# Antimicrobial assays of three native British plants used in Anglo-Saxon medicine for wound healing formulations in 10th century England

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Abstract

*Ethnopharmacological relevance*: Three important Anglo-Saxon medical texts from the 10th century contain herbal formulations for over 250 plant species, many of which have yet to be evaluated for their phytochemical and/or pharmacological properties. In this study, three native British plants were selected to determine antimicrobial activity relevant to treating bacterial infections and wounds.

*Materials and methods*: Several preparations of *Agrimonia eupatoria* L., *Arctium minus* (Hill) Bernh. and *Potentilla reptans* L. were screened for antimicrobial activity against selected Gram-positive and Gram-negative bacteria of relevance in wounds using a 96 well plate microdilution method (200, 40 and 8 µg/mL). Minimum inhibitory concentration (MIC) values were determined for the most potent extracts from 2-0.004 mg/mL and HPLC chromatograms examined by multivariate analysis. Principle components analysis (PCA) was used to identify chemical differences between antimicrobial activity of the crude extracts.

*Results*: The HPLC-PCA score plots attributed HPLC peaks to the antimicrobial activity with all three plants inhibiting growth of Gram-positive *Staphylococcus aureus* by >50% in four or more extracts. The first two principal components (PC) represented 87% of the dataset variance. The *P. reptans* 75% ethanol root extract exhibited the greatest range of activity with $MIC_{50}$ at 31.25 µg/mL to a total MIC that was also the minimum bactericidal concentration (MBC) at 1 mg/mL. Additionally, the root of *P. reptans* inhibited growth of Gram-negative bacteria with the 75% ethanol extract having a $MIC_{50}$ at 1 mg/mL against *Pseudomonas aeruginosa* and the decoction a $MIC_{50}$ at 3.9 µg/mL against *Escherichia coli*.

*Conclusions*: The results indicate a moderate antimicrobial activity against common wound pathogens for *P.reptans* suggesting it may well have been effective for treating wound and bacterial infections. Anglo-Saxon literary heritage may provide a credible basis for researching new antimicrobial formulations. Our approach encompassing advanced analytical technologies and chemometric models paves the way for systematic investigation of Anglo-Saxon medical literature for further therapeutic indications to uncover knowledge of native British plants, some of which are currently lost to modern Western herbal medicine.

## 1. **Introduction**

Three important Anglo-Saxon medical texts based on herbal formulations were compiled in England during the 10$^{th}$ century. The *Old English herbarium,* a classical Latin text (4-5$^{th}$ century) containing many 'simples' or single formulations was reordered and translated into Old English. Bald's *Leechbook* and the *Lacnunga*, by contrast, were written in the vernacular with no known Latin variants and include formulations from a variety of sources including Latin and Greek; Old Irish and Aramaic (Voigts, 1979; Pettit, 2001 and Cameron, 2006). Bald's *Leechbook* combines the best of classical and indigenous teachings into a coherent text suitable for attending the royal household whereas the *Lacnunga* is considered to be a personal collection of formulations used by a lay practitioner. (Talbot,1967; Pettit, 2001, Pollington, 2008 and Meaney, 1984). Later European herbals from 16$^{th}$ and 17$^{th}$ centuries have been reviewed for rheumatism treatments (Adams et al., 2009) with reported activities that seem to support at least some of these historical formulations that have been handed down through the centuries.

Despite a rich history of medicinal plant use throughout the British Isles, much of the native flora listed in the Anglo-Saxon medicinal literature has yet to be evaluated for pharmacological and medicinal applications (Watkins et al., 2011). Following Cockayne's translation of all the major Anglo-Saxon medical literature (1864-1865) many of the formulations were dismissed by medical scholars as having 'little or no value to medical understanding' (Cameron, 2006). There have been a number of calls to research the ancient medical literature (Riddle, 1985; Holland,1996 and Buenz et al., 2004) but to date little has resulted in scientific investigation of the Anglo-Saxon herbal texts. Cameron (2006, 2008) sought to review the scientific literature in support of his hypothesis that the Anglo-Saxons were more advanced in their medical treatments than previously thought. However, no primary experimental data was provided by Cameron and more recent examination of the historical medical texts has fallen short of scientific scrutiny in a laboratory setting (Buenz et al., 2004, Thomas, 2011). Brennessel et al. (2005) reported poor antimicrobial activity for selected Anglo-Saxon medicinal preparations however, the report does not provide all the concentrations at which these were tested and full experimental methods and data have not since been published. The objective of this study is to experimentally determine the *in vitro* antimicrobial activity of selected plants used by the Anglo-Saxon herbal practitioner to treat wounds and bacterial infections.

