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Bogland plant Tormentil inhibits multidrug-resistant pathogen growth and potentiates antibiotics by disrupting iron homeostasis

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Abstract

The antibiotic resistance crisis has created an urgent need for the development of novel therapeutic strategies to tackle recalcitrant infections. In this study, we identified Tormentil [*Potentilla erecta* (L.) Raeusch.], a plant that has been used in traditional medicine for centuries, as being capable of effectively inhibiting the growth of carbapenem-resistant *Acinetobacter baumannii*. We identified agrimoniin and ellagic acid as two bioactive components with antibacterial activity present within Tormentil extracts. Combinatorial RNA-seq analysis revealed that these compounds were able to inhibit the growth of *A. baumannii* by disrupting intracellular iron homeostasis, an effect that could be reversed through exogenous iron supplementation. We also demonstrated that Tormentil could potentiate the activity of the last-resort antibiotic, colistin. Overall, these findings valorize the centuries-old traditional use of Tormentil to treat infection and highlight how its bioactive constituents could be exploited to prolong the lifespan of our last line of antibiotic defence, colistin.

DATA AVAILABILITY

Transcriptomics datasets obtained through this work have been deposited at the Gene Expression Omnibus repository of the National Centre for Biotechnology Information under the accession number GSE253367. The site where each plant is available is detailed in Table 1.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a major threat to modern medicine as the lack of available effective frontline antibiotics can compromise the success of many routine and complex medical procedures [1, 2]. As a result, AMR has been classified as one of the top ten life-threatening global health problems needing multilevel attention and global cooperation [3]. One of the drivers of the AMR crisis is the lack of novel antimicrobials progressing through the clinical development pipeline. This is driving researchers to explore new reservoirs to identify compounds with antimicrobial potential.

Plants represent a particularly promising reservoir for compounds with antimicrobial potential, as, over millennia of selective evolution, they have developed chemical defence strategies to control or prevent pathogen colonization [4, 5]. As part of a study to identify novel plant compounds that could inhibit pathogen growth, we assessed the library of plant extracts from Irish boglands generated as part of the ‘Unlocking Nature’s Pharmacy from Bogland Species’ (UNPBS) Project (grant number DOJProject209825) held at NatPro, Trinity College Dublin, for potential antimicrobial activity against carbapenem-resistant *Acinetobacter baumannii*. *A. baumannii* is a Gram-negative opportunistic pathogen that displays a remarkable capacity to

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Abbreviations: AMR, antimicrobial resistance; ESKAPE, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*; GSEA, Gene Set Enrichment Analysis; OD_{600}, OD at 600 nm; PDA, photodiode array detection; RNA-seq, RNA sequencing.

Five supplementary tables and one supplementary figure are available with the online version of this article.

Table 1. Tormentil collections, growing locations, plant parts and corresponding MeOH extracts prepared by Soxhlet extraction

Plant material collection ID	Growing location	Plant parts	% yield MeOH extract (Soxhlet)	MeOH extract ID
NTP127	Mountain bog, Oldboleys, Co. Wicklow, Ireland East (mountain blanket bog)	Aerial (leaf, stem and flower)	22.6%	Wicklow aerial
NTP127	Mountain bog, Oldboleys, Co. Wicklow, Ireland East (mountain blanket bog)	Underground (rhizome and root)	35.3%	Wicklow root
NTP218	Cummeragh River Bog, Co. Kerry, Ireland Southwest (lowland blanket bog)	Aerial (leaf, stem and flower)	18.5%	Kerry aerial
NTP218	Cummeragh River Bog, Co. Kerry, Ireland Southwest (lowland blanket bog)	Underground (rhizome and root)	22.7%	Kerry root
NTP236	Woodville Bog, Co. Tipperary, Ireland midlands (raised bog)	Aerial (leaf, stem and flower)	25.9%	Tipperary aerial
NTP236	Woodville Bog, Co. Tipperary, Ireland midlands (raised bog)	Underground (rhizome and root)	36.7%	Tipperary root

overcome antibiotic therapy through a wide array of acquired and intrinsic antibiotic resistance mechanisms [6]. This capacity for resistance has resulted in *A. baumannii* ascending to the top of the World Health Organization's priority pathogen list. It is associated with a range of infections, both in hospital settings and the wider community, and is predominantly recognized for causing wound infections, bloodstream infections and pneumonia [7]. Multidrug resistance among clinical isolates of *A. baumannii* has been reported at rates as high as 70% in certain regions [8], underscoring the urgent need for novel therapeutics [9]. Due to limited treatment options, clinical reliance on the last-resort antibiotic colistin has increased, ultimately leading to a concerning rise in colistin-resistant clinical strains [10–12].

Through our screening, we identified that extracts of *Potentilla erecta* (L.) Raeusch. had antimicrobial effects against a multidrug-resistant strain of *A. baumannii*. *P. erecta* (L.) Raeusch., commonly known as Tormentil, is an acid peat-loving member of the Rosaceae family native to Europe and Western Asia. It is a small clump-forming plant with bright yellow four-petalled flowers blooming from May to September, and the leaves are sessile and trifoliate. The rhizomatous root is thick, turning a dark red colour when cut. The *Potentilla* genus has been used in herbal medicine since ancient times by the Greek physician Dioscorides, and *Potentilla* species are prominent plants in European herbal texts through the centuries [13]. The use of Tormentil to treat diarrhoea and dysentery and as an antiseptic for the mouth and throat is consistently reported. In Ireland, reports of ethnomedical uses of Tormentil as a cure for diarrhoea in humans or animals can be found in the Schools' Manuscript Collection of the National Folklore Collection compiled by pupils between 1937 and 1939 with the objective of preserving Irish oral heritage, including entries on local cures and herbs [14, 15].

Today, the European Medicines Agency's Committee on Herbal Medicinal Products recognizes *P. erecta* (L.) Raeusch. rhizome (Tormentil rhizome) for the symptomatic treatment of mild diarrhoea and minor inflammations of the oral mucosa based on long-standing use [16]. According to the European Pharmacopoeia monograph, the rhizome contains not less than 7% tannins, expressed as pyrogallol ($C_6H_6O_3$, Mr 126.1) with reference to the dried herbal substance, and the high tannin content makes medicinal use in the proposed indications plausible [17]. Tannins are astringent polyphenols with probable roles in protection against animals, pathogens and abiotic stresses such as temperature extremes and UV light in the plants that produce them [18, 19]. In addition to conferring antioxidant activity, tannin hydroxyl groups can interact with moieties on proteins and polysaccharides to tighten mucous membranes, serving to increase barrier function and reduce permeability. Due to this astringency effect, preparations of Tormentil were also traditionally used externally for haemostasis, prosthetic pressure points, frostbite, burns, haemorrhoids and poorly healing wounds [20].

This study focuses on uncovering the antibacterial mechanism of action that has underpinned the historical and ethnomedical use of Tormentil to treat infection in *A. baumannii*. By combining HPLC, for the quantification of both ellagic acid and agrimoniin, and RNA-seq analysis, we identify that exposure to Tormentil or its bioactive constituents is associated with disruption of the overlapping regulatory pathways responsible for maintaining iron homeostasis within *A. baumannii*, as evidenced by induction of iron starvation-associated transcriptional responses. Given that the regulation of iron homeostasis within bacteria is an essential process to avoid excess iron toxicity [21], disrupting this process reveals a metabolic vulnerability that can be therapeutically targeted [22]. Furthermore, we explore the potential of Tormentil and its constituents as antibiotic potentiators.

METHODS

Bacterial strain

A. baumannii, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* spp. were used throughout this study and routinely grown in Lysogeny Broth (LB) media (Miller) at 37 °C, under aerobic shaking conditions (180 r.p.m.).

The *A. baumannii* strain AB5075 used was sourced from the Manoil Lab (University of Washington, Seattle, USA).

Plant material collection and extraction

Tormentil plant material was collected from three locations in Ireland in the summer (Table 1). The collected samples were cleaned, allowed to dry at room temperature and separated into aerial (leaf, stem and flower) and underground (rhizome and root) parts. Voucher samples were prepared for collection NTP127 from Wicklow and lodged at the National Botanic Gardens of Ireland in Glasnevin, Dublin. Accession code DBN0007669 was obtained for the aerial plant parts, and DBN0007670 was obtained for the underground plant parts. Methanol extractions of the dried, separated and milled plant material were carried out using a Buchi Soxhlet extraction system B-811 in 'Soxhlet Standard' mode. The methanol solvent was removed under vacuum to yield the six extracts, three from aerial plant parts and three from underground plant parts.

Plant extract screen for antibacterial activity and titration assays

A. baumannii overnight cultures were diluted in LB to an OD at 600 nm (OD_{600}) of 0.1 to get a concentration of ~1×10^7 c.f.u. ml^{-1}. The bacterial suspension (199 µl) was added to each well on a 96-well plate, followed by the addition of 1 µl of each plant extract from a stock concentration of 10 mg ml^{-1}. Each test and control condition was added to the microtiter plate in three separate wells. DMSO (1 µl) was added as a vehicle control. OD_{600} readings were taken every 10 min over 12 h with a Clariostar Plus plate reader (BMG LabTech), at 37 °C, 200 r.p.m. Endpoint OD_{600} was measured after 12 h. This methodology was also used to assess the potential antimicrobial effects against other ESKAPE (*E. faecium*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *Enterobacter cloacae*) pathogens (Supporting Information Fig. S1, available in the online Supplementary Material).

