Artemisia annua L and investigating elicitation effects on the hairy roots biomass production. International Journal of Agriculture: Research and Review, vol. 2, pp 1005-1013
74. Suryanarayanan T S, Thirunavukkarasu N, Govindarajulu M B, Sasse F, Jansen R, Murali T S (2009) Fungal endophytes and bioprospecting. Fungal Biology Reviews 23:9-19. doi: 10.1016/j.fbr.2009.07.001
75. Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, Van Der Lelie D (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl. Environ. Microbiol. 75:748–757. doi: 10.1128/AEM.02239-08
76. Tan W D, Shen M J, Qiu H J, Zeng F L, Huang J H, Huang R S, Luo W G, Liu Y X (2013) Effects of different phosphorus treatments on Arbuscular mycorrhizal formation, growth and artemisinin content of Artemisia annua. Journal of Southern Agriculture, 2013 Vol.44 No.8 pp.1303-1307
77. Tang X, Demiray M, Wirth T, Allemann R K (2018) Concise synthesis of artemisinin from a farnesyl diphosphate analogue. Bioorg Med Chem. 26(7): 1314–1319. doi: 10.1016/j.bmc.2017.03.068
78. Torres M, White J F (2012) Endophytic fungi. Encyclopedia of Science & Technology, McGraw-Hill Education, pp 241-269. doi: 10.13140/2.1.3835.2169
79. Varma A, Bakshi M, Lou B, Hartmann A, Ouelmueller R (2012) Piriformospora indica: a novel plant growth-promoting mycorrhizal fungus. Agric Res. 1(2):117–131. doi: 10.1007/s40003-012-0019-5
80. Wang B, Sui J, Yu Z, Zhu L (2011) Screening the hemostatic active fraction of Artemisia annua L. In-vitro. Iran J Pharm Res 10:57–62.
81. Wang J W, Xia Z H, Tan R X (2002) Elicitation on artemisinin biosynthesis in Artemisia annua hairy roots by the oligosaccharide extract from the endophytic Colletotrichum sp. B501. Acta Botanica Sinica 44(10):1233-1238
82. Wang J W, Zhang Z, Tan R X (2001) Stimulation of artemisinin production in Artemisia annua hairy roots by the elicitor from the endophytic Colletotrichum sp. Biotechnology Letters, Volume 23, Issue 11, pp 857–860. doi: 10.1023/A:1010535001943
83. Wang J W, Zheng L P, Tan R X (2006) The preparation of an elicitor from a fungal endophyte to enhance artemisinin production in hairy root cultures of

Artemisia annua L. Sheng Wu Gong Cheng Xue Bao, 22(5):829-34.

84. White NJ (2008) Qinghaosu (Artemisinin) The price of success. Science 320:331–334. doi:10.1126/science.1155165
85. WHO (2017) World Malaria Report. Luxemburg: Marketing and Dissemination, World Health Organization
86. Zabel F, Putzenlechner B, Mauser W (2014) Global agricultural land resources – a high resolution suitability evaluation and its perspectives until 2100 under climate change conditions, PLoS ONE 9(9): e107522. doi: 10.1371/journal.pone.0107522
87. Zeng Y, Guo L P, Chen B D, Hao Z P, Wang J W, Huang L Q, Yang G, Cui X M et al., (2013) Arbuscular mycorrhizal symbiosis and active ingredients of medicinal plants: current research status and prospectives. Mycorrhiza, 23(4):253–65. doi: 10.1007/s00572-013-0484-0
88. Zhang H W, Zhang J, Hu S, Zhang Z J, Zhu C J, Ng S W, Tan R X (2010) Ardeemins and cytochalasins from Aspergillus terreus residing in Artemisia annua. Planta Med 2010; 76:1616–1621. doi: 0.1055/s-0030-1249781
89. Zhang H, Bai X, Wu B (2012) Evaluation of antimicrobial activities of extracts of endophytic fungi from Artemisia annua. Bangladesh J Pharmacol 7: 120-123. doi: 10.3329/bjp.v7i2.10951
90. Zhao G Z, Li J, Zhu W J, Klenk H P, Xu L H, Li W J (2011) (a) Nocardia artemisiae sp. nov., an endophytic actinobacterium isolated from a surface-sterilized stem of Artemisia annua L. International Journal of Systematic and Evolutionary Microbiology, 61, 2933–2937. doi: 10.1099/ijs.0.029306-0
91. Zhao G Z, Li J, Zhu W J, Li X P, Tian S Z, Zhao L X, Xu L H, Li W J (2011) (b) Pseudonocardia bannaensis sp. nov., a novel actinomycete isolated from the surface-sterilized roots of Artemisia annua L. Antonie van Leeuwenhoek 100:35–42. doi: 10.1007/s10482-011-9562-5
92. Zhao G Z, Li J, Zhu W J, Tian S Z, Zhao L X, Yang L L, Xu L H, Li W J (2012) (a) Rhodococcus artemisiae sp. nov., an endophytic actinobacterium isolated from the pharmaceutical plant Artemisia annua L. International Journal of Systematic and Evolutionary Microbiology, 62, 900–905. doi: 10.1099/ijs.0.031930-0
93. Zhao G Z, Li J, Zhu W J, Wei D Q, Zhang J L, Xu L H, Li W J (2012) (b) Pseudonocardia xishanensis sp. nov., an endophytic actinomycete isolated from the roots of Artemisia annua L. International Journal of Systematic and Evolutionary Microbiology 62, 2395–2399. doi: 10.1099/ijs.0.037028-0
94. Zheng L P, Tian H, Yuan F Y, Wang W J (2016) The influence of endophytic Penicillium oxalicum B4 on growth and artemisinin biosynthesis of in vitro propagated plantlets of Artemisia annua L. Plant Growth Regulation, Volume 80, Issue 1, pp 93–102. doi: 10.1007/s10725-016-0162-2

ORIGINAL PAPER

THE MEDICINAL IMPORTANCE OF WILD PLANTS FROM THE SURROUNDINGS OF ULIEȘ VILLAGE, MUREȘ COUNTY

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Abstract: The village of Ulieş belongs to Râciu Commune. It is located in the south-eastern part of the Transylvanian Plain (N46°41'30" E24°23'56") in a hilly region crossed by wide valleys. It is situated at 24 km distance from Tîrgu Mureş, in the contact area of Mădăraş Hills and Comlod Hills, on the middle stream of the Comlod River (or Lechința) and its tributaries. The paper aims were the study of the flora from the surroundings of Ulieş Village, the investigation of possible medicinal use of the encountered plants species, and the preservation of plant diversity from the area. Fieldworks were conducted in 2014-2017. A total of 415 vascular plant taxa belonging to 76 families were identified. From these, 185 species are medicinal plants with certain content of active principles. Plants containing tannins (13.51%) were in higher percentage followed by those with essential oils (12.97%), saponins (10.81%), alkaloids (8.10%), flavonoids (7.56%), coumarins (7.02%), mucilages (5.94%), iridoids (5.40%), phenolic glycosides (3.78%), anthraquinone derivatives and cardiotonic glycosides (3.24), organic acids, vitamins and provitamins (3.78%), bitter principles (2.70), bitter-aromatic principles (2.16%), etc.

Keywords: spontaneous flora, medicinal plants, active principles, remedies for human diseases, Mureş County.

1. Introduction

Ulieş Village (**Fig. 1A, B**) was attested since 1321 under the name "Wlues, Wleus, Ulves". The relief is characterized by marl, marly shales, clays and sands, conglomerates and volcanic tuffs. Altitude reaches 350-450 m. The low hills and plateaus belong to the temperate continental climate (Şoneriu and Mac, 1973; Conțiu and Conțiu, 2010). The average annual temperature is 9°C and the average annual rainfall is between 550 and 650 mm. In the last years a decrease in the volume of rainfall can be observed. The hydrographic network density is low (0.3-0.5 km/km^2). Ulieş Village is crossed by the right tributary of Comlod, named Ulieş River, with the following rivulets: Izvor, Cetegău, Techeniş and Valea Hegmenilor. The main identified soil classes are: cernisols, luvisols, protisols, hidrisols and antrisols. In the meadows and terraces aluviosols can be found which are rich in nutrients and are favorable to crops (corn, sugar beet, vegetables, potatoes, etc.) (Conțiu, 2010; Florea and Munteanu 2012).

Fig. 1. Ulieș Village and surroundings: **A.** general view; **B.** Glimee-type landslides (original)

Although many researches were carried out on the flora and vegetation of Mureș County, there are still unstudied white spots on the counties map. The present paper aims were: (1) the study of the flora from the surroundings of Ulieş Village; (2) the investigation of possible medicinal use of the encountered plants species; (3) the preservation of the floristic diversity from the area. This region once was rich in heyfields with great plant diversity, but in present it is affected by the spread of invasive plants mainly resulting from the uncultivated agricultural lands.

2. Materials and Methods

The identification of the taxa was made according to the classical techniques and procedures from the literature. For this the specialized works of "Flora Europaea" (1964-1980) were consulted. The nomenclature of the taxa complies with the rules of the "International Code of Botanical Nomenclature" (Code de Melbourne, 2012) and the book of Sârbu et al. (2013). In the inventory of plant species, the adopted classification system was updated according to the most recent publications (Oroian, 2011; Cristea, 2014). The medicinal plants were grouped according to the dominant active principles for which they are used in traditional medicine and phytotherapy, adopting the grouping of plants after Eşianu and Laczkó-Zöld (2016) as well as the most recent specialized publications (Istudor, 1998, 2001, 2005; Stănescu et al., 2002, 2004). The identification and classification of the protected plants were made on the basis of the specialty literature (Dihoru and Dihoru 1993-1994; Oltean et al., 1994; Bilz et al., 2011; Mihăilescu et al., 2015).

3. Results and discussions

The study area once was covered with wide forests. Also the banks of the streams and the steep hills (with clay, marl and many landslides) were covered with shrubs and the sunny slopes facing south with steppe vegetation (**Fig. 2A**) (Borza, 1929, 1931, 1936; Doniță et al., 1992). The meadows which today are characteristic features of the Transylvanian Plain have gradually evolved as forests were cleared to obtain place for agricultural lands (**Fig. 2B**). These productive farmlands now are abandoned by the aging villagers and weeds are spreading on the uncultivated lands (**Fig. 2C, D**). The edges of the cereal fields are often full of the vivid colors of forking larkspur (*Consolida regalis*) (**Fig. 2E**), common poppy (*Papaver rhoeas*) and cornflower (*Centaurea cyanus*) (**Fig. 2F**).

In this study 415 vascular plant taxa belonging to 76 families were identified (see in **Table 1** of the Supplementary Material). Pteridophytes (2 species) and Gimnosperms (1 species) are very poorly represented in the territory.

Most of the taxa belong to Angiosperms from the classes of Dicotyledoneae (342 taxa) and Monocotyledoneae (69 taxa). The families with the most numerous species are: Asteraceae (53 species from 36 genera), Lamiaceae (39 species from 21 genera), Fabaceae (33 species from 15 genera), Poaceae (28 species from 22 genera), Rosaceae (20 species from 12 genera) Ranunculaceae (19 species from 11 genera), Apiaceae and Scrophulariaceae (each with 16 species from 14 and 9 genera, respectively), Brassicaceae and Liliaceae (each with 12 species from 12 and 11 genera, respectively), Caryophyllaceae and Polygonaceae (each with 10 species from 7 and 3 genera, respectively), etc. The analysis of the species number revealed that vascular plant diversity is higher in open land than in forests and shrubs. In forests there are more vernal and annual species, and also geophytes (G). The genera with the most numerous species in the territory are: *Trifolium* (with 9 species), *Veronica* (with 7 species), *Galium*, *Salvia* (each with 6 species), *Campanula*, *Linum*, *Potentilla*, *Rumex* and *Stachys* (each with 5 species), *Ajuga*, *Artemisia*, *Astragalus*, *Centaurea*, *Euphorbia*, *Medicago*, *Ranunculus*, *Viola* (each with 4 species), the remaining genera having one, two or three species.

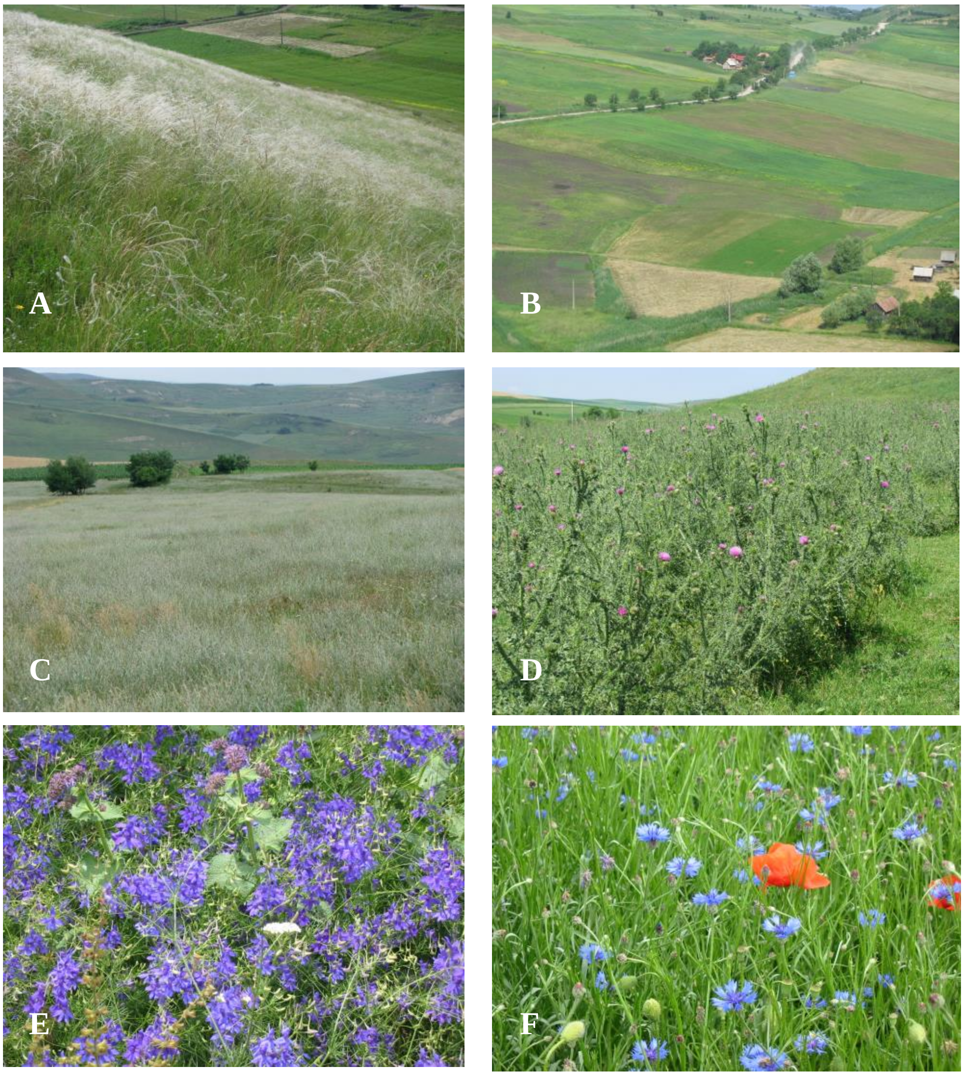

Fig. 2. The surroundings of Ulieș Village: **A.** Steppe vegetation; **B.** Agricultural lands; **C.** *Xeranthemum annuum*; **D.** Abandoned pasture; **E.** *Consolida regalis*; **F.** *Papaver rhoeas* (original)

In the ecological study the species peculiarities were analyzed in respect to abiotic factors such as: edaphic humidity (U), soil reaction (R), air temperature (T), light intensity (L), soil nitrogen content (N) (**Fig. 3**) (Oroian et al., 2014, 2018; Nagy 2018). The spectrum of ecological categories based on edaphic humidity shows that most of the species prefer dry and moderately moist soils (65.84%, $U_3+U_4+U_5$). The spectrum of ecological categories based on temperature presents that plants of plains and hilly areas are in high number (51.59%, $T_5+T_6+T_7$). The euriterm species are present in a proportion of 46.92% (T_x). Regarding the soil reaction slightly acid-neutrophylous (35.96 %, R_7) and euryionic (53.44 %, R_x) species are in higher proportions. The analysis of the bioforms (**Fig. 4**) is an important element in flora characterization, as they represent adaptation strategies of the Cormophytes to the succession of seasons. The high percentage of hemicryptophytes (51.70%, H) indicates the temperate climate and it is related to the large areas occupied by meadows and the presence of the herbaceous layer in the forests. Therophytes (23.79%, T) indicate a more or less arid climate. Their distribution is strongly conditioned by anthropo-zoogenic influences and the existence of territories where the plant cover is discontinuous, occupied by annual plants. Phanerophytes (10.44%, Ph) and geophytes (9.47%, G) are specific to forests and are less present in meadows. The spectrum of geoelements (**Fig. 5**) provides information about the genetic pool richness of the phytocoenosis. The Euro-Asian (Eua) element represents the highest percentage (46.75%), followed by the European (26.51%, Eur), Circumboreal (6.27%, Circumbor), Ponto-Pannonian (6.27%, Pont-Pann), Mediterranean (1.69%, Med) and Carpathian (1.20%, Carp) elements, etc.

