ANTIBACTERIAL PROPERTIES OF *EPILOBIUM* AND OTHER PLANT SPECIES: A SYSTEMATIC REVIEW OF THE LITERATURE

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ABSTRACT

Background and Significance: The emergence of new pathogens and the increase in number of multi-drug resistant strains unaffected by currently available antibiotics is a major growing public health concern globally. The current lack of research into the development of new antibiotics by large pharmaceutical companies is largely due to poor financial returns. Herbal and plant based sources, such as the *Epilobium angustifolium*, may possess antibacterial properties, and therefore provide a viable and cost effective source for the development of new antibiotics.

Methods: A systematic review of the literature was undertaken to locate articles describing potential antimicrobial properties of *E. angustifolium* and similar plant species. A data extraction template was employed, and three databases were searched: (i) PubMed (January 1992 to November 2018), (ii) Web of Science (January 2008 to November 2018), and (iii) Scopus (January 2000 to November 2018).

Results: A total of 84 potential articles were identified; 65 were redundant sources, and a total of 19 studies met the inclusion criteria. These studies consisted primarily of serial dilution, micro dilution, disk diffusion, minimum inhibitory concentration, minimum cytocidal concentration, high performance thin-layer chromatography or zone of inhibition methods.

Conclusion: There is preliminary evidence to suggest that *E. angustifolium* and related plant species may possess both gram positive and gram negative antibacterial properties that warrant further investigation. Specifically, dose-response studies are required as well as the need to isolate and identify the active biochemical compounds present in these plant species.

KEY WORDS: *Epilobium angustifolium*, Willow herb, antimicrobial resistance, plant-based antibiotics, antibiotic resistance.

Abstract word count: 239

INTRODUCTION

The emergence of new pathogens and the increase in the number of stains resistant to currently available antibiotics on the market on the market, is a major and growing public health concern globally [1,2,3]. The World Health Organization (WHO) defines antibiotic resistance (ABR) as a change in a microorganism that prevents antibiotics from inhibiting its growth and function [4]. In fact, as a result of ABR, 40 new diseases and microorganisms have come to existence in the past 30 years [5].

The contributing factors associated with the development of ABR include the over-prescription of antibiotics, lack of adherence to antibiotic treatment, over use of antibiotics in the food industry, hospital acquired infections, inappropriate prescription of antibiotics (e.g. for viruses), poor sanitation, lack of new antibiotic development and regulatory challenges[2, 6]. For example, it is estimated that approximately 50% of antibiotics in the United States alone are prescribed incorrectly or inappropriately [3, 7]. Similarly, nearly 23.6 million antibiotics were prescribed in 2013 in Canada alone, of which 50% were prescribed inappropriately [8]. The other major public health challenge entails the uneven supply and availability of new classes of antibiotics to combat drug resistant strains. A recent discovery of a new antibiotic called Teixobactin has demonstrated antibacterial properties targeting gram positive bacteria including *S. aureus* and *Mycobacterium tuberculosis* [9]. However, limited new classes of antibiotics have been researched and developed over the years recently [1, 10].

In 2013, CDC published a comprehensive analysis outlining the top 18 antibiotic resistant threats in the United States entitled "*Antibiotic Resistance Threats in the United States, 2013"* (AR Threats Report) [11]. This report highlighted the growing public health concern reporting that notably, 2 million individuals in the US develop an ABR infection, and at least 23,000 people die as a result[11]. It may be further argued that the loss of effective antibiotics will undermine our ability to fight infectious diseases and manage the resulting complications common in vulnerable patients such as those undergoing chemotherapy for cancer, dialysis for renal failure, organ transplants, and surgery for which the ability to treat secondary infections is crucial. Lastly, when first-line and second-line antibiotic treatment options are hampered by ABR, or are simply unavailable, healthcare professionals are forced to employ antibiotics that may be more toxic to the patient, and are frequently more expensive and less effective [2, 10, 11].

In response to the emerging public health crisis related to ABR in Canada and abroad, it

is critical that we identify potential new sources and classes of antibiotics, as well as evaluate their potential clinical uses. Herbal and plant based sources reported to possess antibacterial properties may provide a viable and cost-effective source for the development of new antibiotics such as the Willow herb plant (*E. angustifolium)* [12, 13]. Anecdotal evidence, for example, reveals that Willow herb has been used for centuries by First Nations people in Canada to prevent and treat wound infections [14]. Accordingly, a systematic review of the literature related to Willow herb and associated plant species was undertaken to identify potential new plant based extracts and compounds with potential antibiotic properties.

MATERIALS AND METHODS

A data extraction template was employed to locate peer-reviewed articles related to the Willow herb (*E. angustifolium)* and similar plant species. The template consisted of the following information: (i) author(s) name; (ii) year of publication and country of origin; (iii) study design and methods, and (iv) major results and outcomes. Peer-reviewed articles on Willow herb (*E. angustifolium)* and similar plant species that may possess potential antibacterial properties, were searched using the following three electronic databases: PubMed, Web of Science and Scopus. The PubMed database was searched from January 1992 to November 2018, the Web of Science database was searched from January 2008 to November 2018, and the Scopus database was searched from January 2000 to November 2018**. [Insert Figure 1 approximately here- Appendix A]**

Inclusion criteria consisted of all peer-reviewed published clinical and/or laboratory based studies published in English only. Editorials, letters to the editor, and/or other non-peer reviewed reports were excluded. Once sources were identified and located, their reference lists were also assessed for potential secondary sources not noted in the electronic databases searched. The literature search included the following key terms searched alone and in combinations: *Epilobium* antibiotic, *Epilobium* antibiotic AND plants, *Epilobium angustifolium* AND (antibacterial OR antimicrobial), *Epilobium angustifolium* OR *Epilobium* AND (antibiotic OR antimicrobial) AND (gram positive bacteria OR gram negative bacteria). The literature search process and search results are highlighted in the form of a flowchart show in Figure 1 (Appendix A). The evidence-based medicine (EBM) and evidence-based practice (EBP) strength-ranking hierarchy for published research articles, was used to rank the studies found in this review ranging from level 1- highest ranking, to level 7 – lowest ranking[1]. Level 1

includes systematic reviews of randomized control trials (RCTs), and nonrandomized control trials, while level 2 consists of single RCT and non-randomized trails [1]. Level 3 consists of systematic reviews of observational and/or correlational studies and level 4 includes single observational or correlational studies [1]. Level 5 comprises of systematic review of a physiological, descriptive or qualitative study while level 6 involves studies that are physiological, descriptive or qualitative in nature [1]. Level 7 ranking comprises of opinions by experts, panels or committees [1].