For growth titration assays, 200 µl of the bacterial suspension in LB was added to a 96-well plate with a maximum volume of 350 µl. To the first well, 0.5 µl of the treatment was added from a 10 mg ml^{-1} stock solution. The following wells had an increased volume of extract added, increasing by 0.5 µl each time. As controls, 200 µl of the bacterial solution and 200 µl of sterile LB were added to the microtiter plate in three independent wells. A control assay was performed in parallel using equivalent volumes of DMSO as vehicle controls. Each test and control condition was added to the microtiter plate in three independent wells. The 96-well plate was then incubated at 37 °C, 200 r.p.m. Endpoint OD_{600} was measured after 16 h using a Clariostar Plus plate reader (BMG LabTech). The same assay conditions were used for testing agrimoniin and ellagic acid, using stock solutions of 10 mM and 463.25 µM in DMSO, respectively. The stock solution concentration of ellagic acid was limited by its solubility. The increase in the volume of extract added to each well results in a higher final concentration of extract. The observed OD_{600} values reflect the extract's concentration and the volume added. To account for potential dilution effects, the OD_{600} readings from wells containing LB medium, the bacterial suspension and DMSO were used to normalize and compare the OD_{600} values.

The results represent the average of three biological replicates±SEM. For biofilm titration testing, the same experimental setup as for growth titration assessment was followed. Following incubation, the biofilm was stained using the crystal violet method [23], with a modification (washes were performed by pipetting). The absorbance of ethanol-solubilized crystal violet was then read at 600 nm using a Clariostar Plus plate reader (BMG LabTech). The results represent the average of three biological replicates±SEM.

Colistin-potentiating assay

To test the ability of agrimoniin, ellagic acid and Wicklow Tormentil root extract to potentiate the activity of colistin, we screened the treatments against *A. baumannii* in LB media that was supplemented with the sub-MIC of colistin (1.22 µg ml^{-1}, ~0.6× MIC based on the MIC of 2 µg ml^{-1} from Gadar *et al.* [22]). *A. baumannii* overnight cultures were diluted in supplemented LB media to OD_{600} of 0.1. The bacterial suspension (199 µl) was added to each well on a 96-well plate followed by the addition of 1 µl of each treatment from a stock concentration of 10 mM for agrimoniin, 463.25 µM for ellagic acid and 10 mg ml^{-1} for Wicklow Tormentil root extract. DMSO (1 µl) was added to vehicle control wells, and all test wells and control wells were added to the microtiter plate in three independent wells. OD_{600} readings were taken every 10 min over 12 h with a Clariostar Plus plate reader (BMG LabTech), at 37 °C, 200 r.p.m. The results represent the average of three biological replicates±SEM.

High-performance liquid chromatography with photodiode array detection determination of agrimoniin and ellagic acid in the Tormentil methanol extracts

HPLC with photodiode array detection (PDA) analysis of the Tormentil methanol extracts was performed based on a reported method for the quantification of tannins and related polyphenols in commercial products of Tormentil with some modifications [24]. A Waters HPLC system consisting of a Waters 600 Controller featuring a quaternary pump and a Waters 717 Plus Autosampler coupled with a Waters 2996 Photo Diode Array (PDA) detector under the control of Empower® 3 software was used for measurements (Milford, MA, USA). Separations were carried out using a Phenomenex C18 column, 5 µM particle size, 250 mm×4.6 mm. Gradient elution at a flow rate of 1.2 ml min^{-1} was used, and the composition of mobile phase A was acetonitrile+1.5% v/v formic acid, and mobile phase B was H_2O+1.5% v/v formic acid. The elution programme was 0–30% A in B from 0 to 30 min, 30–70% A in B from 30 to 33 min and a holding step of 70% A in B from 33 to 40 min before equilibration to 0% A in B from 40 to 45 min. All samples were filtered through a 0.45 µM filter before analysis, and the injection volume was 10 µl. The chromatographic profiles were extracted at the optimized wavelength of 280 nm. Agrimoniin and ellagic acid peaks were identified by comparison with commercially purchased standards, and quantification was performed based on standard curves. All sample solutions and standard solutions for HPLC analysis were prepared and analysed in triplicate. Agrimoniin was purchased from Wuhan ChemNorm Biotech (product no. TBW01481), and ellagic acid was purchased from Merck (product no. 14668).

RNA-seq and Gene Set Enrichment Analysis

Triplicate samples of *A. baumannii* AB5075 cells were grown in 20 ml LB to mid-exponential phase (OD_{600} 0.6) in either the presence of 50 µM agrimoniin, 2.3 µM ellagic acid, 0.05 mg ml^{-1} of Wicklow Tormentil root extract or DMSO. The RNA was then isolated using the RNeasy Kit with in-column DNase digestion (Qiagen). The RNA integrity of each sample was determined using a Bioanalyzer (Agilent 2100 Bioanalyzer and Agilent RNA 6000 Nano Kit), according to the amplitude and sharpness of the peaks corresponding to the 23S and 16S rRNAs. Sequencing and downstream analyses were performed at the Microbial Genome Sequencing Centre (Pittsburgh, PA, USA), using an Illumina MiSeq, with 12 million reads per sample, using ribodepletion RNA-seq with 150 bp paired-end reads. Quality control and adapter trimming were performed with bclfastq. Read mapping was performed with HISAT.

Differential expression analysis was performed using edgeR's exact test for differences between two groups of negative-binomial counts with an estimated dispersion value of 0.1, using the *A. baumannii* AB5075-UW genome annotation as a reference [25]. Genes with a log fold change ≥1 or ≤ −1 were considered differentially expressed. We used a significance threshold of *P*-value ≤ 0.05 for differential expression. *P*-values were adjusted for multiple comparisons using the false discovery rate method, and genes with an adjusted *P*-value ≤ 0.05 were considered statistically significant. The volcano plot was generated using R, by plotting the log fold change on the x-axis and -log10(*P*-value) on the y-axis. A total of 417, 636 and 518 genes were identified as differentially expressed in response to agrimoniin, ellagic acid and Wicklow Tormentil root extract, respectively, based on the criteria above. A Gene Set Enrichment Analysis (GSEA) was performed using FUNAGE-Pro with the default parameters [26].

Iron supplementation assay

A. baumannii AB5075 overnight cultures were diluted in LB to an OD_{600} of 0.1. Treatments were performed in a 96-well plate and consisted of 50 µM of agrimoniin, 2.3 µM of ellagic acid, 0.05 mg ml^{-1} of Wicklow Tormentil root extract or DMSO vehicle control. The media was supplemented with $FeCl_3$ or $FeCl_2$ to achieve a final concentration of 100 µM or H_2O in equivalent volume, as the vehicle control. The plate was then incubated at 37 °C, 200 r.p.m., in a Clariostar Plus plate reader (BMG LabTech), where an OD_{600} reading was taken every 10 min for 12 h. The results represent the average of three technical replicates and three biological replicates±SEM.

Checkerboard assay

Checkerboard assays were performed in accordance with the protocol outlined by Bellio *et al.* [27]. Briefly, *A. baumannii* AB5075 overnight cultures were diluted in LB to an OD_{600} of 0.1. Each well was filled with 100 µl of 2× Cation Adjusted Muller Hinton Broth (CAMHB). A stock concentration of colistin was prepared and dispensed into the appropriate wells and diluted from row

A to G using a multichannel pipette. Stock concentrations of ellagic acid or agrimoniin were then prepared and dispensed into the appropriate wells and diluted from columns 12 to 2. Then, 100 µl of the bacterial suspension was added to each well. A mirror plate, without bacteria and with the same reagents, was prepared to obtain a no-growth control and an OD_{600} background for data analysis. The plate was then incubated at 37 °C, 200 r.p.m., for 18 h. Following this incubation time, a reading at OD_{600} was taken using the Clariostar Plus plate reader (BMG LabTech). The percentage growth in each well was calculated as (OD_{600} drug combination well−OD_{600} background)/(OD_{600} drug-free well H1−OD_{600} background)×100.

RESULTS

Aerial and underground plant parts of Tormentil exhibit antibacterial activity against *A. baumannii*

In pursuit of phytochemicals that can inhibit the growth of *A. baumannii*, the UNPBS library of over 70 plant extracts derived from Irish bogland species was screened against *A. baumannii* AB5075, a highly virulent MDR clinical isolate, which is a well-characterized model MDR strain of *A. baumannii* [25]. From the initial screening, we found that methanol (MeOH) extracts derived from Tormentil demonstrated the most robust and reproducible antibacterial effects (based on OD_{600} measurements), and therefore, Tormentil was selected for further characterization.