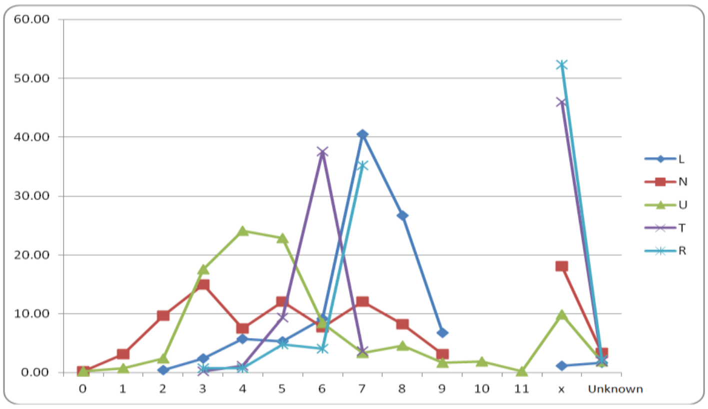

Fig. 3. The ecological categories spectrum of flora from the surroundings of Ulieș Village

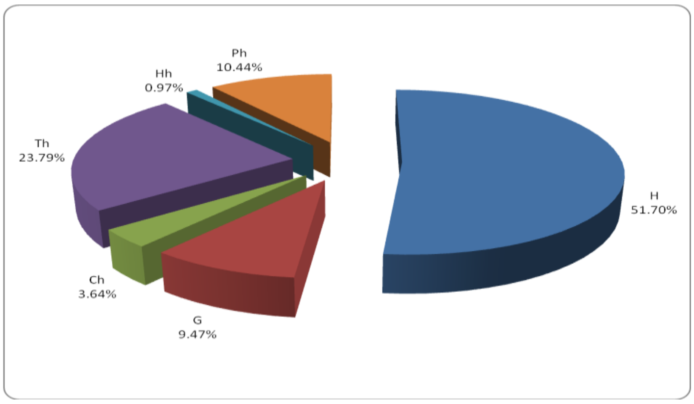

Fig. 4. The bioforms spectrum of flora from the surroundings of Ulieș Village

The Cosmopolitan element is also well represented (7.23%). Most of the medicinal plants have Eurasian, European, Ponto-Pannonian and Circumboreal origins. The higher number of diploids (**Fig. 6**) reveals the long-standing presence of flora, while the poliploids provide resistance to the unfavorable ecological conditions. From the reported taxa, 185 species are medicinal plants with certain content of active principles. The medicinal plants were grouped according to Eşianu and Laczkó-Zöld (2016), Eşianu and Şefănescu (2016) and also on the base of the most recent specialized publications (Rácz et al., 2012; Yberrt and Delesalle, 2013, Domokos et al., 2018). Plants containing tannins (13.51%) were in higher percentage followed by those with essential oils (12.97%), saponins (10.81%), alkaloids (8.10%), flavonoids (7.56%), coumarins (7.02%), mucilages (5.94%), iridoids (5.40%), phenolic glycosides (3.78%), anthraquinone derivatives and cardiotonic glycosides (3.24%), organic acids, vitamins and provitamins (3.78%), bitter principles (2.70%), bitter-aromatic principles (2.16%), etc. (**Fig. 7**). The most commonly used species in traditional medicine and phytotherapy are presented in **Table 2**. Most of the medicinal plants are used in digestive (53 sp.), respiratory (27 sp.), skin (23 sp.), locomotor (17 sp.), genitourinary (19 sp.) and cardiovascular (8 sp. each) disorders. A total of 40 species have monographs in the Romanian Pharmacopoeia (1994) and in the European Pharmacopoeia (2018).

During the study 10 taxa with special scientific value and rare species (in Romania and Europe) were identified. Some of them are considered also medicinal plants.

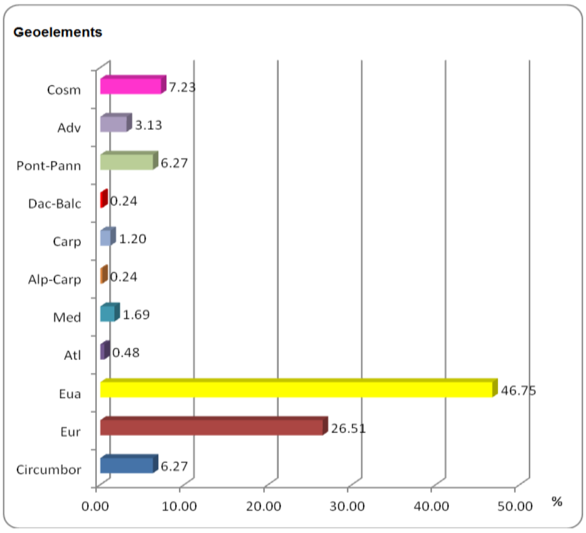

Fig. 5. The geoelements spectrum of flora from the surroundings of Ulieş Village

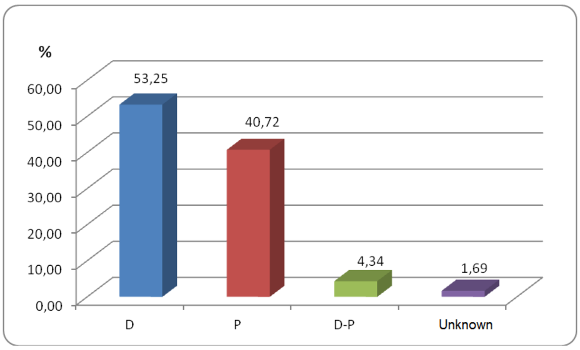

Fig. 6. The caryologic spectrum of flora from the surroundings of Ulieş Village

Thus, 4 endemic and subendemic taxa were found: *Aconitum lycoctonum* ssp. *moldavicum* (see **Fig. 8A** from the Supplementary Material), *Astragalus exscapus* ssp. *transilvanicus* (**Fig. 8C**), *Cephalaria radiata* and *Jurinea mollis* ssp. *transylvanica*. The taxa included in the European and National Red Lists are: *Adonis vernalis* (**Fig. 8B**; V, Dihoru and Dihoru, 1994), *Galanthus nivalis* (**Fig. 8E**; EGO 57/2007, Annex 5 A; Council Directive 92/43/EEC, Annex 5b/VU), *Gymnadenia conopsea* (**Fig. 8D**; R, Oltean et al., 1994), *Orchis morio* (**Fig. 8F**; R, Oltean et al., 1994), *Salvia nutans* (V, Dihoru and Dihoru, 1994) (Bilz et al., 2011; Mihăilescu et al., 2015; Oroian et al., 2017; Sămărghițan et al., 2017).

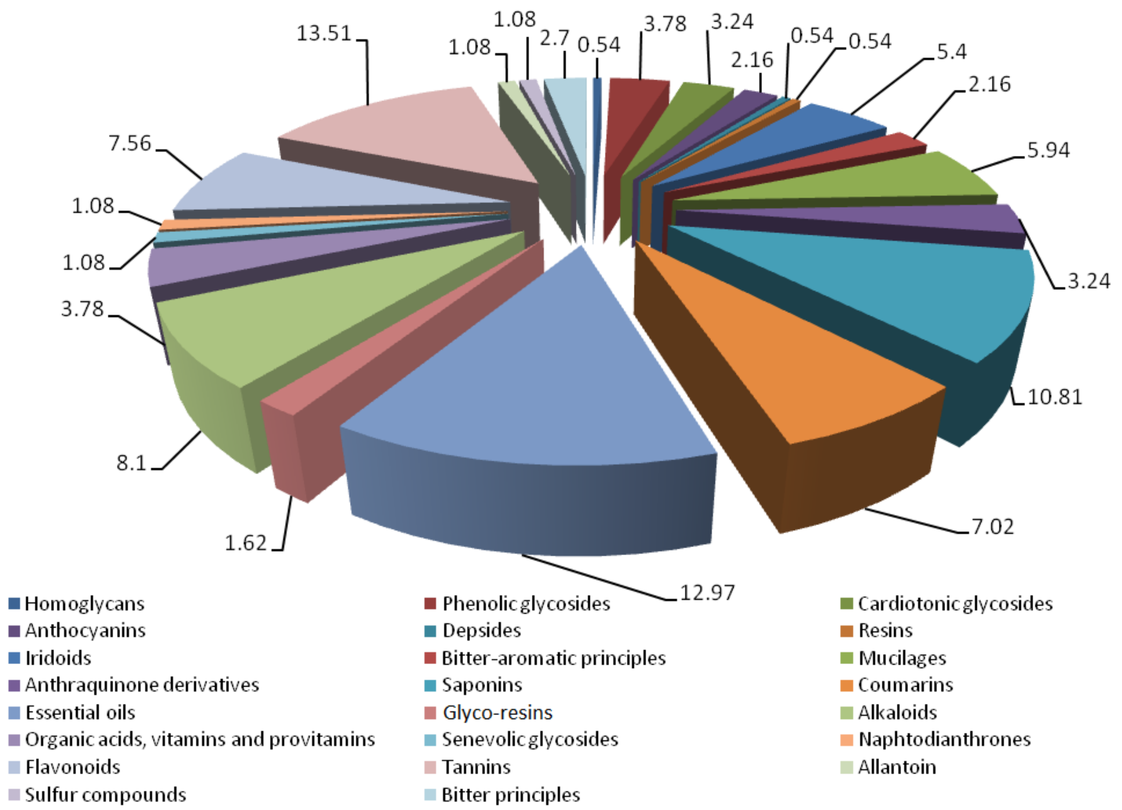

Fig. 7. The spectrum of the dominant active principles present in medicinal plants from the surroundings of Ulieș Village

Table 2. The dominant active principles of medicinal plants from the surroundings of Ulieș Village used in various human diseases

Dominant active principles	**Taxa/ Presence in the Pharmacopoeia**	**Drugs**	**Phytotherapy for human disease/Disorders of various systems**
Homoglycans	*Arctium lappa*	Radix	Dermatological problems: acne, eczema
Mucilages	*Hibiscus trionum* (see **Fig. 9C**, in the Supplementary Material)	Herba	Urogenital system disorders: diuretic/ aquaretic
	Malva sylvestris (**Fig. 9B**) **Eur. Ph.**	Flos et folium	Diseases of the mouth; respiratory system disorders (antitussives); dermatological problems (eczema)

	Orchis morio	Tuber	-
	Plantago lanceolata **Eur. Ph.** *Plantago major* *Plantago media*	Folium	Digestive system disorders: hyperacid gastritis and ulcer; respiratory system disorders (antitussives); dermatological problems: wounds
	Tussilago farfara	Folium	Respiratory system disorders (antitussives)
Senevolic glycosides	*Raphanus raphanistrum*	Herba	Hypoglycemic
	Sisymbrium officinale	Herba	Respiratory system disorders: expectorants
Phenolic glycosides	*Populus nigra* *Populus tremula*	Gemma	Dermatological disorders: wounds, superficial burns; locomotor system problems: anti-inflammatory/analgesic action
	Pyrus pyraster	Folium	Urogenital system disorders: antimicrobial
	Salix alba **Eur. Ph.** ***contains tannins** *Salix cinerea*	Cortex	Locomotor system problems: anti-inflammatory/analgesic action, arthrosis, rheumatoid arthritis
	Viburnum lantana	Cortex	Central nervous system disorders: sleep disturbances, nervousness
Anthraquinone derivatives	*Frangula alnus* **Eur. Ph., Rom. Ph.** *Rhamnus catharticus*	Cortex	Digestive system disorders: constipation
	Rumex acetosa	Herba	Digestive system disorders: constipation
	Rumex acetosella	Herba	
	Rumex conglomeratus	Rhizoma	
	Rumex crispus	Rhizoma	
Naphtodianthrons	*Hypericum perforatum* **(Fig. 8A)** **Eur. Ph., Rom. Ph.**	Herba	Digestive system disorders: hyperacid gastritis and ulcer, acute and chronic liver disorders, functional disorders of the bladder and biliary tract; respiratory system disorders: immunostimulants; dermatological problems: wounds, superficial burns; locomotor system problems: anti-inflammatory/analgesic action; central nervous system disorders (depressions)
Cardiotonic glycosides	*Adonis vernalis*	Herba	Cardiovascular system disorders: heart failure
	Convallaria majalis	Herba	Cardiovascular system disorders: heart failure
	Digitalis grandiflora	Folium	Heart failure
	Helleborus purpurascens	Rhizoma et radix	Cardiovascular system disorders: heart failure
	Leonurus cardiaca **Eur. Ph.**	Herba	Cardiovascular disease: cardiac neurosis
Saponins	*Anagallis arvensis*	Herba	Urogenital and locomotive disorders
	Bupleurum falcatum **Eur. Ph.**	Radix	Digestive system disorders: hyperacid gastritis and ulcer, fatty liver
	Eryngium planum	Herba	Respiratory system disorders: bronchodilators

	Equisetum arvense **Eur. Ph., Rom. Ph.**	Herba	Diseases of the mouth, hyperacid gastritis and ulcer; respiratory system disorders: immunostimulants; urogenital disorders: diuretic/ aquaretic
	Hedera helix **Eur. Ph.**	Herba	Respiratory system disorders: bronchodilators
	Ononis arvensis *Ononis spinosa*	Radix	Urogenital system disorders: diuretic/aquaretic
	Primula veris **Eur. Ph., Rom. Ph.**	Rhizoma cum radicibus	Respiratory system disorders: expectorants
	Saponaria officinalis	Radix	Respiratory system disorders: expectorants
	Solidago virgaurea **Eur. Ph.**	Radix	Urogenital system disorders: diuretic/aquaretic, antimicrobial; locomotor system problems: antirheumatic teas
	Trifolium pratense	Flos	Menopausal disorders
	Trifolium repens	Herba	Menopausal disorders
	Viola odorata	Herba	Respiratory system disorders: expectorants
	Viola tricolor **Eur. Ph.**	Herba	Urogenital disorders: diuretic/ aquaretic; dermatological problems: acne, eczema
Flavonoids	*Capsella bursa-pastoris*	Herba	Dermatological problems: wounds; gynecological disorders: metrorrhagia
	Crataegus monogyna **Eur. Ph., Rom. Ph.**	Folium,fructus, flos	Cardiovascular system disorders: cardiac neurosis, angina pectoris
	Prunella vulgaris **Eur. Ph.**	Herba	Digestive system disorders: chronic gingivitis; allergies; diabetes, etc.
	Prunus avium	Stipites	Urogenital system disorders: diuretic/aquaretic
	Sambucus nigra **Eur. Ph.**	Flos	Digestive system disorders: constipation; locomotor system problems: antirheumatic teas
Anthocyanins	*Papaver rhoeas* **Eur. Ph.**	Flos	Respiratory system disorders; central nervous system disorders
	Rosa gallica	Flos	Urogenital system disorders: diuretic/aquaretic
Coumarins	*Fraxinus excelsior* **Eur. Ph.**	Folium	Digestive system disorders: constipation
	Galium aparine *Galium verum*	Herba	Urogenital system disorders: diuretic/aquaretic; dermatological disorders: eczema, psoriasis
	Medicago falcata *Medicago lupulina*	Herba	Gynecological disorders: menopausal disorders
	Medicago sativa	Herba	Digestive system disorders: hyperacid gastritis and ulcer; menopausal disorders
	Melilotus officinalis **Eur. Ph.**	Flos et herba	Digestive system disorders: hyperacid gastritis and ulcer
	Pastinaca sativa	Radix	Digestive system disorders: diseases of the mouth, functional disorders of

			the bladder and biliary tract
Tannins	*Agrimonia eupatoria* **Eur. Ph.**	Herba	Digestive system disorders: diseases of the mouth, functional disorders of the bladder and biliary tract, diarrhea
	Epilobium hirsutum *Epilobium parviflorum*	Herba Herba	Urogenital disorders: benign prostatic hyperplasia
	Geranium robertianum	Herba	Digestive system disorders: irritated colon, hemorrhoids
	Geum urbanum	Rhizoma	Digestive system disorders: diseases of the mouth, diarrhea
	Juglans regia	Folium, pericarpium	Digestive system disorders: diseases of the mouth, diarrhea; respiratory system disorders: immunostimulants; dermatological disorders: eczema
	Lysimachia nummularia *Lysimachia vulgaris*	Herba	Digestive system disorders: diseases of the mouth
	Lythrum salicaria **Eur. Ph.**	Herba	Digestive system disorders: diseases of the mouth, diarrhea
	Polygonum aviculare	Herba	Urogenital system disorders: diuretic/aquaretic
	Potentilla anserina	Herba	Digestive system disorders: diseases of the mouth, diarrhea; gynecological disorders: dysmenorrhea
	Prunus spinosa	Flos, fructus	Digestive system disorders: constipation
	Quercus robur **Eur. Ph.** *Quercus petraea*	Cortex	Digestive system disorders: diseases of the mouth, diarrhea
Depsids	*Cichorium intybus* **Eur. Ph.**	Herba et radix	Digestive system disorders: functional disorders of the bladder and biliary tract, constipation
Essential oils	*Achillea millefolium* **Eur. Ph., Rom. Ph.**	Flos	Digestive system disorders: diseases of the mouth, functional disorders of the bladder and biliary tract, diarrhea, abdominal colic, helminthiasis (anthelmintic); respiratory system disorders: immunostimulants; dermatological disorders: eczema, dermato-mycoses, contusions
	Carum carvi **Eur. Ph.**	Fructus	Digestive system disorders: meteorism
	Matricaria chamomilla **Eur. Ph.**	Flos	Digestive system disorders: diseases of the mouth, hyperacid gastritis and ulcer, diarrhea, abdominal colic, meteorism; respiratory system disorders: immunostimulants, expectorants; dermatological problems: eczema, wounds, superficial burns, frostbite; locomotor system problems: anti-inflammatory/analgesic action; gynecological disorders: dysmenorrhea
	Mentha arvensis	Folium	Digestive system disorders: functional

	Mentha longifolia		disorders of the bladder and biliary tract, vomiting, nausea, abdominal colic, meteorism; locomotor system problems: hiperemiant
	Origanum vulgare **Eur. Ph.**	Herba	Respiratory system disorders: asthma
	Picea abies	Turiones	Respiratory system disorders: expectorants; locomotor system problems: hiperemiant
	Pinus sylvestris **Eur. Ph.**	Turiones	Respiratory system disorders: disinfectant of the airway, expectorants; locomotor system problems hiperemiant
	Thymus glabrescens *Thymus pannonicus* *Thymus pulegioides*	Herba	Digestive system disorders: mouth diseases, helminthiasis; dermatological problems: dermato-mycoses
	Tilia cordata **Eur. Ph., Rom. Ph.** *Tilia plathyphyllos*	Flos	Respiratory system disorders: respiratory tract infections, bronchitis; central nervous system disorders: sleep disorders, nervousness
	Valeriana officinalis **Eur. Ph., Rom. Ph.**	Radix	Digestive system disorders: hyperacid gastritis and ulcer, vomiting, nausea; cardiovascular disorders: cardiac neurosis; central nervous system disorders: sleep disorders, nervousness
	Xanthium spinosum	Herba	Urogenital system disorders: micturition disorders
	Symphytum officinale	Radix	Digestive system disorders: mouth diseases, hyperacid gastritis and ulcer; dermatological disorders: wounds, contusions
Resins	*Humulus lupulus* **Eur. Ph.**	Strobuli	Central nervous system disorders: nervousness; menopausal disorders
Glyco-resins	*Convolvulus arvensis* *Calystegia sepium*	Herba	Digestive system disorders: constipation
	Euphorbia cyparissias	Herba	Dermatological diseases: verrucosis
Sulfur compounds	*Armoracia rusticana*	Radix	Urogenital system disorders: antimicrobial
Iridoids	*Ajuga genevensis* *Ajuga reptans*	Herba	Respiratory system disorders: asthma
	Euphrasia rostkoviana	Herba	Ophthalmic disorders
	Lamium album	Herba et flos	Urogenital system disorders: diuretic/aquaretic
	Verbena officinalis **Eur. Ph**	Herba	Digestive system disorders: tonic; in convalescence; central nervous system disorders: headaches, migraines
Alkaloids	*Chelidonium majus* **Eur. Ph., Rom. Ph.**	Herba	Digestive system disorders: functional disorders of the bladder and biliary tract; dermatological diseases: verrucosis
	Datura stramonium	Folium	Respiratory system disorders: bronchodilators