RESULTS AND DISCUSSION

A total of 84 potential articles were identified; 64 were redundant sources, and a total of 19 studies met the inclusion criteria. These studies consisted primarily of serial dilution, micro-dilution, disk diffusion, minimum inhibitory concentration, minimum cytocidal concentration, high performance thin-layer chromatography or zone of inhibition methods. **[Insert Table 1 approximately here -Appendix B]**

Among the 19 articles that met the inclusion criteria, 10 articles included the antimicrobial study of the *E. angustifolium* plant. 8 of these articles examined the antimicrobial properties of *E. angustifolium* as well as other plants on both gram positive, and gram negative microorganisms in their studies. Specifically, three studies [14, 22, 26] compared the antimicrobial properties of various *Epilobium* plant species, to some conventional commercial antibiotics. Of the 19 studies, three were carried out in Turkey, three in Romania, and three in the U.S.A. Two studies were carried out in Finland while one was carried out in each of Italy, India, Czech Republic, Bulgaria, South Africa, Canada, Croatia, and Russia. Geographical location, soil properties, climate and seasonal conditions could likely effect the chemical composition and concentration of active antimicrobial components, and thereby produce a variation in antimicrobial properties between the same species of plants.

Among the 19 articles, 9 were quantitative laboratory-based experimental studies with controls. These studies primarily consisted of experiments in cell cultures. In addition, a total of 8 comparative studies, and 2 review articles were included in the literature search. The plant extracts were investigated in dry, aqueous and/or ethanolic forms. Eleven studies used combinations of a serial dilution method, disk diffusion method, minimum inhibitory concentration, and zone of inhibition methods to measure the antimicrobial efficiency of various plant species [14-18, 20, 22, 25-29]. Two studies used the DPPH assay, ABTS assay [23, 24], while two

other studies used gas chromatography-mass spectroscopy [15, 20]. One study used biomonitoring assay for determining the impact on quorum sensing activity of bacteria [31]. The tested plant extracts including *E. angustifolium, Tanacetum balsamita, Quercus frainetto and Quercus robur,* demonstrated quorum sensing inhibition and a decrease in violacein production by the bacteria [31]. One study used high performance thin layer chromatography (HPLTC) [25] and another study used the MCC method of analysis [17].

Gram negative and positive bacteria

Various combinations of these gram positive and gram negative bacteria were used throughout the literature, as detailed in Table 2 below**.** The five most common gram positive bacteria used in the studies were *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*. Specifically, 14 studies used *S. aureus*, 5 studies used methicillin resistant *Staphylococcus aureus* (MRSA) and *B. subtilis*. 4 studies used *S. epidermidis,* and 4 studies used *E. faecalis* to investigate antibacterial effects of various plant extracts. The three most common gram negative bacteria used in the studies were *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. A total of 14 studies used *E. coli*, 9 studies used *Pseudomonas aeruginosa* and 5 studies investigated the antimicrobial properties of plant extracts on *K. pneumonia*. The two most common fungi used in the studies were *Candida albicans* and *Saccharomyces cerevisiae*. A total of 8 studies investigated antifungal activity of plant extracts against *C. albicans*, while 3 studies investigated the antimicrobial properties of plant extracts tested on *S. cerevisiae*. **[Insert Table 2 approximately here- Appendix C].**

The microorganisms that were most susceptible to the plant extracts investigated were *S. aureus*, MRSA, *B. subtilis*, *E. coli*, *P. aeruginosa* and *C. albicans.* In particular, *E. angustifolium* inhibited the broadest range of microorganisms [17]. *E. angustifolium* extracts inhibited the growth of *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans,* suggesting a broad range of activity [16, 28]. Additionally, *E. angustifolium,* when prepared in a honey mixture form, inhibited the growth of *S. pyogenes*, *S. aureus* and MRSA [18]. One study concluded that the *E. angustifolium* extract inhibited growth of *Micrococcus luteus* and *S. aureus* more than that of *E. coli* and *P. aeruginosa* [14]. Overall, *E. angustifolium* plant extracts demonstrated stronger inhibition of gram positive bacteria in comparison to gram negative bacteria.

It was also observed that the *Epilobium* extracts demonstrated greater inhibitory effects

on bacteria compared to their effects on fungi/yeasts. Interestingly, *C. albicans* was reported to be most resistant to the investigated extracts [22]. *E. parviflorium* had greater antimicrobial activity on gram negative bacteria and yeast, compared to gram positive bacteria [15]. One study demonstrated that *E. montanum* demonstrated antimicrobial activity against all tested microorganisms except *E. coli,* [20]. Specifically, an experimental study demonstrated that *E. angustifolium* inhibited gram negative bacteria, such as *E. coli* ($p<0.001$) and *P. aeruginosa* ($p<0.001$) more effectively than gram positive bacteria such as *M. luteus* ($p<0.01$) and *S. aureus* ($p<0.05$) [14]. Additionally, it was found that *E. angustifolium* and *E. rosmarinfolium* exhibited the broadest range of inhibitory activity among all *Epilobium* plant extracts investigated including *E. anustifolium*, *E. hirsutum*, *E. palustre*, *E. tetragonum* and *E. rosmarinifolium*, with an MIC range of 162-325 µg/mL [17].