Growing location and habitat are factors influencing secondary metabolite production in plants and may result in quantitative and qualitative differences in phytochemical profile that affect biological activity. For this reason, Tormentil plant material was collected for analysis from three locations: mountain blanket bog in Co. Wicklow, raised bog in Co. Tipperary and lowland blanket bog in Co. Kerry. Phytochemical profile is also dependent on the plant part, and ethnomedical use places emphasis on the medicinal properties of the underground parts of Tormentil preparations. To explore this, we collected both the underground and aerial plant parts. MeOH extracts were prepared by Soxhlet extraction of the separated and dried aerial and underground plant parts to yield six separate Tormentil extracts (Fig. 1a, Table 1). We found that MeOH extracts derived from Tormentil from each location demonstrated antibacterial effects against *A. baumannii* when compared to the DMSO vehicle control (Fig. 1b).

The aerial and root extracts of Tormentil from Wicklow, Tipperary and Kerry demonstrated significant growth inhibitory effects against *A. baumannii*. Specifically, growth inhibition was seen at concentrations of 0.35 mg ml^{-1} and 0.65 mg ml^{-1} by ~20% and ~50%, respectively, in LB media for all six extracts (Fig. 1b). Titration assays revealed that all six extracts displayed a dose-dependent antibacterial effect within the concentration range tested (Fig. 1c-h), demonstrating conservation in antimicrobial activity across sites and comparable activity between extracts from aerial plant parts and underground plant parts. Concentrations above 0.65 mg ml^{-1} were also tested, but the results were not significant compared to the DMSO control, which exhibited major inhibitory effects on bacterial growth at these higher concentrations. This antimicrobial effect is not uniformly retained across all other ESKAPE pathogens. Despite this, Wicklow Tormentil aerial, Wicklow Tormentil root and Tipperary Tormentil root extracts significantly affect the growth of Gram-negative and Gram-positive pathogens (Supporting Information Fig. S1). While our data suggest a more pronounced effect on *A. baumannii*, this does suggest a potentially more broad-spectrum application of these extracts in combating a range of bacterial infections. Further exploration of the specific mechanisms underlying these antimicrobial properties, including potential organism-specific pathways, is required to better understand the therapeutic potential and extent of their activity across various bacterial species.

Select constituents of Tormentil extract, agrimoniin and ellagic acid, exhibit antibacterial effects against MDR *A. baumannii*

Potentilla spp. contain high concentrations of tannins, which have recognized antimicrobial action regardless of plant source [18]. Tormentil contains condensed tannins and hydrolysable tannins, and while more associated with the rhizome, they are also found in the aerial parts [13]. Tormentil's condensed tannins consist of dimeric and trimeric type B proanthocyanidins together with biosynthetic precursors. The main hydrolysable tannin is agrimoniin, a dimeric ellagitannin. The presence of agrimoniin and its hydrolysis product ellagic acid was confirmed and quantified in the six Tormentil extracts by HPLC in comparison to commercial standards (Fig. 2a–d). Interestingly, the relative concentrations of agrimoniin and ellagic (Fig. 2a) did not strictly align with levels of antimicrobial activity (Fig. 1), suggesting that additional compounds present in the extracts may have antimicrobial activity. It is also possible that additional metabolites present may influence susceptibility to agrimoniin or ellagic acid either by acting on the bacteria or through interactions with agrimoniin or ellagic acid.

Agrimoniin and ellagic acid have previously demonstrated antimicrobial activity against the gastric pathogen *Helicobacter pylori*. Agrimoniin displays an MIC in the range of 12.5–50 µg ml^{-1} [28] and ellagic acid in the range of 5–30 µg ml^{-1} [29]. However, the mechanisms of action of these antimicrobial effects have yet to be determined. Given that both agrimoniin and ellagic acid have shown antimicrobial effects against Gram-negative bacteria and are both present in all the Tormentil extracts tested, we explored the possibility that these compounds could inhibit MDR *A. baumannii* growth. The treatment activity was compared to identical final solvent volumes of DMSO, allowing the treatment's effects to be distinguished from any solvent-associated toxicity. Titration assays, using a standard of agrimoniin and ellagic acid, revealed that both compounds could inhibit the growth of *A. baumannii*. However,

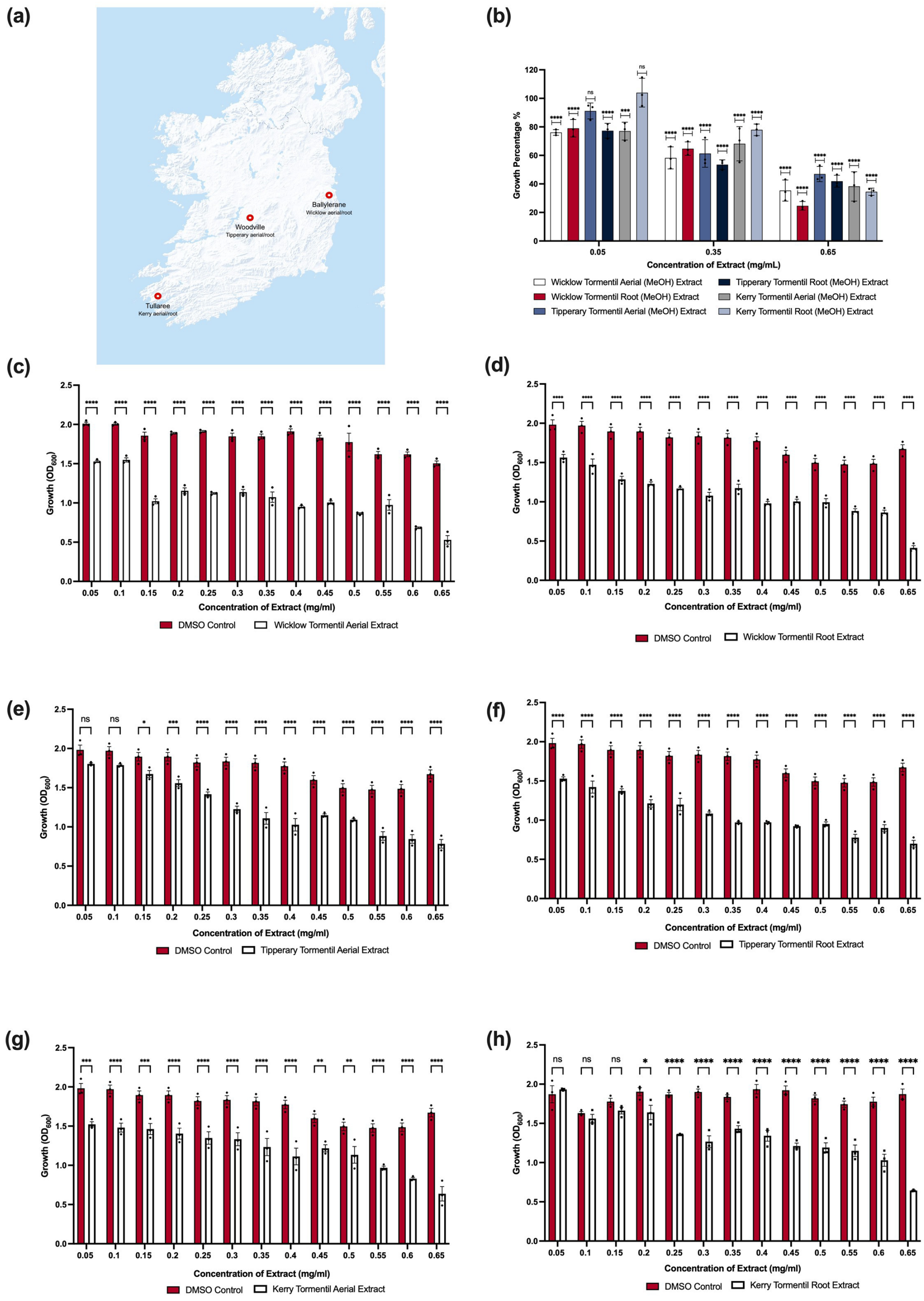
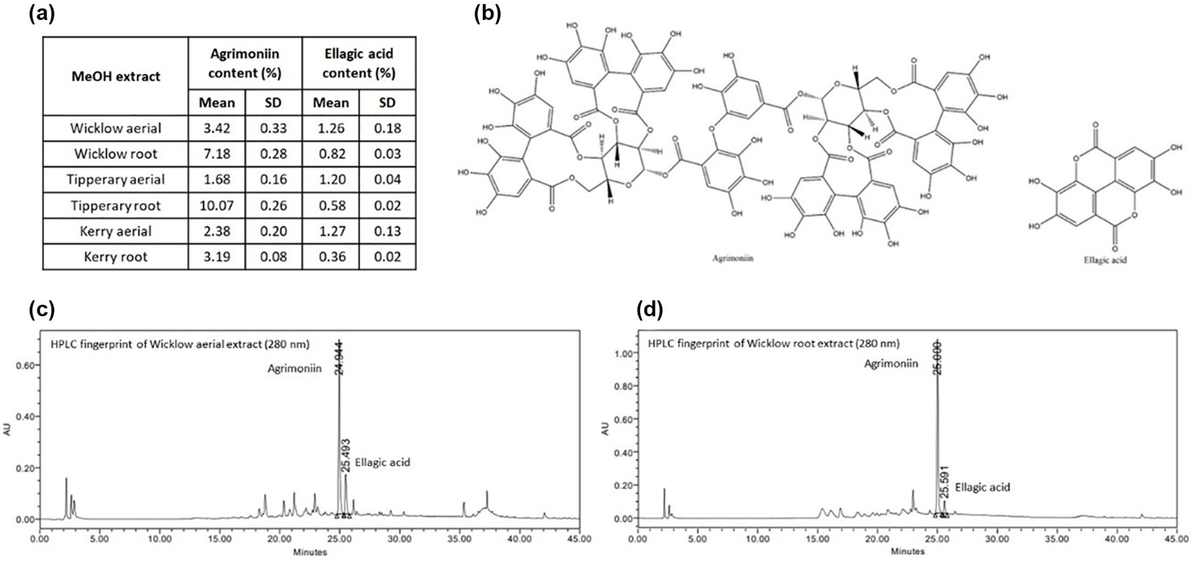