	Echium vulgare	Herba	Urogenital system disorders: diuretic/aquaretic and transpiration stimulant; respiratory system disorders: expectorant; dermatological diseases: wound healing (cicatrization)
	Fumaria officinalis **Eur. Ph.**	Herba	Digestive system disorders: mouth diseases, acute and chronic liver disorders, functional disorders of the bladder and biliary tract
	Solanum dulcamara	Stipites	Dermatological diseases: eczema; locomotor system disorders: anti-rheumatic teas
	Vinca minor **Rom. Ph.**	Herba	Cardiovascular system disorders: hypertension
Bitter principles	*Ballota nigra* **Eur. Ph.**	Herba	Digestive, locomotor and central nervous system disorders
	Centaurium erythraea **Eur. Ph.**	Herba	Digestive system disorders: hypoacidity-dyspepsia, anorexia
	Marrubium vulgare	Herba	Digestive system disorders: hypoacidity-dyspepsia, anorexia, functional disorders of the bladder and biliary tract; respiratory system disorders: immunostimulators
	Taraxacum officinale **Eur. Ph.**	Radix et herba	Digestive system disorders: acute and chronic liver disorders, functional disorders of the bladder and biliary tract; urogenital system disorders: diuretic/aquaretic; Dermatological diseases: acne, eczema; locomotor system disorders: anti-rheumatic teas
Bitter-aromatic principles	*Artemisia absinthium* **Eur. Ph.**	Herba	Digestive system disorders: hypoacidity-dyspepsia, anorexia, functional disorders of the bladder and biliary tract
	Tanacetum vulgare	Herba	Digestive system disorders: helminthiasis
Organic acids, vitamins and provitamins	*Hippophaë rhamnoides*	Fructus	Dermatological diseases: eczema
	Rosa canina **Eur. Ph.**	Fructus	Digestive system disorders: helminthiasis; urogenital system disorders: urolithiasis
	Rubus caesius	Folium	Digestive system disorders: diarrhea
	Urtica dioica **Eur. Ph.**	Folium, Radix	Urogenital system disorders: diuretic/aquaretic, micturition disorders; dermatological diseases: *Alopecia areata*; locomotor system problems: anti-inflammatory/analgesic action, anti-rheumatic teas, arthrosis, rheumatoid arthritis; urogenital system disorders: benign prostatic hyperplasia

Conclusions

The floristic inventory revealed the presence of 415 taxa included in 76 families. There were identified 185 medicinal plant taxa. Plants containing tannins (13.51%) were in higher proportion followed by those with essential oils (12.97%), saponins (10.81%) and alkaloids (8.10%). A total of 40 species have monographs in the Romanian Pharmacopoeia and in the European Pharmacopoeia. Most of the medicinal plants are used in digestive, respiratory, skin, locomotor, genitourinary and cardiovascular disorders. The area gives shelter for 10 rare, endemic and subendemic plant taxa.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Bilz M. Kell SP, Maxted N, Richard V. Lansdown R (2011) European Red List of Vascular Plants, Luxembourg: Publications Office of the European Union, IUCN Publications Services
2. Borza A (1929) Vegetaţia şi flora Ardealului, Schiţă geobotanică, Atelierele grafice „Cultura Naţională", Bucureşti
3. Borza A (1931) Botanic excursion through „The Câmpia", Guide de la sixieme excursion phytogeographique internationale, Roumaine, Institutul de Literatură şi Tipografie „Minerva" SA Cluj
4. Borza A (1936) Câmpia Ardealului. Studiu geobotanic, Tipografia cărţilor bisericeşti, Bucureşti
5. Conţiu VH, Conţiu V (2010) Comuna Râciu. Spaţiu de dăinuire multimilenară. Studiu monografic, Ed. Casa Cărţii de Ştiinţă, Cluj-Napoca, 1-156.
6. Cristea V (2014) Plante vasculare: diversitate, sistematică, ecologie și importanță, Ed. Presa Universitară Clujeană, Cluj Napoca
7. Dihoru G, Dihoru A (1994) Plante rare, periclitate și endemice în flora României-Lista roșie, Acta Botanica Horti Bucurestiensis, 173-197
8. Domokos E, Csizmadia B, Elekes T, Kárp A, Domokos J (2018), The characteristic medicinal plants of different vegetation types from the Niraj Valley, Romania, Acta Biologica Marisiensis, 1(1): 10-17
9. Doniţă N, Ivan D, Coldea G, Sanda V, Popescu A, Chifu T, Paucă-Comănescu M, Mititelu D, Boşcaiu N (1992) Vegetaţia României, Ed. Tehnică Agricolă, Bucureşti
10. Eşianu S, Laczkó-Zöld E (2016) Manual de Farmacognozie, vol. I and II, Ed. University Press, Tîrgu Mureş
11. Eşianu S, Ştefănescu R (2016) Fitoterapie University Press, Tîrgu Mureş
12. Florea N, Munteanu I (2012) Sistemul Român de Taxonomie a Solurilor (SRTS), Institutul Naţional de Cercetăre-Dezvoltare pentru pedologie, agrochimie şi protecţia mediului-ICPA Bucureşti
13. Istudor V (1998) Farmacognozie, Fitochimie, Fitoterapie, vol. 1, Ed. Medicală, Bucureşti
14. Istudor V (2001) Farmacognozie, Fitochimie, Fitoterapie, vol. 2. Ed. Medicală, Bucureşti
15. Istudor V (2005) Farmacognozie, Fitochimie, Fitoterapie, vol. III, Ed. Medicală Bucureşti
16. Mihăilescu S, Anastasiu P, Popescu A (2015) Ghidul de monitorizare a speciilor de plante de interes comunitar din România

17. Nagy ZS (2018) Pharmaco-botanical mapping and evaluation of the medicinal flora – potencial along the Niraj and Târnava Mică Rivers, Acta Biologica Marisiensis, 1(1): 37-52
18. Oltean M, Negrean G, Popescu A, Roman N, Dihoru G, Sanda V, Mihăilescu S (1994) Lista Roşie a plantelor superioare din România, Instit. de biologie, Studii, sinteze, Bucureşti
19. Oroian S (2011) Botanică Farmaceutică, Ed. University Press, Tîrgu-Mureş
20. Oroian S, Sămărghiţan M (2014) Contribution to the study of spontaneous vascular plants from „Câmpia Transilvaniei" (Mureş Country), Marisia Studii şi Mat Studia Scient Nat, Tîrgu Mureş, 33/34: 9-80
21. Oroian S, Sămărghiţan M, Tănase C (2017) Plants species of community interest identified in the flora of the Transylvanian plain (Mureş County), Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii Vol. 27 issue 2:121-126
22. Sămărghiţan M, Oroian S, Tănase C (2017), Endemic species identified in the flora of the Transylvanian plain (Mureş County), Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii, Vol. 27 issue 2:127-134
23. Oroian S, Sămărghiţan M (2018) Flora from Fărăgău area (Mureş County) as potential source of medicinal plants, Acta Biologica Marisiensis, 1(1): 60-70
24. Rácz G, Rácz-KE, Szabó LGy (2012) Gyógynövények ismerete. A fitoterápia és az alternativ medicina alapjai. Ed. Galenus, Budapesta
25. Sârbu I, Ştefan N, Oprea A (2013) Plante vasculare din România - Determinator ilustrat de teren. Ed. VictorBVictor, Bucureşti
26. Stănescu U, Miron A, Hănceanu M, Aprotosoaie C (2002) Bazele farmaceutice, farmacologice şi clinice ale fitoterapiei, vol. I, Ed. „Gr.T.Popa", UMF, Iaşi
27. Stănescu U, Miron A, Hănceanu M, Aprotosoaie C (2002) Bazele farmaceutice, farmacologice şi clinice ale fitoterapiei, vol. II, Ed. „Gr.T.Popa", UMF, Iaşi
28. Stănescu U, Miron A, Hăncianu M, et al. (2004) Plante medicinale de la A la Z. Monografii ale produselor de interes terapeutic, vol. I -II, Ed. Gr. T. Popa, Iași
29. Şoneriu I, Mac I (1973) Judeţele patriei. Judeţul Mureş, Ed. Acad. RSR, Bucureşti
30. Tutin TG, (1991) Flora Europaea, vol**.** I, edit. II, Cambridge University Press
31. Tutin TG et al (1964-1980) Flora Europaea, I-V, Cambridge University Press
32. Yberrt E, Delesalle-FT (coord.) (2013) Larousse des Plantes Medicinales-Identification, preparation, soins, Franţa, Ed. Encyclopedia of Medicinal Plans, edit. II
33. ***Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora
34. ***Emergency Government Ordinance No. 57/2007 on the regime of protected natural areas, conservation of natural habitats, wild flora and fauna, with further amendments
35. ***European Pharmacopoeia Online 9.3 http://online6.edqm.eu/ep903/. Accessed 16 June 2018
36. ***Farmacopeea Română (1993) Ed. a X-a, Ed. Medicală, București
37. ***International Code of Nomenclature for algae, fungi, and plants (Melbourne Code), Regnum Vegetabile 154. Koeltz Scientific Books, 2012, 1-8

ORIGINAL PAPER

THE HERBARIUM FILES OF GYULA ERAZMUS NYÁRÁDY WHICH GOT ABROAD

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Abstract: The herbarium of Gyula Erazmus Nyárády has been assessed to contain around 90.000 sheets. During the past thirty years, the legacy of the famed botanist still found in public institutions and private collection across Romania and Hungary has been studied and processed by Kálmán Váczy, Sándor Bartha and especially Katalin Bartók. However, nothing is known about the sheets that had ended up outside Hungary or Romania. This study proposes to fill this gap to some extent: firstly, it draws attention to sheets from Nyárády's herbarium found in the inventory of the world's biggest botanical gardens, museums and universities of natural science; secondly, it goes into details about the circumstances of collecting the prepared taxa, and the conclusions that can be drawn from this; it presents the reasons why some sheets got abroad; and it adds more data to reconstruct Nyárády's social network. It also indicates that the heritage, now known to consist of around 90.000 sheets, can be completed with the sheets found abroad.

Keywords: herbarium, Gyula Erazmus Nyárády, public institutions, inventory, international and local collections, history of botany.

1. Introduction

Two bigger monographies of the life and work of botanist Gyula Erazmus Nyárády have been written in the past decades (Váczy and Bartha, 1988; Bartók, 2016), but since there are no information about the sheets which got abroad, this study tries to fill this gap in some way. This work draws attention to those sheets, which in the present are in the inventory of the world's biggest botanical gardens, museums and universities of natural science. The study details the circumstances of collecting the prepared taxa, presents the reason why some sheets got abroad, provides data about the scientific social network of Nyárády, and points out that the heritage known to contain 90.000 sheets (Bartók, 2016a; Nagy, 2018) can be completed with the sheets which got abroad.

The professional career of Nyárády was accompanied by his passion for botanizing. He started to collect herbaria in a more serious way when he was only a career entrant, and starting with 1922, when he became the curator of the Museum of Cluj, he could prepare herbaria in a more systematic way, since his passion became his duty and work assignment, he could devote more time.

Since 1921, the Botanical Garden of Cluj published the volumes of *Flora Romaniae Exsiccata* (a thematical collection published in many copies, containing duplicates, which were to be sent out to various institutes), that is the herbal collection of Romania, each volume of it containing 100 herbarium sheets, and was made in 70 copies. With these they could manage to make advantageous transactions with 70 botanical institutes and botanical gardens functioning across the continent (Váczy, 2016). Gyula Erazmus Nyárády participated in this work from the beginnings, and starting with the third volume, he carried the main responsibility of the collection, compilation and sending-out of the volumes, which resulted in his sheets getting abroad.

The reason why his sheets got abroad could be the fact that Nyárády had relationships with Romanian scientists, but with many European botanists, and later, with the assignment of the Academy, he completed foreign missions as well, and he was the member of several foreign botanical associations. Unfortunately, his scientific correspondence, with the help of which his foreign social network could be easily reconstructed, mainly because of the war events in 1944, after his death, all letters perished (in 1983 his widow wiped out their house, and moved to their daughter abroad). Only a small notebook remained, which contains the abstract of his correspondences between 1904 and 1950.

2. Materials and Methods

Following the methods of Katalin Bartók (Bartók, 2016a), who revealed the materials from Romania and Hungary, this study searches the answer to the question: Which foreign collections contain taxa prepared and identified by Gyula Erazmus Nyárády?

Since there are approximately 3000 herbariums in the World (Nagy et al., 2017), the study did not have the opportunity to ask information from all public collections. The results only indicate, that the Romanian and Hungarian Nyárády collection estimated to have 90.000 sheets can and should be completed with the sheets which got abroad.

3. Results and discussions

3.1 Herbarium sheets in Austrian public collections

The herbarium of the Naturhistorisches Museum Wien according to our present knowledge manages the biggest Nyárády collection which got abroad. The number of the sheets found here exceeds one hundred (104), three quarters (76 sheets) of which stem from Gyula Erazmus Nyárády' individual collecting, and one quarter (28 sheets) from collective collectings (collected together with Alexandru Borza (1887–1971), Emil Pop (1897–1974), Eustach Woloszczak (1835–1918) and others). The sheets were made during 1905 and 1930, period which includes the time when Nyárády was in Kežmarok and Târgu Mureș, and the first part of the decades spent in Cluj. Most of the plants come from Slovakia, but the collection contains many sheets from Hungary and Romania, furthermore 3 sheets from Bulgarian and 1 from Polish territory. The taxa represent 6 families of plant: Poaceae, Brassicaceae (7 species of *Alyssum*), Asteraceae (with species of *Hieracium* and *Centaurea*), Potamogetonaceae, Lamiaceae and Salicaceae. The last one contains about 69 species of *Salix*, which during his time in Kežmarok was collected by Nyárády and botanist Eustach Woloszczak, who at that time was already in his seventies. All the *Salix* preparatums of Nyárády were revisioned by the scientist of Ukrainian origin, but who was researching in Vienna, and who in 1917 named a species after Nyárády (*Salix nyárádyi* Woloszcz.), fact that confirms that the young career entrant gained the respect of the times' most acclaimed botanists' at a very young age.

Also in Vienna there are additional 11 herbarium sheets related to Nyárády, kept in the herbarium of Universität Wien (Faculty Center Botany). The sheets were made between 1913 and 1926, 7 of them stem from individual, 4 of them from collective collectings with Emil Pop and were collected from Romania (7), Bulgaria (3) and Hungary (1). The taxa represent 3 families of plant: Brassicaceae (with 9 species of *Alyssum*), Saxifragaceae and Salicaceae (with 1–1 species).

The collection of Karl-Franzens-Universität Graz (Herbarium) also has 2 herbarium sheets with the name of Gyula Erazmus Nyárády. One of them has its origins in 1907 and was collected in Slovakia, and the other one was collected in 1918 in Romania, both being individual collectings (a *Ligularia* and a *Ranunculus* species).

3.2 The Carnegie Museum of Natural History's herbarium in Pittsburgh (USA)

The Carnegie Museum of Natural History's herbarium in Pittsburgh, Pennsylvania according to present knowledge, owns the biggest Nyárády collection of those sheets which got outside of Europe. The 89 herbarium sheets found here provide a hand-picked 'abstract' of the time period when Nyárády was in Cluj. It contains different taxa (81 species) classable in about 25 families of plant (Poaceae, Asteraceae, Caryophyllaceae, Fabaceae, Brassicaceae, Ericaceae, Amaryllidaceae, Ranunculaceae, Apiaceae, Orobanchaceae, Cyperaceae, Cystopteridaceae, Amaranthaceae, Fagaceae, Ceratophyllaceae, Papaveraceae, Cistaceae, Boraginaceae, Asparagaceae, Linaceae, Plantaginaceae, Lamiaceae, Apiaceae, Primulaceae, Rosaceae), which stem from individual collectings made in Romania between 1923 and 1947. Also in this collection there is a herbarium sheet of Nyárády Antal (1920–1982), one of Nyárády's son.

In the inventory of Harvard University (Cambridge, Massachusetts) Herbaria and Libraries Collection there are 4 additional 'Nyárády' sheets. At this time it is still unknown if these sheets were collected by Gyula Erazmus Nyárády, or his son, Antal.

3.3 Nyárády sheets in the collections of Commonwealth of Australia

At present, there is little knowledge about the American relations of Gyula Erazmus Nyárády, and even fewer information about his Australian relationships. The research of this in the present can be done almost solely based on the herbarium sheets. Nyárády's sheets can be found in public botanical collections of three states of the Commonwealth of Australia (Victoria, New South Wales and Tasmania).

The collection of the Royal Botanic Gardens Melbourne (National Herbarium) manages 23 sheets from 1911 and 1947, 16 stem from individual and 7 from collective collectings (with Emil Pop, Eugen Victor Ghișa (1909–1984), Alexandru Borza, George Bujorean (1893–1971), Traian Ștefureac (1908–1986)). The plants collected from Bulgaria (16), Romania (6) and Republic of Moldova (1) belong to 10 families including *Berberis*, *Celtis*, *Ceratodon*, *Ficus* and other species.

The Royal Botanic Garden Sydney's herbarium (National Herbarium of New South Wales) owns 17 herbarium sheets originating from Nyárády's fieldwork in 1911 carried out in Romania (16) and Hungary (1). More than half of the taxa are *Centaurea*.

The collection of the Tasmanian Museum and Art Gallery (Tasmanian Herbarium) in Hobart can be proud of 1 'Nyárády' sheet. According to the data written on the label, the sheet is a *Genista* species collected in 1925 in Romania, which presumably did not get to the institution directly from Nyárády, but thanks to the inter-institutional exchanges in Australia.

3.4 Nyárády's legacy in the United Kingdom

The Royal Botanic Gardens Kew's herbarium in London owns 25 Nyárády sheets. Most of them were collected between 1920 and 1931 in Romania – 20 individual and 5 collective (with George Bujorean, Emil Pop and Alexandru Borza) collectings. The taxa represent 7 families: from the Asteraceae family 3 *Centaurea*, from the Brassicaceae family 6 *Alyssum* and from the Poaceae family 5 *Poa* species are to be found in the collection; the remaining taxa are from the Cupressaceae, Pinaceae, Rosaceae and Violaceae families.

Also in London, the inventory of collection of the Natural History Museum (Herbarium) contains 40 additional 'Nyárády' sheets, however there is no further information about this at present.

