Published in final edited form as:

Phytother Res. 2016 August ; 30(8): 1287–1297. doi:10.1002/ptr.5648.

Therapeutic Potential of Polyphenols from *Epilobium angustifolium* (Fireweed)

Igor A. Schepetkin, Andrew G. Ramstead, Liliya N. Kirpotina, Jovanka M. Voyich, Mark A. Jutila, and Mark T. Quinn

Department of Microbiology and Immunology, Montana State University, Bozeman, MT, 59717

Abstract

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 oenothien 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 oenothien 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; oenothien 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 also 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

Address for correspondence: Dr. Mark T. Quinn, Department of Microbiology and Immunology, Montana State University, Bozeman, MT 59717, Phone: (406) 994-4707; Fax: (406) 994-4303, mquinn@montana.edu.

Conflict of Interest

There are no actual conflicts of interest for the authors.

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. Since 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 (SMT). 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 SMT was increased ~5 fold (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 1321N1 (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 (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 -3, tissue inhibitor of matrix metalloproteinases-1 and -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 to that of well-known antioxidants and flavonoids (Hevesi Tóth et al., 2009). Indeed, aqueous extracts of *E. angustifolium* are able to scavenge superoxide anion ($O_2^{\cdot-}$) and hydroxyl radicals, as well as inhibit ROS production by stimulated neutrophils (Myagmar, 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; Rimmel et al., 2012; Ruszova et al., 2013). In fact, the content of oenothien 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; Rimmel et al., 2012; Ruszova et al., 2013). Flavonoids include flavonol aglycones (quercetin, kaempferol, myricetin) and flavonoid glycosides, such as afzelin (kaempferol-3-*O*-rhamnoside), juglalin (kaempferol-3-*O*-arabinofuranoside), avicularin (quercetin-3-*O*-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 a 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 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., 2015; Kaskoniene et al., 2015b). Oenothien B is comprised of two tellimagrandin I monomers linked between the hexahydroxydiphenoyl groups and the galloyl groups on the glucopyranose ring (Figure 1). Several other oligomeric tannins have also been isolated from *E. angustifolium* extracts, including oenothien A (trimer), and tellimagrandin I-based heptameric ellagitannins (Sasov et al., 2010; Baert et al., 2015). Overall, oenothien 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).

OENOTHEN B

Oenothien 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 oenothien 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 oenothien B has 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). Oenothien 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, oenothien 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 oenothien B may benefit for individuals with prostate disorders, including prostate cancer, through the modulation of prostate enzyme activity. Oenothien B was also recently reported to be an α -glucosidase inhibitor (Kawakami et al., 2014).

In addition to its effects on prostate cells, oenothien B was also found to inhibit tumor growth *in vivo* (Miyamoto et al., 1993a; Miyamoto et al., 1993b). Although oenothien 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, oenothien 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 oenothien B in murine models (Miyamoto et al., 1993a; Miyamoto et al., 1993b). In support of this idea, we found that oenothien B activated both mouse and human neutrophils and monocytes/macrophages. Among the neutrophil responses enhanced by oenothien B were intracellular calcium flux and ROS production (Schepetkin et al., 2009). In addition, oenothien B stimulated monocyte nuclear factor NF- κ B 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 oenothien B may contribute to the antitumor properties of *Epilobium* extracts (Vitalone et al., 2003b), possibly through synergistic mechanisms. While oenothien B was found to enhance neutrophil ROS production, this compound can also directly scavenge O_2^- and H_2O_2 (Schepetkin et al., 2009; Kiss et al., 2011; Granica et al., 2015). Indeed, oenothien 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; Bazylo 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 oenothien B to optimally provide therapeutic benefits.

In addition to effects on myeloid cells and neutrophils, we have also shown that oenothien B activates lymphocytes, including NK cells, $\alpha\beta$ T cells, and $\gamma\delta$ T cells, resulting in increased expression of the activation marker CD69 (Ramstead et al., 2012). It should be noted that the effect of oenothien B on CD69 expression in $\gamma\delta$ 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 oenothien B also enhanced interferon γ (IFN γ) production by $\alpha\beta$ T cells, $\gamma\delta$ T cells, and NK cells in response to secondary stimuli, including IL-18 and a tumor cell line (Ramstead et al., 2012). Oenothien B activated T cells from both young and adult individuals, although higher levels of IFN γ were produced by T cells from adults compared to those from young individuals after oenothien B treatment (Ramstead et al., 2015). Furthermore, oenothien B induced more IFN γ production by CD45RO⁺ memory T cells compared to naïve T cells (Ramstead et al., 2015). Thus, it is clear that oenothien 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 oenothien B. For example, Kiss et al. (Kiss et al., 2011) reported that myeloperoxidase release and production of ROS by activated neutrophils were inhibited by oenothien B. Likewise, oenothien B has been reported to inhibit nitric oxide production, NF- κ B activity, and the production of IL-1 β , 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- κ B signaling pathways (Peng et al., 2015). Moreover, oenothien B inhibited IL-1 β 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 oenothien 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 oenothien 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 oenothien B, the authors used pre-stimulated, activated cells (Kiss et al., 2011; Schmid et al., 2012; Yoshimura et al., 2013). These data suggest that oenothien 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 oenothien B on immunity and how these effects contribute to the proposed health benefits associated with oenothien 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 oenothien 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 oenothien B, as it is a large polyphenol (Schepetkin et al., 2009).

In addition to immunomodulatory effects, oenothien 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 oenothien B directly inhibited *Staphylococcus aureus* growth *in vitro* with an IC₅₀ of ~0.7 μ M (Figure 2A) and also enhanced *S. aureus* killing by human neutrophils (Figure 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, Gallin, 2000). Therefore, our finding that oenothien B significantly enhanced staphylocidal activity of human neutrophils is promising, and future studies will investigate the therapeutic potential of oenothien B *in vivo* and in combination with antibiotics to see if bacterial clearance can be improved by this compound.

Oenothien 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 MMTV, oenothien 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). Since 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 oenothien B could be related to its tellimagrandin I substructures. Finally, oenothien 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 oenothien B may be beneficial during certain bacterial, viral, and fungal infections.

Not much is known regarding the cellular binding properties of oenothien B. We found that serum levels of IL-6 in TLR4 knockout mice after intraperitoneal injection of oenothien B were similar to those in TLR2 knockout and wild-type mice. These data suggest that oenothien B binding could be mediated via TLR2/TLR4-independent signaling pathways. Recent studies have demonstrated that the binding of ellagitannins, including oenothien B, to albumin increases in strength and affinity for the larger tannins (dimers) compared to their monomer forms and that bond rotational flexibility of oenothien 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 oenothien 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), oenothien B has strong metal-chelating properties. Recently Tahara et al. (Tahara et al., 2014) reported that oenothien B binds Al(III) ions and suggested that formation of Al(III) complexes with oenothien B in roots could contribute to high aluminum resistance of *E. camaldulensis* (Tahara et al., 2014). We found that oenothien 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 (Figure 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 oenothien B-copper complexes could have altered antioxidant or other biological properties compared with native oenothien B.