## 2. Materials and Methods

### *2.1. Anglo-Saxon Medical Texts*

An initial search of the Anglo-Saxon medical literature was conducted via the British Library online catalogue using keywords Anglo-Saxon medicine (29 hits), Anglo-Saxon herbals (4) and Anglo-Saxon medical texts (6) in order to find modern

English translations of the surviving Anglo-Saxon medical literature. Further searches using the same keywords were undertaken in the academic journal databases JSTOR, Science Direct and ISIS Web of Knowledge. The *Old English herbarium* was chosen as the initial data source and three translations consulted: *Leechdoms, wortcunning, and starcraft of early England* (Cockayne, 1864); *Medieval herbal remedies: the Old English Herbarium and Anglo-Saxon medicine* (van Arsdall, 2002) and *Leechcraft: early English charms, plant-lore and healing* (Pollington, 2008). Both Bald's *Leechbook* and the *Lacnunga* were reviewed for plant names in single plant and combination formulations used to treat bacterial infections and wounds. Two translations of Bald's *Leechbook* were examined (Cockayne, 1864 and Pollington, 2008) and three works reviewing the texts consulted (Meaney, 1984; Deegan, 1988 and Cameron, 2008). A comprehensive translation of the *Lacnunga* by Pettit (2001) titled *Anglo-Saxon remedies, charms and prayers from British Library MS Harley 585* was used and supported by evidence from three other works (Cockayne, 1864; Cameron 2008 and Pollington, 2008).

*2.2. Plant selection criteria*

Native British plants not subject to any conservation orders were selected if they were assigned both an Anglo-Saxon and Latin name in the *Old English Herbarium* and listed for the treatment of at least two external conditions whereby bacteria would have been present including a puncture wound (battle injury, animal or insect bite), burn, ear, nose or throat infection and boil or ulcer. A study of plant names from medieval manuscripts (Hunt, 1989) was used to confirm common and vernacular names and then cross referenced in Bald's *Leechbook* and the *Lacnunga* for medicinal use in herbal formulations (Cockayne, 1864; Pettit, 2001; Pollington,

2008). A phytochemistry literature search was conducted in Isis Web of Knowledge, Pub Med and Science Direct databases followed by specific journal searches in antimicrobial and phytochemical publications for potential plant candidates using keywords, Latin and/or common plant names + 'antimicrobial'. The Napralert[SM] relational database (University of Illinois at Chicago, USA) was accessed for published research in English on ethno-medical studies, biological tests and known phytochemical compounds from which three plants of under reported phytochemistry were chosen for the antimicrobial assays.

*2.3. Plant collection*

Potential locations were sourced from '*Flora of Hertfordshire*' (James, 2010) and field trips conducted in June - August 2010 and 2011 to identify viable plant colonies, obtain landowner permissions (according to 1981 Wildlife and Countryside Act) and subsequently harvest the plant material. Chosen sites were within a 25 mile radius of Watford, Hertfordshire in areas typical of where an Anglo-Saxon may have collected wild specimens. Depending on plant size, 3-9 specimens of each species were harvested at one or more locations for laboratory analysis and data recorded for the herbarium label (see supplementary data) including geographic location, elevation, date and time of collection. Additionally, two specimens were pressed and dry mounted; authenticated by an independent botanist and deposited as voucher specimens in the herbarium at Kew Royal Botanic Gardens: *Arctium minus* (Hill) Bernh. (Asteraceae) - FMW003was collected at TQ05907 in August 2010 and *Agrimonia eupatoria* L. (Rosaceae) -FMW001 and *Potentilla reptans* L. (Rosaceae) - FMW002 were collected in August 2010 and 2011 at TL06526 and TL06398 respectively.