In Edinburgh, Scotland, the Royal Botanic Garden Edinburgh's herbarium owns the herbarium sheets of *Alyssum* species (11) which were collected and first identified by Nyárády. Further 6 sheets contain 1–1 exemplar of plant genus *Alnus*, *Corylus*, *Cardamine*, *Campanula*, *Saxifraga* and *Tournefortia*, which were collected between 1923 and 1926 in Hungary and Romania. The collection, a total of 17 sheets, is the result of individual (14 sheets) and collective (3 sheets collected with George Bujorean, Alexandru Borza) collectings.

3.5 The Herbarium of Botanic Garden Meise (Belgium)

The Nyárády collection of the Herbarium of Botanic Garden Meise with its 18 sheets (16 taxa) is the most valuable fraction of the sheets which got abroad, as (with the exception of 2 sheets), it is a considerable fungi-preparatum collection. Until now Nyárády was not known to be a mycologist and plant pathologist in the scientific field. The heritage from the Kingdom of Belgium is the witness of the fact that the scientist while working in Cluj, before he was elected academic (between 1923 and 1947), was quite interested in those fungi genera, which are very important from the perspective of plant pathology (*Puccinia*, *Erysiphe*, *Anthracoidea*, *Golovinomyces*, *Podosphaera* etc.), and conducted researches in this field in territories of Romania and Republic of Moldova.

3.6 Nyárády sheets kept in Czech and Ukrainian universities

Nyárády and his institution had relationships with many Central and Eastern European universities. The proof of this can be found in the herbarium of two Czech and two Ukrainian universities.

The collection of the Masaryk University (Herbarium – Department of Botany and Zoology), in the city of Brno has 11 Nyárády sheets collected between 1912 and 1939 from Hungarian and Romanian territories. A total of 6 sheets stem from individual and 5 from collective (Alexandru Buia (1911–1964), Alexandru Borza and Péterfi Ștefan (1906–1978)) collectings: species of *Bifora*, *Caucalis*, *Chaerophyllum*, *Prangos* (2), *Hieracium* (3), *Leucanthemum*, *Riccia* and *Stipa*.

The Charles University in Prague's herbarium (Department of Botany) enriches the Czech Nyárády collection by 2 additional taxa: a *Taraxacum* species collected and identified together with George Bujorean in 1923, and a *Doronicum* species collected and identified together with Alexandru Borza in 1933.

The inventory of the Ukrainian Yu. Fedecovich Chernivtsi State University's herbarium (Botany Department) in the city of Chernivtsi contains 8 sheets under the name of Gyula Erazmus Nyárády. Some stem from individual collecting carried out in territories of

Romania (7) and Bulgaria (1) between 1926 and 1930: 5 species of *Alyssum* and 3 species of *Hieracium*.

The herbarium of the likewise Ukrainian Ivan Franko National University of Lviv owns 4 sheets, which were created between 1923 and 1925 (individual collectings from Romania, from families of plants Asteraceae and Poaceae).

3.7 Smaller collections (Republic of Armenia, Federal Republic of Germany)

During this research many collections were found, which, for now, own just a few sheets which can be related to Erazmus Nyárády. Some of these points out that Nyárády had scientific relationships even in the countries of Asia. For example in the herbarium of the Institute of Botany of the National Academy of Sciences of Armenia in Yerevan, the capital city of Armenia, there is only one *Picris* (Asteraceae) from Nyárády's individual collecting from 1925 in Romania.

Likewise, the German public collections have only a few Nyárády sheets in their property. The Botanischer Garten und Botanisches Museum Berlin's herbarium (Herbarium Berolinense) has only 6 sheets (from the period 1924 and 1932, from Romanian and Bulgarian territories, individual collectings), even though we know that Nyárády was in a constant relationship with the workers of the botanical garden and museum of Berlin (Friedrich Markgraf (1897–1987), Friedrich Ludwig Emil Diels (1874–1945), Robert Knud Friedrich Pilger (1876–1953)), beeing a good german speaker, and in 1932 he even published an article in the institutional magazine *Notzblatt des Königl* about the ambiguous *Alyssum* species (Nyárády, 1932). In this same institution 3 herbarium sheets can be found with the name of Nyárády Antal.

The herbarium of Friedrich-Schiller-Universität Jena (Herbarium Haussknecht) in Central Germany owns 3 sheets, which are related to the name of Nyárády. The three sheets are individual and collective (with Emil Pop) collectings made between 1926 and 1928 in Romania.

Lastly, it is worth to mention the Leibniz Institute of Plant Genetics and Crop Plant Research's herbarium in Gatersleben, where next to the two *Allium* species herbarium sheets of Erazmus Gyula Nyárády which are from an individual collecting from 1949 in Romania, there is a significant *Allium* collection made of 15 sheets by Antal Nyárády.

3.8 General aspects about the foreign herbarium collections

The 18 foreign herbarium collections presented in this study altogether own around five hundred Nyárády sheets (218 taxon) in 9 countries all over the Globe (**Fig. 1**). The research should be continued, since there is known that Nyárády had relationships in cities like Belgrade, Athens, Leningrad, Sofia, Geneva, Basel, Zürich, Turin, Cagliari, Bremen, Weimar, Zagreb, Amsterdam, Krakow, Uppsala and other relations (Váczy, 2016a) (**Fig. 1**).

Concerning the appearance of the sheets, it is important to mention that the sheets of Nyárády were of regular size, made of board, and on each sheet there was only one species. A label was glued on the right bottom of the sheet, containing the scientific name of the plant, the place it was collected, its exposal, a short ecological characterization, and the time of the collecting, the name of the collector and the name of the identifier.

The waste majority of the sheets which got abroad are sterling exemplars, since the scientist placed every important part of the plant with the help of which it can be identified and the taxon was sent with its scientific name and the place and time of collecting (year, month, day).

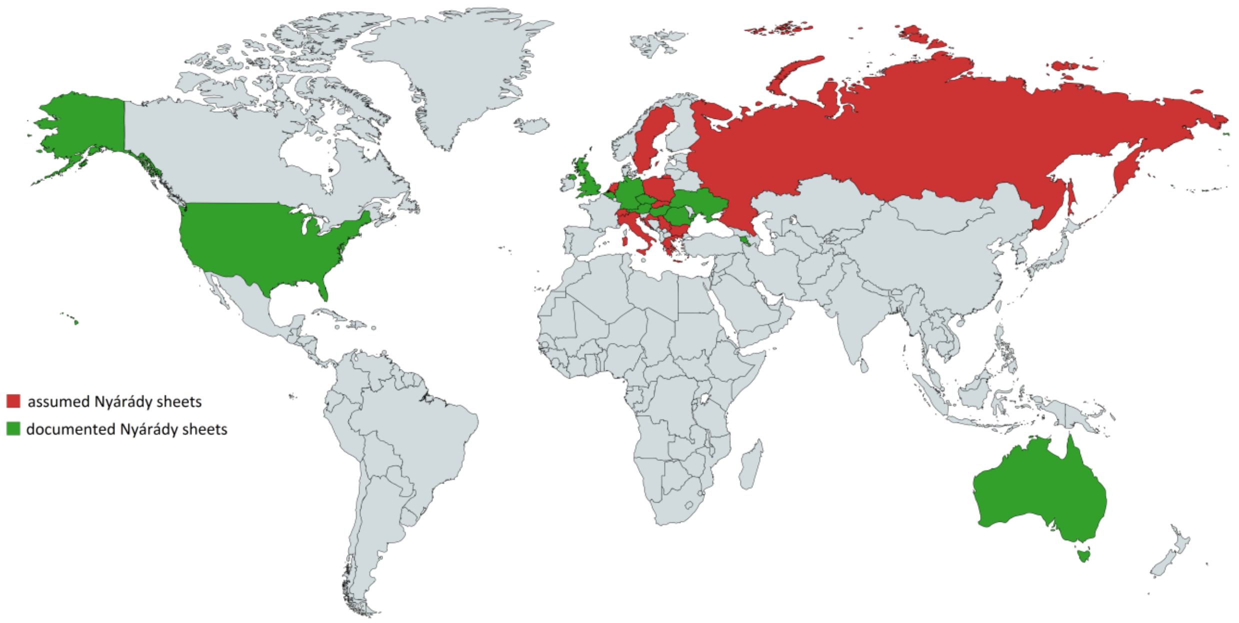

Fig. 1. Countries in the public collections of which, according to the present knowledge there are Nyárády sheets and countries where presumably additional sheets could be found (original, 2018)

Some sheets contain latter revision labels which reflect the changes of the taxonomy and nomenclature in the last half century, since at large Nyárády identified correctly the plants he collected. Thanks to the attachment of this accurate collecting data, other information (botanical descriptions, ethnobotanical knowledge, etc.), his herbarium sheets are of great significance not only in phytogeography, taxonomy and nomenclature research but also in other fields of science such as ethnobotanics, ethnogeobotanics, ethnopharmacobotanics and cultural history. They provide essential information regarding the folk names and uses of certain plants, data (dates, locations) of researchers' activities, life stories, research pathways, inter-institutional relations and the reconstruction of the formation of public collections.

Questioning the relevance of this herbarium research and exploration should be of no use, knowing that in the last decade the results of the Google Academic search engine for the „herbarium" word reached 60.000 (Takács et al., 2017), furthermore, the natural science collections, the herbarium was the central topic of the 12th Advances in research on the flora and vegetation of the Carpato-Pannonian region international conference (debates, presentations etc.) (Molnár, 2018).

Conclusions

The herbarium sheets of Nyárády which got abroad have great importance in the history of science. The public collections in Europe, America, Asia and Australia are just a 'resume', the 'essence' of the diversified botanical work and the inland herbarium – containing 90.000 sheets – of the natural scientist. Nyárády was attentive that those species he considered important (in many cases these were discovered and identified by him) to be sent in form of herbarium to botanical gardens, museums of natural science and the herbariums of universities.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Bartók K (ed.) (2016) Nyárády Erazmus Gyula emlékezete, Kriterion Könyvkiadó, Kolozsvár
2. Bartók K (2016a) Nyárády herbáriumának és hagyatékának sorsa. In: Bartók K (ed.) Nyárády Erazmus Gyula emlékezete, Kriterion Könyvkiadó, Kolozsvár, pp 78–94
3. Nagy T, Takács A, Molnár VA (2017) A herbáriumok históriája és haszna 1. A szárított kerttől a modern gyűjteményekig. Élet és Tudomány 62(22):687–690
4. Nagy Zs (2018) Nyárády Erazmus Gyula külföldre került hagyatéka. Erdélyi Múzeum 80(2):36–52
5. Nyárády EGy (1932) Die Klarstellung zweier zweifelhafter Alyssum-Arten. Notizblatt des Königl 11(107):631–635
6. Molnár VA, Sonkoly J, Takács A (eds.) (2018) Programme and Abstracts. 12th International Conference Advances in research on the flora and vegetation of the Carpato-Pannonian region, University of Debrecen, Department of Botany, Debrecen
7. Takács A, Nagy T, Molnár VA (2017) A herbáriumok históriája és haszna 2. Mire jók a növénygyűjtemények? Élet és Tudomány 62(24):752–754
8. Váczy K (2016) A kiteljesedés évei (1922–1948). In: Bartók K (ed.) Nyárády Erazmus Gyula emlékezete, Kriterion Könyvkiadó, Kolozsvár, pp 42–46
9. Váczy K (2016a) Levelezése külföldi botanikusokkal (1903–1948). In: Bartók K (ed.) Nyárády Erazmus Gyula emlékezete, Kriterion Könyvkiadó, Kolozsvár, pp 144–145
10. Váczy K, Bartha S (1988) Nyárády Erazmus Gyula, a természettudós, Kriterion Könyvkiadó, Bukarest

ORIGINAL PAPER

HEMP SHIVES AS NATURAL AMENDMENTS IN CROPS OF RAPESEED (*BRASSICA NAPUS*) AND FLAX (*LINUM USITATISSIMUM*)

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Abstract The aim of this study is to evaluate the possibility of using hemp shives as natural amendments in a phytoremediation sequence. Thus, plant growth tests were conducted for rapeseed (*Brassica napus*) and flax (*Linum usitatissimum*) in vegetation pots with sandy soil. These were seeded in a cadmium artificial contaminated environment with or without hemp shives. The efficiency of hemp shives as natural amendments was evaluated after 40 days of initiating experimental model, by evaluation of plant growth and development of plants through biometric and gravimetric measurements. Also, the concentration of assimilatory pigments was determined. In order to establish bioaccumulation capacity, degree of recovery and translocation factor, it was determined the concentration of cadmium metal ions absorbed in various parts of rapeseed and flax plants. It has been found that the development of the root system is more pronounced in the presence of hemp shives both in contamination with cadmium ions and in the absence. By determining cadmium concentrations accumulate in various parts of the plant have been shown that supplementing growth media with hemp shives, improves the bioaccumulation process of cadmium ions. According to the results, the hemp shives can be used as amendments in phytoremediation process, enhancing bioaccumulation process.

Keywords: amendments, cadmium, hemp shives

1. Introduction

The concentrations of heavy metals in the soil are currently increasing as a result of industrial activities. These, can cause severe damage to biochemical and physiological process in humans and animals (Jankaite and Vasarevičius 2005; Bech et al., 2012; Choi et al., 2013).

Cadmium (Cd) is a heavy metal that is of great concern in the soil with higher toxicity in living systems. Cadmium toxicity especially affects humans, because of their longevity and the accumulation of Cd in their organs by eating contaminated food (Tudoreanu and Phillips 2004). The pathways by which Cd enters in the environment are through industrial waste from processes such as manufacturing of plastics, mining, electroplating etc. (Adriano 2001; Cordero et al., 2004).

In order to ensure the protection of soil, several researches indicated technologies and methods of phytoremediation to neutralize or block the flow of pollutants and to obtain an efficient protection of soil quality (Stingu et al., 2009, 2011). These include physical remediation, chemical remediation, phytoremediation, and agro-ecological engineering techniques (Chen et al., 1999; Chen 2000). Many of this researches indicated that the uptake of heavy metals by plants can be influenced by soil pH (Eriksson 1989), soil organic matter content and plant species (Bingham et al., 1979).

There has been increasing interest in phytoremediation through the use of plants to reduce concentration of pollutants. Phytoremediation is a cost effective and eco-friendly “green” remediation technology. This technique refers to the use of plants to detoxify a polluted environment (Brooks 1998a). The advantages of phytoremediation are that the procedure is carried out in situ and it is inexpensive compared to other technologies. To remediate metal-contaminated soils, usually the method is phytoextraction. The technology relies on plants that translocate heavy metals from soil through their uptake and accumulate heavy metals (Robinson et al., 2000; do Nascimento et al., 2006). This technique is effective only if the plants accumulate high concentrations of metals in shoots (Stingu et al., 2011) and a reasonable amount of biomass is produced (McGrath and Zhao 2003). The plant biomass is removed from the site and can be burned to reduce its volume.

The use of wastes as amendments to improve soil organic matter level and long-term soil fertility and productivity is gaining importance (Kelechi et al., 2018). Phytostabilization technique is to establish a vegetation cover and causing in situ inactivation of trace elements by combining the use of metal-tolerant plants with soil amendments. The results are decrease of mobility and toxicity of pollutants, increase soil fertility and improvement of plant establishment. The plants recommended for phytostabilization should retain the metals at the root level restricting their transport to aerial parts thus avoiding the further transfer into the food chain (Wenzel et al., 1999, Lou et al., 2018). Another approach is the use of soil amendments: material added to a soil to improve its physical properties as water retention, permeability, water infiltration, drainage, aeration and structure to provide a better environment for roots development (Davis and Wilson 2005). In this context, it is necessary to improve methods for removing, reducing or mitigating toxic substances introduced into soil via human activity.

Novel techniques of phytoremediation involve biostimulation of both plant growth and its remediating potential using extracts and exudates obtained from various biological materials. There is evidence that crude plant extracts or exudates may improve the growth and development of plants potentially exploited in phytoremediation (Hanus-Fajerska et al., 2012; Wiszniewska et al., 2013). Stingu et al., (2012) proposed to exploit natural amendments in phytoremediation of Cd. This study has concluded that polyphenolic extracts from *Picea abies* bark and *Asclepia syriaca* plant stimulated cadmium bioaccumulation, promoting the translocation of heavy metals to the stem and leaves.

In other studies (Tanase et al., 2014) a multi-metal contaminated soil from a site located close to an energy power plant was used to study a phytoremediation process using rapeseed plants, in both the absence and presence of hemp shives as natural amendments. It was found that presence of hemp shives in growth medium, stimulated lead and copper bioaccumulation in rapeseed, and promoted the translocation of heavy metals to the aerial part of the plant. The conclusions were that polyphenolic compounds eluted from hemp shives could properly participate in phytoremediation as natural amendments, increasing the solubilization of heavy metals and improving the phytoextraction process. Another effect could be the reduction of Pb and Cu mobility, decreasing thus translocation, and determining in situ inactivation of heavy metal ions. All these make hemp shives suitable for phytostabilization. The mode of the action and effectiveness of numerous amendments in soil remediation is well known.

The aim of this study is to evaluate the possibility of using hemp shives as natural amendments in a phytoremediation sequence with *Brassica napus* L. and *Linum usitatissimum* L. plants, cultivated on sandy soils.

2. Materials and Methods

2.1 Materials

The hemp shives used in this study are wastes resulted after milling process of hemp stem. After, 70-80% of the material is rejected as shives. Sometimes hemp shives are used as low quality construction materials (tiles that contain fiber, gypsum, cement), as bedding for composting in agriculture, or are converted into fuel briquettes. However, the accessibility of these products led currently to their usage as soil amendment in phytoremediation. For the research, the usage of a sandy soil was proposed in order to eliminate the complicated transformations that could take place in a common soil, and to highlight the influence of the amendment used. Rapeseed and flax seeds were purchased from the "Unisem" seed distribution company, Romania.

2.2 Experimental assessment

Sandy soils contaminated with cadmium chloride $CdCl_2.H_2O$ were tested in different conditions (**Table 1**), both in the absence (RC0, RCd, IC0, ICd) and presence (RCp, RCd+p, ICp, ICd+p) of hemp shives (1g/30cm^2). The plants have been cultivated in greenhouse conditions using three samples of contaminated sand (200g/pot). *Brassica napus* and *Linum usitatissimum* seeds were directly sown into pots. Each sample was replicated in ten pots, and three uniform plants have been spaced evenly in each pot and were allowed to grow. The sand used for cultivation was wetted daily with 10 mL tap water for one week, until germination of the plants was observed. From this point, rapeseed plants were wetted every two days with the same amount of water.