Clear data regarding the bioavailability of ingested oenothien 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 oenothien B are gallic and ellagic acids, and recent *in vitro* and *in vivo* experiments have revealed that ellagic acid elicits, 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). Since 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; Pukhalskaya et al., 1975). It is likely that oenothien 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, 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 Jul. 16, 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 oenothien 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 oenothien 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- and anti-inflammatory immunomodulation. Although oenothien 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).

Acknowledgments

This research was supported in part by National Institutes of Health IDeA Program COBRE grant GM110732, a USDA National Institute of Food and Agriculture Hatch project, Montana University System Research Initiative: 51040-MUSRI2015-03, and the Montana State University Agricultural Experiment Station.

References

- Al-Shalmani S, Suri S, Hughes DA, Kroon PA, Needs PW, Taylor MA, Tribolo S, Wilson VG. Quercetin and its principal metabolites, but not myricetin, oppose lipopolysaccharide-induced hyporesponsiveness of the porcine isolated coronary artery. *Br J Pharmacol*. 2011; 162:1485–1497. [PubMed: 21375526]
- Andrade RG Jr, Dalvi LT, Silva JM Jr, Lopes GK, Alonso A, Hermes-Lima M. The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals. *Arch Biochem Biophys*. 2005; 437:1–9. [PubMed: 15820211]
- Aoki K, Maruta H, Uchiumi F, Hatano T, Yoshida T, Tanuma S. A macrocyclic ellagitannin, oenothien B, suppresses mouse mammary tumor gene expression via inhibition of poly(ADP-ribose) glycohydrolase. *Biochem Biophys Res Commun*. 1995; 210:329–337. [PubMed: 7755607]
- Ayissi VBO, Ebrahimi A, Schluesener H. Epigenetic effects of natural polyphenols: A focus on SIRT1-mediated mechanisms. *Mol Nutr Food Res*. 2014; 58:22–32. [PubMed: 23881751]
- Baert N, Karonen M, Salminen JP. Isolation, characterisation and quantification of the main oligomeric macrocyclic ellagitannins in *Epilobium angustifolium* by ultra-high performance chromatography with diode array detection and electrospray tandem mass spectrometry. *J Chromatogr A*. 2015; 1419:26–36. [PubMed: 26455285]
- Bartfay WJ, Bartfay E, Johnson JG. Gram-negative and gram-positive antibacterial properties of the whole plant extract of willow herb (*Epilobium angustifolium*). *Biol Res Nurs*. 2012; 14:85–89. [PubMed: 21208973]
- Battinelli L, Tita B, Evandri MG, Mazzanti G. Antimicrobial activity of *Epilobium* spp. extracts. *Farmaco*. 2001; 56:345–348. [PubMed: 11482755]

- Bazylko A, Kiss AK, Kowalski J. High-performance thin-layer chromatography method for quantitative determination of oenothien B and quercetin glucuronide in aqueous extract of *Epilobii angustifolii* herba. *J Chromatogr A*. 2007; 1173:146–150. [PubMed: 17980376]
- Bazylko A, Piwowarski JP, Filipek A, Bonarewicz J, Tomczyk M. In vitro antioxidant and anti-inflammatory activities of extracts from *Potentilla recta* and its main ellagitannin, agrimoniin. *J Ethnopharmacol*. 2013; 149:222–227. [PubMed: 23811215]
- Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. *Ann Intern Med*. 1966; 64:328–340. [PubMed: 5216294]
- Bruning A. Inhibition of mTOR Signaling by Quercetin in Cancer Treatment and Prevention. *Anti-Cancer Agents in Med Chem*. 2013; 13:1025–1031.
- Bubeck Wardenburg J, Bae T, Otto M, Deleo FR, Schneewind O. Poring over pores: alpha-hemolysin and Panton-Valentine leukocidin in *Staphylococcus aureus* pneumonia. *Nat Med*. 2007; 13:1405–1406. [PubMed: 18064027]
- Calderon-Montano JM, Burgos-Moron E, Perez-Guerrero C, Lopez-Lazaro M. A review on the dietary flavonoid kaempferol. *Mini Rev Med Chem*. 2011; 11:298–344. [PubMed: 21428901]
- Carpentier JL, Dayer J-M, Lang V, silvermann R, Orchi L, Gorden P. Down regulation and recycling of insulin receptors: Effect of monensin in IM-9 lymphocytes and U937 monocyte-like cells. *J Biol Chem*. 1984; 259:14190–14195. [PubMed: 6389542]
- Chen AY, Chen YC. A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. *Food Chem*. 2013; 138:2099–2107. [PubMed: 23497863]
- Chirumbolo S. Dietary assumption of plant polyphenols and prevention of allergy. *Curr Pharm Des*. 2014; 20:811–839. [PubMed: 23701556]
- Chung SK, Nam JA, Jeon SY, Kim SI, Lee HJ, Chung TH, Song KS. A prolyl endopeptidase-inhibiting antioxidant from *Phyllanthus ussuriensis*. *Arch of Pharmacol Res*. 2003; 26:1024–1028.
- Cruz EA, Da-Silva SAG, Muzitano MF, Silva PMR, Costa SS, Rossi-Bergmann B. Immunomodulatory pretreatment with *Kalanchoe pinnata* extract and its quercitrin flavonoid effectively protects mice against fatal anaphylactic shock. *Int Immunopharmacol*. 2008; 8:1616–1621. [PubMed: 18675940]
- Cuccioloni M, Bonfili L, Mozzicafreddo M, Cecarini V, Eleuteri AM, Angeletti M. Sanguisorba minor extract suppresses plasmin-mediated mechanisms of cancer cell migration. *Biochim Biophys Acta*. 2012; 1820:1027–1034. [PubMed: 22348918]
- Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents*. 2005; 26:343–356. [PubMed: 16323269]
- D'Archivio M, Filesi C, Vari R, Scaccocchio B, Masella R. Bioavailability of the polyphenols: status and controversies. *Int J Mol Sci*. 2010; 11:1321–1342. [PubMed: 20480022]
- da Silva ER, Maquiaveli CD, Magalhaes PP. The leishmanicidal flavonols quercetin and quercitrin target *Leishmania* (Leishmania) *amazonensis* arginase. *Exp Parasitol*. 2012; 130:183–188. [PubMed: 22327179]
- Daglia M, Di Lorenzo A, Nabavi SF, Talas ZS, Nabavi SM. Polyphenols: well beyond the antioxidant capacity: gallic acid and related compounds as neuroprotective agents: you are what you eat! *Curr Pharm Biotechnol*. 2014; 15:362–372. [PubMed: 24938889]
- Dajas F. Life or death: neuroprotective and anticancer effects of quercetin. *J Ethnopharmacol*. 2012; 143:383–396. [PubMed: 22820241]
- Dale DC, Guerry Dt, Wewerka JR, Bull JM, Chusid MJ. Chronic neutropenia. *Medicine* (Baltimore). 1979; 58:128–144. [PubMed: 431399]
- Daughenbaugh KF, Holderness J, Graff JC, Hedges JF, Freedman B, Graff JW, Jutila MA. Contribution of transcript stability to a conserved procyanidin-induced cytokine response in gd T cells. *Genes Immun*. 2011; 12:378–389. [PubMed: 21307878]
- Derewenda U, Artamonov M, Szukalska G, Utepbergenov D, Olekhovich N, Parikh HI, Kellogg GE, Somlyo AV, Derewenda ZS. Identification of quercitrin as an inhibitor of the p90 S6 ribosomal kinase (RSK): structure of its complex with the N-terminal domain of RSK2 at 1.8 angstrom resolution. *Acta Crystallographica Section D-Biological Crystallography*. 2013; 69:266–275.