***Epilobium* plant extracts**

There are many different variations and species of *Epilobium* plants that have been employed to date. Collectively, these investigations suggest that several species within the genus *Epilobium* possess AB properties against both gram positive and gram negative bacteria. For example, *E. hirsutum* extracts demonstrated a larger zone of inhibition against several bacteria types compared to Gentamycin, Nystatin and dimethyl sulfoxide (DMSO) solvent controls [22]. Promising antimicrobial plant species include *E. angustifolium*[13, 14, 16-18, 21, 24, 28, 29, 31], *E. hirsutum* [17, 19, 22, 23, 25-27], *E. parviflorum* [15, 30], *Epilobium romarinfolium* [17], *Epilobium montanum* [20], *Epilobium palustre* [17], and *Epilobium tetragonum* [17]. A total of 10 articles investigated the antimicrobial properties of *E. angustifolium*, 7 articles investigated *E. hirsutum*, while 2 articles investigated the activity of *E. parviflorum*. One article compared the activity of preparations from *Epilobium romarinfolium*, *Epilobium montanum*, *Epilobium palustre* and *Epilobium tetragonum* plants.

While many different studies used similar *Epilobium* plant species, the difference in geographical location in which the plants were grown, variations in extract preparation including seasonal conditions and methods of collection, as well as variations in experimental procedures and analysis, are factors likely affecting the demonstrated antibacterial properties of these plants. To illustrate this point, *E. angustifolium* can be taken as an example. In one study *E. angustifolium* plants originating from Minnesota and Wisconsin were prepared using the stem, leaves, flowers and roots in an ethanolic extract form, and analyzed via the zone of inhibition

method [16]. By comparison, another study used *E. angustifolium* from Mt. Velebit in Croatia, tested preparations of ethanolic extracts of flowers and leaves only, and analyzed these using diffusion and micro dilution assays [21]. While both studies use the same species of *Epilobium*, the latter study demonstrates AB properties against a greater range of microorganisms, whereas the extracts tested in the former study had few or no AB properties. Hence, the sourcing of these compounds is a critical factor to consider and evaluate when comparing studies. While this literature search primarily focused on the species of the genus *Epilobium*, many other effective antibacterial plant extracts have also been described in the literature (see Table 1).

Plant region and antimicrobial properties

In a study examining antimicrobial activity against a range of bacteria and fungi, there was no significant difference between the ethanolic extracts of leaves versus flowers of *E. angustifolium* in terms of AB properties [21]. However, some studies found that the flower part of the various plants were more effective than the stem and leaf parts of the plants on *Staph. epidermis, S. aureus, Shigella ssp.* and *E. coli* [22]. Specifically, a study in the Journal of Medicinal Plants Research tested antimicrobial activity against *Staph. aureus, E. coli, P. aeruginosa* and *C. albicans* demonstrated that the inhibition zone produced by the *E. angustifolium* leaf extract was 7-15 mm and the inhibition zone of the flowering aerial extract was 7-20 mm [16]. These findings suggest that different plant parts (e.g. roots, stems, flowers) need to be investigated alone and in combination to better elucidate their AB properties.

Synergistic effects

While some studies demonstrated the antibacterial properties of a number of different plants, a total of only three studies described synergistic effects of different plants and conventional antibiotics used together [26, 27, 29]. It was found that when *Epilobi Hirsuti* Herba (EHE) extract was used in combination with tetracycline and/or ampicillin, a greater inhibitory effect -was measured on *P. aeruginosa* and *E. coli*, compared to the antimicrobial effects of tetracycline alone [26]. Specifically, tetracycline in combination with EHE demonstrated a 19%-55% increase, while ampicillin in combination with EHE produced a 16-61% increase in growth inhibition, antimicrobial activity, in contrast to the use of antibiotics alone [26]. Interestingly, combining *E. hirutum* extracts and ampicillin and/or tetracycline appeared to produce greater inhibitory effects, in comparison to single administration of these agents. Similarly, extracts of *E. hirutum, E. angustifolium, Filipendula vulgaris, Tanacetum vulgare* and *Serratula coronate*

plants also demonstrated synergistic effects in combination with antibiotics in E. coli [29]. These effects should be investigated further to determine the optimal combination of plant extracts and conventional antibiotics and to analyze synergistic mechanisms of antimicrobial activity.

DISCUSSION

It may be argued that the antibiotic resistance crisis is global in nature, reflecting the worldwide overuse of these drugs and the lack of development of new antibiotic agents by pharmaceutical companies to address this growing and costly public health issue. Antibiotic resistant infections place a substantial health and economic burden on the health care system and population. Additionally, large pharmaceutical companies are no longer investing in the research and development of new antibiotics, due to the low return on investment in the field of antibiotics [32]. To address limited treatment options to control multi-drug resistant diseases (MDRD) around the world [33], new potential sources of antibiotics need to be identified and investigated. If new classes of antibiotics are not discovered, antibiotic resistance will continue to rise and lead to a public health catastrophe [34].

