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REVIEW PAPER

Pharmacological properties of fireweed (*Epilobium angustifolium* L.) and bioavailability of ellagitannins. A review

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Summary

Fireweed (*Epilobium angustifolium* L.) is a well-known medicinal plant traditionally used in the treatment of urogenital diseases, stomach and liver disorders, skin problems, etc. *E. angustifolium* extracts show anti-androgenic, antiproliferative, cytotoxic, antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial activities. The unique combination of biological properties demonstrated by the results of some studies indicates that fireweed has a positive effect in benign prostatic hyperplasia (BPH) and potentially in the prostate cancer chemoprevention. However, the efficacy of *E. angustifolium* phytotherapy is still poorly tested in clinical trials, while numerous beneficial effects of extracts have been documented in the *in vitro* and *in vivo* tests. Fireweed is rich in polyphenolic compounds, particularly ellagitannins. Currently, polyphenols are considered to be modulators of beneficial gut microbiota. The literature data support the use of ellagitannins in the prostate cancer chemoprevention, but caution is advised due to the highly variable production of urolithins by the individual microbiota. A better understanding of the microbiota's role and the mechanisms of its action are crucial for an optimal therapeutic effect. This paper aims to summarize and discuss experimental data concerning pharmacological properties of *E. angustifolium* and bioavailability of ellagitannins – important bioactive compounds of this plant.

Key words: ***rosebay willowherb, pharmacology, bioavailability, benign prostatic hyperplasia (BPH), oenothein B, urolithins, gut microbiota***

Słowa kluczowe: ***wierzbówka kiprzyca, farmakologia, biodostępność, łagodny rozrost prostaty (BPH), oenoteina B, urolityny, mikroflora jelitowa***

INTRODUCTION

Fireweed (*Epilobium angustifolium* L., syn. *Chamerion angustifolium* /L./ Holub) is a medicinal plant from the *Onagraceae* family traditionally used in the treatment of urogenital diseases, stomach disorders, liver inflammation, burns, wounds, and skin problems [1]. This species has an abundance of polyphenolic compounds, especially ellagitannins, and also phenolic acids and flavonoids. Among other bioactive constituents of fireweed, sterols, triterpenes, fatty acids, and essential oils have been found. The therapeutic effects of *E. angustifolium* include antiandrogenic, antiproliferative, cytotoxic, antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial activities (fig. 1) [1]. The European Medicines Agency Assessment Report [2] on *E. angustifolium* and *E. parviflorum* concludes that the aerial parts of these herbs are useful in alleviating symptoms of lower urinary tracks related to benign prostatic hyperplasia (BPH) and assumes their safety in this respect.

BPH is an age-related disease which develops after the age of 40 and among the 60-year-old men it affects more than 50% [3]. BPH is mainly caused by changes in hormone balance and cell-growth factors in the prostate gland. Androgen deficiency condition occurring in elderly men as well as previous inflammation of the prostate gland and positive family history are the risk factors contributing to BPH development and progression. Although the molecular mechanism of BPH development is still not fully understood, the common accepted theory postulates the key role of enzymes: steroid 5α-reductase (5α-R) and aromatase (5α-A) and growth factors in this process [4]. The 5α-R transforms testosterone into dihydrotestosterone (DHT) in prostate cells. DHT binds androgen receptors with a higher affinity than testosterone and it directly stimulates the growth and differentiation of prostate cells. Binding to androgen receptors triggers a transduction cascade of growth factors (epidermal growth factors – EGF, insulin-like growth factors – IGF and others) leading to abnormal prostate cell proliferation. Estrogens act synergistically with androgens and promote the development of BPH. Increased expression of the 5α-A that catalases the conversion of androgens into estrogens was observed in hypertrophic prostates [4].

BPH is a good target for preventive phytotherapy due to the long latency of this disease, and it is usually well tolerated by patients. It is also a cost-effective alternative. The effectiveness of phytotherapy in the BPH prevention is still being discussed, mainly due to limited double-blind clinical trials or difficulties associated with different extraction and formulation methods, variability in the chemical composition and the lack of raw material standardization. Currently, there are no clinical studies regarding the effectiveness of *E. angustifolium*, although a number of preclinical trials have revealed the anti-proliferative, anti-inflammatory, analgesic, antioxidant and antimicrobial effects of its extracts [5].

