Current knowledge on phytochemical profile of *Epilobium* sp. raw materials and extracts. Potential benefits in nutrition and phytotherapy of age-related diseases

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Summary

As a consequence of ageing of populations and increase of life expectancy a growing interest in searching novel, safe and effective food preparations, plant origin dietary supplements or plant derived drugs, containing numerous substances that may counteract the emergence of age related disorders is observed. A clearly visible example of searching abovementioned herbal preparations is a growing interest among men suffering for urogenital disorders on preparations based in herbal plants such as: *Serrenoa repens*, *Pygeum africanum*, *Urtica dioica*, *Cucurbita pepo*, *Hypoxis rooperi*, *Secale cereale*, *Zea mays*. In recent years dietary supplements based on *Epilobium* genus representatives became very popular among patients with lower urinary tract symptoms (LUTS) and suffering for benign prostate hyperplasia (BPH).
For the confirmation or justification of the use of preparations based on *Epilobium* sp. in folk medicine or diet supplementation we summarized the current knowledge on biological activities of herbal extracts and oils derived from this genus and variations of phytochemical compositions. The literature analysis reveals that herb was the substance most often subjected to phytochemical analysis (leaves or stems), the next one was seed oil of Epilobiium sp. representatives. In extracts from herb flavonoids (flavonol 3-O-glycosides based on kaempferol, quercetin, myricetin or guaiaverin aglycones), tannins (macrocyclic ellagitannins – oenothein A and B) and trace amounts of sterols as well as fatty acids were identified, while in extracts from seed oil sterols (β-sitosterol, kaempesterol, stigmasterol and brassicasterol) and fatty acids (mainly stearic, palmitic, oleic, linoleic, linolenic and caproic) were most often present.

The conclusions of this paper may be helpful in further extensive research concerning the evaluation the effectiveness and safety of preparations based on the basis of *Epilobium* sp. and *Onagraceae* family representatives. A knowledge of bioactive compounds in *Epilobium* sp. could be especially needed in designing new, safe and effective formulations of nutriceuticals and phytotherapeutics containing medicinal parts of these plants.

Key words: *herbal raw material, Epilobium* sp.*, phytochemical profiling, bioactive substances, flavonoids, polyphenols, sterols, fatty acids, nutrition, phytotherapy*

INTRODUCTION

The ageing of populations and increase of life expectancy leads to more frequent occurrence of age-related diseases (i.e. hormonal disorders such as urogenital disorders associated with andropause (benign prostatic hyperplasia – BPH or prostatitis) or menopause, hyperglycemias, muscosceletal or cardiovascular systems disorders) as well as cancer. Observed noticeable progress in knowledge aiming the clarification of the mechanisms of effect and safety of food preparations, dietary supplements of plant origin (mono–preparations or mixtures) or plant-derived drugs, containing bioactive metabolites, contributes to the increase of effectiveness of prophylaxis and treatment of these diseases, evaluation of new kind of therapeutic strategies and to the development of novel dietary

formulations and phytotherapeutics [1]. A clearly visible example of searching the above mentioned herbal preparations is a rising interest on preparations based on herbal plants such as: *Serrenoa repens*, *Pygeum africanum*, *Urtica dioica*, *Cucurbita pepo*, *Hypoxis rooperi*, *Secale cereale*, *Zea mays* in men suffering from lower urinary tract symptoms (LUTS) and benign prostate enlargement [2]. Recently, especially in Central Europe, more and more popular are becoming preparations based on the *Epilobium* sp. representatives resulting in the emergence of increasing number of formulations on the market, used mainly as dietary supplements - in Poland, for example, on the market appeared such formulations as: "Viking Plus Fix" and "Wierzbownica" based mainly on herb of *Epilobium* parviflorum are gaining a popularity.

Intensive phytochemical studies of herbal raw materials, extracts and preparations have contributed to the determination of plant secondary metabolites, for example, phytosterols, polyphenols, flavonoids and tannins, with potential health benefits, including anticancer [3-5], antioxidant [4] or endocrine normalization properties [6].