In the past, certain plants have been characterized for their antibacterial properties; notably, the willow herb plant (*E. angustifolium*) has been employed for its medicinal characteristics by First Nations people and others [1]. This systematic review examined the published literature around AB properties, effects and efficacy of *E. angustifolium* as well as other *Epilobium* species. Current evidence indicates that the antimicrobial properties of *E. angustifolim* are largely due to the presence of polyphenols such as oenothein B, which are responsible for the antibacterial properties of this plant [13, 19, 24, 29]. It has been suggested that *E. angustifolium* can be used to treat many infections or diseases, such as benign prostatic hyperplasia [21] and diabetic foot syndrome [23]. However, further research needs to take place before any firm conclusions can be made. Other promising plant species described in the literature included *Betula papyrifera, Centaurea maculosa, Hypericum perforatum, Lythrum salicaria,* and *Rhus glabra* among others. Most of the literature consisted of experimental *in vitro* (i.e. cell culture) studies conducted in a range of countries. These plant extracts were tested on various gram positive and gram negative bacteria for measurement of their antibacterial and inhibitory effects. The methods of analysis included serial dilution, diffusion, minimum inhibitory concentration, thin layer chromatography or zone of inhibition methods. Additionally, to determine the chemical composition of the plants, gas chromatography and mass spectroscopy

methods were employed. One study used a biomonitoring assay to determine the effects of the plant extracts on the quorum sensing activity of *Chromobacterium violaceum* gram negative bacteria [31].

It was reported that in most cases, *Epilobium* extracts demonstrated greater inhibitory effects on gram positive bacteria when compared to gram negative bacteria and yeasts/fungi [14, 15, 20, 22]. According to the 19 studies, the most microorganisms most susceptible to inhibition by the plant extracts were S. aureus, MRSA, *Bacillus subtilis, E. coli, P. aeruginosa* and the yeast *C. albicans.* Based on the literature reviewed, the most effective antimicrobial plant species were determined to be *E. angustifolium, E. hirsutum* and *E. parviflorum*. Additionally, it was found that *E. angustifolium* and *E. rosmarinfolium* exhibited the broadest range of growth inhibition among all other plant extracts, with an MIC range of 81-650 µg/mL when tested against bacteria, yeasts and fungi [17]. While a study suggested that there was no significant difference between the leaf and flower extracts of *E. angustifolium* species, it is important to note that some studies found that the flower region of the plant produced greater antimicrobial effect through a larger zone of inhibition than the leaf region [16]. One study demonstrated bacterial protective properties of some plant extracts including *E. angustifolium* such that the antibacterial effect of the ciprofloxacin antibiotic was significantly reduced [29]. It is therefore also important to screen plant extracts for the potential to impeded antibiotic efficacy. Synergistic effects of various plant species and conventional antibiotics were also noted in only a limited number of studies. It was found that EHE, *E. hirutum* and *E. angustifolium* demonstrated synergistic effects when combined with standard antibiotics. EHE and *E. hirutum* in combination with tetracycline or ampicillin antibiotic produced a greater antimicrobial effect compared to the antimicrobial effect with the antibiotic alone [26, 27]. These synergistic effects warrant further investigation.

CONCLUSION

In conclusion, there is preliminary evidence to suggest that plant-based extracts from *E. angustifolium* may possess both AB properties against both gram positive and gram negative bacteria. Further research is warranted to elucidate which part of these plant species (e.g. roots, stem, and leaves) actually possess these AB properties. Lastly, the synergistic effects of combining conventional antibiotics (e.g. penicillin) with these plant extracts need to be investigated.

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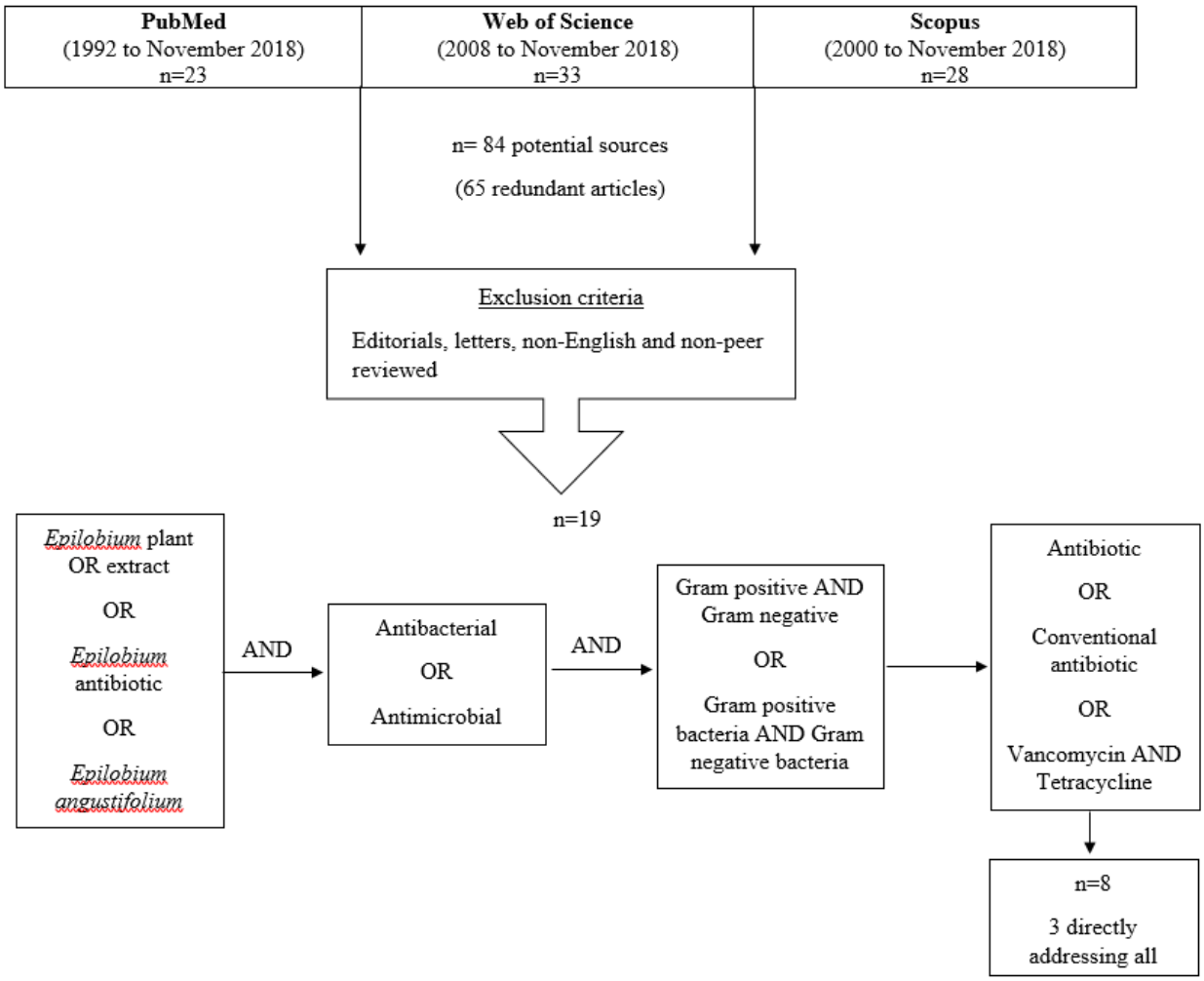
APPENDIX A