Table 1. Experimental variants

Plants treated	Experimental variants (symbol)	Conditions
Rapeseed *Brassica napus*	Control (RC0)	200g sand, 3 seeds/pot, water
	Control with hemp shives (RCp)	200g sand, 1g hemp shives, 3 seeds/pot, water
	Cadmium (RCd)	200g sand, 100µg Cd (II)/ g sand, 3 seeds/pot, water
	Cadmium with hemp shives (RCd+p)	200g sand, 100µg Cd (II)/ g sand, 1g hemp shives, 3 seeds/pot, water
Flax *Linum usitatissimum*	Control (IC0)	200g sand, 3 seeds/pot, water
	Control with hemp shives (ICp)	200g sand, 1g hemp shives/pot, 3 seeds/pot, water
	Cadmium (Icd)	200g sand, 100µg Cd (II)/ g sand, 3 seeds/pot, water
	Cadmium with hemp shives (ICd+p)	200g sand, 100µg Cd (II)/ g sand, 1g hemp shives, 3 seeds/pot, water

2.3 Plantlet analysis

Plant growth and development - To evaluate the influence of natural amendment on plant growth and development in Cd induced stress conditions, after 40 days, the rapeseed and flax plants were separated into roots, stems and primary leaves, followed by biometric measurements of plant elongation and quantitative determinations of biomass. Samples of separated plant have been oven dried at 70°C until constant values of mass were reached and the dry biomass was quantified.

Determination of Cd concentration in plants - For Cd ions analysis, plant tissues (root, stem and leaves) were digested with a mixture of nitric and hydrochloric acids (10.5 mL HCl 12 mol/L and 3.5 mL HNO_3 15.8 mol/L) for 12 hours and, subsequently, they were kept on a hot plate for another 2 hours. The concentrations of cadmium were determined by calibration curves obtained using standards solutions of pure cadmium ions (Fisher Scientific). Calibration curves were generated using three replicates per metal concentration (0.02-5.00 µg/mL) by reading the absorbance at fixed wavelength. Determination of the cadmium ions concentration was carried out by reading a fixed wavelength at 228.80 nm, using the right calibration equation:

$y = 0.128x + 0.0039$, $R^2 = 0.9995$,

obtained for concentrations interval between 0.01-1.8.00 µg/mL.

The concentrations of cadmium ions were determined using a GBC 2008 Avanta Atomic Absorption Spectrophotometer. The following parameters have been calculated:

Translocation factor (TF) = metal concentration in shoots (mg/kg)/metal concentration in roots (mg/kg) (Sun et al., 2009).

Photosynthetic pigments assay - The chlorophyll was extracted in 80% acetone and determined spectrophotometrically by measuring the absorbance at fixed wavelengths of 470, 646, 663 nm. The concentrations of chlorophyll pigments were calculated by using the specific coefficients suggested by Lichtenthaler and Wellburn (1983):

Chlorophyll a (µg/mL) = 12.21 (A 663) – 2.81 (A 646)

Chlorophyll b (µg/mL) = 20.31 (A 646) 5.03 (A 663)

Carotenoids (µg/mL) = (100 x A 470 – 3.27 [Chl a] – 104 [Chl b])/22.

2.4 Statistical analysis

All the results are expressed as mean ± standard error, where n = 3. Comparison of the means was performed by Kruskal-Wallis test and Mann-Whitney U test (post-hoc). Sampling and chemical analyses were examined in triplicate, in order to decrease the experimental errors and to increase the experimental reproducibility.

3. Results and discussions

3.1 Characteristics of hemp shives

In a previous paper was found that the total polyphenols content of hemp shives aqueous extract was 16.41 ± 0.62 g/100g (Tanase et al., 2014). The chromatographic analysis revealed the following components: catechin, caffeic acid, p-coumaric acid and ferulic acid. Thus, the amount of catechins was 5.21 g/100 g plant material and caffeic acid was present in a higher concentration (9.68 g/100g) compared with those of other identified phenolic acids.

3.2 The seed germination of rapeseed and flax

When the growth medium previously contaminated with cadmium ions was supplemented with hemp shives, an increase for rapeseed germination energy and capacity was observed. This shows that the introduction of hemp shives in cadmium ions contaminated environment improves germination rate of rapeseed (**Fig. 1**). In the case of flax, the presence of hemp shives in an environment contaminated with cadmium ions has stimulated the germination energy and capacity. When the medium was contaminated with cadmium ions a decrease of the value for this two studied parameters could be observed (**Fig. 1**). The number of germinated seeds in the environment contaminated with cadmium ions was 12% higher in the presence of hemp shives.

3.3 Growth and development of rapeseed and flax

The development of rapeseed root system was more evident in the presence of hemp shives with or without artificial contamination by cadmium ions (**Fig. 2**). Following biometric measurements for flax, no significant differences were found. When hemp shives were introduced in the soil as amendments, there was an increase in the average length of root and stem.

Concerning the amount of plant biomass accumulated in rapeseed, stimulating effects could be seen in case of plants grown on hemp shives supplemented medium (**Fig. 3** and **4**). The percentages for accumulated biomass in treated plants were over those of the control in all experimental variants (**Fig. 4**). It was found that the soil enriched with hemp shives favored vegetative biomass accumulation in all organs of the plant (rapeseed or flax), especially in the roots (**Fig. 3**) and leaves (**Fig. 4**). When hemp shives were added in Cd contaminated environment, an increase in biomass accumulation in all vegetative organs of the plant was observed.

The concentration of chlorophyll pigments (Chl a + b) synthesized in the leaves was higher in rapeseed and flax grown on hemp shives supplemented environment, compared with the control. The same results were obtained for carotenoids. Analysing the variation of photosynthetic pigments concentration in flax plants, there was a stimulation process for all experimental variants unless Cd ion contaminated soil was mixed with hemp shives. In this case the synthesis of chlorophyll-a and carotenoids was higher compared to the control (with 41.85% and 37%, respectively).

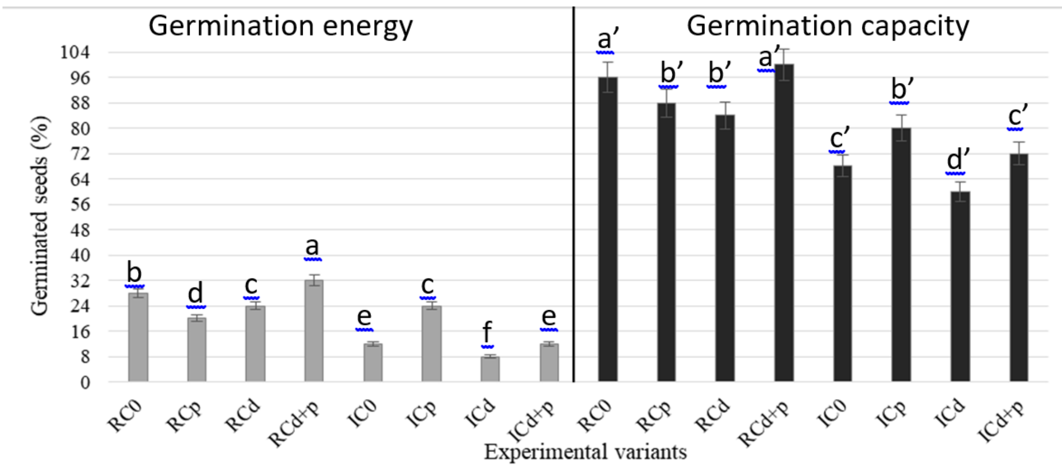

Fig. 1. The influence of hemp shives on germination energy and capacity of rapeseed and flax seeds in cadmium contaminated environment. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means ($n = 3$). The explanations of experimental variants are in Table 1.

Table 2. Variation of photosynthetic pigments content in leaves of rapeseed and flax plants

Experimental variants	Chl a µg/g	Chl b µg/g	Caroten µg/g	Chl a+b	Chl a/b
RC0	782.75±12.54 g	143.60±2.21 e	187.40±4.7 g	926.35	5.45
RCp	989.57±21.74 e	196.02±3.54 b	209.59±5.44 f	1185.60	5.05
RCd	800.65±10.21 f,g	136.60±5.57 e	183.22±7.41 g	937.25	5.86
RCd+p	900.73±5.22 e,f	170.30±9.71 c	207.24±9.11 f	1071.03	5.29
IC0	1116.91±8.41 c	187.46±8.55 c	317.41±4.54 c	1304.37	5.96
ICp	1269.76±9.55 b	242.18±2.22 a	346.13±3.87 b	1511.94	5.24
ICd	945.29±11.33 e	137.09±5.87 e	284.61±5.21 d	1082.38	6.90
ICd+p	1340.90±22.87 a	201.93±5.11 b	389.93±6.17 a	1542.83	6.64

Different letters means statistical significant differences at $p \leq 0.05$. The explanations of experimental variants are in Table 1.

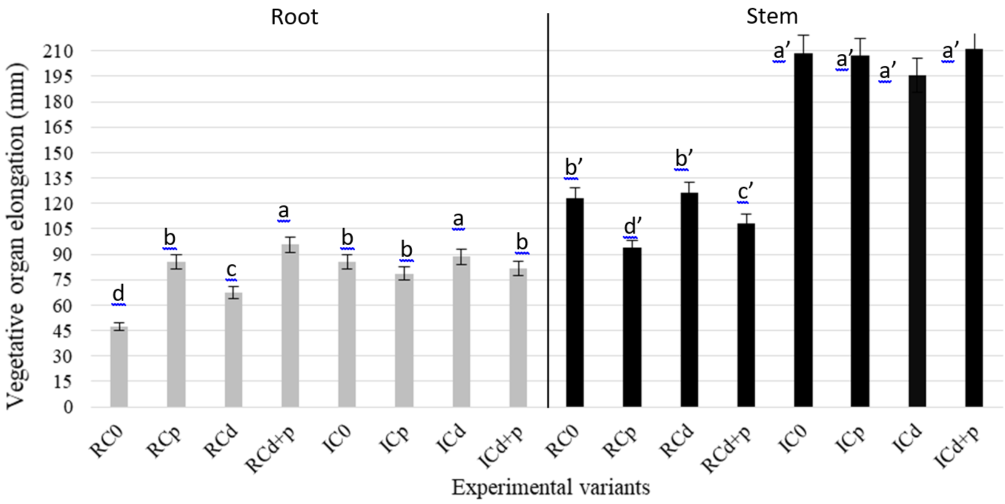

Fig. 2. The influence of hemp shives on root and stem elongation of rapeseed and flax seeds in cadmium contaminated environment. Different letters means statistical significant differences $p \leq 0.05$. Error bars represent the standard deviation of means ($n = 3$). The explanations of experimental variants are in Table 1.

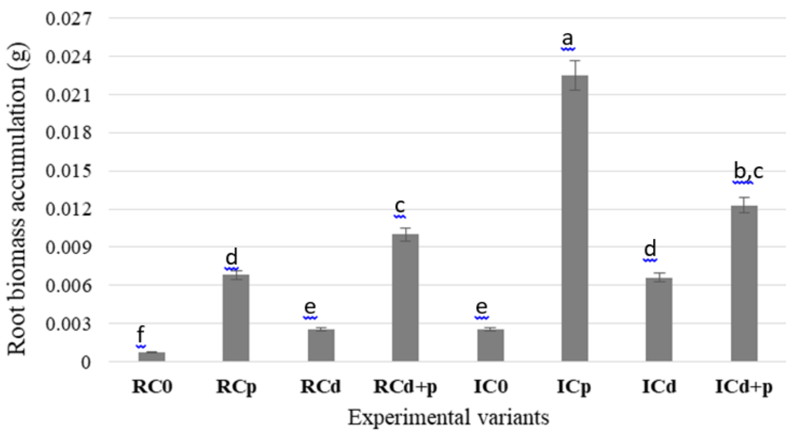

Fig. 3. The influence of hemp shives on rapeseed and flax root biomass accumulation in cadmium contaminated environment. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means ($n = 3$). The explanations of experimental variants are in Table 1.

3.4 Bioaccumulation of cadmium in rapeseed and flax

The determination of cadmium ions accumulated in various parts of the rapeseed and flax plant, showed that soil supplementing with hemp shives improve the bioaccumulation process of metal ions. The presence of the natural amendments (1 g hemp shives) in a contaminated environment with 100µg Cd (II)/g sand stimulated the bioaccumulation process of Cd ions with 6.77% in the case of flax and 42.19% in the case of rapeseed (**Fig. 5**). The same results were obtained for quantification of Cd recovery degree by determining the recovery rate (%).

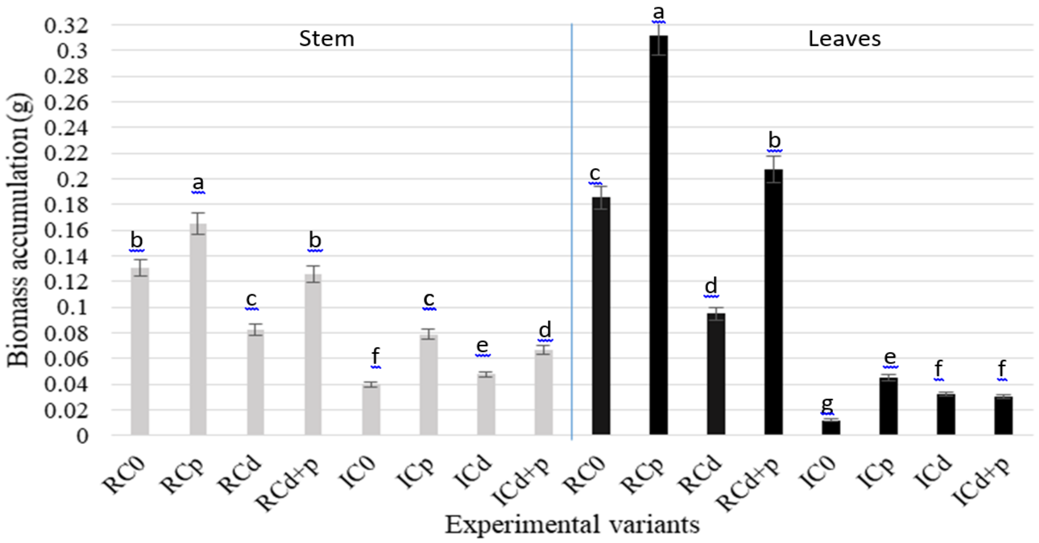

Fig. 4. The influence of hemp shives on rapeseed and flax biomass accumulation in cadmium contaminated environment. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3). The explanations of experimental variants are in Table 1.

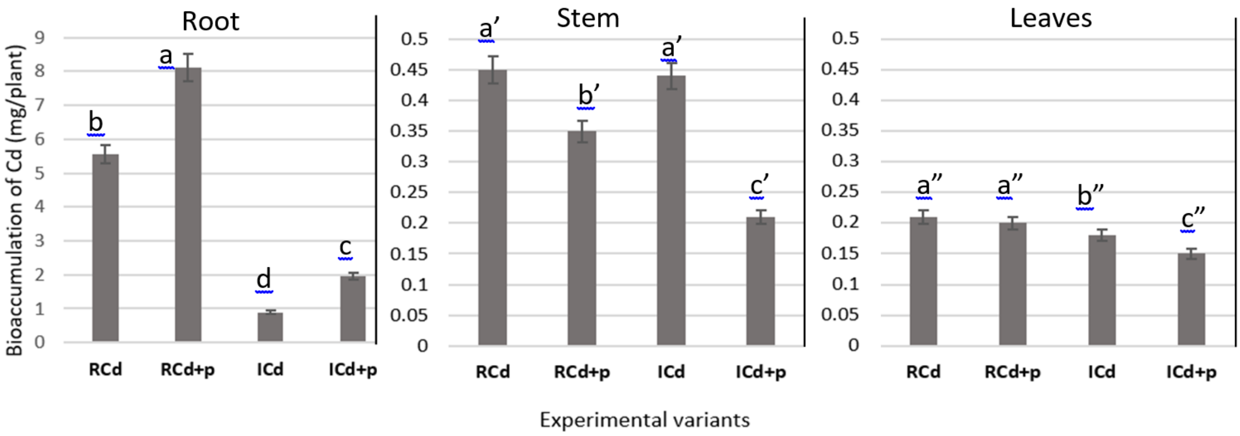

Fig. 5. Cadmium bioaccumulation in rapeseed and flax plants under the influence of hemp shives. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3). The explanations of experimental variants are in Table 1.

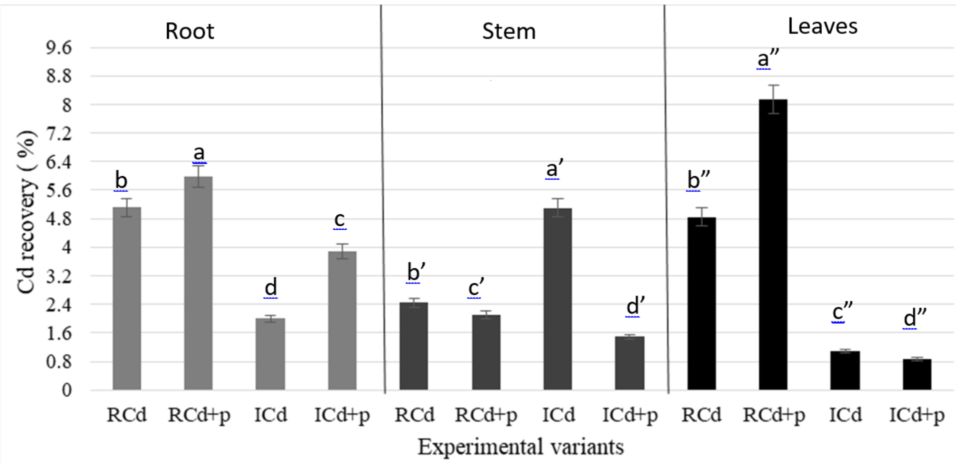

Fig. 6. Cadmium recovery (%) in rapeseed and flax plants under the influence of hemp shives. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3). The explanations of experimental variants are in Table 1.

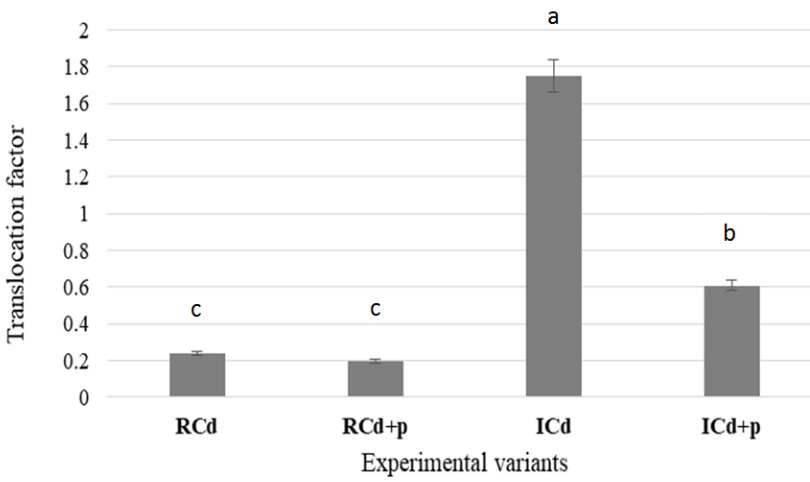

Fig. 7. Translocation factor of cadmium ions in rapeseed and flax plants under the influence of hemp shives. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3). The explanations of experimental variants are in Table 1.