Various beneficial effects of fireweed have been attributed to the phenolic compounds, particularly to macrocyclic ellagitannin – oenothein B [1, 6, 7]. The opinion that the regular consumption of ellagitannin-rich products can help reduce the risk of some chronic diseases, and may be useful in the cancer prevention is commonly accepted [8, 9]. Bioavailability of ellagitannins is poorly understood, but it has been intensively studied since last decade. The gastrointestinal microbiota is an important modulator and contributor to human health, and it is involved in the management of host metabolism of polyphenols, including ellagitannins. Nowadays, the ecology of gut microbiota and its health benefits are hot topics in research on metabolic syndrome, obesity-related diseases, inflammatory bowel disease and other conditions [10, 11]. According to the new concept of prebiotics, polyphenols are modulators of beneficial microbiota and therefore great hopes are associated with their use in the prevention of cancer as well as metabolic or cardiovascular diseases.

The aim of the present work was to summarize and discuss the current literature data concerning pharmacological properties of *E. angustifolium* and bioavailability of ellagitannins – important bioactive compounds of this species. This overview is a continuation of our prior article on botany, phytochemistry and traditional uses of fireweed [1].

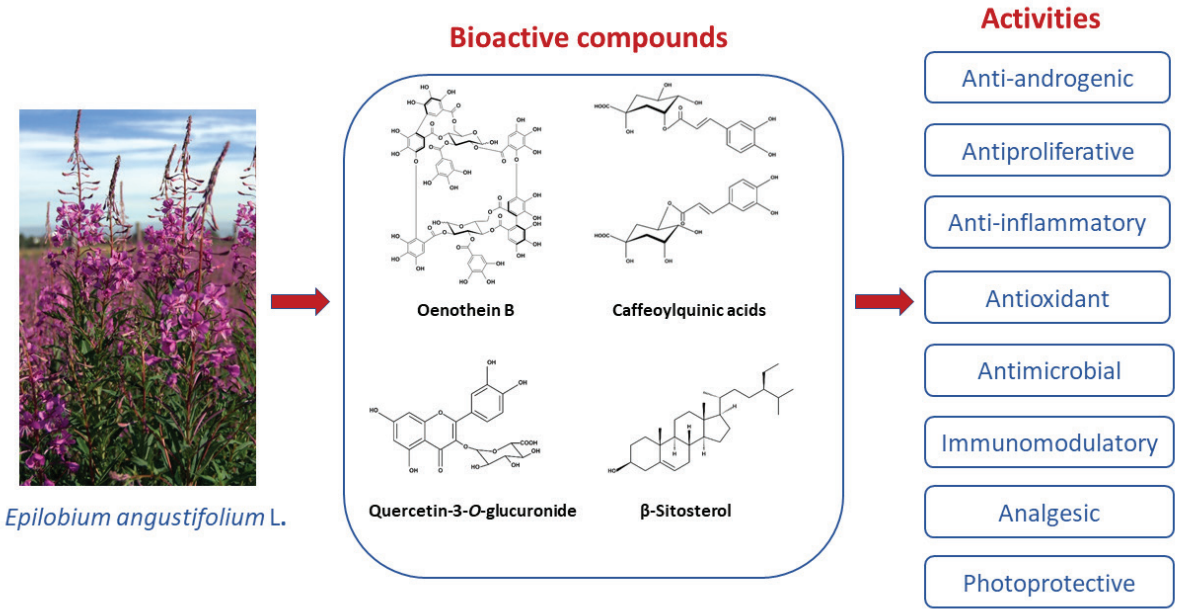

Figure 1
Pharmacological properties of *Epilobium angustifolium*

PHARMACOLOGICAL PROPERTIES

Anti-androgenic activity

The anti-androgenic effect of *E. angustifolium* extract was confirmed in animal tests [12] (tab. 1). Orally administered water extract (dose: 40 mg/kg bw) decreased the weight of vesicles in intact rats, whereas in castrated and testosterone-stimulated rats an increase in prostate vesicle weight was observed (pro-androgenic effect). Although the mechanism of action was not ascertained, it was suggested that the extract contains compounds which enhances bioactivity of the applied androgens. The inhibitory activity of oenothein A and B against 5α-reductase and aromatase has been reported in other studies [13, 14]. More recent work reported antiproliferative and anti-androgen activities of *E. angustifolium* water extract and oenothein B in *in vitro* and *in vivo* models [15]. Oral supplementation of rats implanted with LNCaP cells with extract (dose: 50–200 mg/kg bw) resulted in a significant reduction of prostatic adenoma by up to 13%. Recently, anti-androgenic effect of *n*-butanol extract against testosterone propionate-induced BPH rats *via* down-regulation of androgen level and the suppression of NF-κB (nuclear transcription factor kappa B) expression has been reported [16]. The decreased level of dihydrotestosterone and activity of aromatase in a dose-dependent manner was demonstrated after oral administration of extract (100–400 mg/kg bw). The therapeutic effect was similar to finasteride treated group (3 mg/kg b.w). Gallic acid and avicularin (quercetin 3-*O*-α-L-arabinofuranoside) were the most active compounds in the inhibition of prostate specific antigen (PSA) secretion with the level of 21.82 and 33.19%, respectively.