According to "PDR for Herbal Medicines" [7] in medicinal parts of *Epilobium* genus plants the presence of flavonoids, polyphenols, sterols and palmitic acid has been identified. Moreover, past years have shown a number of phytochemical and biological studies with representatives of *Epilobium* sp. yielding a wide spectrum of identified metabolites and number of biological properties explaining, confirming or justifying their use in folk medicine or diet supplementation.

In this paper we have attempted to make a comprehensive overview of the current knowledge concerning the phytochemical compounds of potential properties in the prevention and treatment of diseases or rationale nutrition of humans. The conclusions of this paper may help researchers in further extensive research to evaluate the effectiveness and safety of preparations based on *Epilobium* sp. and *Onagraceae* family representatives.

BIOLOGICAL ACTIVITIES AND STUDIES ON PHYTOCHEMICAL PROFILE OF *EPILOBIUM SP.* RAW MATERIALS AND EXTRACTS

Biological activities of *Epilobium* sp. extracts

Biological or pharmacological screening of the substances of potential health benefits, for example anti-aging or chemopreventive activity and their phytochemical profiling, should be systematic and of wide range.

Recently, a growing interest in members of the genus *Epilobium* sp. (family *Onagraceae*) consisting of nearly 185 species worldwide. In Europe this genus, with about 28 species, is divided in two sections (*Epilobium* and *Chamaenerion*) [8, 9]. Most species are commonly known as Willow herb. Tea or ethanolic extracts from fresh aerial parts of this plant are used in folk medicine for the treatment

of prostatic disorders, including BPH, in fever, rheumatic complaints, headache, in pain relief [7]. The mechanism of action of herbal preparations derived from *Epilobium* sp. is not fully understood, but it has been reported, in general, to have anti-proliferative (inhibition of 5α-reductase enzyme), antiandrogenic and antiestrogenic activities [10, 11], and inhibition of cell proliferation [12-16], anti-inflammatory [17-20], antiphlogistic [21, 22], anti-microbial [23-27], and anti-oxidant properties [9, 20, 27-28].

Phytochemistry of *Epilobium* sp. raw materials and extracts

The above mentioned wide range of biological properties and the difficulties occurring during the botanical identification of representatives of the genus *Epilobium* had forced researchers to conduct intensive studies attempting to identify and quantify the active substances in extracts based on these plants.

Flavonoids in *Epilobium* sp. representatives

One of the first studies aimed to identify the presence of flavonoids in *Epilobium* sp. was performed by Averett et al. who have found several variations in the pattern of 7 flavonol 3-O-glycosides based on kaempferol, quercetin, or myricetin aglycones identified in nearly 100 populations of *Boisduvalia* and *Epilobium* genera [29]. Denford et al. in a large survey of 239 populations of *Epilobium* representing 17 taxa in Canada (*Epilobium latifolium*, *E. angustifolium*, *E. glandulosum*, *E. paniculatum*, *E. hirsutum*, *anagallidifolium*, *E. platyphyllum*, *E. hornemanii*, *E. clavatum*, *E. lactiflorum*, *E. alpinum*, *E. luteum*, *E. davuricum*, *E. leptophyllum*, *E. palustre*, *E. palustre* var. *Grammadophyllum*, *E. palustre* var. *Monticolor*) have found following flavonol glycosides: myricetin 3-O-arabinoside; 3-O-glucoside; 3-O-rhamnoside; quercetin 3-O-arabinoside; 3-O-glucoside; 3-O-diglucoside; 3-O-rhamnoside; kaempferol 3-O-glucoside; and 3-O-rhamnoside [30]. In early 80's paper and thin layer chromatography performed by Rządkowska-Bodalska et al. [31] and Hiermann [32] confirmed the presence of kaempferol, myricetin, quercetin and their 3-O-glycosides such as kaempferol-3-O-rhamnoside, kaempferol-3-O-gluciside, myricetin-3-O-rhamnoside, quercetin-3-α-L-arabopyranoside, quercetin-3-O-rhamnoside, 3-O-galactoside and 3-O-glucoside in one of the fractions in the ether extract of *Epilobium parviflorum*. In subsequent researches coordinated by Hiermann several flavonoids in extracts from *Epilobium* sp. were also identified, i.e. in aqueous and methanolic herbal extracts of *E. angustifolium* and *E. parviflorum* [18], in water-alcohol extract of E. hirsutum [23], in aerial parts of *E. dodanaei* [33] and, moreover, in leaves of *E. angustifolium* a novel flavonoid – myricetin-3-O-β-D-glucuronide [21, 34]. Slacanin et al. have also separated and identified several flavonol glycosides from *Epilobium parviflorum* [35]. In early 90's a large analysis of the flavonol