Fig. 1. Tormentil extracts exhibit antibacterial properties against *A. baumannii*. (a) Map of Ireland indicating the Tormentil collection sites: Oldboleys, Co. Wicklow; Woodville Bog, Co. Tipperary; Cummeragh River Bog, Co. Kerry. (b) Impact of Tormentil extracts at a concentration of 0.05 mg ml^{-1}, 0.35 mg ml^{-1} and 0.65 mg ml^{-1} on the growth of *A. baumannii* in LB media. Growth percentage and significance were calculated by comparing each sample reading to their respective DMSO vehicle control growth percentage. (c–h) Inhibition of *A. baumannii* growth was dose-dependent in the concentration range of 0.05 mg ml^{-1}–0.65 mg ml^{-1} for all extracts tested at 12 h. Assays were carried out in biological triplicate, each performed in technical triplicate. Statistical analysis consisted of two-way ANOVA for panels b–h, between the Tormentil-treated samples and the DMSO vehicle controls. Average values±SEM are represented. Significance is indicated as *=$P\leq0.05$, **=$P\leq0.01$, ***=$P\leq0.001$ and ****=$P\leq0.0001$.



MeOH extract	Agrimoniin content (%)		Ellagic acid content (%)	
	Mean	SD	Mean	SD
Wicklow aerial	3.42	0.33	1.26	0.18
Wicklow root	7.18	0.28	0.82	0.03
Tipperary aerial	1.68	0.16	1.20	0.04
Tipperary root	10.07	0.26	0.58	0.02
Kerry aerial	2.38	0.20	1.27	0.13
Kerry root	3.19	0.08	0.36	0.02

Fig. 2. Tormentil methanol extracts contain agrimoniin and ellagic acid. (a) Percentage content of agrimoniin and ellagic acid in the Tormentil methanol extracts. Percentage content is the mean of samples prepared in triplicate and quantified by an HPLC-based standard curve. (b) Structures of agrimoniin and ellagic acid. (c, d) Representative HPLC chromatograms: (c) HPLC fingerprint of the Wicklow aerial extract at 280 nm; (d) HPLC fingerprint of the Wicklow root extract at 280 nm.

only the impact of ellagic acid was seen to be dose-dependent over the concentration ranges tested (Fig. 3a, b). This may be due to the larger molecular weight of agrimoniin and issues with crossing the cell envelope as a result.

Biofilm formation, a method of growth where bacterial cells attach to each other and/or a surface and encase themselves in a polysaccharide matrix, is a key defence mechanism adopted by *A. baumannii* to overcome treatments and the rigours of the host immune system [30–33]. Given the challenges posed by biofilm-associated *A. baumannii* infections, we wanted to investigate whether agrimoniin and ellagic acid also harbour antibiofilm properties along with their antimicrobial potential. We found that agrimoniin and ellagic acid both display dose-dependent effects against *A. baumannii* biofilm formation (Fig. 3c, d). In the context of agrimoniin, particularly at the concentration of 500 µM, a much greater impact on biofilm formation was seen compared to bacterial viability (Fig. 3a, c). This suggests that agrimoniin displays next-generation antimicrobial activity, possessing antivirulence properties at a concentration that does not compromise bacterial viability further [34]. However, the antibiofilm effects caused by ellagic acid are likely at least in part mediated through its antimicrobial activity. In comparison to the Wicklow Tormentil root extracts' antibiofilm activity (Fig. 3e), which also exhibited a significant, dose-dependent reduction in biofilm formation, the constituents have a more pronounced effect at the lower concentrations. This suggests that while the Wicklow Tormentil root extract contains compounds that contribute to the antibiofilm activity, agrimoniin and ellagic acid may be the primary active constituents responsible for this effect.

Agrimoniin, ellagic acid and Wicklow Tormentil root extract alter global gene expression in *A. baumannii* with iron-associated pathways primarily impacted

To understand the mechanism behind the growth and biofilm inhibitory effects of agrimoniin and ellagic acid on *A. baumannii* growth and biofilm formation, we performed an RNA sequencing (RNA-seq) experiment. Wicklow Tormentil root extract was also selected for testing based on its availability and high levels of antimicrobial activity (Fig. 1d).

We treated the cells separately with agrimoniin, ellagic acid and Wicklow Tormentil root extract at the lowest concentrations that displayed a statistically significant reduction in growth in the previously tested ranges, 50 µM, 2.3 µM and 0.05 mg ml^{-1}, respectively. The gene expression profiles were then compared to bacterial cells exposed to the DMSO vehicle control only. Our RNA-seq analysis revealed significant changes in gene expression, some of which are conserved amongst all three datasets (Fig. 4a). Agrimoniin elicited a response that led to significant differential expression in 417 genes, with 199 showing upregulation and 218 showing downregulation (Fig. 4b). Ellagic acid exerted an even more pronounced effect, with 636 genes exhibiting significant differential expression; among these, 181 were upregulated, and 455 were downregulated (Fig. 4c). Similarly, Wicklow Tormentil root extract impacted gene expression, resulting in significant differential expression in 518 genes, wherein 139 were upregulated and 379 were downregulated (Fig. 4d) (Supporting Information Datasets 1–3). To gain further insights into the functional implications of the differentially expressed genes, we performed GSEA with FUNAGE-Pro using the annotated genome of *A. baumannii* AB5075-UW as a reference (Supporting Information Datasets 4–5).