*2.4. Preparation of crude extracts*

Plant material was separated into aerial parts and roots for each species and air dried in a cool dark room for five days followed by 48 h in a fan assisted oven (Gallenkamp) at 40°C; cooled and ground to a rough powder using an electric blender and refined using a 500 μm sieve. Two grams of aerial parts and roots were separately macerated for 24 h in 20 mL solvent (red wine, 25% or 75% EtOH in $H_2O$) making a total of four ethanol and two wine extractions for each plant. A 2007 Cabernet Sauvignon at 14% proof from Northern Israel (Barkan Classic, barcode: 7290000023809) was used to represent a berry wine which can reach 18% proof when made with mead (Hagen, 2006). This red wine blank was used as a control. The infusion was prepared by adding 40 mL boiling $H_2O$ to 2 g of dried aerial parts and left for 10 min. For the decoction, 100 mL $H_2O$ was added to 2 g of roots, boiled then simmered for 20 min and reduced to a third. The supernatant for each crude extract was filtered and evaporated to 5 mL using reduced pressure (Buchi Syncore) at 45°C, transferred to a weighed vial and placed on a drying block (Techne Dri-block) at 40°C for 1-2 days to yield a dried crude extract. Stock solutions of crude extract were prepared at 20 mg/mL in DMSO using an ultrasonic water bath (QHKerry) for 30 min and stored at 4°C for further use.

*2.5 Antimicrobial assay*

Chloramphenicol, dimethyl sulphoxide (DMSO), formic acid, HPLC grade ethanol and methanol were obtained from Fisher Scientific UK Ltd; nutrient broth (CM0001) and nutrient agar (CM0003) from Oxoid Limited and distilled water purified using a reverse osmosis system (Purelab Option S). The bacteria strains used for testing the antimicrobial activity were Gram-positive *Staphylococcus aureus* (NCTC

7447) and *Bacillus subtilis* (NCTC 3610) and Gram-negative bacteria *Escherichia coli (*UEL 57) and *Pseudomonas aeruginosa* (NCIB 8295). Four to five colonies were transferred from nutrient agar plates to 5 mL sterile nutrient broth and incubated at 37°C overnight. The bacteria concentration was standardised by using a spectrometer (Jenway 6305) for optical density readings of 0.1 at 600 $_{nm}$ equivalent to 1.5 x $10^6$ colony forming units (CFUs) and diluting inoculums in sterile nutrient broth (Kuete et al., 2009). The four bacteria were screened for antimicrobial activity using the microdilution method in round bottomed 96 well microtitre plates (NCCLS, 2009). Wells were filled with 100 µL plant extract, 95 µL nutrient broth and 5 µL inoculum with final concentrations of 200, 40 and 8 µg/mL (Rios and Reico, 2005 and Cos et al., 2006). Each experiment was run in quadruplicate and repeated on three independent days (n=3). Bacterial growth was determined by taking optical density readings (Multiskan Spectrum) at 600 $_{nm}$ at 0 h ($t_1$) and 18 h ($t_2$) following incubation at 37°C. The percentage of bacterial growth inhibition was calculated by $t_2$-$t_1$/mean of inoculum and nutrient broth cells x 100.

The MIC values of the most potent crude plant extracts from the antimicrobial screening were determined by the microdilution method. Dried extract (48 mg) was reconstituted in 1 mL DMSO and sonicated for 30 min, diluted in 11 mL sterile nutrient broth and serially diluted to give ten final well concentrations ranging from 2–0.004 mg/mL. Chloramphenicol was used as the postive control at the same concentrations and nutrient broth with 1% DMSO, the negative control. Each dilution was performed in quadruplicate and repeated on three different days (n=3). MIC values were determined by using optical density readings at 600 $_{nm}$ taken at 0 h and 18 h. A percentage inhibition was calculated using the same formula as for the antimicrobial screening with >99% inhibition determined as a full MIC value; $MIC_{50}$

and $MIC_{90}$ being 50% and 90% inhibition of bacterial growth. To confirm MBC a loop was inserted into the wells with no visible growth and streaked onto an agar plate, incubated at 37°C for 18 h and checked for any bacterial growth.