glycoside patterns of 13 *Epilobium species* (from section of *Chamaenerion*: *Epilobium angustifolium*, *E. dodonaei*; from section of *Epilobium*: *Schyzostigma: E. campestre*, *E. hirsutum*, *E. hirsurtum*, *E. montanum*, *E. parviflorum*, and *Synstigma: E. alpinum*, *E. roseum*, *E. salignum*, *E. stereophyllum*, *E. tetragonum*, and a hybrid of *E. roseum* and *E. parviflorum*) was carried out by Ducrey et al. using thermospray liquid chromatography-mass spectrometry (TSP/LC-MS) and high performance liquid chromatography coupled with a photodiode array detector (LC-UV) techniques, which enabled the identification of nineteen flavonol glycosides [36]. All detected flavonols were monoglycosides with substitution at the C-3 position, while the aglycones were kaempferol, quercetin and myricetin. Furthermore, thirteen of those compounds were isolated and determined, including the presence of quercetin, guajaverin, hyperosid, isoquercetin, myricetin, isomyricetin and other. In all analyzed species 3-O-glycosides of above mentioned flavonoids (kaempferol, quercetin and myricetin) were detected, while glycosidic groups were as follows: rhamnose, glucose, galloyl-6-glucose [36]. Flavonoids such as quercetin-3-O-glucuronide and myricetin-3-O-rhamnoside were also detected in different extracts of *E. angustifoium*, *E. hirsutum*, *E. parviflorum* [20].

A large contribution to the understanding of the phytochemical composition of individuals of *Epilobium* sp. was given by the research team of Hevesi et al. [37]. Their results revealed that flavonoid composition could be suitable for authentication of native to Hungary *Epilobium* species and commercial samples as well as a differential flavonoid profile in all analyzed aerial parts of *Epilobium* sp. (*Epilobium parviflorum*, *E. hirsutum* (cultivated plant material), *E. roseum, E. montanum, E. roseum, E. tetragonum*) and commercial samples of *Epilobi herba* drugs have been determined. In all samples, with two exceptions, the main flavonoid was a myricetin (concentration over 40% (%=(AUC/TAUC)*100). Moreover only in *E. hirsutum*, *E. parviflorum* and in three commercial samples a myricetin-glycoside was found (concentration over 40% (%=(AUC/TAUC)*100), while the presence of compound No.6 (not determined) was characteristic for the *E. tetragonum*. Summarizing these results the differential profile of analyzed flavonid-aglycones was not as large as for flavonol-glycosides [37]. Another experimental work confirmed a variety of composition of flavonoid-glycisides compounds (myricetin-, quercetin-, kaempferol-glycosides) in methanolic extracts of five *Epilobium* species occurring in Hungary: E. *parviflorum, E. angustifolium, E. montanum, E. tetragonum, E. roseum* and two *E. parviflorum* samples [7]. Among analyzed species a myricetin-3-O-hexose-gallate, myricetin-3-O-hexoside, quercetin-3-O-hexose-gallate, myricetin-3-O-pentoside, myricerin-3-O-rhamnoside, quercetin-3-O-glucuronide, quercetin-3-O-pentoside, kaempferol-3-O-hexoside, kaempferol-7-O-glucuronide, quercetin-3-O-rhamnoside, kaempferol-3-O-rhamnoside were detected. Furthermore, the higher differences in flavonoids profile have been observed between *E. angustifolium* and other species - a quercetin-glucuronide and kaempferol-glucuronide were present only in *E. angustifolium*, the first one seemed to be major compound in this species, while in other species it was myricetin [7].