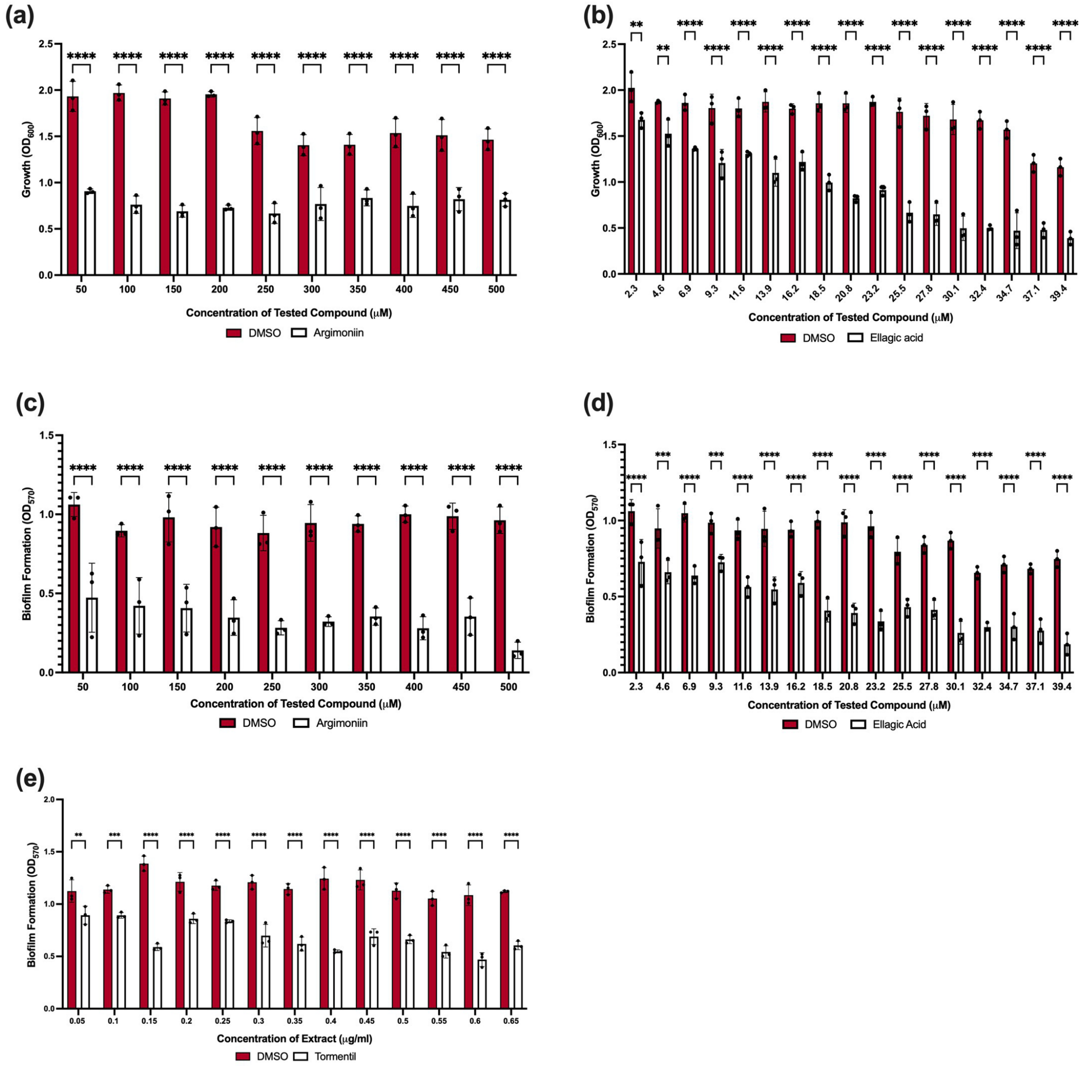


Fig. 3. Agrimoniin and ellagic acid impact the growth of *A. baumannii*. (a) Inhibition of *A. baumannii* growth by agrimoniin. Inhibition effects were not dose-dependent in the tested concentration range. (b) Inhibition of *A. baumannii* growth by ellagic acid. Inhibition effects are shown to be dose-dependent. (c, d) Inhibition of *A. baumannii* biofilm formation by agrimoniin (c) and ellagic acid (d). (e) Inhibition of *A. baumannii* biofilm formation by Wicklow Tormentil root extract. The effect was dose-dependent for both agrimoniin and ellagic acid. Assays for all panels were carried out in biological triplicate, each performed in technical triplicate. Statistical analysis consisted of two-way ANOVA for panels a–e between the treated samples and DMSO vehicle control. Average values±SEM are represented. Significance is indicated as *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$ and ****=$P \leq 0.0001$.

Based on the functional GSEA clustering, the RNA-seq analysis unveiled dysregulation in distinct gene clusters that significantly impact *A. baumannii*'s iron homeostasis across all three conditions under investigation. Notably, there was consistent downregulation observed in bacterioferritin orthologues, *bfr* (ABUW_0306) and *bfrA* (ABUW_3125), which are pivotal for intracellular iron storage [35]. Conversely, the gene clusters involved in the biosynthesis of siderophore proteins exhibited an upregulation in response to exposure to all three conditions. Specifically, a notable upregulation was observed in the expression of the *bar-bas-bau* gene cluster. This cluster mediates the export, biosynthesis and transport pathways of the iron-scavenging siderophore acinetobactin (ABUW_1168–1180). Consequently, the upregulation of this cluster improves the bacterium's ability to scavenge iron from its environment [36]. This suggests that *A. baumannii* may be upregulating its iron acquisition systems as it senses that iron is less available. The consistency of this transcriptional response across all three conditions would suggest that the antimicrobial mechanism may be conserved and linked to iron availability. This aligns with the observed upregulation in genes associated with various functional groups linked to iron uptake, including the *feoB* Fe^{2+} transporter encoding gene (ABUW_3632), which has increased expression in all three datasets (Fig. 4a). Furthermore, a consistent upregulation was observed in the expression of *foxA* ferrioxamine receptor genes (ABUW_1800) across all three conditions (Fig. 4a). An upregulation of *foxA* expression is likely aimed at increasing intracellular iron levels and enhancing the activity of its encoded high-affinity receptor for iron–siderophore complexes. The global transcriptional response suggests that

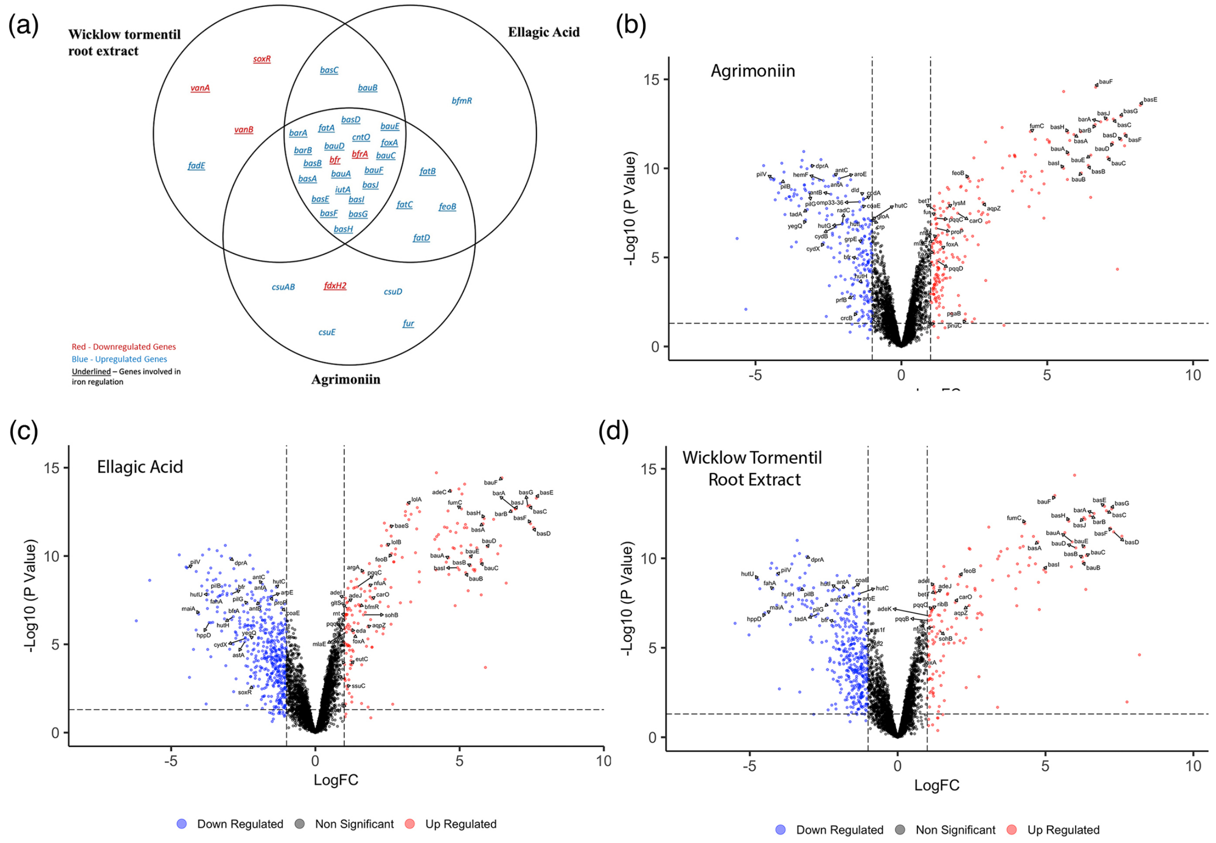


Fig. 4. Alteration of the iron signature in *A. baumannii* by agrimoniin, ellagic acid and Wicklow Tormentil root extract. (a) Venn diagram depicting the commonalities and differences in differentially expressed genes related to iron and biofilm, between the three datasets: agrimoniin, ellagic acid and Wicklow Tormentil root extract. Downregulated genes are represented in red, and upregulated genes are represented in blue. Genes that are involved in iron processes are underlined, and genes that are involved in biofilm processes are not. (b–d) Volcano plots representing the results of the RNA-seq analysis performed on cells exposed to (b) agrimoniin, (c) ellagic acid and (d) Wicklow Tormentil root extract compared to cells exposed to DMSO vehicle control. Genes with no change in their expression levels are shown in black, whereas significantly downregulated and upregulated genes are shown in blue and red, respectively. Representative genes related to iron in panels b–d are labelled, for example, downregulated genes related to iron storage, such as bacterioferritin (*bfr*, ABUW_0306) and *bfrA* (ABUW_3125), upregulated genes related to siderophore biosynthesis, transport and export, such as the acinetobactin (*bar-bas-bau* cluster, ABUW_1168–1188) biosynthetic pathway and the biofilm formation pathway (*csu* cluster, ABUW_1487–1488). The volcano plots were generated using R by plotting the log fold change on the x-axis and the *P*-value on the y-axis. A total of 417, 636 and 518 genes were differentially expressed in agrimoniin, ellagic acid and Wicklow Tormentil root extract, respectively, based on a log fold change ≥1 and *P*-value<0.05 (adjusted *P*-value).

when exposed to Tormentil extracts or its constituents, *A. baumannii* faces substantial iron stress. Specifically, the decrease in iron availability may lead to a restriction in intracellular iron concentrations, thereby limiting essential cellular functions that rely on iron [37]. It is also well established that biofilm formation is impacted by iron availability, suggesting that the antibiofilm effect displayed by Tormentil, agrimoniin and ellagic acid (Fig. 3c, d) can be attributed at least in part to their capacity to induce iron stress within the bacterium [37].