In the case of rapeseed there was an improvement with 26% of Cd recovering possibility from environment where hemp shives were added, compared to growth medium without amendments (**Fig. 6**). In the case of flax, growth in the sand with hemp shives, previously contaminated with cadmium ions imparts a positive effect (24.24%) of the recovery degree.

Calculation of a translocation factor - It has been revealed that the presence of hemp shives in sand contaminated with cadmium ions, lead to a reduction in the transport of metal ions to the upper parts of the plants (**Fig. 7**) only in case of flax plants.

It can be concluded that bioavailability of Cd is modulated by plant root exudates and it is attributed to substances from roots, known to contain small organic acids (Nigam et al., 2001). Mobility and bioavailability of Cd and other metals can be controlled by the addition of organic amendment as hemp shives. In soil solution the bioavailability of metals is decreasing with increasing solubilization of soil organic matter, possibly due to the formation of organo-metal complexes (Hernandez-Soriano and Jimenez-Lopez 2012; Wang et al., 2014). In another study, (Stirk and van Staden 2000) cadmium removal by algal biomass was examined.

Hattab et al., (2014a), observed the limited translocation of some metal ions into primary leaves of dwarf bean plants in the result of compost and dolomite limestone addition. Thus, there is evidence that application of organic amendments in the contaminated sites facilitates germination capacity, biomass production, plant survival and productivity for a long-term period and increases plant diversity (Mench et al., 2010).

Sometimes using organic amendments enriches the soil with biochelators. These components can increase mobility and bioavailability of contaminated soil by increasing phytoextraction efficiency.

Doichinova and Velizarova (2013) cultivated seedlings of *Quercus rubra* L. and *Pinus nigra* Arn. in contaminated soil in the presence of paper industry wastes. They observed that amendments improved plant growth conditions in terms of C/N ratio. Despite high concentration of lead and cadmium in the mixture of soil and amendments, alkaline solution limits their uptake by seedlings. For this, agro-industrial wastes could be considered suitable for reducing bioavailability of toxic elements.

Thus, the use of hemp shives as amendments in phytoremediation can lead to enhance the efficiency of the soil cleanup process. However, the quantification and characterization of the natural materials used in phytoremediation are essential. Besides, composition of natural amendments may affect the metabolism of soil microbiota. Studies that include such interactions would be of a significant importance for further evaluation of this approach. It should be highlighted that some waste of both agriculture and industrial origin contain significant amounts of trace metals, and other contaminants detrimental to soils or to the environment (McBride 2003). Therefore, contaminant levels in wastes should be monitored and decision on the use of such products as soil amendments should be preceded by risk assessment.

Conclusions

It has been found that the development of the root system of the studied plants is more pronounced in the presence of hemp shives both in contamination with cadmium ions and in its absence. Soil contaminated with Cd and enriched with hemp shives favors vegetative biomass accumulation in all organs of the rape or flax plant. The concentrations of cadmium ions accumulate in various parts of the plant show that supplementation of growth media with hemp shives, improves the bioaccumulation process of cadmium metal ions. The presence of hemp shives in sand contaminated with cadmium ions, lead to a reduction in the transport of metal ions to the upper parts of the plants. The results obtained allow stating the hypothesis that, hemp shives modulates the bioaccumulation process of cadmium ions in the rapeseed and flax plant. The mechanism is not fully understood but further studies will provide additional information regarding natural amendments involving in bioaccumulation process of metal ions. The hemp shives can be successfully used as amendments in phytoremediation process, enhancing bioaccumulation process.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interst.

References

1. Adriano DC. 2001. Trace Elements in Terrestrial Environments. Biogeochemistry, Bioavailability, and Risks of Metals. Springer-Verlag, New York. p.867.
2. Bech J, Roca N, Barcel´o J, Duran P, Tume P, Poschenrieder C. 2012. Soil and plant contamination by lead mining in Bellmunt (Western Mediterranean Area). J Geochem Explor 113:94-99.
3. Bingham FT, Page AL, Mitchell GA, Strong JE. 1979. Effects of liming an acid soil amended with sewage sludge enriched with Cd, Cu, Ni and Zn on yield and Cd content of wheat grain. J Environ Qual 8:202-207.
4. Brooks RR. 1998a. General introduction. In: Brooks, R.R. (Ed.), Plants That Hyperaccumulate Heavy Metals. CAB International, Wallingford, Oxon, United Kingdom, p.1-14.
5. Brooks RR. 1998b. Geobotany and hyperaccumulators. In: Brooks, R.R. (Ed.), Plants That Hyperaccumulate Heavy Metals. CAB International, Wallingford, Oxon, United Kingdom. p 55-94.
6. Chen HM, Zheng CR, Tu C, Zhu YG. 1999. Heavy metals pollution in soils in China: status and countermeasures. Ambio 28:130-134.
7. Chen XT, Wang G, Zhang CH, Fang L. 2001. Effects of lime and peat on the growth and element uptake of Brassica chinensis in Cd, Pb, and Zn contaminated soil. Soil and Environmental Sciences 11(1):17-21.
8. Choi JW, Yoo EJ, Kim JY, Hwang JY, Lee K, Lee WS, Han JS, Lee KS. 2013. Pb concentrations and isotopic compositions in the soil and sediments around abandoned mine in southwest of Korea. J Agr Chem Environ 2:101-108.
9. Cordero B, Lodeiro P, Herrero R, Esteban Sastre de Vicente M. 2004. Biosorption of cadmium by *Fucus spiralis*. Environmental Chemistry 1, 180-187.
10. Davis JG, Wilson CR. 2005. Choosing a soil amendment. Colorado State University. Fact Sheet 7, 235.
11. do Nascimento CWA, Amarasiriwardena D, Xing BS. 2006. Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. Environ Pollut 140:114-123.
12. Doichinova V, Velizarova E. 2013. Reuse of paper industry wastes as additives in phytoremediation of heavy metals polluted substrates from the spoil banks of the Kremikovtsi region, Bulgaria. Procedia Environ Sci 18:731-736.
13. Eriksson JE. 1989. The influence of pH, soil type and time on adsorption and plant uptake by plants of Cd added to the soil. Water Air Soil Pollut 48:317-335.
14. Hanus-Fajerska E, Wiszniewska A, Muszynska E. 2012. In vit- ro multiplication and acclimatization of Biscutella laevigata (Brassicaceae) to cultivation in greenhouse conditions. BioTechnologia 93:97-101.
15. Hattab N, Motelica-Heino M, Bourrat X, Mench M. 2014a. Mobility and phytoavailability of Cu, Cr, Zn, and As in a contaminated soil at a wood preservation site after 4 years of aided phytostabilization. Environ Sci Pollut R 21:10307-10319.
16. Hernandez-Soriano MC, Jimenez-Lopez JC. 2012. Effects of soil water content and organic matter addition on the speciation and bioavailability of heavy metals. Sci Total Environ 423:55-61.
17. Jankaite A, Vasarevicius S. 2005. Remediation Technologies for soils contaminated with heavy metals. J Env Engin Land Manag 2(2), 109-113.
18. Kelechi EA, Sakrabania R, Patchigollab K, Mouazen AM. 2018. Critical evaluation of oil palm fresh fruit bunch solid wastes as soil amendments: Prospects and challenges. Resources, Conservation and Recycling 136, 399-409.
19. Lichtenthaler HK, Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in

different solvents, Biochem Soc Trans 11:591-593.
20. Luo J, Wu J, Huo S, Qi S, Gu SX. 2018. A real scale phytoremediation of multi-metal contaminated e-waste recycling site with *Eucalyptus globulus* assisted by electrical fields, Chemosphere, 201, 262-268,
21. McBride MB. 2003. Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks? Adv Environ Res 8:5-19.
22. McGrath SP, Zhao FJ. 2003. Phytoextraction of metals and metalloids from contaminated soils. Current Opinion in Biotechnology 14, 277-282.
23. Mench M, Lepp N, Bert V, Schwitzguebel JP, Gawronski SW, Schroder P, Vangronsveld J. 2010. Successes and limitations of phytotechnologies at field scale: Outcomes, assessment and outlook from COST Action 859. J Soil Sediments 10:1039-1070.
24. Mulatya JM, Wilson J. 2002. Root architecture of provenances, seedlings, and cuttings of Melia volkensii: implications for crop yield in dryland agroforestry. Agroforestry Systems 56(1):65-72.
25. Nigam R, Srivastava S, Prakash S, Srivastava MM. 2001. Cadmium mobilisation and plant availability the impact of organic acids commonly exuded from roots. Plant Soil. 230:107-113.
26. Robinson BH, Mills TM, Petit D, Fung LE, Green SR, Clothier BE. 2000. Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation. Plant and Soil 227, 301-306.
27. Stirk WA, van Staden J. 2000. Removal of heavy metals from solution using dried brown seaweed material. Bot Mar 43:467-473.
28. Stingu A, Volf I, Popa VI. 2009. Study of copper and cadmium accumulation by bean, Env Eng Manag J 5:1247-1252.
29. Stingu A, Volf I, Popa VI, Gostin I. 2011. New approaches concerning the utilization of natural amendments in cadmium phytoremediation, Ind Crop Prod 35: 53-60.
30. Stingu A, Volf I, Popa VI, Gostin I. 2012. New approaches concerning the utilization of natural amendments in cadmium phytoremediation. Ind Crop Prod 35:53-60.
31. Sun Y, Zhou Q, Liu W, An J, Xu Z, Wang L. 2009. Joint effects of arsenic and cadmium on plant growth and metal bioaccumulation: a potential Cd hyperaccumulator and as excluder Bidens pilosa L, Journal of Hazardous Materials 161:808-814.
32. Tanase C, Volf I, Popa IV. 2014. Enhancing copper and lead bioaccumulation in rapeseed by adding hemp shives as soil natural amendments. J Env Engin Land Manag 22 (4), 245-253.
33. Tudoreanu L, Phillips CJC. 2004. Modeling cadmium uptake and accumulation in plants. Advances in Agronomy 84, 121-157.
34. Wang FL, Ouyang W, Hao FH, Lin CY, Song NN. 2014. In situ remediation of cadmium-polluted soil reusing four byproducts individually and in combination. J Soil Sediments. 14:451-461.
35. Wenzel WW, Lombi E, Adriano DC. 1999. Biogeochemical processes in the rhizosphere: role in phytoremediation of metal-polluted soils. In: Prasad, M.N.V. and Hagemeyer, J. eds. Heavy metal stress in plants - From molecules to ecosystems. Berlin, Heidelberg, New York, Springer-Verlag p. 271-303.
36. Wiszniewska A, Hanus-Fajerska E, Grabski K, Tukaj Z. 2013. Promoting effects of organic medium supplements on the micropropagation of promisingornamental Daphne species (Thymelaeaceae). In Vitro Cell Dev-Pl. 49: 51–59.

REVIEW PAPER

FERULIC ACID – A VERSATILE MOLECULE

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Abstract: The review summarizes the main roles of ferulic acid (4-hydroxy-3-methoxy-cinnamic acid), a phenolic compound widespread in the vegetable world, being present in cereals (rice, wheat, oats), coffee, tomatoes, nuts or corn, but also in a range of plants used in Traditional Chinese Medicine. It plays a vital role in ensuring cell wall rigidity and also in the formation of other important organic compounds for plants. Ferulic acid has a wide variety of biological activities such as: antioxidant, anti-inflammatory, antimicrobial, antiallergic, anticancer, antithrombotic, antiviral activities, vasodilator, hepatoprotective and metal chelation actions, enzymatic activity modulation, and wound healing activity. The most important action is the antioxidant one, being studies that demonstrate that ferulic acid acts synergistically with other antioxidants. Also, the antioxidant effect of ferulic acid is enhanced by skin exposure to ultraviolet light, making it a good ingredient for sunscreen cosmetics. It is one of the most powerful natural antioxidant that has the potential to neutralize free radicals, to slow down the aging process of the skin, to accelerate skin regeneration, to heal the skin wounds and also to preserve the health and beauty of the skin.

Keywords: ferulic acid, skin health, antioxidant activity, free radicals, photoprotection.

1. Introduction

Ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) is a phenolic compound found in plant cell walls as a component of lignocelluloses. It plays a key role in the plants self preservation mechanism, ensures cell wall rigidity, protection against microbial invasion as well as sun damage protection. Ferulic acid was isolated first in 1866 from *Ferula foetida* (Order Apiales, Family Apiaceae), its name being based on the botanical name of plant. It is a phenolic derivative of cinnamic acid present in plant cell wall components as covalent side chains (Zhao and Moghadasian, 2008). It has been found in vegetables, fruits, flowers, coffee and cereals both in free and conjugated forms. Mainly, the conjugated forms are esters with the specific polysaccharides, alcohols, sterols, acids etc.

Ferulic acid is present in several plants, including Poaceae, Solanaceae and

Chenopodiaceae, in high concentrations. It is commonly found in cereal seeds (rice, wheat, oats, rye, barley), whole grains, spinach, parsley, grapes, banana, orange, grapefruit, rhubarb, artichoke, beans, berries, pineapple, coffee seeds, peanut and nuts (Drăgan et al., 2018). Cereals and a variety of vegetables and fruits contain 0.5-2% ferulic acid in free and esterified form (Mathew and Abraham, 2004). In **Table 1** the ferulic acid content is presented in different sources (Kumar and Pruthi, 2014; Boz, 2015).

In 1925 it was chemically synthesized (Dutt, 1925), and later the structure was confirmed by spectroscopic techniques (Nethaji et al., 1988), revealing the existence of both *cis* and *trans* isomeric forms (**Fig. 1)**.

Table 1. Ferulic acid content from various known sources

Source	**Ferulic acid content (mg/100 g)**
Bamboo shots	243.6
Soyabean	12
Peanut	8.7
Red beet	25
Spinach	7.4
Eggplant	7.3-35
Red cabbages	6.3-6.5
Radish	4.6
Broccoli	4.1
Tomato	0.29-6
Grapefruit	10.7-11.6
Orange	9.2-9.9
Banana	5.4
Coffee	9.1-14.3
Maize kernels	174
Maize flour	38
Whole-wheat flour	89
Whole-wheat	64-127
Rye bran	280
Rye flour	86
Oat bran	33
Oat flakes	25-52
Whole oats	25-35
Whole brown rice	42

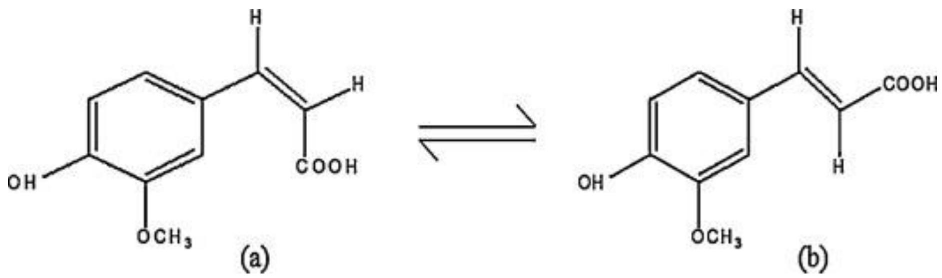

Fig. 1. Ferulic acid *cis-trans* isomers

The aim of this study is to attract attention to ferulic acid, to give summary of obtaining methods, its chemical and pharmacological properties and to better understand its multiple biological effects. Also, the article highlights antioxidant potential, anti-inflammatory, antimutagenic, antiproliferative, antimicrobial, antifungic, antihypertensive, antithrombotic, antihyperglycemic, photoprotective and wound healing activities.

2. Obtaining methods for ferulic acid

Ferulic acid can be obtained by chemically or enzymatically hydrolysis of natural sources. Chemical hydrolysis can be performed as alkaline or acidic methods, in order to extract ferulic acid from different natural sources (Kim et al., 2006; Anvar and Mazza, 2009). Due to the lower yields of such chemical methods, optimization of parameters is required (temperature, pH, time of extraction, concentration of acid or alkali etc.).

A more specific method is enzymatic hydrolysis. Many researchers studied different types of enzymes which can be used to extract ferulic acid with higher yields, using a gentle process. Such enzymes are feruloyl esterases isolated from different organisms or microorganism, such as *Streptomyces viridosporus*, *Streptomyces olivochromogenes*, *Pseudomonas fluorescens* subsp. *cellulosa*, *Penicillium pinophilum*, *Schizophyllum commune*, *Aspergillus niger* and *Clostridium thermocellum* (Deobald and Crawford, 1987; Brézillon et al., 1996; Donaghy and McKay, 1997; Blum et al., 2000b) grown on complex substrates such as xylan, pectin, wheat bran or sugar beet pulp. Nowadays, over 30 microbial feruloyl esterases have already been identified. It had been observed that feruloyl esterases act synergistically with other hemicellulases such as xylanases and pectinases in order to maximize the hydrolyzation of the ester bond between ferulic acid and hemicellulose present in plant cell walls. The yield of ferulic acid was highly dependent on the source of xylanase.

3. Properties of ferulic acid

Ferulic acid is a crystalline powder, insoluble in water at room temperature (0.78 g/L), soluble in water at high temperature, soluble in organic solvents. It has a melting point of 168-172°C and a pK_a value of 4.61.

Multiple chromatographic methods can be used for qualitative and quantitative analysis of ferulic acid: high-performance liquid chromatography (Kováčová and Malinová, 2007; Laokuldilok et al., 2011), thin-layer chromatography (Sharma et al., 1998), high-performance thin layer chromatography (Sharma et al., 2007), capillary tube electrophoresis (Sharma et al., 2007; Aturki et al., 2008) and colorimetry (Garcia et al., 2002; Tee-ngam et al., 2013).

From all these methods high-performance liquid chromatography is the most used technique for ferulic acid analysis. For both qualitative and quantitative analysis several researchers proposed high-performance thin layer chromatography, which is useful for routine assays in pharmaceutical industry (Hingse et al., 2014).

4. Pharmacokinetic profile of ferulic acid

Ferulic acid crosses the intestinal barrier, a major absorption site, by passive transcellular diffusion and facilitated transport mainly as a free form (Konishi et al., 2006; Silberberg et al., 2006). A small percent of ferulic acid is absorbed like conjugates as feruloyl-glucuronide, sulfate and dihydroferulic acid. The free and conjugated forms of ferulic acid are further metabolized by the liver under the action of sulfotransferases and UDP-glucuronosyl transferases. The excretion of ferulic acid in human urine is as free form or conjugated one as feruloyl-glucuronide (Bourne and Rice-Evans, 1998; Poquet et al., 2008). A small percentage of ferulic acid is excreted in the bile, which explains the presence of free and conjugated ferulic acid in the feces.