The presence of phytosterols in the *Epilobium* sp.

In extracts from representatives of *Epilobium* species phytosterols and its derivatives were also observed [29, 35]. Hiermann et al. have detected β-sitosterol and its esters in a neutral fraction of *E. parviflorum* [38]. Chromatographic analysis of *Epilobium angustifolium* herb revealed a high concentration of β-sitosterol (50.2 mg% determined as weight percentage expressed as acetate), and a much smaller quantities of kaempesterol, stigmasterol and cholesterol (0.9 mg%; 0.3 mg% and trace amounts, respectively) were detected [39]. The content and composition of sterols were also determined by Pelc et al. in the seed oil of two willow herb species (*Epilobium tetragonum* L., *E. hirsutum* L.) [40]. Total oil content for *E. tetragonum* was 22.3%, for the *E. hirsutum* – 28.1%. In seed oil samples derived from all analyzed *Epilobium* sp. five phytosterols were determined, namely: cholesterol, brassicasterol, kaempesterol, stigmasterol and β-sitosterol (1%, 4.29%, 0.26%, 0.92%, 8.65% in *E. tetragonum,* and 0.12%, 3.44%, 0.29%, 4.02%, 6.65% in *E. hirsutum,* respectively) [40]. Results obtained from this scientific group suggest that β-sitosterol and brassicasterol appeared to be the main sterols in the seed oil of these two plants [40]. A relatively small level of sterols during whole time of vegetational stage of *Epilobium angustifolium* (study period from June to September) was observed by Mrozikiewicz et al. [41].

The presence of polyphenolic acids and tannins in the *Epilobium* sp.

Another group of chemical compounds found in raw materials and extracts derived from above mentioned genera, are polyphenolic compounds, namely polyphenolic acids and tannins [38, 42]. One of the bio-active tannin – oenothein B – has been reported and isolated from aqueous and alcohol extracts of aerial parts of *Epilobium hirsutum* by e.g. Ivancheva et al. [23], from the butanol fraction of *E. angustifolium* herb (phenolic acids such as gallic and ellagic acids were also isolated from the ethyl acetate fraction of this herb) [11] and from stems and leaves of *E. parviflorum* [10, 21]. The concentration of oenothein B has also been determined by another authors. Bazylko et al. identified this macrocyclic ellagitannin in water extract of *E. angustifolium* (the mean concentration of oenothein B was 152.46±4.92 mg/g [43]. In another experiment, carried out by Kiss et al., this substance was determined in water extracts of this herb and two other species of *Epilobium* (*E. parviflorum, E. hirsutum*), resulting in its mean concentrations 225.8±7.6; 326.7±6.2; 333.6±24.8 mg/g, respectively, or above mentioned species [20]. Furthermore, the research team led by Vitalone compared the concentration of oenothein B in water extracts of two different *E. angustifolium* (Linné denomination of *E. spicatum* – from Europe) and *E. angustifolium* from Canada. Results obtained in their work have shown that the amount of oenothein B in *E. angustifolium* from Canada was approximately 40-fold higher than that of *E. spicatum* produced in Europe (1.34% vs. 0.03%,