Figure 1: Systematic Review Search Results



APPENDIX B

Table 1: Key outcomes for articles identified.

Author(s), & Year	Country of origin	Study Design/Methodology	Ranking	Major Findings and Outcomes
Bajer et al. 2017	Czech Republic	-Comparative research study - *E. parviflorum* oil prepared using dry leaves and stems by a hydro-distillation process. -Antibacterial activity of the oil and aromatic water was measured using a micro dilution method on S. *aureus, E. faecalis, E. coli, P. aeruginosa* and *C. albicans.* -GC-MS analysis used to determine the major constituents of the oil and aromatic water.	Level 4	-Essential oil inhibited growth of all tested bacteria: MIC (%) values between 10-40 µg mL^{-1} - Yeast MIC (%) value of 5 mg mL^{-1}. - Distilled aromatic water has greater antimicrobial activity on Gram negative bacteria and yeast compared to Gram positive bacteria types. -Major constituents of the oil were palmitic acid (30.8%), α-linolenic acid (10.8%) and linoleic acid (12.5%), while the aromatic water had monoterpenoids (38.9%).
Borchardt et al. 2008	USA	- Experimental study with controls - Antimicrobial activity of 597 aqueous ethanol extracts from the stems, leaves, flowers and roots of several different plants from Minnesota and Wisconsin (USA). -Plant samples were immediately frozen upon sample collection and extracts tested within 48 hours. - Extracts tested for antimicrobial activity against *S. aureus, E. coli, P. aeruginosa and C. albicans*. -Negative control: evaporated solvent -Positive control (antibiotic): ticarcillin 75 mcg, or chloramphenicol 30 mcg - Disk diffusion technique used to measure the	Level 4	- 142 extracts demonstrated AB activity - Plant extracts demonstrating antimicrobial activity against all four microorganisms tested included *E. angustifolium, B. papyrifera, C. maculosa, H. perforatum, L. salicaria,* and *R. glabra*. - *E. angustifolium L.* extracts demonstrated inhibition zones of 6-18 mm (against the 4 microorganisms tested) - *E. angustifolium L.* leaf extract inhibition zones: 7-15 mm, and flowering aerial extract inhibition zones: 7-20 mm.

		zone of inhibition for each extract.		
Bartfay, Bartfay, Johnson. 2012.	Canada	-Comparative, quantitative experimental study with controls conducted in cell cultures. - Study of antimicrobial proprieties of *E. angustifolium* whole plant extract on microorganisms. -Extracts tested on *S. aureus, M. luteus, E. coli, P.aeruginosa*. -Negative controls: Media only, and combination of media and bacteria. -Positive controls: Vancomycin and Tetracycline. -Antimicrobial activity measured using the MIC approach.	Level 4	-*E. angustifolium* demonstrates antibacterial properties on both gram positive and gram negative bacteria. - *E. angustifolium* inhibited *M. luteus* ($p < .01$) *S. aureus* ($p < .05$) *E. coli* ($p < .001$) *P.aeruginosa* ($p < .001$) -*E. angustifolium* was more effective in inhibiting growth of bacteria upon comparison to vancomycin ($p < .05$) and tetracycline ($p < .004$).
Battinelli et al. 2001	Italy	-Comparative study design -Experimental in-vitro study of ethanolic extracts of various *Epilobium* species to measure antimicrobial activity and cytotoxicity. -Extracts of *E. angustifolium*, *E. hirsutum*, *E. palustre*, *E. tetragonum* and *E. rosmarinifolium* used. -Extracts tested for antimicrobial activity against *S. aureus, S. pyogenes, S. sanguis, B. subtilis, E. faecalis, L. monocytogenes, E. coli, K. pneumonia, P. aeruginosa, S. flexneri and S. enteritidis*, yeasts and fungi. -Standard antibiotic used for comparison of MIC and MCC values with *Epilobium* plants. -Antibiotics: Tetracycline used for bacteria and miconazole used for yeasts, fungi. - Antimicrobial activity evaluated by MIC and	Level 4	-Overall, all extracts possessed antimicrobial activity with MIC range of 10-650 µg/mL. -All *Epilobium* plant extracts demonstrated antimicrobial activity against gram positive and gram negative bacteria; MIC range of 81-650 µg/mL. -Only *E. angustifolium* and *E. rosmarinfolium* had broadest inhibition of yeasts; MIC range of 162-325 µg/mL - *E. angustifolium* and *E. rosmarinfolium* exhibited broadest range of inhibition among all other plant extracts. -All *Epilobium* plant extracts inhibited fungi; MIC range of 81-650 µg/mL.

