Therapeutic Potential of Polyphenols from Epilobium Angustifolium (Fireweed)

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Epilobium angustifolium **is a medicinal plant used around the world in traditional medicine for the treatment of many disorders and ailments. Experimental studies have demonstrated that *Epilobium* extracts possess a broad range of pharmacological and therapeutic effects, including antioxidant, anti-proliferative, anti-inflammatory, antibacterial, and anti-aging properties. Flavonoids and ellagitannins, such as oenothein B, are among the compounds considered to be the primary biologically active components in *Epilobium* extracts. In this review, we focus on the biological properties and the potential clinical usefulness of oenothein B, flavonoids, and other polyphenols derived from *E. angustifolium*. Understanding the biochemical properties and therapeutic effects of polyphenols present in *E. angustifolium* extracts will benefit further development of therapeutic treatments from this plant.**

Keywords: Epilobium angustifolium; polyphenol; ellagitannin; oenothein B.

INTRODUCTION

Polyphenols are a structural class of organic chemicals characterized by the presence of more than one phenolic ring. The number and characteristics of these phenol structures influences the physical, chemical, and biological properties of various classes of these compounds, which include flavonoids, phenolic acids, lignans, coumarins, stilbenes, and tannins (Quideau *et al.,* 2011). Polyphenols are mainly natural but can also be synthetic or semisynthetic. Plant-derived polyphenols exhibit beneficial effects on human health because of their anti-inflammatory, anti-allergic, anti-atherogenic, anti-microbial, anti-viral, anti-proliferative, and immunomodulatory properties (Feldman, 2005; Okuda, 2005; Holderness *et al.*, 2008; Stagos *et al.*, 2012; Gollucke *et al.*, 2013; Korkina *et al.*, 2013; Chirumbolo, 2014; Ratz-Lyko *et al.*, 2015). The ability of natural polyphenols to modulate certain immune responses may explain, in part, some of beneficial effects of various medicinal plants. In addition, polyphenols exhibit antioxidant properties because of their ability to scavenge reactive oxygen species (ROS) and chelate metal ions (Rice-Evans *et al.*, 1995; Rice-Evans *et al.*, 1996). Polyphenols isolated from various medicinal plants play an important role in the prevention and therapy of a variety of ailments and chronic diseases, and the study of polyphenols has become an increasingly important area of human nutrition research (Landete, 2012; Kishimoto *et al.*, 2013). For example, some dietary polyphenols have been reported to influence the colonic flora via prebiotic effects (Landete, 2012). Polyphenols have also been shown to modulate the immune system by rapidly inducing lymphocyte gene transcription, leading to cytokine production and increased responsiveness to secondary signals (Holderness *et al.*, 2007; Holderness *et al.*, 2008). *In vivo* studies have demonstrated the lifespan-extending properties of polyphenol-containing plants (Uysal *et al.*, 2013), and certain polyphenols may protect against Alzheimer's disease-type cognitive deterioration and neurodegeneration during brain aging and dementia (Pasinetti, 2012; Schaffer *et al.*, 2012). Recent reports also indicate strong epigenetic effects of polyphenols (Joven *et al.*, 2013; Ayissi *et al.*, 2014).

Among the *Epilobium* species, *E.* (*Chamerion*) *angustifolium* is one of the best known medicinal plants and has been used worldwide in traditional medicine. It is also commonly known as fireweed or rosebay willow-herb. Extracts obtained from fireweed are known in folk medicine to exhibit a variety of pharmacological effects (Vitalone *et al.*, 2001; Vitalone *et al.*, 2003a; Vitalone *et al.*, 2003b). Based on the importance of *E. angustifolium* in traditional medicine and the potential for therapeutic development of its constituents in modern medicine, it is important to understand the composition and pharmacological properties of *E. angustifolium* extracts. Because polyphenols are among the most abundant medically active constituents, we have focused this review on the contribution of these compounds to the pharmacological properties of *E. angustifolium* extracts and medicinal preparations.

PHARMACOLOGICAL EFFECTS OF EPILOBIUM EXTRACTS

Therapeutic properties of *Epilobium* extracts have been described in various pharmacological studies. Traditional use of fireweed includes an infusion or tea, which has been reported as a treatment for migraine headaches, insomnia, anemia, delirium tremens, infections, and colds. *E. angustifolium* extracts have been reported to be effective treatments for gastric ulcer; duodenal ulcer; gastritis; colitis; various gastrointestinal disorders, such as dysentery and diarrhea; and prostate or urinary problems, such as urethral inflammation, micturition disorders, prostatic adenoma, and benign prostatic hyperplasia (BPH) (Vitalone *et al*., 2001; Vitalone *et al*., 2003a; Vitalone *et al*., 2003b). *E. angustifolium* has also been used topically as a cleansing, soothing, antiseptic, and healing agent to treat minor burns, skin rashes, ulcers, and infections, and for treatment of inflammation of the ear, nose, and throat (Vogl *et al*., 2013). Experimentally, fireweed extracts have been reported to exhibit analgesic properties using hot plate and writhing tests (Tita *et al*., 2001). Aqueous extracts of the herb have also been reported to have anti-inflammatory properties and reduced carrageenan-induced paw edema (Hiermann *et al*., 1986; Juan *et al*., 1988). Extracts of *E. angustifolium* also have been shown to have bactericidal and antifungal effects (Moskalenko, 1986; Jones *et al*., 2000; Rauha *et al*., 2000; Battinelli *et al*., 2001; Webster *et al*., 2008; Bartfay *et al*., 2012; Kosalec *et al*., 2013). Moreover, administration of *E. angustifolium* extracts prior to influenza virus exposure reduced mortality and increased survival mean time. These effects were even more striking when infection occurred seven days after the last administration of the extract, where mortality rate was reduced by 50% and survival mean time was increased ~fivefold (Vila *et al*., 1989).