5. Pharmacological profile and therapeutic applications of ferulic acid

Free radicals and reactive oxygen species causes serious diseases in human body. Ferulic acid is a powerful antioxidant and has a high radical scavenger effects for free radicals such as hydrogen peroxide, superoxide, hydroxyl radical, and nitrogen dioxide (Zhang et al., 1998; Ou and Kwok, 2004; Réblová, 2012; Abdel-Aal and Rabalski, 2013; Wolszleger et al., 2015; Stan et al., 2016).

The protective effect of ferulic acid against heart disease is due to its antioxidant effects (Price et al., 2008; Boz, 2015). Also, ferulic acid has an antihypertensive effect, reduces left ventricular diastolic rigidity, attenuates the infiltration of inflammatory cells and the collagen deposition in the left ventricle (Alam et al., 2013; Drăgan et al., 2016; Drăgan et al., 2018).

Other recent studies showed that ferulic acid decreases the serum lipids, inhibits platelet aggregation and prevents thrombus formation (Wang et al., 2004; Pagidipati and Gaziano, 2013; Zhang, 2014).

Ferulic acid have an important chemopreventive activity by means of antimutagenic and antiproliferative effects which supports the potential adjuvant role of ferulic acid in cancer therapy (Kroon et al., 1997; Ferguson et al., 2003; Sakthi et al., 2015; Fong et al., 2016).

Researchers revealed that it is possible to use ferulic acid in treating diabetes mellitus, due to decrease blood glucose levels and increase insulin plasma concentration activities (Nomura et al., 2003; Jung et al., 2007; Roy et al., 2013).

Ferulic acid has a photoprotective role for skin structures, beeing a strong UV absorber (Staniforth et al., 2012). It protects the skin against UVB induced erythema. It is used in skin aging and photoaging, hyperpigmentation, seborrheic skin and acne (Bezerra et al., 2017; Zduńska et al., 2018). It has also the ability to inhibit tyrosinase so it can be used in anti-blemish cosmetic formulations.

Ferulic acid accelerates the regeneration of skin, increase epithelialization and healing of wounds (Sangeeta et al., 2015; Zduńska et al., 2018). Recent studies showed that ferulic acid increased synthesis of amino acids involved in wound healing. Also it can inhibit lipid peroxidation and increases catalase, superoxide dismutase and glutathione, these processes significantly accelerates shrinkage of the wound (Ghaisas et al., 2014; Zduńska et al., 2018).

Ferulic acid has an antimicrobial effect against Gram-negative and Gram-positive bacteria, as well as yeasts e.g.: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Citrobacter koseri*, *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Shigella sonnei*, *Bacillus subtilis* and *Streptococcus pneumoniae* (Jeong et al., 2000; Tsou et al., 2000; Ou & Kwok 2004; Mathew and Abraham, 2004; Boz, 2015). It has also antifungal effect against *Sclerotinia sclerotiorum*, *Fusarium oxy-sporum*, *Alternia* sp., *Botrytis cinerea*, and *Penicillium digitatum* (Ou and Kwok, 2004; Boz, 2015).

All these pharmacological roles and medical applications of ferulic acid are represented in **Table 2**.

Table 2. Possible applications of ferulic acid

Crt. no.	Effect of ferulic acid
1.	Antimicrobial
2.	Antiviral
3.	Antidiabetic
4.	Antiageing
5.	Anticarcinogenic
6.	Anti-inflammatory
7.	Antiallergic
8.	Antioxidant
9.	Antiatherogenic
10.	Antiapoptotic
11.	Antihypertensive
12.	Antithrombotic
13.	Antiarrythmic
14.	Photoprotective
15.	Neuroprotective
16.	Hepatoprotective
17.	Cardioprotective
18.	Free radical scavenger activity
19.	Lipid lowering activity
20.	Metal chelation activity
21.	Enzymatic activity modulation
22.	Increase NO synthesis
23.	Regeneration and wound healing activity

Conclusions

Our study has shown that ferulic acid, a widely spread natural bioactive compound, reduces the risk of serious human diseases. Ferulic acid proved to be a strong antioxidant, anti-inflammatory, antimicrobial, cytotoxic, antithrombotic, antihypertensive and antidiabetic molecule. It helps to protect cellular structures, being used in skin disorders like erythema, aging and photoaging processes, acne, hyperpigmentation, wounds and also to reduce fine wrinkles. For such a skin protection role nowadays, ferulic acid is a compound increasingly used in cosmetology and aesthetic dermatology.

In light of current knowledge and literature data, ferulic acid promise to be used in wound healing in diabetic patients, in cancer therapy and in chronic and acute inflammatory diseases. Researchers also suggested that ferulic acid might be of interest as a new compound for development of an antiviral, antimicrobial, or antiparasitic drug.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Abdel-Aal ESM, Rabalski I (2013) Effect of baking on free and bound phenolic acids in wholegrain bakery products. J Cereal Sci 57(3):312-318.
2. Alam MA, Sernia C, Brown L (2013) Ferulic acid improves cardiovascular and kidney structure and func-tion in

hypertensive rats. J Cardiovasc Pharmacol 61:240-249.

3. Anvar UB, Mazza G (2009) Extraction and purification of ferulic acid from flax shives, wheat and corn bran by alkaline hydrolysis and pressurised solvents. Food Chemistry 115:1542-1548.
4. Aturki Z, Fanali S, D'orazio G, Rocco A, Rosati C (2008) Analysis of phenolic compounds in extra virgin olive oil by using reversed-phase capillary electrochromatography. Electrophoresis 29:1643-1650.
5. Bartolome B, Garcia-Conesa MT, Williamson G (1996) Release of the bioactive compound, ferulic acid, from malt extracts. Biochem Soc Trans 24:379S.
6. Bezerra G, Pereira M, Ostrosky E, Barbosa E, Moura M, Ferrari M, Aragao C, Gomes A (2017) Compatibility study between ferulic acid and excipients used in cosmetic formulations by TG/DTG, DSC and FTIR. J Therm Anal Calorim 127:1683-1691.
7. Blum DL, Schubot FD, Ljungdahl LG, Rose JP, Wang BC (2000b) Crystallisation and preliminary X-ray analysis of the Clostridium thermocellum cellulosome xylanase Z feruloyl esterase domain. Acta Crystallographica D 56:1027-1029.
8. Bourne LC, Rice-Evans C (1998) Bioavailability of ferulic acid. Biochem Biophys Res Commun 253:222-227.
9. Boz H (2015) Ferulic Acid in Cereals – a Review. Czech Journal of Food Sciences 33(1):1-7.
10. Brézillon C, Kroon PA, Faulds CB, Brett GM, Williamson G (1996) Novel ferulic acid esterases are induced by growth of Aspergillus niger on sugar-beet pulp. Appl Microbiol Biotechnol 45(3):371-376.
11. Deobald LA, Crawford DL (1987) Activities of cellulase and other extracellular enzymes during lignin solubilization by Streptomyces viridosporus. Appl Microbiol Biotechnol 26(2):158-163.
12. Donaghy J, McKay AM (1997) Purification and characterization of a feruloyl esterase from the fungus Penicillium expansum. J Appl Microbiol 83:718-726.
13. Drăgan M, Stan CD, Iacob AT, Dragostin O, Profire L (2018) Ferulic acid: potential therapeutic applications. Rev Med Chir Soc Med Nat Iaşi 122(2):388-395.
14. Drăgan M, Stan CD, Pânzariu A, Profire L (2016) Assessment of in vitro antioxidant and antiinflamatory activities of new azetidin-2-ones derivatives of ferulic acid. Farmacia 64(5):717-721.
15. Drăgan M, Stan CD, Pânzariu A, Profire L (2016) Evaluation of anti-inflammatory potential of some new ferulic acid derivatives. Farmacia 64(2):194-197.
16. Dutt S (1925) General synthesis of a-unsaturated acids from malonic acid. Q J Chem Soc 1:297-301.
17. Ferguson LR, Lim IF, Pearson AE, Ralph J, Harris PJ (2003) Bacterial antimutagenesis by hydroxycinnamic acids from plant cell walls. Mutation Research 542:49-58.
18. Fong Y, Tang CC, Hu HT, Fang HY et al. (2016) Inhibitory effect of trans-ferulic acid on proliferation and migration of human lung cancer cells accompanied with increased endogenous reactive oxygen species and β-catenin instability. Chin Med 11:45-50.
19. Garcia R, Rakotozafy L, Telef N, Potus J, Nicolas J (2002) Oxidation of ferulic acid or arabinose-esterified ferulic acid by wheat germ peroxidase. J Agric Food Chem 50:3290-3298
20. Ghaisas M, Kshirsagar S, Sahane R (2014) Evaluation of wound healing activity of ferulic acid in diabetic rats. Int Wound J 11:523-532.
21. Hingse SS, Digole SB, Annapure US (2014) Method development for

simultaneous detection of ferulic acid and vanillin using high-performance thin layer chromatography. J Anal Sci Tech 5:21-27.

22. Jeong YC, Jae HM, Keun HP (2000) Isolation and identification of 3-methoxy-4-hydroxybenzoic acid and 3-methoxy-4-hydroxycinnamic acid from hot water extracts of Hovenia dulcis Thumb and confirmation of their antioxidative and antimicrobial activity. Korean J Food Sci Tech 32:1403-1408.
23. Jung EH, Kim SR, Hawang IK, Ha TY (2007) Hypoglicemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db mice. J Agric Food Chem 55:9800-9804.
24. Konishi Y, Zhao Z, Shimizu M (2006) Phenolic acids are absorbed from the rat stomach with different absorption rates. J Agric Food Chem 54:7539-7543.
25. Kováčová M, Malinová E (2007) Ferulic and coumaric acids, total phenolic compounds and their correlation in selected oat genotypes. Czech Journal of Food Sciences 25(6):325-332.
26. Kim KH, Tsao R, Yang R, Gnu SW (2006) Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chemistry 95:466-473.
27. Kroon PA, Faulds CB, Brézillon C, Williamson G (1997) Methyl phenylalkanoates as substrates to probe the active sites of esterases. Eur J Biochem 248(1):245-251.
28. Kumar N, Pruthi V (2014) Potential applications of ferulic acid from natural sources. Biotechnol Rep (Amst) 4:86-93.
29. Laokuldilok T, Shoemaker CF, Jongkaewwattana S, Tulyathan V (2011) Antioxidants and Antioxidant Activity of Several Pigmented Rice Brans. J Agric Food Chem 59(1):193-199.
30. Mathew S, Abraham TE (2004) Ferulic acid: an antioxidant found naturally in plant cell walls and feruloyl esterases involved in its release and their applications. Crit Rev Biotechnol 24(2-3):59-83.
31. Nethaji M, Pattabhi V, Desiraju GR (1988) Structure of 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid (ferulic acid). Acta Cryst C 44:275-277.
32. Nomura E, Kashiwada A, Hosoda A, Nakamura K, Morishita H, Tsuno T, Taniguchi H (2003) Synthesis of amide compounds of ferulic acid and their stimulatory effects on insulin secretion in vitro. Bioorg Med Chem 11:3807-3813.
33. Ou S, Kwok KC (2004) Ferulic acid: Pharmaceutical functions, preparation and applications in foods. J Sci Food Agric 84(11):1261-1269.
34. Pagidipati NJ, Gaziano TA (2013) Estimating deaths from cardiovascular disease: a review of global meth-odologies of mortality measurement. Circulation 127:749-756.
35. Poquet L, Clifford MN, Williamson G (2008) Transport and Metabolism of Ferulic Acid through the Colonic Epithelium. Drug Metab Dispos 36(1):190-197.
36. Price RK, Welch RW, Lee-Manion AM, Bradbury I, Strain JJ (2008) Total phenolics and antioxidant potential in plasma and urine of humans after consumption of wheat bran. Cereal Chemistry 85:152-157.
37. Réblová Z (2012) Effect of temperature on the antioxidant activity of phenolic acids. Czech J Food Sci 30:171-177.
38. Roy S, Metya SK, Sannigrahi S, Raharnan N, Ahmed F (2013) Treatment with ferulic acid to rats with streptozotocin-induced diabets: effects on oxidative stress, pro-inflammatory cytokines and apoptosis in the pancreatic β cell. Endocrine 44:369-379.

39. Sakthi PS, Vijayalakshmi G (2015) Therapeutical properties of ferulic acid and bioavailability enhancement through feruloyl esterase. J Funct Foods 17:656-666.
40. Sangeeta D, Digvijay S, Pradeep TD, Rupesh S, Rahul T (2015) Healing potential of ferulic acid on dermal wound in diabetic animals. Asian J Molec Model 1:1-16.
41. Sharma UK, Sharma N, Gupta AP, Kumar V, Sinha AK (2007) RP-HPTLC densitometric determination and validation of vanillin and related phenolic compounds in accelerated solvent extract of Vanilla planifolia. J Sep Sci 30:3174-3180.
42. Silberberg M, Morand C, Mathevon T, Besson C, Manach C, Scalbert A, Remesy C (2006) The bioavailability of polyphenols is highly governed by the capacity of the intestine and of the liver to secrete conjugated metabolites. Eur J Nutr 45(2):88-96.
43. Stan CD, Drăgan M, Pânzariu A, Profire L (2016) Assessment of in vitro antioxidant activity of some new ferulic acid derivatives. Rev Med Chir Soc Med Nat Iaşi 120(3):727-731.
44. Staniforth V, Huang W, Aravindaram K, Yang N (2012) Ferulic acid, a phenolic phytochemical, inhibits UVB-induced matrix metalloproteinases in mouse skin via posttranslational mechanisms. J Nutr Biochem 23:443-451.
45. Tee-ngam P, Nunant N, Rattanarant P, Siangproh W, Chailapakul O (2013) Simple and rapid determination of ferulic acid levels in food and cosmetic samples using paper-based platforms. Sensors 13:13039-13053.
46. Tsou MF, Hung CF, Lu HF, Wu LT, Chang SH, Chang HL, Chen GW, Chung JG (2000) Effects of caffeic acid, chlorogenic acid and ferulic acid on growth and arylamine N-acetyltransferase activity in Shigella sonnei (group D). Microbios 101:37-46.
47. Wang B, Ouyang J, Liu Y, Yang J, Wei L, Li K, Yang H (2004) Sodium ferulate inhibits atherosclerogenesis in hyperlipidemia rabbits. J Cardiovasc Pharmacol 43:549-554.
48. Wolszleger (Drăgan) M, Stan CD, Pânzariu A, Jităreanu A, Profire L (2015) New thiazolidin-4-ones of ferulic acid with antioxidant potential. Farmacia 63(1):150-154.
49. Zhao Z, Moghadasian MH (2008) Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. Food Chemistry 109(4):691-702.
50. Zduńska K, Agnieszka D, Kolodziejczak A, Rotsztejn H (2018) Antioxidant Properties of Ferulic Acid and Its Possible Application. Skin Pharmacol Physiol 31:332-336.
51. Zhang J, Chen J, Yang J, Xu C, Ding J, Yang J, Guo Q, Hu Q, Jiang H (2014) Sodium Ferulate Inhibits Neointimal Hyperplasia in Rat Balloon Injury Model. PLoS One 9(1):e87561. doi: 10.1371/journal.pone.0087561
52. Zhang ZE, Yao SD, Lin WZ, Wang WF, Jin YZ, Lin NY (1998) Mechanism of reaction of nitrogen dioxide radical with hydroxycinnamic acid derivatives: a pulse radiolysis study. Free Radical Research 29:13-16.

REVIEW PAPER

ISOFLAVONOIDS – DUAL ACTION ON ESTROGEN RECEPTORS

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Abstract: Isoflavonoids are phytoestrogenic compounds found mainly in plants from the Fabaceae family and also in soy-based foods. Isoflavonoids exhibit (anti)estrogenic effects, acting on estrogen receptors due to the structural similarities with estrogenic hormones (17β-estradiol). The aim of our minireview is to highlight the pharmacokinetic and pharmacodynamic properties of isoflavonoids, in order to sustain the beneficial effects in different pathologies (osteoporosis associated with menopause, breast cancer, prostate cancer, protective cognitive functions) but, at the same time, to aware about the possible adverse effects on long-term administration.

Keywords: isoflavonoids, estrogen, receptors, menopause, SERM.

1. Introduction

Nowadays, there is an increased research interest in phytoestrogen compounds as they are widely used by vegetarians (through the consumption of soy, soy-based foods and vegetable products) and also by women at menopause (as dietary supplements) due to the beneficial effects produced in this physiological state, improving both somatic and psychological symptoms (Dong et al., 2011; Ahsan et al. 2017; Setchell, 2017).

Phytoestrogens are compounds of vegetal origin, classified according to their chemical structure in four classes: isoflavonoids, flavonoids, coumestans and lignans. Isoflavonoids and flavonoids represent the active substances with an important role in phytotherapy based on pharmacological studies. Isoflavonoids are found mainly in plants from the Fabaceae family, the most important source being represented by soybean (*Glycine max*). Actually, the food sources of isoflavonoids are soy milk, tofu, roasted soybeans etc. (Rietjens et al., 2017).

Isoflavonoids are benefic in pathologies like breast cancer, prostate cancer, cardiovascular diseases, metabolic syndrome associated with type II diabetes, but also in hypothyroidism and in cognitive dysfunctions (Cano and Garcia-Peres, 2010; Colacurci et al., 2013; Delmanto et al., 2013; Sathyapalan et al., 2018).

2. Pharmacokinetic aspects

Regarding the absorption and metabolism of isoflavonoids, after oral administration, glycosides are enzymatic hydrolysed to aglycone and carbohydrates by *β*-glucosidase and other enzymes in the gastrointestinal tract, but also by intestinal bacteria, then are absorbed through enterocytes into blood stream.

After absorption, isoflavonoids are -glucurono or sulfo-conjugated. Bacterial intestinal flora play a vital role in the isoflavonoids metabolism, producing both hydrolysis and transformation into the most important metabolites (from genistein and daidzein: dehydrodaidzein, dehydrogenistein, equol or secondary metabolites: *p*-ethylphenol from genistein and final transformation into CO_2) (Franke et al., 2014).

The scientific literature indicates that isoflavonoids have different effects depending on individual microbial flora. The isoflavonoid metabolites have grater estrogenic effects than the `parent` molecules (i.e. S-equol, the intestinal flora metabolite of daidzein, presents higher estrogenic activity than daidzein). Microbial flora produces enzymes capable of metabolizing food, xenobiotics and isoflavonoids, and differs from one person to another. There are notable interindividual differences, only 30-50% of individuals are reported to have the ability to turn isoflavones into equol, depending on ethnicity and lifestyle (Reverri et al., 2017; Kolátorová, et al., 2018).