		MCC. -Cytotoxicity evaluated using *Artemia salina* test.		
Canli et al. 2017	Turkey	- Experimental study with control. - Negative control: sterile disks and ethanol solvent. - Testing antimicrobial activity of *E. montanum* samples on *Bacillus, Enterobacter, Enterococcus, Escherichia, Klebsiella, Listeria, Pseudomonas, Salmonella, Staphylococcus* and *Candida* spp. - Antimicrobial activity analyzed using disk diffusion method. - Chemical composition of *E. montanum* analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS).	Level 4	- *E. montanum* demonstrated antimicrobial activity against all tested microorganisms except *E. coli*. -p value of 0.05 used to determine significance. -Compared to gram negative bacteria, gram positive bacteria types demonstrate less resistance against aromatic plants as indicated by larger zones of inhibition. -GC-MS revealed 17 major chemical components in *E. montanum*.
Huttunen et al. 2013	Finland	- Comparative, quantitative study design - Testing antimicrobial activity and phenolic makeup of five Finnish honeys, at various concentrations, on gram positive bacteria: *S. pyogenes, S. pneumoniae, S. aureus,* MRSA. -Antimicrobial testing was conducted using the micro-broth dilution assay method.	Level 4	- Willow herb (*E. angustifolium*), heather (*Calluna vulgaris*), and buckwheat (*Fagopyrum esculentum*) honeys yielded significant antimicrobial activity with $p < 0.01$ - Significant antimicrobial activity against all gram positive bacteria tested.
Ivancheva et al. 1992	Bulgaria	- Comparative study of the anti-infectious activity of 3 polyphenolic Bulgarian plants -Testing the aqueous and ethanolic extracts against various microorganisms for their anti-infectious activities. - In vitro, in ovo and in vivo testing of the polyphenolic mixture of *E. hirsutum* reproduction of influenza virus was	Level 4	- Water and alcohol extracts of *E. hirsutum* demonstrated inhibitory effect on influenza virus - Only study that demonstrated antiviral activity of *Epilobium* plant.

		determined. -Microorganisms include: *K. pneumoniae, Proteus vulgaris, E. coli, P. aeruginosa, S. aureus, and C. albicans* -These organisms were studied for in vitro growth inhibition. -G*eranium macrorrhizum*, *G. sanguineum*, and *Epilobium hirsutum* plants were chemically analyzed.		
Kadam, Patil, Yadav. 2018	India	- Review article -Focus on *E. angustifolium* L. properties, uses and chemical composition of this plant. -Information collected through Medline, PubMed, Science Direct, journals, books and reports.	Level 5	- *E. angustifolium* plant part of the Onagraceae family of plants - *E. angustifolium* possesses high levels of active polyphenols and oenothein B which hold its antibacterial properties. *E. angustifolium* demonstrated antibacterial, anti-inflammatory, antioxidant and anti-aging properties, suggesting it has potential to be used in treatment of many diseases.
Kosalec, Kopjar, Kremer. 2013	Croatia	-Comparative quantitative study -*in vitro* comparison of the antimicrobial activity of the flowers and leaves of *E. angustifolium* from Mt. Velebit, Crotia, on various microorganisms: - *S. aureus*, MRSA *B. subtilis*, *E. coli, P. aeruginosa*, *Proteus mirabilis*, *C. albicans*, *Candida tropicalis*, *Candida dubliniensis* and *Saccharomyces cerevisiae.* -Antimicrobial activity measured using broth micro dilution assay. -Viability of *C. albicans* after exposure to	Level 4	-*E. angustifolium* demonstrates growth inhibitory activity on all tested microorganisms with MIC values between 4.6±0.2 and 18.2±0.8 mg/mL. - No significant difference between ethanol extracts of leaves and flowers of *E. angustifolium* was demonstrated.

		willow herb was analyzed using quantitative fluorescent assay.		
Kunduhoglu, Pilatin, Caliskan. 2011	Turkey	- Comparative, experimental study with controls - Antimicrobial activity of 178 crude extracts from 22 different Turkish medicinal plants -Extracts prepared in solvents such as ethanol, acetone and ether. - Antibiotic controls: Gentamycin, Nystatin. -Microorganism include: *B. subtilis*, *Bacillus cereus*, *S. aureus*, *S. epidermidis*, *Sarcina lutea*, *E. coli*, *E. aerogenes*, *Shigella ssp*., *Salmonella typhimurium*, *C. albicans*, *Pichia membranifaciens*, *S. cerevisiae*, *Shizosaccharomyces pombe*, *Zygosaccharomyces rouxii*. -Antimicrobial activity measured using disk diffusion method.	Level 4	-All tested medicinal plants demonstrated antimicrobial effects -Inhibition zones ranged between 7-30 mm -Plants that demonstrated greatest antimicrobial range on all microorganisms include: *E. hirsutum, T. sibthorpii. H. confertum subsp. confertum, G. verum subsp. verum, A. tinctoria, M. pulegium, I. montbretiana, P. peregrina, T. longicaulis* and *C. creticus1 and 2*. -These plants demonstrated a larger zone of inhibition compared to the control group. -The most susceptible microorganisms included: *S. epidermis, S. aureus, Shigella* ssp and *E. coli*, -The most resistant microorganism was *C. albicans.* - Ethanol was found to be the most effective solvent. - In contrast to the leave and stem, the flower parts of the plants were found to be more effective.
Kustova et al. 2014	USA	- Correlational, comparative study - Study of antimicrobial activity of crude extracts from plants in Kazakhstan. - Extracts prepared in solvents such as dichloromethane and ethanol.	Level 4	-Extracts demonstrating antifungal activity against *Candida glabrata* in extracts where IC_{50} less than 3µg/ml: *E. hirsutum, Rhodiola quadrifida, Rumex confertus, Glycyrrhiza Uralensis*