E. angustifolium extracts have also been reported to exhibit anti-tumor properties, including inhibition of human prostate epithelial cell PZ-HPV-7 growth (Vitalone *et al*., 2001). Likewise, treatment of androgen-sensitive human prostate adenocarcinoma cells LNCaP with *Epilobium* extracts (20–70 μg/ml) resulted in a significant increase in the number of apoptotic cells (Stolarczyk *et al*., 2013a). Various *Epilobium* extracts, including extracts from *E. angustifolium*, caused a similar inhibitory effect on the proliferation of human cancer cell lines and inhibited DNA synthesis in human astrocytoma cells 1321 N1 (Vitalone *et al*., 2003b). In addition, *E. angustifolium* aqueous extracts (Kiss *et al*., 2006b; Kiss *et al*., 2006a) demonstrated higher antiproliferative activity than ethanol extracts (Vitalone *et al*., 2003a; Vitalone *et al*., 2003b). *E. hirsutum* extracts also exhibited antitumor properties in a mouse model of leukemia (P388 cells) and ascites tumor (Voynova *et al*., 1991), and small doses of this extract (1–3 mg/kg) prolonged the lifespan of mice with tumors over 150% (Voynova *et al*., 1991).

Epilobium extracts may also exhibit anti-aging properties, and treatment of human dermal fibroblasts with 10 μg/ml extract down-regulated UV-induced release of matrix metalloproteinase-1 and matrix metalloproteinase-3, tissue inhibitor of matrix metalloproteinases-1 and matrix metalloproteinase-2, and hyaluronidase 2 gene expression (Ruszova *et al*., 2013). The authors suggested that polyphenols might account for these benefits. In addition, *Epilobium* extracts have high antioxidant activity, which is comparable with that of well-known antioxidants and flavonoids (Hevesi Tóth *et al*., 2009). Indeed, aqueous extracts of *E. angustifolium* are able to scavenge superoxide anion (O_2^-) and hydroxyl radicals, as well as inhibit ROS production by stimulated neutrophils (Myagmar and Aniya, 2000).

POLYPHENOLIC COMPOUNDS

Over 100 compounds have been identified in different materials from the *Epilobium* genus [reviewed in (Granica *et al*., 2014)], with polyphenols being the predominant constituent. Indeed, it is thought that polyphenols of *E. angustifolium* could explain, at least in part, many of the therapeutic and beneficial properties of this plant because of the known immunomodulatory properties of polyphenols (Holderness *et al*., 2007; Ramiro-Puig *et al*., 2008; Schepetkin *et al*., 2009; Daughenbaugh *et al*., 2011; Holderness *et al*., 2011; Skyberg *et al*., 2011; Ramstead *et al*., 2012; Ramstead *et al*., 2015).

High concentrations of polyphenols are present in members of the genus *Epilobium* L. (Onagraceae), which consists of over 200 species found worldwide. Secondary metabolites have been characterized in approximately 25% of the species from this genus, and flavonoids and tannins have been found to be the principal bioactive constituents in *E. hirsutum* L., *E. parviflorum* Schreb., *E. montanum* L., *E. tetragonum* L., *E. roseum* L., *E. adenocaulon* Hausskn., *E. palustre* L., and *E. angustifolium* L. (Ivancheva *et al*., 1992; Lesuisse *et al*., 1996; Kiss *et al*., 2006a; Hevesi Tóth *et al*., 2009; Schepetkin *et al*., 2009; Kiss *et al*., 2011; Jurgenson *et al*., 2012; Remmel *et al*., 2012; Ruszova *et al*., 2013). In fact, the content of oenothein B and quercetin-3-*O*-glucuronide has been suggested as a basis for the standardization of commercially available *Epilobium* products (Bazylko *et al*., 2007; Hevesi Tóth *et al*., 2009; Granica *et al*., 2012; Monschein *et al*., 2015).

E. angustifolium contains a variety of polyphenols (Jurgenson *et al*., 2012). Phytochemical analyses of *E. angustifolium* extracts have identified three major polyphenol groups: flavonoids, phenolic acids, and ellagitannins (Ducrey *et al*., 1997; Shikov *et al*., 2006; Remmel *et al*., 2012; Ruszova *et al*., 2013). Flavonoids include flavonol aglycones (quercetin, kaempferol, and myricetin) and flavonoid glycosides, such as afzelin (kaempferol-3-*O*-rhamnoside), juglalin (kaempferol-3-*O*-arabinofuranoside), avicularin (quercetin-3-*O*-α-arabinofuranoside), hyperoside (quercetin-3-*O*-galactoside), isoquercetin (quercetin-3-*O*-glucoside), quercitrin (quercetin-3-*O*-rhamnoside), and miquelianin (quercetin-3-*O*-glucuronide) (**Table** 1). Among the flavonoid glycosides that have been identified in *Epilobium* species, miquelianin is the main flavonoid in *E. angustifolium*, whereas myricitrin (myricetin-3-*O*-rhamnoside) was found to be the main flavonoid in other species (Hevesi Tóth *et al*., 2009). Some of these compounds are active constituents of many medicinal plants that are used in traditional medicines for their neuroprotective, anti-inflammatory, antioxidant, anti-proliferative, and other pharmacological properties (**Table** 2). Miquelianin is a major flavonoid glycoside from *E. angustifolium*. The activity of miquelianin seems to be primarily due to its