Antiproliferative and cytotoxic activities

E. angustifolium extracts showed antiproliferative activities towards different cell lines, including human prostate epithelial cells (PZ-HPV-7 line), human astrocytoma cells (1321N1), human prostate adenocarcinoma cells (LNCaP), and normal mammary cells (HMEC) [17, 18] (tab. 1). The antiproliferative effect was not specific to prostate cells and the inhibition was similar (in IC_{50} value) for both androgen-sensitive (LNCaP) and androgen non-responsive cells (PZ-HPV-7). The above-mentioned effect was attributed to the high amount of oenothein B in ethanolic extract. The ability of *E. angustifolium* extracts to induce metallopeptidase activity in a PC-3 cell model (androgen-independent prostate cancer line) was also documented [19-21].

Stolarczyk *et al.* [22] demonstrated the inhibition

Table 1.

Literature overview on the main biological activities of *Epilobium angustifolium* extracts

Plant material/extracts/ compounds	Experimental model/assays	Effect	Reference
E. angustifolium herb/ aqueous and hexane extracts	*In vivo*; anti-androgen assay on intact and testosterone stimulated castrated rats	Anti-androgenic effect of aqueous extract (40 mg/kg/day) on intact rats	[12]
E. angustifolium/aqueous extract	*In vitro*; LNCaP, PZ-HPV-7 and Du 145 (human prostate carcinoma cells). *In vivo*; male rats implanted with LNCaP cells	Anti-androgenic and selective anti-proliferative effect of extract and oenothein B	[15]
E. angustifolium leaves, stems, flowers/ethyl acetate and *n*-butanol extracts	*In vivo*; testosterone propionate stimulated castrated rats. *In vitro*; human prostate cells (BPH-1 and LNCaP lines); cell proliferation (MTT assay) and cytotoxicity assay; PSA (prostate specific antigen) assay	Anti-androgen activity of *n*-butanol extracts. Gallic acid was the strongest inhibitor of PSA secretion and showed the most potent anti-proliferative activity	[16]
Commercial *E. angustifolium* ethanolic extract	*In vitro*; human prostatic epithelial cells (PZ-HPV-7). Cell proliferation (MTT assay) and cytotoxicity assay (LDH)	Anti-proliferative effect at concentration 1900 μg/ml after 24 and 48 h	[17]
E. angustifolium, *E. rosmarinifolium, E. tetragonum* aerial parts/ ethanolic extracts/ oenothein B	*In vitro*; human prostate cells (PZ-HPV-7, LNCaP); human mammary epithelial cells (AG 11132), human astrocytoma cells (1321N1). Cell proliferation assay (flow cytometry); determination of cell viability (trypan blue exclusion assay) and measurement of DNA synthesis	Anti-proliferative effect on different prostate cells, strongly correlated with oenothein B concentration	[18]
E. angustifolium herb/ methanolic, ethyl acetate, butanolic, chloroform extracts/oenothein B	*In vitro*; neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE), aminopeptidase (AMP) activity	Inhibition of metallopeptidases activity. Oenothein B exhibited inhibition of NEP and ACE with IC_{50}=20 μM and IC_{50}=250 μM, respectively	[19]
E. angustifolium herb/ aqueous extract/ oenothein B	*In vitro*; human androgen independent prostate cells (PC-3) and human neuroblastoma cells (SK-N-SH). NEP activity; cell proliferation assay (Hoechst 33258)	Inhibition of metallopeptidases and anti-proliferative effect. Flavonoids showed much slighter activity on NEP than oenothein B	[20]
E. angustifolium herb/ aqueous and methanolic extracts	*In vitro*; PC-3 cells; NEP and ACE activity, cell proliferation assay (Hoechst 33258), cell cytotoxicity	Inhibition of metallopeptidases and anti-proliferative effect. Oenothein B induced NEP activity in dose-dependent manner	[21]
E. angustifolium, *E. hirsutum*, *E. parviflorum* aerial parts/ aqueous extracts/oenothein B, quercetin-*3*-*O*-glucuronide, myricetin-*3*-*O*-rhamnoside, urolithins	*In vitro*; LNCaP cells. PSA assay and arginase activity assay	Anti-proliferative effect. *Epilobium* extracts inhibited cell proliferation with IC_{50} value of 32.2–44.6 μg/ml. Oenothein B was the strongest inhibitor of cell proliferation (IC_{50}=7.8 μM)	[22]
E. angustifolium, *E. hirsutum*, *E. parviflorum* aerial parts/aqueous extracts	*In vitro*; LNCaP cells. PSA assay and arginase activity assay	Apoptotic effect of extracts (from 2.86 to 86.6%) by activation of mitochondrial pathway	[23]
E. angustifolium/aqueous extract	*In vitro*; breast cancer cell lines: MCF7, MDA-MB-468, MD-MB-231	Anti-proliferative effect. Dose-dependent inhibition of growth and proliferation of cells	[28]
E. angustifolium herb/ ethanolic extract	*In vitro*; MTT and Comet assay (human hepatocellular carcinoma HepG2 cells). DNA fragmentation assay. Oxygen Radical Absorbance Capacity (ORAC)	Cytotoxic and genotoxic effect. DNA damages in HepG2 cells. Antioxidant effect	[27]
E. angustifolium and *E. parviflorum* herbs/ aqueous extracts	*In vivo*; carrageen induced rat paw edema, perfused rabbit ear. Release of prostaglandins	Anti-inflammatory effect. Reduced release of prostaglandins	[36]