		- Antimicrobial activity measured using a version of CLSI/NCCLS methods - Microorganisms tested include *Candida glabrata*, *S. aureus*, and MRSA. - Antioxidant properties of extracts analyzed using the DPPH and ABTS assay.		- Extracts demonstrating antifungal activity against *S. auerus*: *Rumex confertus, Glycyrrhiza Uralensis and Vexibia alopecuroides.* - Extracts displaying ABTS antioxidant activity include: 6.6µg/ml *E. hirsutum*, 4.5µg/ml *Rumex confertus*, 3.8µg/ml *Rhodiola quadrifida*, 5.7µg/ml *Glycyrrhiza Uralensis.* - Extracts with high antioxidant acitivity greater than 85% inhibition of DPPH include: *E. hirsutum, Rumex confertus*
Pirvu et al. 2014	Romania	-Comparative quantitative research study -Determine antioxidant, antimicrobial activity of 8 whole plant species from Romania -response measured on whole vegetal extracts and aqueous, ethyl acetate and chloroform fractions made from the 8 plant species. - Antioxidant properties analyzed using DPPH method -Antimicrobial properties analyzed using diffusion method on microorganisms. -Microorganisms included: *S. aureus, E. coli*, and *C. albicans.*	Level 4	- Plant whole extracts displaying antimicrobial properties against *S. aureus, E. coli:* greater burdock leaves (*Arctium lappa*), beech leaves (*Fagus sylvatica*) and great willow herb aerial part (*Epilobium hirsutum*) - Plant whole extracts displaying weak antimicrobial properties against *S. aureus, E. coli:* Purple loosestrife aerial part (*Lythrum salicaria)* and sea-buckthorn leaves (*Hippophae rhamnoides).* - Plant whole extracts displaying no antimicrobial properties against *S. aureus, E. coli:* tarragon aerial part (*Artemisia dracunculoides)*, chokeberries leaves (*Aronia melanocarpa*) and quince fruit (*Cydonia oblonga*). - No antimicrobial properties demonstrated on *C. albicans* by any tested plant extract

				- Results indicative of possible synergistic effects between polyphenols, and glycosyl chain that effect antimicrobial properties.
Pirvu et al. 2016	Romania	- Comparative *in vitro* experimental study with controls: Negative control to measure antimicrobial properties: Muller-Hinton Agar (MHA) + propylene glycol PG 20% (4.1 mL + 0.9 mL) - Control to measure synergism: MHA without vegetal extract - Study of the antimicrobial activity of *Epilobi Hirsuti* Herba (EHE) extracts on microorganisms and its effects on common antibiotics. - Microorganisms include: *S. aureus* ATCC 6538, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *S. epidermidis* ATC12228, *P. aeruginosa ATCC 27853*, *Proteus mirabilis ATCC 29245*, *E. coli ATCC 35218*, *E. coli ATCC 11229*, *E. coli ATCC 8739*. - Antibiotics include: Ampicillin (10 µg), Gentamicin (10 µg), Tetracycline (30 µg), Sulfamethoxazole/ Trimethoprim (25 µg), Ciprofloxacin (5 µg), Cefoxitine (30 µg). - Antimicrobial activity measured with MIC values.	Level 4	- EHE greatly inhibited growth of S. *epidermidis* MIC value 50-200 µg GAE mL^{-1}. - *E. coli* and *E. faecalis* ATCC 29212 were resistant to EHE. - Tetracycline and Ampicillin combinations produced inhibition zones 60% larger than antibiotic alone. - Tetracycline in combination with EHE produced 19-55% greater zone of inhibition, in contrast to tetracycline alone. - Ampicillin in combination with EHE produced 16-61% greater zone of inhibition, in contrast to ampicillin alone. - Study demonstrates combinatory influence of EHE and antibiotics on overall antimicrobial activity.
Pirvu et al. 2014	Romania	- Comparative experimental with negative control for antimicrobial assay: propylene glycol PG (20%) - Study to test antimicrobial and synergistic properties of the aerial whole part of *E. hirutum L.* on *S. aureus* and MRSA	Level 4	- All 4 tested *E. hirsutum* extracts (whole, aqueous, ethyl acetic, and chloroformic fraction) demonstrated inhibitory activity against *S. aureus* and MRSA - *E. hirsutum* demonstrated the greatest antimicrobial effect in whole extract and

		- Synergism tested with antibiotics: Gentamicin, Tetracycline, Ampicillin, Nalidixic acid and Ciprofloxacin.		selective aqueous fraction form. -Combinatory antimicrobial effects of *E. hirsutum* and ampicillin or tetracycline produced greater antimicrobial effects (p=0.04). - Reduced antibacterial effects noted when *E. hirsutum* combined with gentamicin, nalidixic acid or ciprofloxacin.
Rauha et al. 2000	Finland	-Comparative study design -Study of the antimicrobial properties of 13 phenolic substances 29 extracts of Finnish plants -Antimicrobial properties analyzed using diffusion methods on various microorganisms -Microorganisms include: *Aspergillus niger, B. subtilis, C. albicans, E. coli, M. luteus, P. aeruginosa, S. cerevisiae, S. aureus and S. epidermidis.*	Level 4	- The compounds found to be effective in inhibiting microorganism growth: Flavone, quercetin and naringenin. -Plants active against bacteria: *E/ angustifolium L., Filipendula ulmaria L. Maxim, Rubus chamaemorus L., Rubus idaeus L.* -Plant active against *C. albicans*: *Lythrum salicaria* - Plants active against *Staphylococcus aureus*: *Betula pubescens Ehrh., Pinus sylvestris L., Solanum tuberosum L.*
Schepetkin et al. 2016	USA	-Exploratory, review study -Review article studying the active components (e.g. polyphenols) of *E. angustifolium.* - Article highlights properties and clinical usefulness of polyphenols of *E. angustifolium.*	Level 6	-Studies demonstrate that *E. angustifolium* demonstrates antibacterial, anti-proliferative, antioxidant, anti-inflammatory and anti-aging properties - Plant contains active polyphenols and flavonoids such as oenothein B -Active polyphenols are responsible for the therapeutic properties of *E. angustifolium.* -The signal pathways induced by the active polyphenols and the clinical safety of *E. angustifolium* need to be studied in the future.
Smirnova et al.	Russia	- Comparative, experimental study with controls	Level 4	- Tested plant extracts demonstrate potential