Table 1. Chemical structures of selected flavonoids and their glycosides found in *E. angustifolium* (Hiermann *et al*., 1991; Ducrey *et al*., 1995; Hevesi Tóth *et al*., 2009)

Compound	Name	R_1	R_2	R_3
1	Kaempferol	H	H	H
2	Quercetin	H	OH	H
3	Myricetin	H	OH	OH
4	Afzelin (kaempferol-3-*O*-rhamnoside)	Rha	H	H
5	Quercitrin (quercetin-3-*O*-rhamnoside)	Rha	OH	H
6	Myricetin-3-*O*-rhamnoside	Rha	OH	OH
7	Juglalin (kaempferol-3-*O*- arabinofuranoside)	Ara	H	H
8	Guajaverin (quercetin 3-*O*-arabinopyranside)	Ara	OH	H
9	Hyperoside (quercetin-3-*O*-galactoside)	Gal	OH	H
10	Isoquercetin (quercetin-3-*O*-glucoside)	Glc	OH	H
11	Isomyricitrin (myricetin-3-*O*-glucoside)	Glc	OH	OH
12	Quercetin-3-*O*-(6"-galloyl)-galactoside	(6"-Gall)Gal	OH	H
13	Miquelianin (quercetin-3-*O*-glucuronide)	GlcA	OH	H
14	Kaempferol-3-*O*-β-glucuronide	GlcA	H	H
15	Myricetin-3-*O*-glucuronide	GlcA	OH	OH
16	Myricetin-3-*O*-galactoside	Gal	OH	OH

bioactive metabolites (Jimenez *et al*., 2015; Messer *et al*., 2015) (**Table** 2).

Phenolic acids identified in *Epilobium* species are gallic acid (3,4,5-trihydroxybenzoic acid) and its methyl ester, protocatechuic acid (3,4-dihydroxybenzoic acid), ellagic acid, octyl gallate, 5-*O*-caffeoylquinic acid, 6-*O*-galloyl-glucose, 1,2,6-*O*-trigalloyl glucose, and 1,2,3,4,6-*O*-pentagalloyl glucose (Hiermann *et al*., 1991; Ducrey *et al*., 1995; Kiss *et al*., 2004; Shikov *et al*., 2006; Bazylko *et al*., 2007; Hevesi Tóth *et al*., 2009; Kiss *et al*., 2011; Stolarczyk *et al*., 2013b; Karakurt *et al*., 2016).

Among the relatively high-molecular weight polyphenols identified are tellimagrandin I-based oligomeric ellagitannins (Ducrey *et al*., 1997; Shikov *et al*., 2006; Bazylko *et al*., 2007; Yoshida *et al*., 2010; Baert *et al*.,

Table 2. Biological properties of selected flavonoids found in *E. angustifolium*

Compound	Biological properties	References
Afzelin (kaempferol-3-*O*-rhamnoside)	Antibacterial, DNA-protective, antioxidant, anti-inflammatory, anti-complement activity, inhibitor of angiotensin converting enzyme (ACE)	(Hansen *et al*., 1996; Min *et al*., 2003; Shin *et al*., 2013; Lee *et al*., 2014)
Hyperoside (quercetin-3-*O*-galactoside)	Suppresses vascular inflammatory, anti-apoptotic and antithrombotic activity, relieves pain and improves cardiovascular functions, neuroprotective, inhibits cytochrome P450 activity	(Zeng *et al*., 2011; Liu *et al*., 2012; Ku *et al*., 2013; Song *et al*., 2013; Zhang *et al*., 2013)
Isoquercetin (quercetin-3-*O*-glucoside)	Antidiabetic, anti-inflammatory, antiviral, neuroprotective, antioxidative, inhibitor of α-glucosidase	(Li *et al*., 2009; Nguyen *et al*., 2009; Kim *et al*., 2010; Zhang *et al*., 2011; Thapa *et al*., 2012; Wang *et al*., 2013)
Kaempferol	Antioxidative, anti-inflammatory, anti-proliferative, antimicrobial, cardioprotective, neuroprotective	(Khlebnikov *et al*., 2007; Calderon-Montano *et al*., 2011; Chen and Chen, 2013; Rajendran *et al*., 2014)
Miquelianin (quercetin-3-*O*-glucuronide)	Immunostimulatory and anti-inflammatory; ameliorates insulin resistance in skeletal cells under inflammatory conditions; suppresses plasmin-mediated mechanisms of cancer cell migration	(Al-Shalmani *et al*., 2011; Cuccioloni *et al*., 2012; Liao and Lin, 2014; Liu *et al*., 2016)
Myricetin	Antioxidant, anti-inflammatory, antimicrobial, anti-proliferative, anti-aging	(Cushnie and Lamb, 2005; Leonarduzzi *et al*., 2010; Ruzic *et al*., 2010)
Quercetin	Anti-proliferative, antioxidative, neuroprotective, and anti-inflammatory, pleiotropic kinase inhibitor, inhibitor of α-glucosidase	(Li *et al*., 2009; Dajas, 2012; Bruning, 2013; Furst and Zundorf, 2014; Russo *et al*., 2014; Sak, 2014)
Quercitrin (quercetin-3-*O*-rhamnoside)	Promotes osteoblast differentiation and inhibits osteoclastogenesis, antioxidative, antileishmanial activity, inhibitor of aldose reductase, p90S6 ribosomal kinase (RSK), AP-1 and MAPK signaling, protects mice against fatal anaphylactic shock	(Khlebnikov *et al*., 2007; Cruz *et al*., 2008; Ding *et al*., 2010; da Silva *et al*., 2012; Derewenda *et al*., 2013; Kim *et al*., 2013; Satue *et al*., 2013; Yin *et al*., 2013)