*2.6. HPLC and Principal Components Analysis (HPLC-PCA)*

The HPLC system (Agilent 1200 series) comprised a Quaternary pump (G1311A), degasser (G13221), autosampler (G1329A), column oven (G1316A) and diode-array detector (G1316A). Two hundred microlitres of stock solution (20 mg/mL) were centrifuged at 13,000 rpm for 5 min and 100 µL transferred to HPLC vial and diluted tenfold in 900 µL starting mobile phase (Sanchez-Medina et al., 2007). Solvent controls were prepared for DMSO, methanol and red wine. UV data were acquired from 200-360 $_{nm}$ with 210 $_{nm}$, 254 $_{nm}$ and 320 $_{nm}$ wavelengths selected for monitoring. A Zorbax Eclipse C18 analytical column (150 x 4.6 mm id) was maintained in the column oven at 25°C using a flow rate of 1 mL/min. The starting mobile phase comprised aqueous 0.01% formic acid in distilled water (A) and methanol (B) with initial conditions set at 25% B with a linear gradient to 90% B at 30 min and retained for 2 min. The gradient returned to 25% B at 34 min and the column was equilibrated for 6 min giving a total gradient cycle of 40 min. The sample injection was 20 µL performed in triplicate. HPLC chromatograms for each crude extract were generated in Agilent 1200 Chemstation software (Rev B.04.01) at 210 $_{nm}$, 254 $_{nm}$ and 320 $_{nm}$ and the data exported as comma separated value files (.CSV) into SIMCA-P+ 12.00 (Umetrics, Sweden) software. The HPLC-PCA profiles or patterns of the secondary metabolites were generated by multivariate analysis. PCA a commonly used algorithm to discern differences between large amounts of data was employed to verify the quality of the HPLC data, correlate the chemistry with the

inhibitory growth of the wound pathogens and identify HPLC peaks attributed to the antimicrobial activity (Gao et al., 2010).

## 3. Results

The broad keywords identified the lack of scientific evidence regarding the Anglo-Saxon medical literature and that a number of translations would have to be read in order to appreciate the context in which the herbal formulations were used. The *Old English Herbarium* was the most appropriate for the basis of the selection criteria in that it comprised mainly single plant preparations whereby outcomes could be attributable to a named plant. The text includes 185 plants of which 140 were assigned both Latin and Anglo-Saxon names. Thirty were native British plants (Stace, 2010) listed for the treatment of puncture wound, open wound, burn, ear nose or throat infection, boil or ulcer; 12 plants were used as simple and in combination formulations in all three texts and following the phytochemical and ethno-medical search (supplementary data) three plants having under reported phytochemistry were selected for the antimicrobial study: *A. minus*, *A. eupatoria* and *P. reptans*. The 10$^{th}$ century literature references for formulations specifying these three plants to treat wounds and bacterial infections compared with modern Western herbal applications are reported in Table 1.

In the antimicrobial screen against Gram-positive organisms all three plants (Fig. 1.) demonstrated >50% inhibition of *S. aureus* in four or more of the eight extracts at 200 µg/mL. Each plant also showed one or more of the ethanol root extracts to inhibit growth at 40 µg/mL with dose response activity across the majority of root extracts. For *A. minus* only the 25% root extract exhibited >60% inhibition of *S. aureus* at 40 µg/mL and >50% activity at 200 ug/mL against *B. subtilis* in leaf and

root extracts (data not shown). *A. eupatoria* demonstrated greater activity against *S. aureus* with >58% inhibition at 200 µg/mL across all the preparations except the wine extracts and >61% inhibition *of B. subtilis* at 200 µg/mL in the ethanol and aqueous root extracts (data not shown). *P. reptans* was the only plant to demonstrate >50% inhibition of *S.aureus* at 8 µg/mL in the 75% ethanol root extract and 68% inhibition at 40 µg/mL in the 25% ethanol root extract (Fig.1.). The greatest activity of *P. reptans* against *B. subtilis* was the 25% ethanol leaf exhibiting 60% inhibition at 40 µg/mL (data not shown). None of the plants demonstrated >50% inhibition of Gram-negative *P. aeruginosa* at the screening concentrations. However, *A. eupatoria* and *P. reptans* both inhibited growth of *E.coli* by >60% at 200 µg/mL in the decoctions and 25% ethanol root extracts.