Table 1. (*continued*)

Plant material/extracts/ tested compounds	Experimental model/assays	Effect	References
E. angustifolium leaves/ aqueous extract/myricetin-*3-O*-glucuronide	*In vivo*; carrageen induced rat paw edema; perfused rabbit ear. Release of prostaglandins.	Anti-inflammatory effect of myricetin-*3-O*-glucuronide	[37]
E. angustifolium and *E. parviflorum* herbs/aqueous and alcoholic extracts	*In vivo*; carrageen or dextran induced rat paw oedema. Release of prostaglandins	Anti-inflammatory effect comparable with indomethacin	[38]
E. angustifolium, E. hirsutum, E. parviflorum herbs/aqueous extracts/ oenothein B, quercetin-*3-O*-glucuronide, myricetin-*3-O*-rhamnoside	*In vitro*; human skin fibroblast (NHDFs), human neutrophils. Lipoxygenase, cyclooxygenase and hyaluronidase assay. Elastase and myeloperoxidase (MPO) release by human neutrophils. Evaluation of ROS production by f-MLP (formylmethionyl-leucyl-phenylalanine) and PMA (phorbol myristate acetate) stimulated neutrophils	Antioxidant effect. ROS (IC_{50}=5 and 25 μg/ml). Extract activity was related with activity of oenothein B, which was the strongest scavenger (IC_{50}=1 μM)	[32]
E. angustifolium aerial parts/commercial water-soluble extract	1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging assay, Iron(III) to Iron (II) Reducing Activity assay, Iron II Chelation activity assay, Ascorbate-Iron(III)-Catalyzed Phospholipid Peroxidation, Site-Specific and Non-Site-specific Hydroxyl Radical-Mediated 2-Deoxy-D-rybose degradation assays	Antioxidant effect correlated with the highest total polyphenol content	[29]
E. angustifolium fresh leaves, stems, and roots extracted with phosphate medium (pH=7)	DPPH assay, Ferric Reducing Antioxidant Power (FRAP) assay, activity of superoxide dismutase, catalase, peroxidase and reduced glutathione assay	Antioxidant effect. The highest antioxidant activity was observed in leaves	[30]
E. angustifolium, E. parviflorum, E. montanum, E. tetragonum, E. roseum/ methanolic extracts	Spectrophotometric method/ABTS assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). Extracts concentration: 10–50 μg/ml	Antioxidant activity comparable with Trolox and ascorbic acid. (IC_{50} value from 1.71 to 3.00 μg/ml)	[31]
E. angustifolium aerial parts collected from 6 different locations (Lithuania)	Online HPLC-DPPH radical scavenging assay	Antioxidant effect. RSA was strongly correlated with amount of oenothein B. Ecotypes of *E. angustifolium* differ in radical scavenging activity	[33]
E. angustifolium at different phase of vegetation/ aqueous and methanolic extracts	DPPH assay	Antioxidant activity. The highest radical scavenging activity showed material collected during massive blooming	[34]
E. angustifolium aerial parts/ethanol, ethyl acetate and petroleum ether extracts	DPPH, ABTS, FRAP and TBARS (thiobarbituric acid reactive substances) assay	Antioxidant effect of ethyl acetate extract. Methyl gallate, quercetin, and gallic acid were the main antioxidant constituents	[35]
29 species including *E. angustifolium* herb/ dry extract dissolved in methanol	Hole-plate and cylinder diffusion methods. Microbial species: *Aspergilus niger*, *Bacillus subtilis, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa, Staphylococcus aureus*, and *S. epidermidi*s	Antimicrobial effect against *S. aureus* and *E. coli*	[41]
E. angustifolium, E. hirsutum, E. palustre, E. tetragonum, E. rosmarinifolium aerial parts/ethanolic extracts	Microdilution method. Microbial species: *Staphylococcus aureus, Streptococcus pyogenes, S. sanguis, Enterococcus faecalis, Listeria monocytogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, Schigella flexneri, Salmonella enteritidis*, *Candida albicans, C. glabrata, C. crusei, Microsporum canis, M. gypseum, Trychophyton rubrum, T. mentagrophytes*	Antimicrobial effect. *E. angustifolium* and *E. hirsutum* were most effective against *Microsporum canis* (MIC=10 µg/ml)	[42]