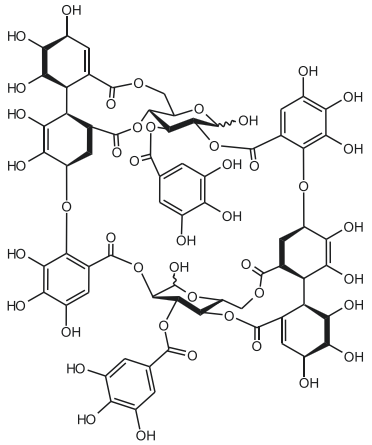

Figure 1. Chemical structure of oenothein B.

2015; Kaskoniene *et al*., 2015b). Oenothein B is comprised of two tellimagrandin I monomers linked between the hexahydroxydiphenoyl groups and the galloyl groups on the glucopyranose ring (Fig. 1). Several other oligomeric tannins have also been isolated from *E. angustifolium* extracts, including oenothein A (trimer), and tellimagrandin I-based heptameric ellagitannins (Sasov *et al*., 2010; Baert *et al*., 2015). Overall, oenothein B is the predominant polyphenol in this plant (14–23%), while flavonoid content was $< 2\%$ (Ducrey *et al*., 1997; Kiss *et al*., 2011).

The majority of plant polyphenols occur in the form of biologically unavailable polymers or glycosides, which are degraded to low molecular weight compounds by intestinal enzymes originating from the host organism or secreted by colonic microflora (D'Archivio *et al*., 2010). Since the biological activity of many of these polyphenols has been established using cell cultures *in vitro*, interpretation of their potential pharmacological effects *in vivo* could be problematic, and further evaluation using *in vivo* studies is warranted. In addition, the activity of polyphenolic compounds can even be influenced by the routes of *in vivo* administration, as the active metabolites generated *in vivo* can differ based on route of administration. For example, neither hyperoside nor its metabolites were detected in rat brain after intragastric administration, whereas both compounds were detected after intraperitoneal administration (Guo *et al*., 2012).

OENOTHIEN B

Oenothein B is a macrocyclic ellagitannin (Hatano *et al*., 1990). It is a major contributor to the biological activity of *Epilobium* extracts and is present at high concentration in these species (Lesuisse *et al*., 1996; Kiss *et al*., 2006b; Kiss *et al*., 2006a; Kiss *et al*., 2011). Biological activities of oenothein B include antioxidant, immunomodulatory, tumor cell cytotoxicity, enzyme inhibition, and enzyme induction (Miyamoto *et al*., 1993a; Miyamoto *et al*., 1993b; Aoki *et al*., 1995; Lesuisse *et al*., 1996; Sakagami *et al*., 2000; Kiss *et al*., 2006b; Kiss *et al*., 2006a; Kiss *et al*., 2011; Stolarczyk *et al*., 2013b) (**Table** 3). Much of the research on oenothein B has

Table 3. Biological properties of oenothein B

Assay	Biological properties	References
In vitro	Anti-proliferative in human neuroblastoma SK-N-SK cells, human prostate tumor cell line PC-3, and human prostate adenocarcinoma LNCaP cells	(Kiss *et al*., 2006b; Kiss *et al*., 2006a; Stolarczyk *et al*., 2013b)
	Reduces prostate specific antigen (PSA) secretion in LNCaP cells	(Stolarczyk *et al*., 2013b)
	Inhibits arginase activity in LNCaP cells	(Stolarczyk *et al*., 2013b)
	Inhibits α-glucosidase	(Kawakami *et al*., 2014)
	Stimulates release of IL-1β from macrophages	(Miyamoto *et al*., 1993a; Miyamoto *et al*., 1993b)
	Stimulate Ca^{2+} flux and ROS production in neutrophils	(Schepetkin *et al*., 2009)
	Stimulated NF-kB activation and production of TNF and IL-6 in human monocytic THP-1 cells	(Schepetkin *et al*., 2009)
	Activates NK cells, αβ T cells, and γδ T cells, resulting in increased expression of the activation marker CD69 and IFNγ production	(Ramstead *et al*., 2012)
	Induces more IFNγ production by $CD45RO^{+}$ memory T cells compared with naïve T cells	(Ramstead *et al*., 2015)
	Scavenger of ROS ($O_2^{-\cdot}$ and H_2O_2)	(Schepetkin *et al*., 2009; Kiss *et al*., 2011; Granica *et al*., 2015)
	Inhibits mouse mammary tumor virus transcription in 34I cells	(Aoki *et al*., 1995)
	Antibacterial activity against *Helicobacter pylori*	(Funatogawa *et al*., 2004).
In vivo	Inhibits growth of MM2 ascites tumors and Meth-A solid type tumor in mice	(Miyamoto *et al*., 1993a; Miyamoto *et al*., 1993b)
	Induces recruitment of neutrophils in peritoneal cavity, which is associated with induction of KC production	(Schepetkin *et al*., 2009)
	Inhibits IL-1β and IL-6 production by activated dendritic cells and attenuates neuroinflammation in response to LPS treatment	(Okuyama *et al*., 2013)