The HPLC-diode array chromatograms in Fig. 2. illustrate HPLC peaks for each of the *P. reptans* crude extracts at the chosen wavelengths (data for other plants not shown). Under the reverse phased separation system at 210 $_{nm}$ the major components appear to be polar compounds eluting in the first 5 min. At 254 $_{nm}$ a further series of smaller peaks are present in the subsequent 20 min. At 320 $_{nm}$ the sensitivity is poor and many of the peaks seen at 254 $_{nm}$ and 210 $_{nm}$ are not visible. The HPLC-PCA map or scores plot of principal component 1 (PC1) versus PC2 at 210 $_{nm}$ represents the distribution of samples in multivariate space showing tight clustering for the triplicates of each extract thus demonstrating good reproducibility between HPLC injections and high quality raw data (Fig. 3.). Each file contained 6000 discrete regions with data acquisition occurring every second from 0.00 to 40.00 min giving a total of 72 samples for each plant (24 per selected wavelength). The distribution of samples shows those with similar composition forming clusters and the distinct samples as separate entities (Gao et al., 2010). Crude extracts were

supervised into three activity groups: those with the most inhibition of growth at 200 µg/mL considered poor to intermediate inhibitors and the more potent inhibition seen in the distinct separations of *P. reptans* 25% ethanol root at 40 µg/mL and the 75% ethanol root at 8 µg/mL. Furthermore, the main variance is due to the 1$^{st}$ principalcomponent (PC1, 60%) with PC2 accounting for a further 27%of the variance. The contributing variables identified in the loadings plot at 210 $_{nm}$ (not shown) located the active metabolites primarily found in the root extracts and as HPLC peaks eluting within the first 5 min. The HPLC-PCA score plots for all plants at 320 $_{nm}$ and 254 $_{nm}$ did not discriminate between active and inactive samples as tightly as those in the 210 $_{nm}$ model.

*P. reptans* was selected for a MIC screen on the basis that it was the most potent plant with under reported phytochemistry. The MICs for several of the extracts are given in Table 2 showing the 75% ethanol root extract having the greatest inhibitory range of 31.25 µg/mL to 1 mg/mL against *S. aureus* and for the decoction, a $MIC_{50}$ value of 3.9 µg/mL against *E. coli*. The wine extractions exhibited the weakest activity against all bacterial strains although the wine blank and root wine extracts demonstrated a $MIC_{50}$ of 250 µg/mL against *S. aureus* as well as the root wine having a $MIC_{50}$ at 1 mg/mL against Gram-negative *E. coli* (Table 2).

## 4. Discussion

This research has been based on modern English translations of the Anglo-Saxon medical texts as the authors do not read the vernacular. The initial literature search revealed three major herbal texts with the *Old English Herbarium* selected as the most appropriate for starting the selection criteria in that it comprises mainly single formulations of plants having multiple applications for different conditions

associated with wounds and bacterial infections. Battle wounds and burns were commonplace in Anglo-Saxon England (van Arsdall, 2002) and mortality rates were high with sword, sling shot and arrow injuries as well as individuals being exposed to potential infections from bloody wounds (Hutley and Green, 2009). In the phytochemical and pharmacological search a number of the Napralert[SM] reports showed the selected plants to have negative antimicrobial assay results that may be interpreted as the plants being inactive; alternatively it may be harsh extraction methods degrading the active compounds of the original formulation resulting in little or no antimicrobial activity.

The precise identification of some plant names assigned in the Anglo-Saxon medical literature is difficult. The same physical descriptions may apply to more than one plant as in burdock and cleavers both having fruiting burs that attach to passers by although the two plants are visually very different (Cockayne, 1864). Plants were assigned a number of synonyms and in some cases, generic names as in 'Personacia' used to describe large leafed plants of different species including burdock, beet and water-lily (Hunt, 1989) or an incorrect illustration being inserted after the text had been compiled for illustrated versions of the *Old English herbarium* (van Arsdall, 2002; Voigts, 1979). Plant locations were selected within a radius of 25 miles of Watford as Oxhey, a village in Watford, was first mentioned in an Anglo-Saxon Charter of 1007 (British Library: Cotton Nero, D.1,ff.149-61). For true botanical authentication all of the plants were harvested whilst in flower during August and September 2010/2011. The *Old English Herbarium* occasionally states the month in which a plant is to be harvested as in the case of *P. reptans* 'you must prepare [collect and save] the plant in August' (van Arsdall, 2002, p.145). Plant extracts were chosen to reflect indigenous preparations of infusion, decoction and

wine; 25% ethanol for tinctures commonly prescribed in Western herbal medicine and the 75% ethanol extracts to mirror those used in classic phytochemical analysis.