14 species including *E. angustifolium* root/aqueous extracts	Microdilution method. Microbial species: *Candida krusei, C. albicans, C. parapsilosis, C. glabrata, C. tropicalis, C. lusitaniae, Saccharomyces cerevisiae, Cryptococcus, neoformans, Trichophyton tonsurans, T. rubrum, Microsporum canis, Epidermophyton floccosum, Aspergillus fumigatus, A. flavus, Fusarium solani, Rhizophus* sp.	Antifungal effect. The strongest activity against *C. glabra* and *C. lusitaniae* (MIC=25 and 50 μg/ml, respectively)	[43]
E. angustifolium whole plant/commercial aqueous extract	Microdilution method. Microbial species: *Staphylococcus aureus, Microsporum luteus, Echerichia coli, Psedomonas aeruginosa*	Antibacterial effect, extracts more effective than vancomycine or tetracycline	[44]
E. angustifolium leaves and flowers/ethanolic extracts	Microdilution and diffusion methods. Microbial species: *Staphylococcus aureus* (including MRSA), *Bacillus subtilis, Escherichia coli* (including p-fimbriae positive strain), *Pseudomonas aeruginosa, Candida albicans, C. tropicalis, C. dubliniensis, Saccharomyces cerevisiae, Proteus mirabilis*	Antibacterial effect. No differences between antimicrobial activity of leaves and flowers	[45]
E. angustifolium/ ethanolic dry extract prepared from tincture (10% w/v)	*In vivo* (mice); hot plate test, writhing test	Analgesic (380 mg/kg s.c.) effect	[46]
E. angustifolium aerial parts/1,3-butanediol extract	*In vitro*; normal human dermal fibroblast. *In vivo*; skin exposure to UV radiation (8 healthy Caucasian volunteers)	Photoprotective (UV-induced erythema *in vivo*) and antioxidant effect	[47]

of prostate specific antigen (PSA) secretion and the arginase activity of *Epilobium* spp. extracts. Aqueous extracts of *E. angustifolium*, *E. parviflorum* and *E. hirsutum* reduced PSA secretion from 325.6 to about 90 ng/ml, and inhibited the arginase activity, which was dependent on the oenothein B concentration. Oenothein B showed a stronger inhibitory effect on cell proliferation (IC_{50}=7.8 μM), PSA secretion (IC_{50}=21.9 μM) and arginase activity (IC_{50}=19.2 μM) compared to the flavonoids (IC_{50}>40 μM for myricetin-3-*O*-rhamnoside). Despite the lack of oenothein B, biological activity was also determined in the case of acetate extract, especially from *E. angustifolium*, but this effect was weaker, and attributed to an unidentified extract compound. Additionally, urolithins (metabolites of ellagitannins transformed by gut microbiota) were also tested, and urolithin C showed the strongest antiproliferative effect (IC_{50}=35.2 μM). However, it was weaker than that of oenothein B. The apoptotic potential of the standardized *E. angustifolium*, *E. parviflorum* and *E. hirsutum* extracts was demonstrated on LNCaP cells [23]. A significant increase in the level of apoptotic cells compared to the controls (86.6 *vs* 2.86%) was observed. All extracts reduced the mitochondrial potential and increased caspase-3 activity over four-fold (0.3 *vs* 1.26 ng/mg of protein). The authors also suggested another mode of action *via* reactive oxygen species (ROS). Cumulative evidence has indicated that oenothein B can stimulate the ROS production and trigger apoptosis in a caspase-independent manner [24-26]. Cytotoxic and apoptotic effects of *E. angustifolium* ethanolic extract were investigated and confirmed by MTT and Comet assays [27]. Extracts at concentration of 50–75 μg/ml reduced cell viability and induced DNA damages in hepatocellular carcinoma.