Iron supplementation can rescue cells from the antimicrobial effects of agrimoniin, ellagic acid and Wicklow Tormentil root extract

Agrimoniin and ellagic acid have previously been shown to bind iron, but crucially, this has not been linked to their antimicrobial activity [38, 39]. Given that our transcriptomic data (Fig. 4b–d and Supporting Information Datasets 1–3) highlight that agrimoniin, ellagic acid and Wicklow Tormentil root extract significantly impact iron homeostasis in *A. baumannii*, we sought to investigate whether this property underpins their antimicrobial and antibiofilm activities. To determine if this disruption was responsible for the antimicrobial and antibiofilm effects of Tormentil, ellagic acid and agrimoniin, we supplemented the treated bacterial cells each with Fe^{2+} or Fe^{3+}. Strikingly, supplementing with either Fe^{2+} or Fe^{3+} led to a complete rescue of both bacterial cell growth and biofilm-forming capabilities (Fig. 5a–d). These findings shed light on the pivotal role of iron chelation in the observed antimicrobial and antibiofilm effects of agrimoniin, ellagic acid and Wicklow Tormentil root extract.

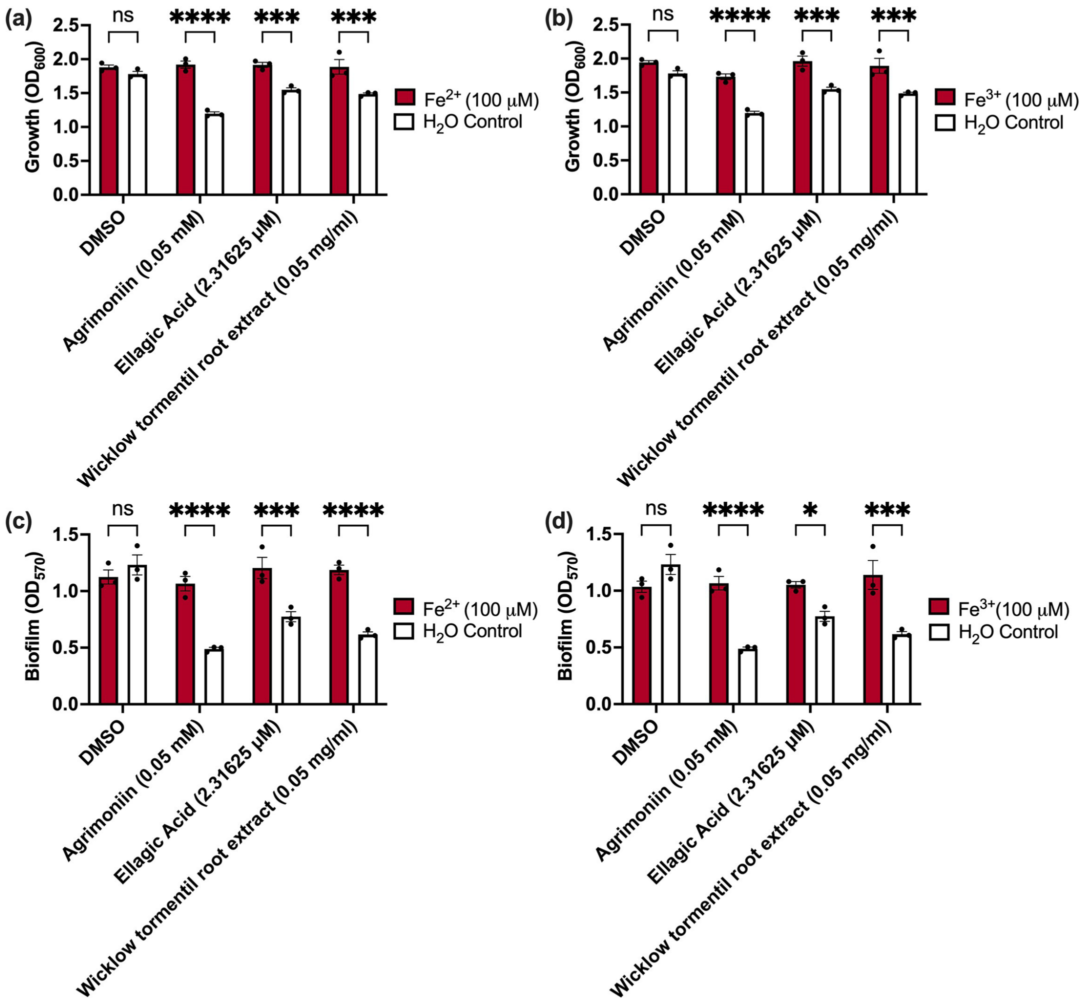
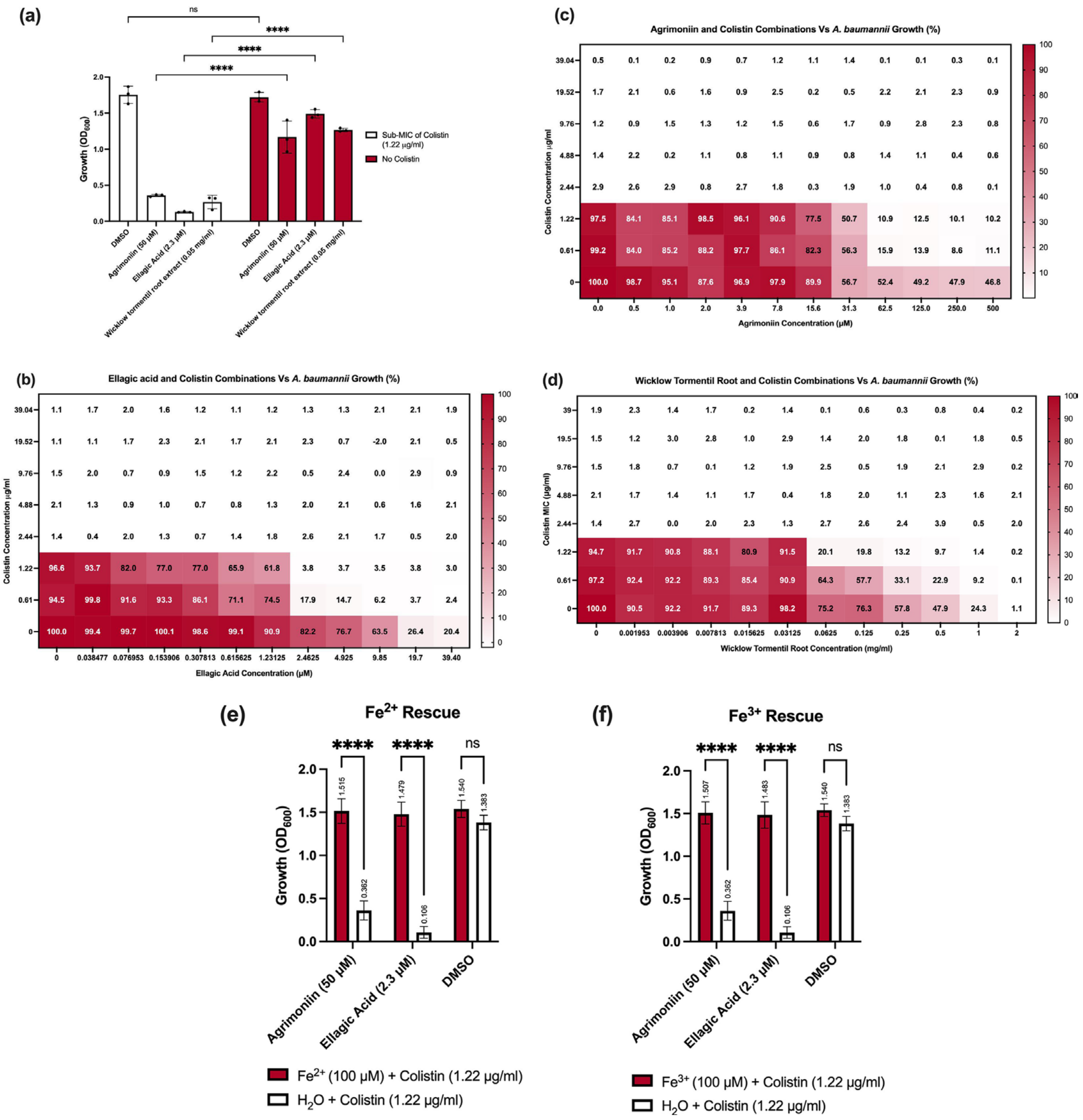


Fig. 5. The *in vitro* antimicrobial and antibiofilm effects of agrimoniin, ellagic acid and Wicklow Tormentil root extract on *A. baumannii* are arrested by iron supplementation. (a, b) Growth inhibition of *A. baumannii* in the presence of 50 µM agrimoniin, 2.3 µM ellagic acid and 0.05 mg ml^{-1} Wicklow Tormentil root extract is rescued by Fe^{2+} (a) and Fe^{3+} (b) supplementation in LB media. (c, d) Biofilm inhibition of *A. baumannii* in the presence of 50 µM agrimoniin, 2.3 µM ellagic acid and 0.05 mg ml^{-1} Wicklow Tormentil root extract is rescued by Fe^{2+} (c) and Fe^{3+} (d) supplementation. For all panels, assays were carried out in biological triplicate, with three technical repeats. The analysis consists of a two-way ANOVA between the treated samples and the treated samples that were supplemented with Fe^{2+} or Fe^{3+}. Average values±SEM are represented. Significance is indicated as ns=non-significant, *=P≤0.05, **=P≤0.01, ***=P≤0.001 and ****=P≤0.0001.