*A. minus* (Lesser Burdock) is a biennial similar in appearance and structure to *A. lappa* although smaller forming a rosette in the first year. Flower heads are purple with hooked bracts forming burrs appearing July to September. The plant is mainly found in lowland central southern England and borders of Wales and is occasionally seen in open woods, grassy verges and scrubland (Stace, 2010). Hunt (1989) attributes 'Lappa Eversa' to both *A. lappa* and *A. minus* and in this study, we selected the latter as having under reported phytochemistry. *A. minus* remains in the medicinal flora (Barker, 2001) although in contemporary Western herbal practice it has been replaced with *A. lappa* with the UK herbal manufacturers only offering dried herb of the aerial parts and 25% ethanol tincture for sale. The plant is currently used topically for boils, acne and eczema which is of similar use to some of the Anglo-Saxon formulations (Table 1). Sanders et al. (1945) report an aqueous juice of *A. minus* aerial parts being active against *B. subtilis* with an inhibitory zone of 15-25 mm compared to an inhibitory zone against *E. coli* of 15-20 mm. Interestingly, they commented that there was a marked stimulation of growth of the test organism in many samples which they attributed to plant extract providing growth factors or similar compounds. In another antimicrobial study of a disc diffusion assay, a 70% ethanol infusion of *A. lappa* flower at 400 µg/disc exhibited 10-15 mm zone of inhibition against *S. aureus* and 15-20 mm against *B. subtilis* with discs of streptomycin and chloramphenicol at 30 µg per disc used as the positive control (Moskalenko, 1986). The reported literature is similar to our study with *A. minus* showing moderate activity against *S. aureus* and *E.coli* as well as evidencing the stimulation of growth in some extracts (Fig 1).

In comparison with lesser burdock more has been reported on *A. eupatoria* although less for antimicrobial activity. *A. eupatoria* (Agrimony) is a hairy herbaceous perennial with erect stems up to 60 cm with yellow flowers appearing in long spikes from June to September. The plant is fairly widespread in the British Isles except Northern Scotland and common in hedgerows, grassy pastures and scrubland (Rose, 2006 and Stace, 2010). Agrimony is used by herbalists practicing Western herbal medicine to treat diarrhoea and urinary and respiratory infections by contrast to the many Anglo-Saxon formulations for treating fresh and infected wounds (Table 1). Copland et al. (2003) reported an *A. eupatoria* seed hexane extract to have a MIC value of 0.75 mg/mL against *B. subtilis* but no activity against *P. aeruginosa* or *E. coli* although in Western herbal medicine it is the herb and not the seed that is used. In another species, isolated agrimol derivatives from *A. pilosa* have been reported to inhibit the growth of *S. aureus* with a MIC range of 3.13-50 µg/mL (Yamaki et al, 1994). In our antimicrobial screening both aerial and root extracts demonstrated moderate inhibition of *S. aureus* whilst only the root extracts showed moderate activity against *B. subtilis* and *E. coli*.

*P. reptans* commonly known as Creeping Cinquefoil is a low growing perennial with slender creeping stems that may be up to 1 m long with small yellow flowers appearing on long stalks from June to September. The plant is common in the British Isles in hedge banks, roadsides, open grasslands and sand-dunes although not so common in Scotland (Stace, 2010). By contrast *P. reptans* use has been superceded by *P. erecta* although still referenced in Barker's medicinal flora (2001)*;* perhaps an example of economic botany with both *A. minus* and *P. reptans* being substituted and later replaced by larger and more abundant counterparts. In the Anglo-Saxon formulations *P. reptans* is considered to be an effective treatment

to ‘prevent a canker spreading’ and with only eight compounds reported to date became the target of our study (Table 1). The review by Tomczyk and Latte (2009) report antimicrobial compounds for the aerial parts of the plant as kaempferol, quercetin-3’-glucoside, quercetin-3,7-*O*-β-D-glucuronide, isosalipurposide, ellagic acid, *p*-coumaric acid, caffeic acid and ferulic acid. By contrast a survey of nine Potentilla species reported aqueous infusions of aerial parts to inhibit *S. aureus* (ATCC 6538) within a MIC range of 12.5 mg to >100 mg/mL and in the same study, *P. argenta* L. was the most potent against Gram-negative bacteria by inhibiting *E.coli* (ATCC 25922) at 50 mg/mL and *P. aeruginosa* (ATCC 27853) at >100 mg/mL (Tomczyk et al., 2008). Antimicrobial activity for *P. reptans* has only been previously reported for the aerial parts but we have shown the root extracts exhibited greater inhibition against *S. aureus* and *E. coli* than reported in the literature for other Potentilla species. The 75% ethanol root extract was the only one to show $MIC_{50}$ activity at 1 mg/mL against Gram-negative bacteria *P. aeruginosa* and the decoction, the only one with a $MIC_{50}$ at 3.90 µg/mL inhibiting growth of *E. coli* (Table 2) highlighting that there may be more than one active antimicrobial compound present in the two different extracts.