- Ding M, Zhao JS, Bowman L, Lu YJ, Shi XL. Inhibition of AP-1 and MAPK signaling and activation of Nrf2/ARE pathway by quercitrin. *International Journal of Oncology*. 2010; 36:59–67. [PubMed: 19956833]
- Dobrev MA, Green RJ, Mueller-Harvey I, Salminen JP, Howlin BJ, Frazier RA. Size and molecular flexibility affect the binding of ellagitannins to bovine serum albumin. *J Agric Food Chem*. 2014; 62:9186–9194. [PubMed: 25162485]
- Ducrey B, Marston A, Gohring S, Hartmann RW, Hostettmann K. Inhibition of 5 α -reductase and aromatase by the ellagitannins oenothin A and oenothin B from *Epilobium* species. *Planta Medica*. 1997; 63:111–114. [PubMed: 9140222]
- Ducrey B, Wolfender JL, Marston A, Hostettmann K. Analysis of Flavonol Glycosides of 13 *Epilobium* Species (Onagraceae) by Lc-Uv and Thermospray Lc-MS. *Phytochemistry*. 1995; 38:129–137.
- Feldman KS. Recent progress in ellagitannin chemistry. *Phytochemistry*. 2005; 66:1984–2000. [PubMed: 16153404]
- Fukuchi K, Sakagami H, Okuda T, Hatano T, Tanuma S, Kitajima K, Inoue Y, Inoue S, Ichikawa S, Nonoyama M. Inhibition of herpes simplex virus infection by tannins and related compounds. *Antiviral Res*. 1989; 11:285–297. [PubMed: 2552915]
- Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, Hirai Y. Antibacterial activity of hydrolyzable tannins derived from medicinal plants against *Helicobacter pylori*. *Microbiol Immunol*. 2004; 48:251–261. [PubMed: 15107535]
- Furst R, Zundorf I. Plant-derived anti-inflammatory compounds: Hopes and disappointments regarding the translation of preclinical knowledge into clinical progress. *Mediat Inflamm*. 2014; 2014:146832.
- Gollucke APB, Aguiar O, Barbisan LF, Ribeiro DA. Use of Grape Polyphenols Against Carcinogenesis: Putative Molecular Mechanisms of Action Using In Vitro and In Vivo Test Systems. *J Med Food*. 2013; 16:199–205. [PubMed: 23477622]
- Granica S, Bazylo A, Kiss AK. Determination of Macrocyclic Ellagitannin Oenothin B in Plant Materials by HPLC-DAD-MS: Method Development and Validation. *Phytochem Analysis*. 2012; 23:582–587.
- Granica S, Piwowarski JP, Czerwinska ME, Kiss AK. Phytochemistry, pharmacology and traditional uses of different *Epilobium* species (Onagraceae): A review. *J Ethnopharmacol*. 2014; 156:316–346. [PubMed: 25196824]
- Granica S, Piwowarski JP, Kiss AK. Ellagitannins modulate the inflammatory response of human neutrophils ex vivo. *Phytomedicine*. 2015; 22:1215–1222. [PubMed: 26655403]
- Guo JM, Lin P, Duan JA, Shang EX, Qian DW, Tang YP. Application of microdialysis for elucidating the existing form of hyperoside in rat brain: Comparison between intragastric and intraperitoneal administration. *J Ethnopharmacol*. 2012; 144:664–670. [PubMed: 23063958]
- Hansen K, Adsersen A, Smitt UW, Nyman U, Christensen SB, Schwartner C, Wagner H. Angiotensin converting enzyme (ACE) inhibitory flavonoids from *Erythroxylum laurifolium*. *Phytomedicine*. 1996; 2:313–317. [PubMed: 23194768]
- Hatano T, Yasuhara T, Matsuda M, Yazaki K, Yoshida T, Okuda T. Oenothin B, a dimeric, hydrolyzable tannin with macrocyclic structure, and accompanying tannins from *Oenothera erythrosepala*. *J Chem Soc-Perkin Trans 1*. 1990; 10:2735–2743.
- Hevesi Tóth B, Blazics B, Kéry A. Polyphenol composition and antioxidant capacity of *Epilobium* species. *J Pharm Biomed Anal*. 2009; 49:26–31.
- Hiermann A, Juan H, Sametz W. Influence of *Epilobium* extracts on prostaglandin biosynthesis and carrageenin induced oedema of the rat paw. *J Ethnopharmacol*. 1986; 17:161–169. [PubMed: 3099091]
- Hiermann A, Reidlinger M, Juan H, Sametz W. Isolation of the antiphlogistic principle from *Epilobium angustifolium*. *Planta Medica*. 1991; 57:357–360. [PubMed: 1775578]
- Holderness J, Hedges JF, Daughenbaugh K, Kimmel E, Graff J, Freedman B, Jutila MA. Response of gd T cells to plant-derived tannins. *Crit Rev Immunol*. 2008; 28:377–402. [PubMed: 19166386]