The inhibition of growth and proliferation of some breast cancer cell lines exposed to *E. angustifolium* extracts was also reported [28]. In turn, antiproliferative activity of fireweed water extract and oenothein B in *in vitro* and *in vivo* models was described by Piwowarski *et al.* [15]. This extract and oenothein B showed a selective effect against cancer cells (LNCaP and PZ-HPV-7), more significantly inhibiting the proliferation of the dihydrotestosterone-stimulated LNCaP cells in comparison with antiandrogens. Recently, *in vitro* screening of 50 isolated compounds from *n*-butanol and acetate extracts revealed that 26 substances induced anti-proliferation in BPH-1 cells, while 36 of them showed PSA (prostate specific antigen) inhibition in LNCaP cells [16]. Among isolated compounds, gallic acid exhibited the most potent antiproliferative effect in both cell lines.

Antioxidant activity

Commercially available water-soluble extracts from *E. angustifolium* leaves showed significant antioxidant activity in *in vitro* assays [29] (tab. 1). The antioxidant properties of different plant parts (roots, leaves, and stems) were evaluated by Štajner *et al.* [30]. The highest activity determined by DPPH and FRAP assays was noted for leaves. The strong antioxidant capacity of *Epilobium* species was attributed to the high ellagitannin content [31] and particularly to oenothein B [32, 33]. The assessment of radical scavenger activity (RSA) of *E. angustifolium* harvested during vegetation with respect to the flavonoid content was reported by Maruška *et al.* [34]. The highest levels of flavonoids (8.71–11.12 mg/g) and RSA were determined for flowers collected during the massive blooming phase. Lipid peroxidation inhibition and antiradical (DPPH, ABTS) activities of ethanolic extracts have been recently confirmed [35]. In these investigations, flavonoids and phenolic acids were the main components of the active fractions of the fireweed extract.

Anti-inflammatory and immunomodulatory activities

In early studies, the reduction in pro-inflammatory cytokines and inhibition of prostaglandin synthesis (PGE_2, PGI_2, PGD_2) were revealed in *in vivo* assays [36, 37] (tab. 1). The *E. angustifolium* extract showed strong anti-inflammatory activity towards carrageenan-induced edema in rats [38]. Additionally, myricetin-3-*O*-glucuronide isolated from the leaves was more than ten times more effective than the nonsteroidal anti-inflammatory drug – indomethacin. Kiss *et al.* [32] compared the anti-inflammatory properties of *E. parviflorum*, *E. hirsutum* and *E. angustifolium* extracts as well as selected compounds: oenothein B, quercetin-3-*O*-glucuronide and myricetin-3-*O*-rhamnoside in an *in vitro* assay. All extracts inhibited hyaluronidase and lipoxygenase activity. Oenothein B was the strongest inhibitor of hyaluronidase (IC_{50}=1.1 μM) and myeloperoxidase (IC_{50}=7.7 μM), whereas flavonoids showed no or a much weaker effect. Oenothein B was also active against cyclooxygenase-1 (COX-1) at a level comparable with indomethacin, while extracts exhibited

weak activity against both enzymes (COX-1 and COX-2).

Activation of phagocytic cells and modulation of immune function by the *E. angustifolium* extract and isolated oenothein B were also reported [24, 39, 40]. Fireweed extract activated ROS production in murine bone marrow leukocytes in a dose-dependent manner and induced NF-κB in human monocytes [24]. Differences in cytokine production by T cells exposed to oenothein B were observed [40]. The authors suggested that the immunostimulant effect of oenothein B could be modulated by T cell age, particularly with respect to the cytokine production.