focused on its effects on abnormal prostate cells, where it inhibits cell proliferation, prostate specific antigen (PSA) secretion, and arginase activity (Stolarczyk *et al*., 2013b). Oenothein B is an inhibitor of the enzyme 5-α-reductase, which is an important target enzyme in certain prostate disorders (Lesuisse *et al*., 1996). In addition, oenothein B induced neutral endopeptidase activity in a prostate tumor cell line, thereby inhibiting cell proliferation (Kiss *et al*., 2006b; Kiss *et al*., 2006a). Therefore, supplements containing oenothein B may benefit individuals with prostate disorders, including prostate cancer, through the modulation of prostate enzyme activity. Oenothein B was also recently reported to be an α-glucosidase inhibitor (Kawakami *et al*., 2014).

In addition to its effects on prostate cells, oenothein B was also found to inhibit tumor growth *in vivo* (Miyamoto *et al*., 1993a; Miyamoto *et al*., 1993b). Although oenothein B can induce apoptosis in tumor cell lines (Sakagami *et al*., 2000), the previously observed inhibition of tumor growth was not believed to be caused by direct cytotoxicity to the tumor cells. Instead, oenothein B was found to stimulate macrophages and promote the production of interleukin (IL) 1, and this was proposed to contribute to the observed antitumor effects of oenothein B in murine models (Miyamoto *et al*., 1993a; Miyamoto *et al*., 1993b). In support of this idea, we found that oenothein B activated both mouse and human neutrophils and monocytes/macrophages. Among the neutrophil responses enhanced by oenothein B were intracellular calcium flux and ROS production (Schepetkin *et al*., 2009). In addition, oenothein B stimulated monocyte NF-κB activation and pro-inflammatory cytokine production, including tumor necrosis factor (TNF) and IL-6 (Schepetkin *et al*., 2009), which may contribute to the antitumor effects. Note, however, that additional compounds besides oenothein B may contribute to the antitumor properties of *Epilobium* extracts (Vitalone *et al*., 2003b), possibly through synergistic mechanisms.

While oenothein B was found to enhance neutrophil ROS production, this compound can also directly scavenge $O_2^{-\cdot}$ and H_2O_2 (Schepetkin *et al*., 2009; Kiss *et al*., 2011; Granica *et al*., 2015). Indeed, oenothein B had the highest radical scavenging activity among other polyphenols in methanol extracts from *E. angustifolium* (Kaskoniene *et al*., 2015a). Antioxidant activity is one of main properties of tannins and has been reported for many other ellagitannins, such as agrimoniin, corilagin, punicalagin, punicalin, and pedunculagin (Lin *et al*., 2001; Chung *et al*., 2003; Marzouk *et al*., 2007; Bazylko *et al*., 2013). In addition, the metabolites of various ellagitannins may have even more potent antioxidant activity than their respective parent compounds (Ishimoto *et al*., 2012). Thus, combined enhancement of innate immune defenses and protection of host tissues through antioxidant effects could allow oenothein B to optimally provide therapeutic benefits.

In addition to effects on myeloid cells and neutrophils, we have also shown that oenothein B activates lymphocytes, including NK cells, αβ T cells, and γδ T cells, resulting in increased expression of the activation marker CD69 (Ramstead *et al*., 2012). It should be noted that the effect of oenothein B on CD69 expression in γδ T cells was similar to the immunomodulatory properties of condensed tannins isolated from *Uncaria tomentosa* (Cat's Claw) and *Malus domestica* (apple) (Holderness *et al*., 2007). Treatment with oenothein B also enhanced interferon γ (IFNγ) production by αβ T cells, γδ T cells, and NK cells in response to secondary stimuli, including IL-18 and a tumor cell line (Ramstead *et al*., 2012). Oenothein B activated T cells from both young and adult individuals, although higher levels of IFNγ were produced by T cells from adults compared with those from young individuals after oenothein B treatment (Ramstead *et al*., 2015). Furthermore, oenothein B induced more IFNγ production by $CD45RO^+$ memory T cells compared with naïve T cells (Ramstead *et al*., 2015). Thus, it is clear that oenothein B is a potent immune cell agonist and can enhance the activity of various types of immune cells.