- Holderness J, Jackiw L, Kimmel E, Kerns H, Radke M, Hedges JF, Petrie C, McCurley P, Glee PM, Palecanda A, Jutila MA. Select plant tannins induce IL-2Ra up-regulation and augment cell division in gd T cells. *J Immunol.* 2007; 179:6468–6478. [PubMed: 17982035]
- Holderness J, Schepetkin IA, Freedman B, Kirpotina LN, Quinn MT, Hedges JF, Jutila MA. Polysaccharides isolated from Acai fruit induce innate immune responses. *PLoS One.* 2011; 6:e17301. [PubMed: 21386979]
- Ishimoto H, Tai A, Yoshimura M, Amakura Y, Yoshida T, Hatano T, Ito H. Antioxidative properties of functional polyphenols and their metabolites assessed by an ORAC assay. *Biosci Biotechnol Biochem.* 2012; 76:395–399. [PubMed: 22313776]
- Ivancheva S, Manolova N, Serkedjieva J, Dimov V, Ivanovska N. Polyphenols from Bulgarian medicinal plants with anti-infectious activity. *Basic Life Sci.* 1992; 59:717–728. [PubMed: 1417697]
- Izzo AA, Hoon-Kim S, Radhakrishnan R, Williamson EM. A critical approach to evaluating clinical efficacy, adverse events and drug interactions of herbal remedies. *Phytother Res.* 2016
- Jimenez R, Lopez-Sepulveda R, Romero M, Toral M, Cogolludo A, Perez-Vizcaino F, Duarte J. Quercetin and its metabolites inhibit the membrane NADPH oxidase activity in vascular smooth muscle cells from normotensive and spontaneously hypertensive rats. *Food Funct.* 2015; 6:409–414. [PubMed: 25562607]
- Jones NP, Arnason JT, Abou-Zaid M, Akpagana K, Sanchez-Vindas P, Smith ML. Antifungal activity of extracts from medicinal plants used by First Nations Peoples of eastern Canada. *J Ethnopharmacol.* 2000; 73:191–198. [PubMed: 11025156]
- Joven J, Rull A, Rodriguez-Gallego E, Camps J, Riera-Borrull M, Hernandez-Aguilera A, Martin-Paredero V, Segura-Carretero A, Micol V, Alonso-Villaverde C, Menendez JA. Multifunctional targets of dietary polyphenols in disease: A case for the chemokine network and energy metabolism. *Food Chem Toxicol.* 2013; 51:267–279. [PubMed: 23063595]
- Juan H, Sametz W, Hiermann A. Anti-inflammatory effects of a substance extracted from *Epilobium angustifolium*. *Agents Actions.* 1988; 23:106–107. [PubMed: 3354371]
- Jurgenson S, Matto V, Raal A. Vegetational variation of phenolic compounds in *Epilobium angustifolium*. *Nat Prod Res.* 2012; 26:1951–1953. [PubMed: 22149976]
- Karakurt S, Semiz A, Celik G, Gencler-Ozkan AM, Sen A, Adali O. Contribution of ellagic acid on the antioxidant potential of medicinal plant *Epilobium hirsutum*. *Nutr Cancer.* 2016; 68:173–183. [PubMed: 26700224]
- Kaskoniene V, Maruska A, Akuneca I, Stankevicius M, Ragazinskiene O, Bartkuvienė V, Kornysova O, Briedis V, Ugenskiene R. Screening of antioxidant activity and volatile compounds composition of *Chamerion angustifolium* (L.) Holub ecotypes grown in Lithuania. *Nat Prod Res.* 2015a; 29:1–9. [PubMed: 25204784]
- Kaskoniene V, Stankevicius M, Drevinskas T, Akuneca I, Kaskonas P, Bimbiraite-Surviliene K, Maruska A, Ragazinskiene O, Kornysova O, Briedis V, Ugenskiene R. Evaluation of phytochemical composition of fresh and dried raw material of introduced *Chamerion angustifolium* L. using chromatographic, spectrophotometric and chemometric techniques. *Phytochemistry.* 2015b; 115:184–193. [PubMed: 25725961]
- Kawakami K, Li P, Uraji M, Hatanaka T, Ito H. Inhibitory effects of pomegranate extracts on recombinant human maltase-glucoamylase. *J Food Sci.* 2014; 79:H1848–H1853. [PubMed: 25154971]
- Khlebnikov AI, Schepetkin IA, Domina NG, Kirpotina LN, Quinn MT. Improved quantitative structure-activity relationship models to predict antioxidant activity of flavonoids in chemical, enzymatic, and cellular systems. *Bioorg Med Chem.* 2007; 15:1749–1770. [PubMed: 17166721]
- Kim HJ, Lee JS, Woo ER, Kim MK, Yang BS, Yu YG, Park H, Lee YS. Isolation of virus-cell fusion inhibitory components from *Eugenia caryophyllata*. *Planta Med.* 2001; 67:277–279. [PubMed: 11345703]
- Kim Y, Narayanan S, Chang KO. Inhibition of influenza virus replication by plant-derived isoquercetin. *Antiviral Res.* 2010; 88:227–235. [PubMed: 20826184]
- Kim YS, Jung DH, Lee IS, Choi SJ, Yu SY, Ku SK, Kim MH, Kim JS. Effects of *Allium victorialis* leaf extracts and its single compounds on aldose reductase, advanced glycation end products and

- TGF-beta 1 expression in mesangial cells. BMC Complementary and Alternative Medicine. 2013; 13:251. [PubMed: 24090434]
- Kishimoto Y, Tani M, Kondo K. Pleiotropic preventive effects of dietary polyphenols in cardiovascular diseases. European Journal of Clinical Nutrition. 2013; 67:532–535. [PubMed: 23403879]
- Kiss A, Kowalski J, Melzig MF. Compounds from *Epilobium angustifolium* inhibit the specific metalloproteinases ACE, NEP and APN. Planta Medica. 2004; 70:919–923. [PubMed: 15490319]
- Kiss A, Kowalski J, Melzig MF. Effect of *Epilobium angustifolium* L. extracts and polyphenols on cell proliferation and neutral endopeptidase activity in selected cell lines. Pharmazie. 2006a; 61:66–69. [PubMed: 16454210]
- Kiss A, Kowalski J, Melzig MF. Induction of neutral endopeptidase activity in PC-3 cells by an aqueous extract of *Epilobium angustifolium* L. and oenothien B. Phytomedicine. 2006b; 13:284–289. [PubMed: 16492533]
- Kiss AK, Bazylko A, Filipek A, Granica S, Jaszewska E, Kiarszys U, Kosmider A, Piwowarski J. Oenothien B's contribution to the anti-inflammatory and antioxidant activity of *Epilobium* sp. Phytomedicine. 2011; 18:557–560. [PubMed: 21112753]
- Korkina LG, Pastore S, Dellambra E, De Luca C. New molecular and cellular targets for chemoprevention and treatment of skin tumors by plant polyphenols: a critical review. Curr Med Chem. 2013; 20:852–868. [PubMed: 23210776]
- Kosalec I, Kopjar N, Kremer D. Antimicrobial activity of Willowherb (*Epilobium angustifolium* L.) leaves and flowers. Curr Drug Targets. 2013; 14:986–991. [PubMed: 23796429]
- Ku SK, Kim TH, Lee S, Kim SM, Bae JS. Antithrombotic and profibrinolytic activities of isorhamnetin-3-O-galactoside and hyperoside. Food and Chemical Toxicology. 2013; 53:197–204. [PubMed: 23220618]
- Landete JM. Updated knowledge about polyphenols: Functions, bioavailability, metabolism, and health. Crit Rev Food Sci Nutr. 2012; 52:936–948. [PubMed: 22747081]
- Larrosa M, Gonzalez-Sarrias A, Garcia-Conesa MT, Tomas-Barberan FA, Espin JC. Urolithins, ellagic acid-derived metabolites produced by human colonic microflora, exhibit estrogenic and antiestrogenic activities. J Agric Food Chem. 2006; 54:1611–1620. [PubMed: 16506809]
- Lee SY, So YJ, Shin MS, Cho JY, Lee J. Antibacterial effects of afzelin isolated from *Cornus macrophylla* on *Pseudomonas aeruginosa*, a leading cause of illness in immunocompromised individuals. Molecules. 2014; 19:3173–3180. [PubMed: 24642906]
- Lekstrom-Himes JA, Gallin JI. Immunodeficiency diseases caused by defects in phagocytes. N Engl J Med. 2000; 343:1703–1714. [PubMed: 11106721]
- Leonarduzzi G, Testa G, Sottero B, Gamba P, Poli G. Design and development of nanovehicle-based delivery systems for preventive or therapeutic supplementation with flavonoids. Curr Med Chem. 2010; 17:74–95. [PubMed: 19941477]
- Lesuisse D, Berjonneau J, Ciot C, Devaux P, Doucet B, Gourvest JF, Khemis B, Lang C, Legrand R, Lowinski M, Maquin P, Parent A, Schoot B, Teutsch G. Determination of oenothien B as the active 5-a-reductase-inhibiting principle of the folk medicine *Epilobium parviflorum*. J Nat Prod. 1996; 59:490–492. [PubMed: 8778238]
- Lewandowska U, Szweczyk K, Hrabec E, Janecka A, Gorlach S. Overview of metabolism and bioavailability enhancement of polyphenols. J Agric Food Chem. 2013; 61:12183–12199. [PubMed: 24295170]
- Li YQ, Zhou FC, Gao F, Bian JS, Shan F. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase. J Agric Food Chem. 2009; 57:11463–11468. [PubMed: 19938837]
- Liao YR, Lin JY. Quercetin, but not its metabolite quercetin-3-glucuronide, exerts prophylactic immunostimulatory activity and therapeutic antiinflammatory effects on lipopolysaccharide-treated mouse peritoneal macrophages ex vivo. J Agric Food Chem. 2014; 62:2872–2880. [PubMed: 24620730]
- Lin CC, Hsu YF, Lin TC. Antioxidant and free radical scavenging effects of the tannins of *Terminalia catappa* L. Anticancer Res. 2001; 21:237–243. [PubMed: 11299741]
- Liu K, Mei F, Wang Y, Xiao N, Yang L, Wang Y, Li J, Huang F, Kou J, Liu B, Qi LW. Quercetin oppositely regulates insulin-mediated glucose disposal in skeletal muscle under normal and