Antimicrobial activity

Antimicrobial activity of *E. angustifolium* extract against *Staphylococcus aureus* and *Escherichia coli* was determined by Rauha *et al.* [41] (tab. 1). In turn, Battinelli *et al.* [42] reported moderate or weak action of ethanolic extract against both Gram-positive and Gram-negative bacteria as well as yeast. The significant antifungal activity of *E. angustifolium* root extract against *Candida glabrata*, *C. lusitaniae*, and *Saccharomyces cerevisiae* (MIC=25–50 μg/ml) was detected by Webster *et al.* [43]. Furthermore, the whole plant ethanolic extracts strongly inhibited the growth of *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Obtained extracts were more effective than antibiotics: vancomycin and tetracycline [44]. Kosalec *et al.* [45] compared the activity of ethanolic extracts from leaves and flowers of *E. angustifolium*, and exhibited their similar effect against various bacterial strains and fungi: *Staphylococcus aureus* (including methicillin-resistant *S. aureus*), *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Candida albicans*, *C. tropicalis*, *C. dubliniensis*, and *Saccharomyces cerevisiae* (MIC=4.6–18.2 mg/ml).

Other properties

Analgesic activity of *E. angustifolium* tincture was determined by the hot plate and writhing test in mice [46] (tab. 1). Additionally, the photoprotective and antiaging properties of fireweed extract have been revealed in human stress-induced fibroblasts in *in vitro* and *in vivo* models [47].

Toxicity and safety

According to the European Medicines Agency (EMA), *E. angustifolium* extracts did not show toxicity and can be considered safe in traditionally used doses [2]. Nevertheless, there are no clinical data about the safety of *E. angustifolium* extracts, and there are only two *in vivo* studies [46, 48]. Tita *et al.* [46] conducted a single-dose toxicity experiment on mice and determined LD_{50}=1.4 g/kg bw (subcutaneously) after 24 h. In a repeated-dose toxicity study hydro-alcoholic extracts of *E. angustifolium*, *E. hirsutum* and *E. parviflorum* were tested on albino male Wistar rats and no toxicity effect was observed in the brain, hypothalamic-hypophyso-adrenal axis, liver, kidneys, spleen or thymus [48]. The results of other studies showed the influence of *E. angustifolium* on the expression of cytochromes [49, 50] – enzymes that metabolize drugs and could cause the potential risk of interactions.

BIOAVAILABILITY OF ELLAGITANNINS

Oenothein B is the most abundant ellagitannin of fireweed with a broad spectrum of biological activities, including anti-androgenic, cytotoxic, antioxidant, anti-inflammatory, immunomodulatory, and neuroprotective effects [6, 7]. However, the bioavailability of ellagitannins is very poor due to the large molecular size and their relatively high polarity. Ellagitannins undergo partial hydrolysis to ellagic acid in gastrointestinal tract and then free ellagic acid and the remaining ellagitannins are metabolized by the colon microbiota to dibenzopyran-6-one derivatives – urolithins [51, 52]. Transformation of ellagic acid starts from Uro-M5 and then leads to the production of different urolithins and their intermediates, while Uro-A and Uro-B are final catabolic products. The presence of urolithins and their conjugate derivatives has been detected at relatively high concentrations in plasma, urine and feces. Currently, *Gordonibacter urolithinfaciens* and *G. pamelaeae* [53, 54] as well as *Ellagibacter isourolithinifaciens* [55] and *Bifidobacterium pseudocatenulatum* (INIA P815) [56] have been identified as urolithin producers.

Interindividual variability in the qualitative and quantitative urolithins production was revealed in humans [58, 59]. Three urolithin metabotypes have been classified: metabotype A, B and 0. Metabotype A is characterized by the excretion of Uro-A

and its conjugates, metabotype B produces Uro-B and IsoUro-A in addition to Uro-A, and metabotype 0 does not produce Uro-A, IsoUro-A, or Uro-B [60]. The relationship between the presence of *Gordonibacter* microbes and individuals who were able to produce Uro-A was observed. Moreover, higher amounts of *Gordonibacter* were found in individuals with metabotype A than in those with metabotype B [52, 60]. It has been considered that interindividual variability is caused by the differences and composition of the intestinal microbiota. Recently, aging has been reported as the main factor that determines urolithin metabotypes with potential consequences for human health [59].

