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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. Copyright © 2016 John Wiley & Sons, Ltd.

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, antimicrobial, 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; Chiurumbolo, 2014; Ratz-Lyko et al., 2015). The ability of natural polyphenols to modulate certain immune responses may explain, in part, some of the 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 diarrhoea; 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., 2008, 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 (Hieermann et al., 1986; Juan et al., 1988). Extracts of *E. angustifolium* also have been shown to have bactericidal and antifungal effects (Mokslenko, 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 of *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 anti-proliferative 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 (Voyanova et al., 1991), and small doses of this extract (1–3 mg/kg) prolonged the lifespan of mice with tumors over 150% (Voyanova 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 (O2−) 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-glucuronic acid 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), jugalin (kaempferol-3-O-arabinofuranoside), avicularin (quercetin-3-O-arabinofuranoside), hyperoside (quercetin-3-O-galactoside), isoquercetin (quercetin-3-O-glucoside), quer cetin (quercetin-3-O-rhamnoside), and miquelianin (quercetin-3-O-glucuronicide) (Table 1). Among the flavonoid glycosides that have been identified in *Epilobium* species, miquelianin is the main flavonoid in *E. angustifolium*, whereas myricetin (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, antioxidiant, 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
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 galactose, and 1,2,3,4,6-O-pentagallloyl 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., 2010).

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)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kaempferol</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>2</td>
<td>Quercetin</td>
<td>H</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>Myricetin</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>4</td>
<td>Afzelin (kaempferol-3-O-rhamnoside)</td>
<td>Rha</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>5</td>
<td>Quercitrin (quercetin-3-O-rhamnoside)</td>
<td>Rha</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>6</td>
<td>Myricetin-3-O-rhamnoside</td>
<td>Rha</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>7</td>
<td>Juglalin (kaempferol-3-O-arabino-furanoside)</td>
<td>Ara</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>8</td>
<td>Guajaverin (quercetin 3-O-arabinopyranoside)</td>
<td>Ara</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>9</td>
<td>Hyperoside (quercetin-3-O-galactoside)</td>
<td>Gal</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>10</td>
<td>Isoquercetin (quercetin-3-O-glucuronide)</td>
<td>Glc</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>11</td>
<td>Isomyricitrin (myricetin-3-O-galactoside)</td>
<td>Glc</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>12</td>
<td>Quercetin-3-0-(6'-galloyl)-galactoside</td>
<td>(6'-Gal)</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>13</td>
<td>Miquelianin (quercetin-3-O-glucuronide)</td>
<td>GlcA</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>14</td>
<td>Kaempferol-3-O-β-glucuronide</td>
<td>GlcA</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>15</td>
<td>Myricetin-3-O-glucuronide</td>
<td>GlcA</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>16</td>
<td>Myricetin-3-O-galactoside</td>
<td>Gal</td>
<td>OH</td>
<td>OH</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Assay</th>
<th>Biological properties</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td>Anti-proliferative in human neuroblastoma</td>
<td>(Kiss et al., 2006b; Stolarczyk et al., 2013b)</td>
</tr>
<tr>
<td></td>
<td>SK-N-SK cells, human prostate tumor cell</td>
<td>Kiss et al., 2006a; Stolarczyk et al., 2013b</td>
</tr>
<tr>
<td></td>
<td>line PC-3, and human prostate</td>
<td></td>
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<tr>
<td></td>
<td>adenocarcinoma LNCaP cells</td>
<td></td>
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<tr>
<td></td>
<td>Reduces prostate specific antigen (PSA) secretion in LNCaP cells</td>
<td>Stolarczyk et al., 2013b</td>
</tr>
<tr>
<td></td>
<td>Inhibits arginase activity in LNCaP cells</td>
<td>Stolarczyk et al., 2013b</td>
</tr>
<tr>
<td></td>
<td>Inhibits α-glucosidase</td>
<td>Kawakami et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Stimulates release of IL-1β from macrophages</td>
<td>Miyamoto et al., 1993a; Miyamoto et al., 1993b</td>
</tr>
<tr>
<td></td>
<td>Stimulate Ca$^{2+}$ flux and ROS production in neutrophils</td>
<td>Schepetkin et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Stimulated NF-κB activation and production of TNF and IL-6 in human monocytic THP-1 cells</td>
<td>Schepetkin et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Activates NK cells, αβ T cells, and γδ T cells, resulting in increased expression of the activation marker</td>
<td>Ramstead et al., 2012</td>
</tr>
<tr>
<td></td>
<td>CD68 and IFNγ production</td>
<td></td>
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<tr>
<td></td>
<td>Induces more IFNγ production by CD45RO$^-$ memory T cells compared with naïve T cells</td>
<td>Ramstead et al., 2015</td>
</tr>
<tr>
<td></td>
<td>Scavenger of ROS (O$_2^-$ and H$_2$O$_2$)</td>
<td>Schepetkin et al., 2009; Kiss et al., 2011; Granica et al., 2015</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td>Inhibits mouse mammary tumor virus transcription in 34l cells</td>
<td>Aoki et al., 1995</td>
</tr>
<tr>
<td></td>
<td>Antibacterial activity against <em>Helicobacter pylori</em></td>
<td>Funatogawa et al., 2004.</td>
</tr>
<tr>
<td></td>
<td>Inhibits growth of MM2 ascites tumors and Meth-A solid type</td>
<td>Miyamoto et al., 1993a; Miyamoto et al., 1993b</td>
</tr>
<tr>
<td></td>
<td>tumor in mice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induces recruitment of neutrophils in peritoneal cavity, which is associated with induction of KC production</td>
<td>Schepetkin et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Inhibits IL-1β and IL-6 production by activated dendritic cells and attenuates neuroinflammation in response to LPS treatment</td>
<td>Okuyama et al., 2013</td>
</tr>
</tbody>
</table>
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-kB 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$_2^-$ and H$_2$O$_2$ (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 agrimonin, 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, γδ 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 γδ 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.

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, casuaricin, 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 (Dobrev 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).

**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; Pukhal’skaya 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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