inflammatory conditions: The dual roles of AMPK activation. *Mol Nutr Food Res*. 2016; 60:551–565. [PubMed: 26627467]

- Liu RL, Xiong QJ, Shu Q, Wu WN, Cheng J, Fu H, Wang F, Chen JG, Hu ZL. Hyperoside protects cortical neurons from oxygen-glucose deprivation-reperfusion induced injury via nitric oxide signal pathway. *Brain Res*. 2012; 1469:164–173. [PubMed: 22771858]
- Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr*. 2005; 81:230S–242S. [PubMed: 15640486]
- Marzouk MSA, Moharram FA, Mohamed MA, Gamal-Eldeen AM, Aboutabl EA. Anticancer and antioxidant tannins from *Pimenta dioica* leaves. *Zeitschrift Fur Naturforschung C-a Journal of Biosciences*. 2007; 62:526–536.
- Messer JG, Hopkins RG, Kipp DE. Quercetin metabolites up-regulate the antioxidant response in osteoblasts isolated from fetal rat calvaria. *J Cell Biochem*. 2015; 116:1857–1866. [PubMed: 25716194]
- Min BS, Lee SY, Kim JH, Lee JK, Kim TJ, Kim DH, Kim YH, Joung H, Lee HK, Nakamura N, Miyashiro H, Hattori M. Anti-complement activity of constituents from the stem-bark of *Juglans mandshurica*. *Biol Pharmaceut Bull*. 2003; 26:1042–1044.
- Mira L, Fernandez MT, Santos M, Rocha R, Florencio MH, Jennings KR. Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. *Free Radic Res*. 2002; 36:1199–1208. [PubMed: 12592672]
- Miyamoto K, Murayama T, Nomura M, Hatano T, Yoshida T, Furukawa T, Koshiura R, Okuda T. Antitumor activity and interleukin-1 induction by tannins. *Anticancer Res*. 1993a; 13:37–42. [PubMed: 8476228]
- Miyamoto K, Nomura M, Sasakura M, Matsui E, Koshiura R, Murayama T, Furukawa T, Hatano T, Yoshida T, Okuda T. Antitumor activity of oenothien B, a unique macrocyclic ellagitannin. *Jpn J Cancer Res*. 1993b; 84:99–103. [PubMed: 8449833]
- Monschein M, Jandl K, Buzimkic S, Bucar F. Content of phenolic compounds in wild populations of *Epilobium angustifolium* growing at different altitudes. *Pharm Biol*. 2015; 53:1576–1582. [PubMed: 25856698]
- Moskalenko SA. Preliminary screening of far-eastern ethnomedicinal plants for antibacterial activity. *J Ethnopharmacol*. 1986; 15:231–259. [PubMed: 3724205]
- Myagmar BE, Aniya Y. Free radical scavenging action of medicinal herbs from Mongolia. *Phytomedicine*. 2000; 7:221–229. [PubMed: 11185733]
- Nguyen XN, Phan VK, Chau VM, Ninh KB, Nguyen XC, Tai BH, Kim YH. Phenylpropanoid glycosides from *Heterosmilax erythrantha* and their antioxidant activity. *Arch Pharmacol Res*. 2009; 32:1373–1377.
- Okuda T. Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry*. 2005; 66:2012–2031. [PubMed: 15982679]
- Okuda T, Yoshida T, Hatano T. Ellagitannins as active constituents of medicinal plants. *Planta Medica*. 1989; 117–122:117–122.
- Okuyama S, Makihata N, Yoshimura M, Amakura Y, Yoshida T, Nakajima M, Furukawa Y. Oenothien B suppresses lipopolysaccharide (LPS)-induced inflammation in the mouse brain. *Int J Mol Sci*. 2013; 14:9767–9778. [PubMed: 23652834]
- Pasinetti GM. Novel role of red wine-derived polyphenols in the prevention of Alzheimer's Disease dementia and brain pathology: Experimental approaches and clinical implications. *Planta Medica*. 2012; 78:1614–1619. [PubMed: 23023952]
- Peng J, Wei D, Fu Z, Li D, Tan Y, Xu T, Zhou J, Zhang T. Punicalagin ameliorates lipopolysaccharide-induced acute respiratory distress syndrome in mice. *Inflammation*. 2015; 38:493–499. [PubMed: 25005005]
- Petrova MF, Pukhal'skaia EC, Gotinskaya LG. A study for structure of Chanerol and characterization of its antitumor activity. *Prob Chemother Tumors Moscow-Kiev*. 1974; 1:49.
- Piowowski JP, Granica S, Kiss AK. Influence of gut microbiota-derived ellagitannins' metabolites urolithins on pro-inflammatory activities of human neutrophils. *Planta Med*. 2014; 80:887–895. [PubMed: 24995502]