2012		- Positive control: Antibiotic - Negative control: Extract -Antibiotics studied: Ciprofloxacin, Kanamycin, Chloramphenicol, Rifampicin, and Ampicillin. - Study to determine how the presence of polyphenols or various plant extracts influence antibiotic activity against E. *coli*. - Measured using MIC.		to reduce susceptibility of *E. coli* to antibiotics. - The polyphenols or plant extracts in tested combination with various antibiotics suggest a protective effect on *E. coli.* - Plant extracts displaying high protective effect: *Chamerion (Epilobium) angustifolium, Filipendula vulgaris, Tanacetum vulgare and Serratula coronata.* -Antibacterial effect of ciprofloxacin was significantly reduced. - Ciprofloxacin MIC increased by four times with the tested extracts. -Positive correlation between antioxidant activity of polyphenols/plant extracts and their protective effects. - High levels of antioxidant activity found in *Chamerion (Epilobium) angustifolium, Filipendula vulgaris, and Serratula coronata.*
Steenkamp et al. 2006	South Africa	-Comparative, experimental study with controls -Positive control: ciprofloxacin -Negative control: Broth without plant extract -Microorganisms studied: *E. coli* - Investigated antibacterial activity, hydroxyl scavenging activity and COX-1 and COX-2 catalysed prostaglandin biosynthesis of 5 herbal remedies for treatment of Benign prostatic hyperplasia (BHP)	Level 4	- Growth of *E. coli* was inhibited in the crude water and ethanolic plant extracts from *Hypoxis hemerocallidea* and *E. parviflorum* - All extracts demonstrated hydroxyl scavenging activity. -Ethanolic plant extracts most effective at inhibiting COX-1 prostaglandin biosynthesis. -Ethanolic extracts of *E. parviflorum* inhibited COX-1, COX-2 prostaglandin biosynthesis, inhibited growth of *E. coli*,

		- Study uses crude water and ethanolic extracts -Micro-well dilution method was used to assess antibacterial activity. -Visual scoring (0-3) of the color intensity relative to controls was used to measure bacterial growth. Visual scoring was made possible after reaction with 50µL of p-iodonitrotetrazolium violet.		demonstrated antioxidant properties.
Yuzbasioglu et al. 2017	Turkey	-Experimental, quantitative study with controls -Study of effects on bacterial growth, and inhibition/induction of Quorum sensing using a biomonitoring assay. -Testing the activity of 36 extracts from 26 Turkish plant species on *Chromobacterium violaceum (*a QS indicator strain)	Level 4	-Plant extracts that demonstrated QS inhibition and a decrease in violacein production by *Chromobacterium violaceum*: *E. angustifolium, Tanacetum balsamita, Quercus frainetto* and *Quercus robur* - *E. angustifolium* decreased violacein production by 41% at 250µg/mL, $p < 0.01$ - *E. angustifolium* decreased violacein production by 57% 500µg/mL, $p < 0.001$

Abbreviations used in Table 1: MIC = Minimum inhibitory concentration (%), MCC= Minimum cytocidal concentration, GC-MS = Gas chromatography-Mass Spectrometry, DMSO = dimethylsulfoxide, CLSI/NCCLS = Clinical & Laboratory Standard Institute, Guidelines, DPPH= 2, 2-diphenyl-1-picrylhydrazyl, ABTS= 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), HPTLC = high-performance thin-layer-chromatography, MHA = Muller-Hinton Agar, BHP = Benign prostatic hyperplasia, COX-1 and COX-2 = Cyclooxygenase-1 and -2, QS = Quorum sensing, AB = Antibacterial

APPENDIX C

Table 2: List of gram positive bacteria, gram-negative bacteria, and fungus/yeast investigated.

Gram positive	**Count (n)**	**Gram negative**	**Count (n)**	**Fungus/Yeast**	**Count (n)**
Aspergillus niger	1	*Chromobacterium violaceum*	1	*Candida albicans*	8
Bacillus cereus	1	*Escherichia coli*	14	*Candida dibliniensis*	1
Bacillus subtilis	5	*Klebsiella pneumonia*	5	*Candida glabrata*	1
Enterococcus durans	1	*Proteus mirabilis*	2	*Candida tropicalis*	1
Enterococcus faecalis	4	*Proteus vulgaris*	1	*Pichia membranifaciens*	1
Enterococcus faecium	1	*Pseudomonas aeruginosa*	9	*Saccharomyces cerevisiae*	3
Listeria monocytogenes	3	*Pseudomonas fluorescens*	1	*Schizosaccharomyces pombe*	1
Micrococcus luteus	2	*Salmonella enteritidis*	3	*Zygosaccharomyces rouxii*	1
Methicillin Resistant Staphylococcus aureus (MRSA)	5	*Shigella flexneri*	2		
Staphylococcus aureus	14	*Salmonella infantis*	1		
Sarcina lutea	1	*Salmonella kentucky*	1		
Staphylococcus epidermidis	4	*Salmonella typhimurium*	2		
Streptococcus pneumoniae	1				
Streptococcus pyogenes	2				
Streptococcus sanguis	1				

Note: The count (n) is the total number of studies that have investigated this microorganism from the 19 identified sources.