respectively) [44]. A content assessment of abovementioned tannin – oenothein B, as well as another macrocylic ellgaitannin – oenothein A (a trimeric form) was made also by Ducrey et al. in aequous-methanol extracts from representatives of *Epilobium* sp. occurring mainly in Switzerland, such as: *E. dodanei, E. hirsutum* (Kenya), *E. stereophyllum, E. salignum, E. montanum, E. parviflorum, E. angustifolium, E. roseum, E. capense, E. hirsutum* (Switzerland). Mean contents of oenothein B for above mentioned species were as follow: 14.0±0.33%; 12.0±0.25%; 10.3±0.21%; 7.0±0.16%; 5.2±0.18%; 5.1±0.18%; 4.5±0.2%; 4.0±0.21%; 3.2±0.23%; 2.4±0.26%, respectively [11]. Moreover, results received by Pelc et al. revealed that herbs of *Epilobium angustifolium* and *E. hirsutum* differ one from another in the total profile of polyphenolic acids and flavonoids in the generative and vegetative phase. In general, the herb of *E. angustifolium* had more polyphenolic acids than the other one species (the content of polyphenolic acids was in the vegetative stage was 3.29%, 1.35%, in the generative stage was 3.63%, 1.78% in *E. angustifolium* and *E. hirsutum,* respectively) [40]. Different profile of tannins in *E. angustifolium* during vegetation and fructification was also observed by Mrozikiewicz et al. [41].

The presence of fatty acids in the *Epilobium* sp.

Another group of phytochemical compounds found in herb extracts and seed oil of *Epilobium* sp. are different saturated and non-saturated fatty acids and their esters. For example, in the oil-etheric fraction WE2 from seed oil of *E. parviflorum* a palmitic (21.5%), palimiticoil (5.7%), stearic (7.0%), oleic (10.5%), arachidinoic (11.0%), arachidoic (15.0%) acids and some non-saturated fatty acids such as: linoleic (18.0%) and linolenic (15.0%) acids were detected [31]. In seed oil of another two species: *Epilobium tetragonum* and *E. hirsutum* six fatty acids (stearic, palmitic, oleic, linoleic, γ-linolenic and α-linolenic acids) were also identified in concentrations of 15.2; 94.0; 28.4; 697.5; 43.5; 13.9 mg/g for *E. tetragonum,* and 25.2; 83.9; 51.6; 747.3; 11.2; 1.7 mg/g for *E. hirsutum,* respectively. In seeds oil of both analyzed species linoleic and palmitic acids were predominant. However, in *E. tetragonum*, the content of both linolenic acids were higher than in the *E. hirsutum,* while the content of linoleic acid in the seed oil of both willow herb species were similar [42]. A high content of palmitic acid in a nonpolar fraction of ethanolic extracts of *E. tetragonum* and *E. hirsutum* seed oils were observed also in analysis performed by Vitalone et al. (94% and 83.9% in *E. tetragonum* and *E. hirsutum,* respectively) [44]. Furthermore, Hiermann et al. identified and quantified 17 fatty acids, including caprooic, caprylic, capric, lauric, myristic, palmitic, stearic, linoleic, linolenic, arachidic, behenic, lignoceric, cerotic, montanic, and mellisic acid, in hexan extract of *Epilobium angutifolium,* in which predominantly linolenic, linoleic, palmitic and cerotic acids have occurred. In general, these results of this study were similar to results obtained from above mentioned studies [45].

CONCLUSIONS

In recent years there is a growing interest observed in searching of herbal substances and their preparations which potentially can be used in prevention and prophylaxis of age-related diseases, prevention of cancer and for widening of an offer of nutritional and dietary supplements containing bioactive substances of plant origin with potential health benefits.

In this paper, we summarized the current knowledge on biological activities of herbal extracts from *Epilobium* genus and their variations of phytochemical composition. The literature analysis reveals that substance most often subjected to phytochemical analysis from a herbal was herb (leaves or stems), less frequently seed oil of *Epilobium* sp. representatives. In extracts from herb flavonoids (flavonol 3-O-glycosides based on kaempferol, quercetin, myricetin or guaiaverin aglycones), tannins (macrocyclic ellagitannins – oenothein A and B) as well as trace amounts of sterols and fatty acids were identified, while in extracts from seed oil sterols (β-sitosterol, kaempesterol, stigmasterol and brassicasterol) and fatty acids (mainly, stearic, palmitic, oleic, linoleic, linolenic and caproic) were most often present.