- Pukhal'skaia E, Petrova MF, Kibal'chich PN, Alieva TA, Denisova SI. Isolation of a polymer from *Chamaenerium angustifolium* and study of its antineoplastic action. *Antibiotiki*. 1970; 15:782–785. [PubMed: 5536919]
- Pukhalskaya EC, Chernyakhovskaya I, Petrova MF, Denisova SI, Alieva TA. Macromolecular antitumor agents from *Chamaenerium angustifolium*. *Neoplasma*. 1975; 22:29–37. [PubMed: 1153036]
- Quideau S, Deffieux D, Douat-Casassus C, Pouysegur L. Plant polyphenols: chemical properties, biological activities, and synthesis. *Angew Chem Int Ed Engl*. 2011; 50:586–621. [PubMed: 21226137]
- Rajendran P, Rengarajan T, Nandakumar N, Palaniswami R, Nishigaki Y, Nishigaki I. Kaempferol, a potential cytostatic and cure for inflammatory disorders. *Eur J Med Chem*. 2014; 86C:103–112. [PubMed: 25147152]
- Ramiro-Puig E, Perez-Cano FJ, Ramos-Romero S, Perez-Berezo T, Castellote C, Permanyer J, Franch A, Izquierdo-Pulido M, Castell M. Intestinal immune system of young rats influenced by cocoa-enriched diet. *J Nutr Biochem*. 2008; 19:555–565. [PubMed: 18061430]
- Ramstead AG, Schepetkin IA, Quinn MT, Jutila MA. Oenothien B, a Cyclic Dimeric Ellagitannin Isolated from *Epilobium angustifolium*, Enhances IFN γ Production by Lymphocytes. *PLoS One*. 2012; 7:e50546. [PubMed: 23226309]
- Ramstead AG, Schepetkin IA, Todd K, Loeffelholz J, Quinn MT, Jutila MA. Aging influences the response of T cells to stimulation by the ellagitannin, oenothien B. *Int Immunopharmacol*. 2015; 26:367–377. [PubMed: 25887271]
- Ratz-Lyko A, Arct J, Majewski S, Pytkowska K. Influence of polyphenols on the physiological processes in the skin. *Phytother Res*. 2015; 29:509–517. [PubMed: 25586195]
- Rauha JP, Remes S, Heinonen M, Hopia A, Kahkonen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol*. 2000; 56:3–12. [PubMed: 10857921]
- Rommel I, Vares L, Toom L, Matto V, Raal A. Phenolic compounds in five *Epilobium* species collected from Estonia. *Nat Prod Commun*. 2012; 7:1323–1324. [PubMed: 23156999]
- Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Res*. 1995; 22:375–383. [PubMed: 7633567]
- Rice-evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med*. 1996; 20:933–956. [PubMed: 8743980]
- Russo GL, Russo M, Spagnuolo C, Tedesco I, Bilotto S, Iannitti R, Palumbo R. Quercetin: A pleiotropic kinase inhibitor against cancer. *Adv Nutr Cancer*. 2014; 159:185–205.
- Ruszova E, Cheel J, Pavsek S, Moravcova M, Hermannova M, Matejkova I, Spilkova J, Velebný V, Kubala L. *Epilobium angustifolium* extract demonstrates multiple effects on dermal fibroblasts in vitro and skin photo-protection in vivo. *Gen Physiol Biophys*. 2013; 32:347–359. [PubMed: 23817638]
- Ruzic I, Skerget M, Knez Z. Potential of phenolic antioxidants. *Acta Chim Slov*. 2010; 57:263–271. [PubMed: 24061721]
- Sak K. Site-specific anticancer effects of dietary flavonoid quercetin. *Nutr Cancer*. 2014; 66:177–193. [PubMed: 24377461]
- Sakagami H, Jiang Y, Kusama K, Atsumi T, Ueha T, Toguchi M, Iwakura I, Satoh K, Ito H, Hatano T, Yoshida T. Cytotoxic activity of hydrolyzable tannins against human oral tumor cell lines--a possible mechanism. *Phytomedicine*. 2000; 7:39–47. [PubMed: 10782489]
- Santos GD, Ferri PH, Santos SC, Bao SN, Soares CMA, Pereira M. Oenothien B inhibits the expression of PbFKS1 transcript and induces morphological changes in *Paracoccidioides brasiliensis*. *Med Mycol*. 2007; 45:609–618. [PubMed: 18033615]
- Sasov SA, Tolkachev VN, Yartseva IV, Tolkachev ON. Macrocyclic tannins of *Chamaenerium angustifolium*. *Problems of Biological, Medical and Pharmaceutical Chemistry*. 2010; 10:24–27.
- Satue M, Arriero MD, Monjo M, Ramis JM. Quercitrin and Taxifolin stimulate osteoblast differentiation in MC3T3-E1 cells and inhibit osteoclastogenesis in RAW 264.7 cells. *Biochem Pharmacol*. 2013; 86:1476–1486. [PubMed: 24060614]

- Schaffer S, Asseburg H, Kuntz S, Muller WE, Eckert GP. Effects of polyphenols on brain ageing and Alzheimer's Disease: Focus on mitochondria. *Mol Neurobiol.* 2012; 46:161–178. [PubMed: 22706880]
- Schepetkin IA, Kirpotina LN, Jakiw L, Khlebnikov AI, Blaskovich CL, Jutila MA, Quinn MT. Immunomodulatory activity of oenothien B isolated from *Epilobium angustifolium*. *J Immunol.* 2009; 183:6754–6766. [PubMed: 19846877]
- Schepetkin IA, Kirpotina LN, Khlebnikov AI, Jutila MA, Quinn MT. Gastrin-releasing peptide/neuromedin B receptor antagonists PD176252, PD168368, and related analogs are potent agonists of human formyl-peptide receptors. *Mol Pharmacol.* 2011; 79:77–90. [PubMed: 20943772]
- Schmid D, Gruber M, Piskaty C, Woehs F, Renner A, Nagy Z, Kaltenboeck A, Wasserscheid T, Bazylko A, Kiss AK, Moeslinger T. Inhibition of NF-kappa B-dependent cytokine and inducible nitric oxide synthesis by the macrocyclic ellagitannin oenothien B in TLR-stimulated RAW 264.7 macrophages. *J Nat Prod.* 2012; 75:870–875. [PubMed: 22560043]
- Shikov AN, Poltanov EA, Dorman HJ, Makarov VG, Tikhonov VP, Hiltunen R. Chemical composition and *in vitro* antioxidant evaluation of commercial water-soluble willow herb (*Epilobium angustifolium* L.) extracts. *J Agric Food Chem.* 2006; 54:3617–3624. [PubMed: 19127734]
- Shin SW, Jung E, Kim S, Kim JH, Kim EG, Lee J, Park D. Antagonizing effects and mechanisms of Afzelin against UVB-induced cell damage. *PLoS One.* 2013; 8:e61971. [PubMed: 23626759]
- Shiota S, Shimizu M, Sugiyama J, Morita Y, Mizushima T, Tsuchiya T. Mechanisms of action of corilagin and tellimagrandin I that remarkably potentiate the activity of beta-lactams against methicillin-resistant *Staphylococcus aureus*. *Microbiol Immunol.* 2004; 48:67–73. [PubMed: 14734860]
- Skyberg JA, Robison A, Golden S, Rollins MF, Callis G, Huarte E, Kochetkova I, Jutila MA, Pascual DW. Apple polyphenols require T cells to ameliorate dextran sulfate sodium-induced colitis and dampen proinflammatory cytokine expression. *J Leukoc Biol.* 2011; 90:1043–1054. [PubMed: 21693591]
- Song M, Hong M, Lee MY, Jee JG, Lee YM, Bae JS, Jeong TC, Lee S. Selective inhibition of the cytochrome P450 isoform by hyperoside and its potent inhibition of CYP2D6. *Food Chem Toxicol.* 2013; 59:549–553. [PubMed: 23835282]
- Spiridonov NA, Arkhipov VV, Foigel AG, Tolkachev ON, Sasov SA, Syrkin AB, Tolkachev VN. The cytotoxicity of *Chamaenerium angustifolium* (L.) Scop. and *Hippophae rhamnoides* L. tannins and their effect on mitochondrial respiration. *Eksp Klin Farmakol.* 1997; 60(4):60–63. [PubMed: 9376762]
- Stagos D, Amoutzias GD, Matakos A, Spyrou A, Tsatsakis AM, Kouretas D. Chemoprevention of liver cancer by plant polyphenols. *Food Chem Toxicol.* 2012; 50:2155–2170. [PubMed: 22521445]
- Stolarczyk M, Naruszewicz M, Kiss AK. Extracts from *Epilobium* sp. herbs induce apoptosis in human hormone-dependent prostate cancer cells by activating the mitochondrial pathway. *J Pharm Pharmacol.* 2013a; 65:1044–1054. [PubMed: 23738732]
- Stolarczyk M, Piwowarski JP, Granica S, Stefanska J, Naruszewicz M, Kiss AK. Extracts from *Epilobium* sp herbs, their components and gut microbiota metabolites of *Epilobium Ellagitannins*, urolithins, inhibit hormone-dependent prostate cancer cells-(LNCaP) proliferation and PSA secretion. *Phytother Res.* 2013b; 27:1842–1848. [PubMed: 23436427]
- Syrkin AB, Konyaeva OI. Pharmacological investigations of some new antineoplastic compounds. *Khimiko-Farmatsevticheskii Zhurnal.* 1984; 18:1172–1180.
- Tahara K, Hashida K, Otsuka Y, Ohara S, Kojima K, Shinohara K. Identification of a hydrolyzable tannin, oenothien B, as an aluminum-detoxifying ligand in a highly aluminum-resistant tree, *Eucalyptus camaldulensis*. *Plant Physiology.* 2014; 164:683–693. [PubMed: 24381064]
- Tarko T, Duda-Chodak A, Zajac N. Digestion and absorption of phenolic compounds assessed by *in vitro* simulation methods. A review. *Rocz Panstw Zakl Hig.* 2013; 64:79–84. [PubMed: 23987074]
- Thapa M, Kim Y, Desper J, Chang KO, Hua DH. Synthesis and antiviral activity of substituted quercetins. *Bioorg Med Chem Lett.* 2012; 22:353–356. [PubMed: 22115591]