Often the Anglo-Saxon formulations specify ‘old or aged wine.’ Hagen (2006) states that wine was only for the elite and the majority of people in England would have used ale or produced fruit wines including mulberry, blackberry, raspberry and elderberry. The extracts were made using wine at room temperature as many of the formulations instruct to mix with herb and use. Some preparations specifically state that the wine must be boiled but this was not tested in this study.

Many of the herbal preparations for treating wounds and bacterial infections appear pragmatic and in context for the given condition and the compilers of the Anglo-Saxon herbal literature were often convinced of a good outcome by stating '...then it soon healeth' at the end of a formulation (Table 1). The main unknown is strength of preparation although it is believed that the Anglo-Saxon practitioner was trained to administer varying doses as seen in the preparation of '*dwale*' a stupefactive (plants not specified) prepared as an anaesthetic and given to drink until the patient falls asleep (Voigts and Hudson, 1994). In line with the British Herbal Pharmacopoeia (1996) a typical daily dose for an infusion would be 2-4 g dried herb three times a day which equates to drinking 2-3 cups (500- 750 mL) of herbal tea. In our study of *P. reptans* an infusion of 2 g of aerial parts yielded 285 mg of dried extract which would equate to 285-570 mg of dried herb per cup compared to the decoction yield of 1232–2464 mg per cup (data not shown).

In this study the *P. reptans* root extracts have demonstrated greater MIC values against *S. aureus, P. aeruginosa* and *E. coli* than those reported for other *Potentilla* species showing that the plants no longer used in practice would have been suitable substitutes. MIC values of 50% and 90% inhibition of growth were used to show the range of inhibitory activity against the wound pathogens. There may be more than one active compound as the mode of antimicrobial activity in the 75% ethanol root extract against *S. aureus* is bactericidal whereas the root decoction at the MIC concentrations exhibited a bacteriostatic action against *E.coli.* To date there is no evidence in the literature reporting which compounds may be responsible for the antimicrobial activity of *P. reptans*. The HPLC-PCA map was used to determine the quality of the data as well as discern different HPLC peaks between extracts that may account for the antimicrobial activity. This technique has been

previously used to group plants based on their chemical profiles in order to determine compounds that contributed to pharmacological activity (Li et al., 2012; Sharma et al., 2012). The loading plot (not shown) confirmed that the differences in activity of the 75% ethanol extract and root decoction were due to the major peaks seen in the first five minutes at 210 $_{nm}$. This suggests that should the HPLC method be optimised to isolate the nominated peaks, the fractions will potentially be active antimicrobial compounds; a method that has been previously demonstrated by Gao et al. (2010) using secondary metabolite mapping to identify inhibitors of lung cancer cells.

Interestingly, the data for this study has been compiled using less than 30 g of dried plant for each species demonstrating that very little material is needed to obtain scientific evidence from modern analytical methods compared to classic phytochemical extraction processes. These typically use 500-1000 g for an initial screening of one plant which clearly has implications for conservation of those less common native plants where the root is specified in the Anglo-Saxon formulations. Based on our own work, the Anglo-Saxon texts have shown to be a good source for rediscovering plants lost to current herbal practice and, in particular, those used to treat bacterial infections and wounds could be further studied for related anti-inflammatory activity.

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## Figure Captions

**Fig. 1.** Antimicrobial screening of *A. eupatoria*, *A. minus* and *P. reptans* extracts expressed as a percentage of inhibition against *S. aureus*.

**Fig. 2.** HPLC-diode array chromatogram overlays of *P. reptans* crude extracts at 320 (top), 254 (middle) and 210 $_{nm}$ (lower).

**Fig. 3.** HPLC-PCA score plot of *P. reptans* extracts at 210 $_{nm}$ (top) confirming the active extracts on the left hand side of the plot and the loadings plot for 75% ethanol extract (bottom).