Regarding the pharmacokinetic parameters, daidzein and genistein reach the maximum concentration range (C_{max}) 2-8 hours after administration, the half-life being about 6-8 hours. An increased intake of isoflavonoid-containing products, for example the consumption of soybean 2-3 times a day, can lead to clinically relevant concentrations (Kano et al., 2006).

3. Mechanism of action related with the chemical structure

The interaction of isoflavonoids with the estrogen receptors (ER) is due to their structural similarity with 17β-estradiol (endogenous agonist), although they do not have a steroidal structure like C_{18}-hormones.

The chemical structures of 17β-estradiol and daidzein, genistein, glicitein (the most important isoflavonoids) are presented in **Fig. 1**. Each structure presents two hydroxyl (-OH) groups on both sides. The key point differentiating 17β-estradiol from isoflavonoids, is the distance between the two hydroxyl groups, that influences the affinities to the ER (Simons et al., 2012).

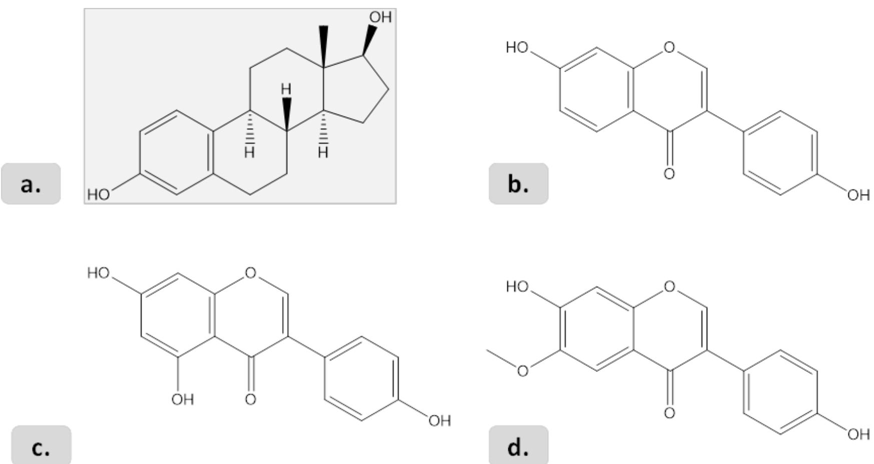

Fig. 1. Chemical structures of estradiol(a), daidzein(b), genistein(c), glicitein(d)

Because of this structure, phytoestrogens may act as agonists, but also as antagonists (in the presence of endogenous agonist), having, at the same time, similar structure with tamoxifen (an antiestrogenic molecule). In fact, these structures act as selective estrogen receptor modulators (SERMs) with comparable affinity for the ER, but a lower intrinsic activity than the endogenous agonist (Sathyapalan et al., 2018).

The ER are part of a superfamiliy of nuclear receptors. There are two subtypes of ER: alpha (ER-α) and beta (ER-β). The ER-α are predominantly found in the urogenital system (endometrium, uterus, kidneys), mammary gland, pituitary gland, while ER-β are found in bone, prostate, brain, cardiovascular system and reproductive organs. The main activity of these receptors, as nuclear receptors, is to regulate the expression of different genes (through the process of transcription and translation) or repressing other genes (Pang et al., 2018).

The beneficial effects of isoflavonoids are due to their interaction with the ER-β subtype. On the other hand, by activating the ER-α receptors, isoflavonoids are responsible for inflammatory and malignant processes. However, it seems that some isoflavonoids such as genistein and daidzein present a selective action on ER-β, producing predominantly the beneficial effects of isoflavonoids.

Regarding the detailed molecular mechanism of action, isoflavonoids cross the cell membrane (being sufficiently lipophilic) and form a complex with the ER. The isoflavonoid-receptor complex traverses the nuclear membrane, attaches itself to DNA, to the estrogen responsive element `ERE`, and initiates transcription processes of the genes with mRNA generation. The mRNA will undergo the ribosomal translation process, finally producing the different estrogen-specific effects. At the same time, during this process, other genes are repressed and the transcription process is inhibited.

The action of isoflavonoids on these receptors is dual, because they act as agonists or partial agonists (antagonists in the presence of endogenous agonist). On the other hand, genistein binds to both estrogen receptors (α and β) but with a 5-6-fold greater affinity for the ER-β. The position and number of hydroxyl groups influences the receptor selectivity. Thus, genistein has increased affinity for ER-β whereas the removal of a hydroxyl group at daidzein decreases the binding affinity (Dixon and Ferreira, 2002). There are studies which demonstrate the affinity of genistein for ER subtypes (Paterni et al., 2014; Jiang et al., 2018). In **Table 1** it can be observed that genistein has a high relative binding affinity (RBA) for the ER-β subtype, influencing minimally the ER-α subtype, compared to 17β-estradiol.

Table 1. The RBA of 17ß-estradiol and genistein for estrogen receptor (ER) subtypes

Compound	**RBA* for ER-α (%)**	**RBA for ER-β (%)**
17ß-estradiol	100	100
Genistein	5	36

Note: *The relative binding affinity (RBA) was calculated as the ratio of the concentrations of agonist and competitor required to reduce the specific ligand-binding by 50% (the ratio of IC_{50} values) (Kuiper et al., 1997; Paterni et al., 2014).

Thus, these isoflavonoids have beneficial effects in menopausal women (with low levels of estrogen), but may have less beneficial or even harmful effects in young women (acting as antagonists). In this regard, the effect of isoflavonoids in pathologies of estrogen-rich tissues, for example the effect on the mammary glandular breast cancer remains to be investigated (Nagat et al., 2014; Senthilkumar et al., 2018).

An additional mechanism of action recently proposed for genistein is the tyrosine-kinase receptor binding and therefore the modulation of the activities of hormones that use tyrosine phosphorylation for intracellular signal transfer (eg. insulin, insulin-like growth factor etc.) (Amanat et al., 2018).

This mechanism of action was also investigated in the study of Glisic et al. (2018), which demonstrated that isoflavonoids would have a beneficial effect on glucose metabolism, reducing the risk of developing type II diabetes in non-diabetic women, lowering insulin resistance. It interferes directly with lipid metabolism (lipogenesis and lipolysis), but indirectly at the level of the intestinal, hepatocyte and skeletal cells. It seems that isoflavonoids suppress the synthesis of genes involved in gluconeogenesis, but also have antioxidant effects (Glisic et al., 2018).

4. Effects on bone tissue

Isoflavonoids produce stimulation of osteoblast activity and decrease osteoclast activity. The presence of a free hydroxyl group in position 7 (for genistein) is very important to exert the antiosteoporotic effect. Synthesis of osteoclasts is inhibited by binding to ER-β in the bone cells. Isoflavonoids increase alkaline phosphatase levels, a marker for bone cell differentiation and proliferation, highlighting the idea of an inhibitory effect on the bone resorption process (Gupta, 2014; Muhammad et al., 2018). Regarding the action on bone resorption, a recent study compared the preventive osteoporotic effect in menopausal women treated with low concentrations of estrogen hormones (replacement therapy) and standardized soy isoflavonoid extract. The conclusion was that there are no major differences between the preventive effects of hormonal therapy and isoflavonoid therapy, both decreasing the bone resorption process (Tit et al., 2018).

5. Effects on adipose tissue

The adipose tissue is influenced by isoflavonoids by decreasing insulin-induced lipogenesis in both adipocytes and preadipocytes and increasing adrenaline-induced lipolysis. It was observed a decrease in the circumference of adipocytes, therefore the decrease in fat deposits occurs by decreasing the size of the cells. At different stages of development, isoflavonoids may also influence the number of adipocyte cells (Harmon et al., 2001).

Thus, isoflavonoids produce qualitative and quantitative decreases in fat deposits. Genistein may cause weight loss by inhibiting mRNA responsible for LPL synthesis, rather than producing lipolysis (Grossini et al., 2018).

6. Other recently discovered benefic effects

Considering that isoflavonoids are acting like SERMs, in a recent study on experimental animals, Fáber et al. (2018) show the protective effect of genistein on the skin flap viability. This effect could be potentially applied in plastic surgery to women undergoing a reconstructive intervention, but the mechanism responsible for these effects is not clearly defined, so further investigations should be conducted (Fáber et al., 2018). There are also studies offered by the scientific literature that suggest the protective cognitive functions of isoflavonoids, but these effects appear to depend on age, comorbidities, plant type and the type of isoflavonoids administered (Roozbeh et al., 2018).

Conclusions

Regarding the major interest among women in phytoestrogen supplementation (especially isoflavonoids) for preventing or treating different pathological conditions and regarding the new trend of population to become vegetarian, more studies following the effects of isoflavonoids should be done in order to evaluate the balance benefit-risk of intake of high amounts of isoflavonoids, because in long-term administration they can act like endocrine disruptors.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Ahsan M, Mallick A (2017) The effect of soy isoflavones on the menopause rating scale scoring in perimenopausal and postmenopausal women: a pilot study. J Clin Diag Res. 11(9):13-6.
2. Amanat S, Eftekhari MH, Fararouei M, Bagheri Lankarani K, Massoumi SJ (2018) Genistein supplementation improves insulin resistance and inflammatory state in non-alcoholic fatty liver patients: a randomized, controlled trial. Clin Nutr. 37:1210-1215.
3. Cano A, Garcia-Perez MA, Tarin JJ (2010) Isoflavones and cardiovascular disease. Maturitas 67:219-226.
4. Colacurci N, De Franciscis P, Atlante M, Mancino P, Monti M, Volpini G, Benvenuti C (2013) Endometrial, breast and liver safety of soy isoflavones plus lactobacillus sporogenes in post-menopausal women. Gynecol Endocrinol 29:209-212.
5. Delmanto A, Nahas-Neto J, Traiman P, Uemura G, Pessoa EC, Nahas EA (2013) Effects of soy isoflavones on mammographic density and breast parenchyma in postmenopausal women: a randomized, double-blind, placebo-controlled clinical trial. Menopause 20(10):1049-54. doi: 10.1097/GME.0b013e3182850270.
6. Dixon RA, Ferreira D (2002) Genistein. Phytochem. 60:205-211.
7. Dong JY, Wang P, He K, Qin LQ (2011) Effect of soy isoflavones on circulating C-reactive protein in postmenopausal women: meta-analysis of randomized controlled trials. Menopause. 18(11):1256-62. doi: 10.1097/gme.0b013e31821bfa24
8. Fáber L, Kováč I, Mitrengová P, Novotný M, Varinská L, Vasilenko T, Kello M, Čoma M, Kuruc T, Petrová K, Miláčková I, Kuczmannová A, Perželʼová V, Mižáková Š, Dosedla E, Sabol F, Luczy J, Nagy M, Majerník J, Koščo M, Mučaji P, Gál P (2018) Genistein improves skin flap viability in rats: a preliminary in vivo and in vitro investigation. Mol. 23:1637; doi:10.3390/molecules23071637.
9. Franke A, Lai J, Halm BM (2014) Absorption, distribution, metabolism, and excretion of isoflavonoids after soy intake. Arch Biochem Biophys. 559:24-28.
10. Glisic M, Kastrati N, Gonzalez-Jaramillo V, Bramer WM, Ahmadizar F, Chowdhury R, Danser AJ, Roks AJ, Voortman T, Franco OH, Muka T (2018) Associations between phytoestrogens, glucose homeostasis and risk of diabetes in women:

A systematic review and meta-analysis. Adv Nutr. 9:726-740.
11. Grossini E, Farruggio S, Rainaet G, Mary D, Deiro G, Gentilli S (2018) Effects of genistein on differentiation and viability of human visceral adipocytes. Nutr. 10:978; doi:10.3390/nu10080978.
12. Gupta C, Prakash D (2014) Phytonutrients as therapeutic agents, J Complement Integr Med. 11(3):151-169.
13. Harmon AW, Harp JB (2001) Differential effects of flavonoids on 3T3-L1 adipogenesis and lipolysis. Am J Physiol Cell Physiol. 280(4):C807-13.
14. Jiang H, Fan J, Cheng L, Hu P, Liu R (2018), The anticancer activity of genistein is increased in estrogen receptor beta 1-positive breast cancer cells. Onco Thargets Ther. 11:8153-8163.
15. Kano M, Takayanagi T, Harada K, Sawada S, Ishikawa F (2006) Bioavailability of isoflavones after ingestion of soy beverages in healthy adults. J. Nutr. 136:2291-2296.
16. Kolátorová L, Lapčík O, Stárka L (2018) Phytoestrogens and the intestinal microbiome. Physiol. Res. 67(3):S401-S408.
17. Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA (1997) Comparison of the Ligand Binding Specificity and Transcript Tissue Distribution of Estrogen Receptors α and β. Endocrinol. 138(3):863-870.
18. Muhammad A, Madaa SB, Malami I, Forcados GE, Erukainure OL, Sani H, Abubakar IB (2018) Postmenopausal osteoporosis and breast cancer: The biochemical links and beneficial effects of functional foods. Biomed Pharmacother. 107:571-582.
19. Nagat C, Mizoue T, Tanaka K, Tsuji I, Tamakoshi A, Matsuo K, Wakai K, Inoue M, Tsugane S, Sasazuki S (2014) Soy intake and breast cancer risk: An evaluation based on a systematic review of epidemiologic evidence among the Japanese population. Jpn. J. Clin. Oncol. 44:282-295.
20. Pang X, Fu W, Wang J, Kang D, Xu L, Zhao Y, Liu AL, Du GH (2018) Identification of estrogen receptor α antagonists from natural products via *in vitro* and *in silico* approaches. Oxid. Med. Cell. Longev. 6040149. doi: 10.1155/2018/604014
21. Paterni I, Granchi C, Katzenellenbogen JA, Minutolo F (2014) Estrogen Receptors Alpha (ERα) and Beta (ERβ): SubtypeSelective Ligands and Clinical Potential. Steroids. 90:13-29.
22. Reverri EJ, Slupsky CM, Mishchuk DO, Steinberg FM (2017) Metabolomics reveals differences between three daidzein metabolizing phenotypes in adults with cardiometabolic risk factors. Mol Nutr Food Res. 6(1). doi: 10.1002/mnfr.201600132 11500815
23. Rietjens I, Louisse J, Beekmann K (2017) The potential health effects of dietary phytoestrogens. Br J Pharmacol. 174:1263-1280.
24. Roozbeh N, Kashef R, Ghazanfarpour M, Kargarfard L, Darvish L, Khadivzadeh T, Dizavandi FR, Afiat M (2018) Overview of the effect of herbal medicines and isoflavones on the treatment of congnitive function. Journal of Menopausal Medicine. 24:113-118.
25. Sathyapalan T, Aye M, Rigby A, Thatcher NJ, Dargham SR, Kilpatrick ES, Atkin SL (2018) Soy isoflavones improve cardiovascular disease risk markers in women during the early menopause. Nutr Metab Cardiovasc Dis. 28(7):691-697.
26. Sathyapalan T, Dawson AJ, Rigby AS, Thatcher NJ, Kilpatrick ES, Atkin SL (2018) The effect of phytoestrogen on thyroid in subclinical hypothyroidism:

randomized, double blind, crossover study. Front Endocrinol. 9:531. doi: 10.3389/fendo.2018.00531

27. Senthilkumar HA, Fata JE, Kennelly EJ (2018) Phytoestrogens: The current state of research emphasizing breast pathophysiology. Phytother Res. 32(9):1707-1719. doi: 10.1002/ptr.6115
28. Setchell K (2017) The history and basic science development of soy isoflavones. Menopause. 24(12):1338-50.
29. Simons R, Gruppen H, Toine F, Verbruggen MA, Vincken JP (2012) Prenylated isoflavonoids from plants as selective estrogen receptor modulators (phytoSERMs). Food Funct. 3:810-827.
30. Tit DM, Bungau S, Iovan C, Nistor Cseppento DC, Endres L, Sava C, Sabau AM, Furau G, Furau C (2018) Effects of the hormone replacement therapy and of soy isoflavones on bone resorption in postmenopause. J. Clin. Med. 7:297; doi:10.3390/jcm7100297

SUPPLEMENTARY MATERIALS

Supplementary material

Table 1. The number of plant taxa for each family from the surroundings of Denteș Village

Family	No. of taxa	Family	No. of taxa
Aceraceae	3	Juglandaceae	1
Aliaceae	1	Juncaceae	4
Alismataceae	1	Lamiaceae	39
Amaranthaceae	1	Lemnaceae	1
Amaryllidaceae	1	Lliliaceae	12
Apiaceae	16	Linaceae	5
Apocynaceae	2	Lythraceae	1
Araceae	1	Malvaceae	3
Araliaceae	1	Moraceae	2
Aristolochiaceae	1	Oleaceae	2
Asclepiadaceae	2	Onagraceae	2
Aspleniaceae	1	Orchidaceae	2
Asteraceae	53	Orobanchaceae	1
Boraginaceae	9	Oxalidaceae	1
Brassicaceae	12	Papaveraceae	3
Campanulaceae	6	Pinaceae	2
Cannabaceae	1	Plantaginaceae	3
Caprifoliaceae	3	Poaceae	28
Caryophyllaceae	10	Polygalaceae	2
Celastraceae	1	Polygonaceae	10
Chenopodiaceae	2	Portulacaceae	1
Convolvulaceae	2	Primulaceae	4
Cornaceae	1	Ranunculaceae	19
Corylaceae	2	Rhamnaceae	2
Cuscutaceae	1	Rosaceae	20
Cyperaceae	4	Rubiaceae	9
Dipsacaceae	5	Salicaceae	4
Elaeagnaceae	2	Scrophulariaceae	16
Equisetaceae	1	Solanaceae	4
Euphorbiaceae	5	Sparganiaceae	1
Fabaceae	33	thymelaeaceae	1
Fagaceae	2	Tiliaceae	2
Fumariaceae	2	Typhaceae	2
Gentianaceae	1	Ulmaceae	1
Geraniaceae	4	Urticaceae	1
Grossulariaceae	1	Valerianaceae	1
Hypericaceae	2	Verbenaceae	1
Iridaceae	2	Violaceae	4
		Total no. of taxa	**415**

Fig. 8. Rare and protected species from the surroundings of Ulieș Village: **A.** *Aconitum lycoctonum* ssp. *moldavicum*; **B.** *Adonis vernalis*; **C.** *Astragalus exscapus* ssp. *transilvanicus*; **D.** *Gymnadenia conopsea*; **E.** *Galanthus nivalis*; **F.** *Orchis morio* (original)

Fig. 9. Medicinal plants from the surroundings of Ulieș Village: **A.** *Hypericum perforatum*; **B.** *Malva sylvestris*; **C**. *Hibiscus trionum* (original)

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