- Tita B, Abdel-Haq H, Vitalone A, Mazzanti G, Saso L. Analgesic properties of *Epilobium angustifolium*, evaluated by the hot plate test and the writhing test. *Farmaco*. 2001; 56:341–343. [PubMed: 11482754]
- Uysal U, Seremet S, Lamping JW, Adams JM, Liu DY, Swerdlow RH, Aires DJ. Consumption of polyphenol plants may slow aging and associated diseases. *Current Pharmaceutical Design*. 2013; 19:6094–6111. [PubMed: 23448445]
- Vila S, Prahoveanu E, Esanu V, Mihaila V, Pintilie G, Niculescu M, Teoteoi N, Colceru S. The action of a *Chamenerion angustifolium* extract in experimental influenza virus infection in mice. *Virologie*. 1989; 40:129–132. [PubMed: 2781726]
- Vitalone A, Bordi F, Baldazzi C, Mazzanti G, Saso L, Tita B. Anti-proliferative effect on a prostatic epithelial cell line (PZ-HPV-7) by *Epilobium angustifolium* L. *Farmaco*. 2001; 56:483–489. [PubMed: 11482783]
- Vitalone A, Guizzetti M, Costa LG, Tita B. Extracts of various species of *Epilobium* inhibit proliferation of human prostate cells. *J Pharm Pharmacol*. 2003a; 55:683–690. [PubMed: 12831512]
- Vitalone A, McColl J, Thome D, Costa LG, Tita B. Characterization of the effect of *Epilobium* extracts on human cell proliferation. *Pharmacology*. 2003b; 69:79–87. [PubMed: 12928581]
- Vogl S, Picker P, Mihaly-Bison J, Fakhrudin N, Atanasov AG, Heiss EH, Wawrosch C, Reznicek G, Dirsch VM, Saukel J, Kopp B. Ethnopharmacological in vitro studies on Austria's folk medicine--an unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. *J Ethnopharmacol*. 2013; 149:750–771. [PubMed: 23770053]
- Voyich JM, Braughton KR, Sturdevant DE, Whitney AR, Said-Salim B, Porcella SF, Long RD, Dorward DW, Gardner DJ, Kreiswirth BN, Musser JM, DeLeo FR. Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. *J Immunol*. 2005; 175:3907–3919. [PubMed: 16148137]
- Voyich JM, Otto M, Mathema B, Braughton KR, Whitney AR, Welty D, Long RD, Dorward DW, Gardner DJ, Lina G, Kreiswirth BN, DeLeo FR. Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? *J Infect Dis*. 2006; 194:1761–1770. [PubMed: 17109350]
- Voyich JM, Vuong C, DeWald M, Nygaard TK, Kocianova S, Griffith S, Jones J, Iverson C, Sturdevant DE, Braughton KR, Whitney AR, Otto M, DeLeo FR. The SaeR/S gene regulatory system is essential for innate immune evasion by *Staphylococcus aureus*. *J Infect Dis*. 2009; 199:1698–1706. [PubMed: 19374556]
- Voynova E, Dimitrova S, Naydenova E, Karadjov P. Inhibitory action of extracts of *Maclura aurantiaca* and *Epilobium hirsutum* on tumour models in mice. *Acta Physiol Pharmacol Bulg*. 1991; 17:50–52. [PubMed: 1841518]
- Wang CP, Li JL, Zhang LZ, Zhang XC, Yu S, Liang XM, Ding F, Wang ZW. Isoquercetin protects cortical neurons from oxygen-glucose deprivation-reperfusion induced injury via suppression of TLR4-NF-kappa B signal pathway. *Neurochem Int*. 2013; 63:741–749. [PubMed: 24099731]
- Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, DeLeo FR, Otto M. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat Med*. 2007; 13:1510–1514. [PubMed: 17994102]
- Webster D, Taschereau P, Belland RJ, Sand C, Rennie RP. Antifungal activity of medicinal plant extracts; preliminary screening studies. *Journal of Ethnopharmacology*. 2008; 115:140–146. [PubMed: 17996411]
- Yamanaka D, Tamiya Y, Motoi M, Ishibashi K, Miura NN, Adachi Y, Ohno N. The effect of enzymatically polymerised polyphenols on CD4 binding and cytokine production in murine splenocytes. *PLoS One*. 2012; 7:e36025. [PubMed: 22540016]
- Yin YQ, Li WQ, Son YO, Sun LJ, Lu J, Kim D, Wang X, Yao H, Wang L, Pratheeshkumar P, Hitron AJ, Luo J, Gao N, Shi XL, Zhang Z. Quercitrin protects skin from UVB-induced oxidative damage. *Toxicol Appl Pharmacol*. 2013; 269:89–99. [PubMed: 23545178]
- Yoshida T, Amakura Y, Yoshimura M. Structural features and biological properties of ellagitannins in some plant families of the order Myrtales. *Int J Mol Sci*. 2010; 11:79–106. [PubMed: 20162003]