In contrast to our studies, others have reported anti-inflammatory effects of oenothein B. For example, Kiss et al. (Kiss *et al*., 2011) reported that myeloperoxidase release and production of ROS by activated neutrophils were inhibited by oenothein B. Likewise, oenothein B has been reported to inhibit nitric oxide production, NF-kB activity, and the production of IL-1β, IL-6, and TNF by a murine macrophage cell line pretreated with Toll-like receptor (TLR) 2 and TLR4 agonists (Schmid *et al*., 2012). A related ellagitannin, punicalagin, was also found to inhibit TLR4-mediated NF-kB signaling pathways (Peng *et al*., 2015). Moreover, oenothein B inhibited IL-1β and IL-6 production by activated dendritic cells and inhibited neuroinflammation in response to systemic lipopolysaccharide treatment (Okuyama *et al*., 2013; Yoshimura *et al*., 2013). Therefore, it appears that oenothein B has a complex influence on innate immune cells, which is similar to what has been observed for other ellagitannins. One possible explanation for the discrepancies in these data is the activation state of the cells at the time of treatment with oenothein B. For much of our research demonstrating activation of immune cells, unstimulated, resting cells were used (Schepetkin *et al*., 2009; Ramstead *et al*., 2012). However, in the studies demonstrating immune suppression by oenothein B, the authors used pre-stimulated, activated cells (Kiss *et al*., 2011; Schmid *et al*., 2012; Yoshimura *et al*., 2013). These data suggest that oenothein B may have differential effects on activated and resting immune cells, suppressing activated cells and stimulating resting ones. Additional research is needed to better understand the complex effects of oenothein B on immunity and how these effects contribute to the proposed health benefits associated with oenothein B and *E. angustifolium* extracts.

One factor that appears to be important for stimulation of T cell cytokine production by polyphenols is the size of the polyphenol molecule. Indeed, molecular subunits of oenothein B with smaller molecular weights do not have the same leukocyte immunomodulatory capacity (Schepetkin *et al*., 2009; Granica *et al*., 2015). Similar observations were made by Yamanaka et al. (Yamanaka *et al*., 2012), who found that stimulation of murine splenocytes by polymerized polyphenols with large molecular weights, but not their corresponding monomers, enhanced T cell cytokine production. Furthermore, our research has found that procyanidin oligomers, but not monomers, stimulate innate lymphocytes (Holderness *et al*., 2008). The importance of molecular size is consistent with the activity of oenothein B, as it is a large polyphenol (Schepetkin *et al*., 2009).

In addition to immunomodulatory effects, oenothein B has been reported to exhibit direct antimicrobial and antiviral activity. For example, this compound has been reported to have antibacterial activity against *Helicobacter pylori* (Funatogawa *et al*., 2004). Likewise, we found that oenothein B directly inhibited *Staphylococcus aureus* growth *in vitro* with an IC_{50} of ~0.7 μM (Fig. 2A) and also enhanced *S. aureus* killing by human neutrophils (Fig. 2B). The ability of *S. aureus* to survive after neutrophil phagocytosis is thought to contribute significantly to the relative virulence of this pathogen (Voyich *et al*., 2005; Voyich *et al*., 2006; Bubeck Wardenburg *et al*., 2007; Wang *et al*., 2007; Voyich *et al*., 2009). This is exemplified by the observed increase in susceptibility to *S. aureus* infections of individuals suffering from defects that alter normal neutrophil function, such as chronic granulomatous disease, leukocyte adhesion deficiency, and neutropenia (Bodey *et al*., 1966; Dale *et al*., 1979; Lekstrom-Himes and Gallin, 2000). Therefore, our finding that oenothein B significantly enhanced staphylocidal activity of human neutrophils is promising, and future studies will investigate the therapeutic potential of oenothein B *in vivo* and in combination with antibiotics to see if bacterial clearance can be improved by this compound.