According to the authors, knowledge presented in this paper may be helpful for further extensive research evaluating the effectiveness and safety of preparations based on plants of *Onagraceae* family, including *Epilobium* sp. representatives, in safe, effective prophylaxis and phytotherapy of age-related diseases and in sound and safe nutrition of humans. A knowledge of bioactive compounds in *Epilobium* sp. is needed especially in designing new, safe and effective formulations of nutriceuticals and phytotherapeutics for humans containing medicinal parts of this plants.

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PODSUMOWANE AKTUALNEGO STANU WIEDZY NA TEMAT ANALIZ FITOCHEMICZNYCH WYCIĄGÓW ROŚLINNYCH Z RODZAJU *EPILOBIUM* Z UWZGLĘDNIENIEM ICH POTENCJALNEGO ZNACZENIA W RACJONALNYM ŻYWIENIU I FITOTERAPII SCHORZEŃ ZWIĄZANYCH Z WIEKIEM

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S t r e s z c z e n i e

W konsekwencji starzenia się populacji oraz wydłużenia życia w ostatnich latach obserwuje się znaczny wzrost zainteresowania przetworami spożywczymi, suplementami diety i lekami pochodzenia roślinnego, zawierającymi liczne substancje czynne, mogącymi przeciwdziałać powstawaniu lub progresji schorzeń postępujących wraz z wiekiem. Szczególnie widocznym przykładem poszukiwania przez konsumentów i pacjentów wspomnianych bioaktywnych preparatów pochodzenia roślinnego jest wzrost zainteresowania u pacjentów cierpiących na schorzenia układu moczowo-płciowego preparatami na bazie następujących surowców zielarskich: *Serrenoa repens, Pygeum africanum, Urtica dioica, Cucurbita pepo, Hypoxis rooperi, Secale cereale, Zea mays*. U mężczyzn z dolegliwościami w dolnych okolicach dróg moczowych i dotkniętych łagodnym przerostem gruczołu krokowego szczególnym zainteresowaniem w ostatnich latach cieszą się preparaty oparte na bazie surowców zielarskich z rodziny *Epilobium* (wierzbownica).
W celu potwierdzenia i uzasadnienia ich stosowania w zielarstwie oraz suplementacji diety dokonaliśmy przeglądu aktualnego stanu wiedzy dotyczącej identyfikacji i określenia stopnia zmienności metabolitów wtórnych zawartych w wyciągach i olejach z nich uzyskanych. W toku analizy literatury wykazano, iż w wyciągach z ziela *Epilobium* sp. wykazano obecność głównie flawonoidów (3-O-glikozydów kemferolu, kwercetyny, myrycetyny i guajaweryny), tanin (makrocyklicznych ellagitanin – oenoteiny A i B) oraz w mniejszych ilościach steroli i kwasów tłuszczowych. W oleju z nasion natomiast wykazano obecność głównie fitosteroli (m.in. β-sitosterolu, kampesterolu, stigmasterolu and brassikasterolu) oraz kwasów tłuszczowych (głównie stearynowego, palmitynowego, oleinowego, linolowego, linolenowego i kapronowego).

Wnioski z niniejszej publikacji mogą być pomocne dla podjęcia dalszych, szeroko zakrojonych badań nad oceną skuteczności i bezpieczeństwa stosowania preparatów żywieniowych i leczniczych produkowanych przy udziale surowców roślinnych z rodziny *Onagraceae*, w tym też poszczególnych surowców zielarskich z rodzaju *Epilobium*. Znajomość występowania związków bioaktywnych w wyciągach może być również przydatna przy projektowaniu nowych, bezpiecznych i efektywnych postaci nutriceutyków i fitoterapeutyków opartych na *bazie* powyższych surowcach zielarskich.

Słowa kluczowe: *surowce zielarskie, Epilobium sp., profilowanie fitochemiczne, substancje bioaktywne, flawonoidy, polifenole, sterole, kwasy tłuszczowe, nutraceutyki, fitoterapeutyki*