- Yoshimura M, Akiyama H, Kondo K, Sakata K, Matsuoka H, Amakura Y, Teshima R, Yoshida T. Immunological effects of oenothien B, an ellagitannin dimer, on dendritic cells. *International Journal of Molecular Sciences*. 2013; 14:46–56. [PubMed: 23344020]
- Yoshioka H, Senba Y, Saito K, Kimura T, Hayakawa F. Spin-trapping study on the hydroxyl radical formed from a tea catechin-Cu(II) system. *Biosci Biotechnol Biochem*. 2001; 65:1697–1706. [PubMed: 11577706]
- Yu HN, Shen SR, Xiong YK. Cytotoxicity of epigallocatechin-3-gallate to LNCaP cells in the presence of Cu²⁺. *J Zhejiang Univ Sci B*. 2005; 6:125–131. [PubMed: 15633248]
- Zambuzzi-Carvalho PF, Tomazett PK, Santos SC, Ferri PH, Borges CL, Martins WS, Soares CMD, Pereira M. Transcriptional profile of *Paracoccidioides* induced by oenothien B, a potential antifungal agent from the Brazilian Cerrado plant *Eugenia uniflora*. *BMC Microbiology*. 2013; 13:227. [PubMed: 24119145]
- Zeng KW, Wang XM, Ko H, Kwon HC, Cha JW, Yang HO. Hyperoside protects primary rat cortical neurons from neurotoxicity induced by amyloid beta-protein via the PI3K/Akt/Bad/Bcl(XL)-regulated mitochondrial apoptotic pathway. *Eur J Pharmacol*. 2011; 672:45–55. [PubMed: 21978835]
- Zhang HM, Zhao L, Li H, Xu H, Chen WW, Tao L. Research progress on the anticarcinogenic actions and mechanisms of ellagic acid. *Cancer Biol Med*. 2014; 11:92–100. [PubMed: 25009751]
- Zhang R, Yao Y, Wang Y, Ren G. Antidiabetic activity of isoquercetin in diabetic KK -Ay mice. *Nutr Metab (Lond)*. 2011; 8:85. [PubMed: 22133267]
- Zhang ZY, Sethiel MS, Shen WZ, Liao ST, Zou YX. Hyperoside downregulates the receptor for advanced glycation end products (RAGE) and promotes proliferation in ECV304 cells via the c-Jun N-terminal kinase (JNK) pathway following stimulation by advanced glycation end-products in vitro. *Int J Mol Sci*. 2013; 14:22697–22707. [PubMed: 24252909]

Figure 1.
Chemical structure of oenothien B.

Figure 2.

Effect of oenothien B on *Staphylococcus aureus* growth and human neutrophil staphylocidal activity. **Panel A.** Direct effect of oenothien B on growth of *Staphylococcus aureus*. *S. aureus* USA300 (2×10^6 in 200 μ l of RPMI) were mixed with the indicated concentrations of oenothien B in microplate wells and incubated at 37°C for 3 hours. Oenothien 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 oenothien B dilutions in RPMI alone were subtracted from each OD₆₀₀ reading, and % growth was calculated as: (OD₆₀₀ of oenothien B-treated *S. aureus*/OD₆₀₀ of control, untreated *S. aureus* in RPMI) $\times$ 100. We subtracted these values from 100 to plot % growth inhibition resulting from the oenothien B concentrations tested. Values are the mean $\pm$ S.D. of triplicate samples from one experiment,

which is representative of three independent experiments. **Panel B.** Effect of oenothien 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 oenothien B or control Hank's balanced-salt solution (HBSS) for 15 min, washed to remove remaining oenothien 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 μ l 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 $\pm$ 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 oenothien B are indicated.

Figure 3.

Binding of Cu(II) to oenothien B. Oenothien 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)-oenothien 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 oenothien 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.

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- <i>O</i> -rhamnoside)	Rha	H	H
5	Quercitrin (quercetin-3- <i>O</i> -rhamnoside)	Rha	OH	H
6	Myricetin-3- <i>O</i> -rhamnoside	Rha	OH	OH
7	Juglalin (kaempferol-3- <i>O</i> -arabinofuranoside)	Ara	H	H
8	Guajaverin (quercetin 3- <i>O</i> -arabinopyranside)	Ara	OH	H
9	Hyperoside (quercetin-3- <i>O</i> -galactoside)	Gal	OH	H
10	Isoquercetin (quercetin-3- <i>O</i> -glucoside)	Glc	OH	H
11	Isomyricitrin (myricetin-3- <i>O</i> -glucoside)	Glc	OH	OH
12	Quercetin-3- <i>O</i> -(6''-galloyl)-galactoside	(6''-Gall)Gal	OH	H
13	Miquelianin (quercetin-3- <i>O</i> -glucuronide)	GlcA	OH	H
14	Kaempferol-3- <i>O</i> -β-glucuronide	GlcA	H	H
15	Myricetin-3- <i>O</i> -glucuronide	GlcA	OH	OH
16	Myricetin-3- <i>O</i> -galactoside	Gal	OH	OH

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

Compound	Biological properties	References
Afzelin (kaempferol-3- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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, Chen, 2013; Rajendran et al., 2014)
Miquelianin (quercetin-3- <i>O</i> -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, Lin, 2014; Liu et al., 2016)
Myricetin Quercetin	Antioxidant, anti-inflammatory, antimicrobial, anti-proliferative, anti-aging Anti-proliferative, antioxidative, neuroprotective, and anti-inflammatory, pleiotropic kinase inhibitor, inhibitor of α -glucosidase	(Cushnie, Lamb, 2005; Leonarduzzi et al., 2010; Ruzic et al., 2010) (Li et al., 2009; Dajas, 2012; Bruning, 2013; Furst, Zundorf, 2014; Russo et al., 2014; Sak, 2014)
Quercitrin (quercetin-3- <i>O</i> -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)

Table 3

Biological properties of oenothien B

Assay	Biological properties	References
<i>In vitro</i>	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-1 β 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- κ B activation and production of TNF and IL-6 in human monocytic THP-1 cells	(Schepetkin et al., 2009)
	Activates NK cells, $\alpha\beta$ T cells, and $\gamma\delta$ T cells, resulting in increased expression of the activation marker CD69 and IFN γ production	(Ramstead et al., 2012)
	Induces more IFN γ production by CD45RO ⁺ compared to naïve T cells memory T cells	(Ramstead et al., 2015)
	Scavenger of ROS (O ₂ ⁻ and H ₂ O 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 <i>Helicobacter pylori</i>	(Funatogawa et al., 2004).
<i>In vivo</i>	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-1 β and IL-6 production by activated dendritic cells and attenuates neuroinflammation in response to LPS treatment	(Okuyama et al., 2013)