Urolithins which have shown antiproliferative, anti-inflammatory and anticancer properties differ in their activities depending on their structure [22, 61, 62]. Furthermore, studies on animal models also revealed the protection role of urolithins in neuronal inflammation and their potential benefit effect for Alzheimer's disease [63] and other degenerative diseases [64, 65]. At present, a topical issue is whether the oral application or consumption of ellagitannin-rich dietary products are able to provide appropriate bioactive concentrations in blood, then transport this to target organs. Animal tests exhibited that urolithins are able to reach some organs: the prostate, intestine, colon, liver, kidneys, lungs, and brain [65-67]. The oral administration of ellagitannins or urolithins in humans led to the occurrence of urolithins in plasma [68], colon [69] and prostate [70, 71].

Dehydroxylation performed by gut microflora affects the urolithin activity level. Stolarczyk *et al.* [22] reported the occurrence of urolithins (Uro-C, -A, and -B) in human feces after incubation with an *E. hirsutum* extract with a high oenothein B content (23.5%). In the same paper, the authors evaluated the antiproliferative activity of both *E. hirsutum* and *E. parviflorum* extracts, oenothein B and synthesized urolithins (Uro-A, -B, -C) in an *in vitro* model on LNCaP prostate cancer cells. These urolithins were active, although not so strong as oenothein B, which exhibited the strongest inhibition activity (IC_{50}=7.8 μM). The inhibition effect of urolithin C (IC_{50}=35.2 μM) was comparable with a synthetic drug (flutamide) used in BPH and prostate cancer therapies, whereas Uro-B showed the weakest activity.

Metabolic fate of oenothein B is still an open question [15, 58]. The differences in gut metabolism of ellagitannins present in *E. angustifolium* extract were recognized in humans and animals [58]. The study revealed that the pure oenothein B is not transformed to urolithins after incubation with human microbiota, in contrary to other ellagitannins. Neither urolithins nor oenothein B were present in feces and urine of rats in the *E. angustifolium* extract-treated group. However, glucuronides-II phase conjugates were identified: nasutin A and its metabolites. Nasutin A was found previously in rats [72] and other animals, but not in humans. Examination of human urine after ingestion of *E. angustifolium* infusion exhibited the presence of urolithins conjugates: urolithin A glucuronide (GUA), GUB, GUC, and iso-urolithin A glucuronide (GUiA). Neither oenothein B nor nasutin and its conjugates were detected. When the extract was incubated with human gut microbiota *ex vivo*, urolithins (Uro-A, Uro-B, and IsoUro-A) were produced. However, incubation with isolated oenothein B did not result in urolithin detection, in contrary to the positive control. These studies may suggest that oenothein B is not prone to degradation by microbiota, but another unknown metabolite is transformed into urolithins or nasutin. Therefore, biological activities of oenothein B and other ellagitannins in *in vitro* and *in vivo* assays have to be interpreted with caution and there is a need of the further, detail studies on their bioavailability. Recently, Stanisławska *et al.* [73, 74] investigated the influence of urolithins on androgen-dependent LNCaP cells. UroA and UroB exhibited antiproliferative and pro-apoptotic activities in LNCaP cells and had an additive antiproliferative effect with an antiandrogen drug bicalutamide [73]. Additionally, the synergic antiproliferative effect of Uro-A and M4 valerolactone was demonstrated on LNCaP cells [74].

CONCLUSIONS

E. angustifolium is an important medicinal plant with valuable properties for human health, including anti-androgenic, antiproliferative, cytotoxic, antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial activities. Some studies indicate that fireweed extracts have a positive effect on BPH and potentially in the prostate cancer chemoprevention. Numerous beneficial effects of this plant have been documented in the *in vitro* and *in vivo* tests, but the efficacy of *E. angustifolium* phytotherapy is still poorly investigated in the clinical trials.

Oenothein B is considered to be one of the main bioactive compounds of fireweed. Despite many research focused on the anti-BPH effect of this plant substance, it seems that also other secondary metabolites, which have not been tested and/or isolated

yet, contribute to the biological activities of *E. angustifolium*. For example, gallic acid has been recently proposed as a candidate for the treatment of BPH. Therefore, extensive and detailed studies are needed, including analyses of chemical constituents, their pharmacological properties and bioavailability.

The bioavailability of ellagitannins is still insufficiently known, but recent investigations on their metabolism in humans suggest that the gut microbiota play a key role and can modulate the therapeutic effects. The experimental results support the use of ellagitannins in the prostate cancer chemoprevention, but caution is advised due to a high variation in the production of urolithins by the individual microbiota. Therefore, a better understanding of the role and action mechanism of gastrointestinal microflora is crucial for effective therapy.

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