Agrimoniin, ellagic acid and Wicklow Tormentil root extract potentiate colistin activity

In our previous work, we demonstrated that disrupting iron homeostasis exposes a metabolic vulnerability that can potentiate the activity of the last resort antibiotic, colistin [22]. Thus, we sought to explore if the iron chelating abilities of agrimoniin and ellagic acid [38, 39] could similarly potentiate colistin. Moreover, our curiosity extended to the colistin-potentiating properties of Wicklow Tormentil root extract.

Our investigation revealed that agrimoniin (50 µM), ellagic acid (2.3 µM) or Wicklow Tormentil root extract (0.05 mg ml^{-1}) combined with the sub-inhibitory concentration (sub-MIC) of colistin (1.22 µg ml^{-1}, ~0.6× MIC based on the MIC of 2 µg ml^{-1} from Gadar *et al.* [22]), exhibited a remarkable ability to inhibit the growth of *A. baumannii* (Fig. 6a). Additionally, checkerboard assays revealed a relationship between the concentration of ellagic acid, agrimoniin and Wicklow Tormentil root extract combined with colistin (Fig. 6b–d). This is demonstrated by the decrease in growth percentage from combined treatments with colistin being greater than the decrease in growth by either compound or extract alone across the heatmap (left to right). This effect further highlights their potential in combating *A. baumannii* infections. However, the fractional inhibitory concentration index could not be calculated to confirm this synergism as MICs could not be determined due to the impact of the DMSO solvent on growth at the higher concentrations of ellagic acid, agrimoniin and Wicklow Tormentil root extract. However, the Wicklow Tormentil root extract appears to be less potent than the individual components (Fig. 6b, c). This could be a result of a proportion of the agrimoniin or ellagic acid already being bound



(b) Ellagic acid and Colistin Combinations Vs *A. baumannii* Growth (%)

Colistin Concentration µg/ml	0	0.038477	0.076953	0.153906	0.307813	0.615625	1.23125	2.4625	4.925	9.85	19.7	39.40
39.04	1.1	1.7	2.0	1.6	1.2	1.1	1.2	1.3	1.3	2.1	2.1	1.9
19.52	1.1	1.1	1.7	2.3	2.1	1.7	2.1	2.3	0.7	-2.0	2.1	0.5
9.76	1.5	2.0	0.7	0.9	1.5	1.2	2.2	0.5	2.4	0.0	2.9	0.9
4.88	2.1	1.3	0.9	1.0	0.7	0.8	1.3	2.0	2.1	0.6	1.6	2.1
2.44	1.4	0.4	2.0	1.3	0.7	1.4	1.8	2.6	2.1	1.7	0.5	2.0
1.22	96.6	93.7	82.0	77.0	77.0	65.9	61.8	3.8	3.7	3.5	3.8	3.0
0.61	94.5	99.8	91.6	93.3	86.1	71.1	74.5	17.9	14.7	6.2	3.7	2.4
0	100.0	99.4	99.7	100.1	98.6	99.1	90.9	82.2	76.7	63.5	26.4	20.4

Ellagic Acid Concentration (µM)

(c) Agrimoniin and Colistin Combinations Vs *A. baumannii* Growth (%)

Colistin Concentration µg/ml	0.0	0.5	1.0	2.0	3.9	7.8	15.6	31.3	62.5	125.0	250.0	500
39.04	0.5	0.1	0.2	0.9	0.7	1.2	1.1	1.4	0.1	0.1	0.3	0.1
19.52	1.7	2.1	0.6	1.6	0.9	2.5	0.2	0.5	2.2	2.1	2.3	0.9
9.76	1.2	0.9	1.5	1.3	1.2	1.5	0.6	1.7	0.9	2.8	2.3	0.8
4.88	1.4	2.2	0.2	1.1	0.8	1.1	0.9	0.8	1.4	1.1	0.4	0.6
2.44	2.9	2.6	2.9	0.8	2.7	1.8	0.3	1.9	1.0	0.4	0.8	0.1
1.22	97.5	84.1	85.1	98.5	96.1	90.6	77.5	50.7	10.9	12.5	10.1	10.2
0.61	99.2	84.0	85.2	88.2	97.7	86.1	82.3	56.3	15.9	13.9	8.6	11.1
0	100.0	98.7	95.1	87.6	96.9	97.9	89.9	56.7	52.4	49.2	47.9	46.8

Agrimoniin Concentration (µM)

(d) Wicklow Tormentil Root and Colistin Combinations Vs *A. baumannii* Growth (%)

Colistin MIC (µg/ml)	0	0.001953	0.003906	0.007813	0.015625	0.03125	0.0625	0.125	0.25	0.5	1	2
39	1.9	2.3	1.4	1.7	0.2	1.4	0.1	0.6	0.3	0.8	0.4	0.2
19.5	1.5	1.2	3.0	2.8	1.0	2.9	1.4	2.0	1.8	0.1	1.8	0.5
9.76	1.5	1.8	0.7	0.1	1.2	1.9	2.5	0.5	1.9	2.1	2.9	0.2
4.88	2.1	1.7	1.4	1.1	1.7	0.4	1.8	2.0	1.1	2.3	1.6	2.1
2.44	1.4	2.7	0.0	2.0	2.3	1.3	2.7	2.6	2.4	3.9	0.5	2.0
1.22	94.7	91.7	90.8	88.1	80.9	91.5	20.1	19.8	13.2	9.7	1.4	0.2
0.61	97.2	92.4	92.2	89.3	85.4	90.9	64.3	57.7	33.1	22.9	9.2	0.1
0	100.0	90.5	92.2	91.7	89.3	98.2	75.2	76.3	57.8	47.9	24.3	1.1

Wicklow Tormentil Root Concentration (mg/ml)

Fig. 6. Agrimoniin, ellagic acid and Wicklow Tormentil root extract potentiate the activity of colistin. (a) Impact of agrimoniin, ellagic acid and Wicklow Tormentil root extract at a concentration of 50 µM, 2.3 µM and 0.05 mg ml^{-1}, respectively, on the growth of *A. baumannii*, when combined with the sub-MIC of colistin (1.22 µg ml^{-1}). (b) Combinations of ellagic acid and colistin against *A. baumannii* exhibit significant activity, with increasing concentrations of both components enhancing the inhibition of *A. baumannii* growth. (c) Concentration combinations of agrimoniin and colistin against *A. baumannii*. (d) Concentration combinations of Wicklow Tormentil root (MeOH) and colistin against *A. baumannii*. Significant activity between increasing concentrations of both components and the Wicklow Tormentil root (MeOH) extract is seen to have effects on the growth of *A. baumannii*. (e, f) Growth inhibition of *A. baumannii* in the presence of 50 µM agrimoniin, 2.3 µM ellagic acid and 0.05 mg ml^{-1} Wicklow Tormentil root extract is rescued by Fe^{2+} (e) and Fe^{3+} (f) supplementation in LB media. Assays were carried out in biological triplicate, with three technical repeats. Statistical analysis consisted of two-way ANOVA between the treated samples and the co-treated samples. Average values±SEM are represented. Significance is indicated as ns=non-significant, *=*P*≤0.05, **=*P*≤0.01, ***=*P*≤0.001 and ****=*P*≤0.0001.

to iron in the neat extract or compounds present in the extract that interfere with the activity of colistin. Similar to the effects of the individual constituents and the Wicklow Tormentil root extract alone, the effects of these combined with the sub-MIC of colistin could be rescued with the addition of Fe^{2+} or Fe^{3+} (Fig. 6e, f).

DISCUSSION

Recent studies have unveiled Tormentil's antimicrobial effects against biofilm-forming strains of *S. aureus*, including antibiotic-resistant variants [40]. Additionally, Tormentil has demonstrated antimicrobial activity against *Bacillus subtilis* ATCC 6633, as well as certain yeast strains such as *Candida lipolytica* KKP 322 and *Hansenula anomala* R 26 [41]. Tormentil extracts have also shown activity against *H. pylori*, and its effectiveness was confirmed *in vivo* through an *H. pylori* SS1-infected mouse model [28, 29]. Despite these previous findings and Tormentil's traditional medicinal use, the antimicrobial mechanism of action of Tormentil is still unknown. Here, we demonstrate the antimicrobial effects against *A. baumannii* exhibited by MeOH extracts of Tormentil from both the aerial and underground plant parts from different regions of Ireland (Fig. 1, Table 1). The bioactivity of extracts is indicative of their ability to elicit biological effects or responses when they are consumed or applied. It provides a measure of the biological or pharmacological activity exhibited by the constituents present within Tormentil derivative extracts. HPLC analysis confirmed that the six extracts contained agrimoniin and ellagic acid (Fig. 2). These compounds were selected for further evaluation in parallel with the Wicklow root extract in an effort to deconvolute the mechanisms contributing to the antimicrobial effects of Tormentil's complex extract.