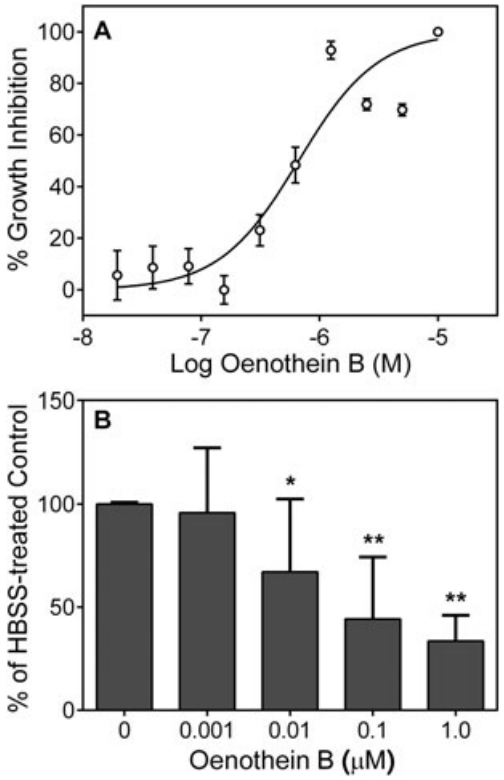

Figure 2. Effect of oenothein B on *Staphylococcus* aureus growth and human neutrophil staphylocidal activity. **Panel A**. Direct effect of oenothein B on growth of *Staphylococcus aureus*. *S. aureus* USA300 (2×10^6 in 200 μl of RPMI) were mixed with the indicated concentrations of oenothein B in microplate wells and incubated at 37 °C for 3 hours. Oenothein B was isolated from *E. angustifolium*, as described previously (Schepetkin *et al.*, 2009). Bacterial growth was monitored spectrophotometrically at 600 nm using a SpectraMax Plus microplate reader. Background measurements of the oenothein B dilutions in RPMI alone were subtracted from each OD_{600} reading, and % growth was calculated as: (OD_{600} of oenothein B-treated *S. aureus*/OD_{600} of control, untreated *S. aureus* in RPMI) × 100. We subtracted these values from 100 to plot % growth inhibition resulting from the oenothein B concentrations tested. Values are the mean ± S.D. of triplicate samples from one experiment, which is representative of three independent experiments. **Panel B**. Effect of oenothein B on human neutrophil bactericidal activity. Human neutrophils were purified from the blood of healthy donors (in accordance with a protocol approved by the Institutional Review Board at Montana State University) using dextran sedimentation, followed by Histopaque 1077 (Sigma-Aldrich) gradient separation and hypotonic lysis of red blood cells, as previously described (Schepetkin *et al.*, 2011). The neutrophils were pre-incubated with the indicated concentrations of oenothein B or control Hank's balanced-salt solution (HBSS) for 15 min, washed to remove remaining oenothein B, and incubated with opsonized *S. aureus* USA300 at a 1:10 ratio of neutrophils to bacteria (10^6 neutrophils and 10^7 bacteria in 200 μl of RPMI). After 3 hours incubation, neutrophils were lysed with 0.1% saponin (Sigma-Aldrich) for 5 min at 4 °C, and the total bacterial CFUs were determined by plating the mixture on tryptic soy agar for 18 hours at 37 °C and then counting colonies. CFU values are presented as the % of bacteria remaining after incubation with control HBSS pre-treated neutrophils (mean ± S.D. of duplicate samples from one experiment, which is representative of three independent experiments). Statistically significant differences (* $p < 0.05$; ** $p < 0.01$) between HBBS-treated neutrophils and cells treated with oenothein B are indicated.

Oenothein B has also been reported to inhibit mouse mammary tumor virus (MMTV) transcription (Aoki *et al*., 1995), which was believed to be due to inhibition of poly(ADP-ribose) glycohydrolase. In addition to mammary tumor virus, oenothein B also inhibited herpes simplex virus, which is similar to other tannins, including coriariin A, rugosin D, cornusiin A, tellimagrandin I, casuarictin, and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (Fukuchi *et al*., 1989; Kim *et al*., 2001). Because tellimagrandin I also has direct antibacterial activity (Funatogawa *et al*., 2004; Shiota *et al*., 2004), it is possible that some antiviral and bactericidal activities of oenothein B could be related to its tellimagrandin I substructures. Finally, oenothein B has been suggested to have antifungal activity based on its inhibition of 1,3-β-glucan synthase transcription in *Paracoccidioides brasiliensis* (Santos *et al*., 2007; Zambuzzi-Carvalho *et al*., 2013). Together, these reports suggest that oenothein B may be beneficial during certain bacterial, viral, and fungal infections.

Not much is known regarding the cellular binding properties of oenothein B. We found that serum levels of IL-6 in TLR4 knockout mice after intraperitoneal injection of oenothein B were similar to those in TLR2 knockout and wild-type mice. These data suggest that oenothein B binding could be mediated via TLR2/TLR4-independent signaling pathways. Recent studies have demonstrated that the binding of ellagitannins, including oenothein B, to albumin increases in strength and affinity for the larger tannins (dimers) compared with their monomer forms and that bond rotational flexibility of oenothein B also plays a role by increasing the strength of interaction and number of stronger (possibly hydrogen bonding) binding sites on the protein surface (Dobreva *et al*., 2014). Clearly, further studies are necessary to elucidate the cellular targets of oenothein B, especially in relation to immune cell regulation.

Similar to tannins and other polyphenols (Yoshioka *et al*., 2001; Mira *et al*., 2002; Andrade *et al*., 2005), oenothein B has strong metal-chelating properties. Recently Tahara et al. (Tahara *et al*., 2014) reported that oenothein B binds Al(III) ions and suggested that formation of Al(III) complexes with oenothein B in roots could contribute to high aluminum resistance of *E. camaldulensis* (Tahara *et al*., 2014). We found that oenothein B was also able to chelate Cu(II) ions, with a 1:1 stoichiometry for the soluble complex, whereas addition of excess Cu(II) initiated the formation of insoluble precipitates (Fig. 3). Previously, it has been reported that Cu(II) complexes of

several other polyphenols altered their biological properties (Yoshioka *et al*., 2001; Mira *et al*., 2002; Yu *et al*., 2005). Thus, it is possible that oenothein B-copper complexes could have altered antioxidant or other biological properties compared with native oenothein B.

Clear data regarding the bioavailability of ingested oenothein B are still missing. Although this dimeric ellagitannin is effective after oral administration (Okuda *et al*., 1989), it is still not clear if the active molecule(s) is the same as the parent. Indeed, most polyphenolic compounds undergo metabolic transformations, which significantly change their biological activities (Lewandowska *et al*., 2013; Tarko *et al*., 2013). For example, the primary products of acid degradation of oenothein B are gallic and ellagic acids, and recent *in vitro* and *in vivo* experiments have revealed that ellagic acid exhibits, for example, antitumor effects by inhibiting tumor cell proliferation, inducing apoptosis, blocking virus infection, and disturbing inflammation, angiogenesis, and drug-resistance processes required for tumor growth and metastasis (Zhang *et al*., 2014). Gallic acid is one of most well-absorbed polyphenols (Manach *et al*., 2005) and has neuroprotective properties in different models of neurotoxicity, neurodegeneration, and oxidative stress (Daglia *et al*., 2014). Likewise, gallic acid has been shown to inhibit carcinogenesis *in vitro* by cancerous cell lines and in animal models (Carpentier *et al*., 1984). Because ellagic acid-derived metabolites produced by human colonic microflora are urolithins, biological effects of dibenzo[b,d]pyran-6-one should be also be considered (Larrosa *et al*., 2006; Piwowarski *et al*., 2014).

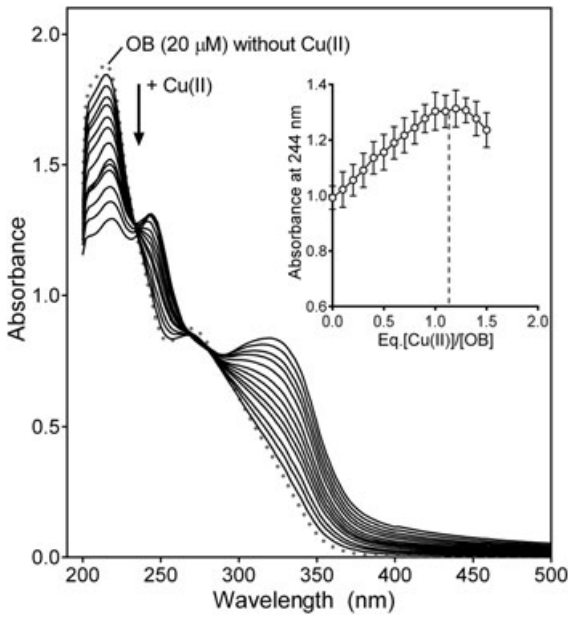

Figure 3. Binding of Cu(II) to oenothein B. Oenothein B (20 μM), isolated from *E. angustifolium* as described previously (Schepetkin *et al.*, 2009), was mixed with increasing concentrations of $CuCl_2$ (Sigma-Aldrich) in 20 mM phosphate buffer (pH 7.5) and spectra of the Cu(II)-oenothein complex were obtained using a SpectraMax Plus microplate reader. The spectra of the complex revealed absorbance peaks at 244 and 322 nm and two isobestic points at 235 and 280 nm. ***Inset***: The increase in Cu(II) concentration caused a linear increase in the absorbance at 244 nm, and saturation occurred at 20 μM Cu(II), suggesting that oenothein B is able to chelate Cu(II) ions, with a 1:1 stoichiometry for the soluble complex. The spectra shown are representative of three independent determinations.

CLINICAL STUDIES

Various fireweed preparations have been developed for clinical use. For example, Chanerol is a complex polyphenolic medicinal drug prepared from the blossoms of fireweed (Pukhal'skaia *et al*., 1970; Petrova *et al*., 1974; Pukhalskaya *et al*., 1975). It is likely that oenothein B is one of main bioactive constituents of Chanerol and could be responsible for its pharmacological activities (Spiridonov *et al*., 1997; Sasov *et al*., 2010), including antitumor activity (Syrkin and Konyaeva, 1984). An aqueous extract of *E. angustifolium* was patented for use as an oral anti-inflammatory treatment (German Patent No. 3,605,250 of 16. July 1987). In addition, skin care treatments containing *E. angustifolium* extract have been patented (WO2011007183) by a Canadian company, Fytokem (Saskatoon, Saskatchewan), that markets several different Willowherb™ extracts with anti-irritant effects. Fytokem claims that the principle bioactive molecules found in Willowherb™ extract are oenothein B and flavonols.

CONCLUSION

E. angustifolium (a.k.a. fireweed) is a medicinal plant widely used in traditional medicine. Extracts from this plant represent a rich source of biologically active polyphenols, such as oenothein B and its metabolites. These polyphenols are responsible for many of the biological responses that contribute to the therapeutic potential of fireweed extracts in a variety of diseases. The therapeutic effects of fireweed polyphenols are mediated by multiple mechanisms, including direct killing of cancer cells and microbes, antioxidant activity, metal chelation, and both pro-inflammatory and anti-inflammatory immunomodulation. Although oenothein B is the predominant polyphenol in *E. angustifolium* responsible for many of its therapeutic properties, its putative receptor and downstream signal transduction pathways are not well understood and will require further research to elucidate. This is essential, as polyphenolic compounds can react with many protein targets. Certainly, a better understanding of fireweed's active molecules and their mechanisms of action is essential for maximizing the therapeutic potential of this interesting plant and ensuring safe use of these compounds as a therapeutics. Although fireweed extract and its components appear to be relatively safe, further clinical studies are also clearly necessary to assess potential adverse effects and interactions with other drugs, as is normally performed for conventional medicines (Izzo *et al*., 2016).

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Conflict of Interest

There are no actual conflicts of interest for the authors.

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