We demonstrate that both agrimoniin and ellagic acid exhibit antibacterial properties against an MDR strain of *A. baumannii* (Fig. 3a–c) and uncover that they can both induce iron stress (Fig. 4). These findings indicate that these compounds may hold potential as novel antibacterial agents. Interestingly, the relative potency/antimicrobial properties of the extracts do not clearly align with the individual component concentrations. For example, Tipperary root has the highest concentration of agrimoniin (Fig. 2a, 10.07%) but has less of an impact on growth as compared to Wicklow Tormentil root extract (Fig. 1d, f), which has a lower concentration of agrimoniin (Fig. 2a, 7.18%). A similar disparity is seen when comparing the concentrations of ellagic acid to the antimicrobial activity of specific extracts, suggesting that other compounds within the extracts are likely to contribute to their bioactivity or work synergistically with agrimoniin/ellagic acid. Or it is feasible that a proportion of the ellagic acid or agrimoniin in the extract is already bound to iron, limiting their antimicrobial activity.

RNA-Seq analysis revealed the downregulation of bacterioferritin orthologues, *bfr* (ABUW_0306) and *bfrA* (ABUW_3125), responsible for iron storage, upon exposure to all three treatments (Fig. 4 and Supporting Information Datasets 1–3). Conversely, the expression of genes involved in the biosynthesis, export and transport of the siderophore acinetobactin (*bar-bas-bau* cluster) was upregulated by all three treatments (Fig. 4). Collectively, our transcriptomics data indicate that the tested treatments all induce iron stress within *A. baumannii* by increasing the acquisition and impeding the storage of this vital nutrient. Previous studies have demonstrated that both agrimoniin and ellagic acid possess the ability to chelate iron ions, ultimately leading to their depletion [38, 39]. These findings, of agrimoniin and ellagic acid's iron chelation abilities, align with our hypothesis that iron stress plays a role in the antimicrobial activity of Tormentil. To further challenge our hypothesis that iron depletion was responsible for the antimicrobial activity of Tormentil and its constituents, we supplemented the media with free iron. This resulted in the rescuing of the cells from the inhibitory effects of Tormentil and its metabolites and further confirmed the role of iron sequestration in their antimicrobial activities (Fig. 5). The evidence that genes associated with acinetobactin were upregulated across all three conditions may also help to explain why *A. baumannii* appears more susceptible to the antimicrobial activity of Tormentil than other pathogens, as acinetobactin is specifically produced by this species, whereas other pathogens may produce siderophores which can compete more effectively with ellagic acid and agrimoniin for iron.

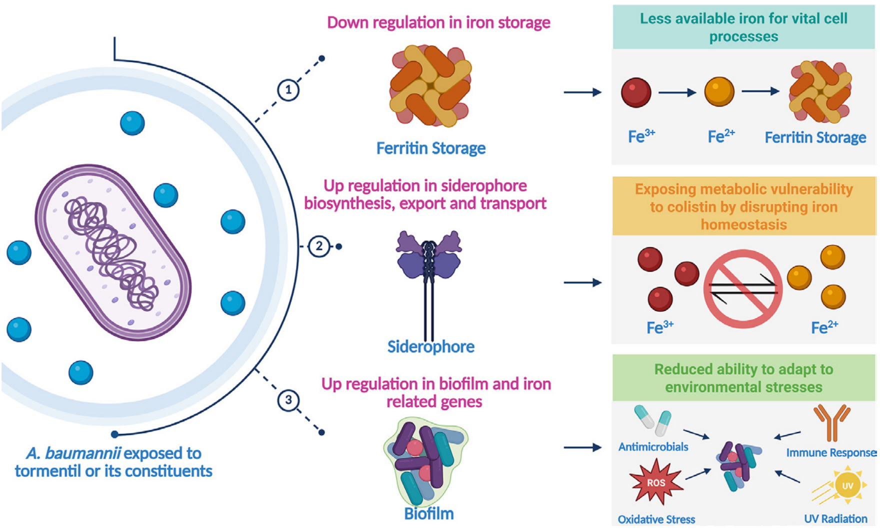


Fig. 7. Summary figure for the potential mechanism of action of Tormentil and its constituents against *A. baumannii*. Specific chemicals within Tormentil and its constituents are able to impact the growth and biofilm formation of *A. baumannii* by acting upon iron homeostasis. These chemicals can downregulate genes involved in iron storage and simultaneously upregulate genes involved in siderophore biosynthesis, export and transport. This also exposes the cell's metabolic vulnerability to the sub-MIC of colistin. Additionally, they are able to functionally reduce biofilm formation, resulting in the bacteria having a reduced ability to adapt to environmental stress. Created using BioRender.

Agrimoniin and ellagic acid also exhibited antibiofilm effects against *A. baumannii* in a dose-dependent manner, while Wicklow Tormentil root extract displayed a less potent impact on biofilm (Fig. 3c–e). Iron availability has been linked to biofilm formation in *A. baumannii* previously [37], indicating that iron stress was likely responsible for the altered biofilm phenotypes. This was further supported by the abrogation of the antibiofilm effect upon iron supplementation (Fig. 5c, d). However, within the transcriptional datasets, there were some genes linked to biofilm formation, although their regulation did not always align with the observed phenotype. For example, there was an upregulation of the *csu* cluster (ABUW_1487–1488), a set of genes essential for biofilm formation, observed solely in the agrimoniin dataset (Fig. 4a, b and Supporting Information Dataset 1), despite the negative impact agrimoniin had on biofilm formation. Interestingly, across all three conditions, there was a downregulation of genes associated with Type IV Pili, which play a key role in *A. baumannii* natural transformation and motility, suggesting a potential additional antivirulence effect of Tormentil, agrimoniin and ellagic acid (Supporting Information Datasets 1–3, 5) which will be explored as part of future work. Additionally, further work at the protein and pathway level will be required to fully validate the suggested mechanistic model.

Our previous study, which focused on the significance of iron homeostasis in colistin susceptibility, highlighted the efficacy of various compounds in augmenting colistin's activity. Kaempferol, along with non-specific iron chelators such as EDTA and specific iron chelators like ExJade and 8-hydroxyquinoline, exhibited promising colistin-potentiating effects [22]. Similarly, a study conducted by Chusri *et al.* (2009) found that ellagic acid can enhance the activity of novobiocin, coumermycin, chlorobiocin, rifampicin and fusidic acid against *A. baumannii* at a concentration of 40 µM [42]. Thus, the iron chelating abilities of agrimoniin and ellagic acid, coupled with the demonstrated potentiating effects of ellagic acid [38, 39, 42], incited us to investigate the colistin-potentiating properties of agrimoniin, ellagic acid and Wicklow Tormentil root extract. Strikingly, our findings indicate that when combined with the sub-MIC of colistin, each tested treatment, at a concentration of 50 µM, 2.3 µM and 0.05 mg ml^{-1}, respectively, significantly reduced the growth of *A. baumannii* (Fig. 6a). These effects were further enhanced by the interactions observed in the checkerboard assays, which demonstrated increased growth inhibition with higher concentrations of either ellagic acid, agrimoniin or Wicklow Tormentil root extract and colistin (Fig. 6b–d). When comparing the extract's activity (Fig. 1c–h) with the checkerboard assays, we observed that the extracts caused greater growth inhibition than expected based on their major components alone. This suggests that there are other active compounds within the extract that contribute to activity. It is also possible that a proportion of agrimoniin and/or ellagic acid is already bound to iron in the neat extract, limiting their activity in these assays. Future work will explore this possibility. However, a key limitation of these checkerboard assays is that a fractional inhibitory concentration index could not be calculated to confirm this synergism as MICs could not be determined due to the impact of the DMSO solvent on growth at the higher concentrations of ellagic acid, Wicklow Tormentil root extract and the non-dose-dependent effect of agrimoniin. It is also important to note that not all iron-binding compounds exhibit antimicrobial activity or function as antimicrobial potentiators, as their effects depend on factors such as bacterial uptake mechanisms, binding affinity and intracellular availability [21, 22]. This potentiation effect was able to be rescued by the addition of Fe^{2+} or Fe^{3+} (Fig. 6e, f). This suggests that iron homeostasis also plays a key role in the mechanism of colistin potentiation by agrimoniin, ellagic acid and Wicklow Tormentil root extract.

Tormentil has a long history of use in traditional medicine, including treatment of microbial infection. In this paper, we valorize the traditional use and uncover the fundamental mechanisms underpinning the antimicrobial and antibiofilm activity associated with this plant (Figs 1 and 7 and Supporting Information Dataset 1). We demonstrate how select active components limit bacterial cell growth by affecting iron homeostasis (Figs 1 and 5 and Supporting Information Dataset 1). Additionally, we demonstrate the ability of Wicklow Tormentil root extract and its bioactive constituents to enhance the efficacy of colistin (Fig. 6). In the current climate, where we are in urgent need of novel compounds with antimicrobial activity against MDR pathogens, this work highlights the potential of plants as a source of novel antimicrobials and identifies compounds with therapeutic potential as standalone antimicrobials or as potentiators of the last-resort antibiotic colistin against MDR *A. baumannii*.

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Author contributions

Conceptualization: H.S. and R.R.M.C. Methodology: K.G., M.P., C.J.G., I.O. and S.N. Investigation: K.G., M.P., C.J.G., I.O. and S.N. Supervision: H.S. and R.R.M.C. Writing–original draft: K.G., M.P. and R.R.M.C. Writing–review and editing: K.G., M.P., C.J.G., I.O., S.N., J.J.W., H.S. and R.R.M.C.

Conflicts of interest

The authors declare no conflicts of interest.

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