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Quorum Sensing Inhibitors for *Staphylococcus aureus* from Italian Medicinal Plants

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Abstract

Morbidity and mortality estimates due to methicillin-resistant *Staphylococcus aureus* (MRSA) infections continue to rise. Therapeutic options are limited by antibiotic resistance. Anti-pathogenic compounds, which inhibit quorum sensing (QS) pathways, may be a useful alternative to antibiotics. Staphylococcal QS is encoded by the *agr* locus and is responsible for the production of δ-hemolysin. Quantification of δ-hemolysin found in culture supernatants permits the analysis of *agr* activity at the translational, rather than transcriptional, level. We employed RP-HPLC techniques to investigate the anti-QS activity of 168 extracts from 104 Italian plants through quantification of δ-hemolysin. Extracts from three medicinal plants (*Ballota nigra*, *Castanea sativa*, and *Sambucus ebulus*) exhibited a dose-dependent response in the production of δ-hemolysin, indicating strong anti-QS activity in a pathogenic MRSA isolate.

Keywords

quorum sensing; MRSA; medicinal plants; δ-toxin; δ-hemolysin; *agr*

Introduction

Emerging infections due to methicillin resistant *Staphylococcus aureus* (MRSA) pose a significant threat to hospital patients as the rates of nosocomial infection steadily rise [1]. Moreover, healthcare-associated MRSA (HA-MRSA) are often multidrug-resistant (MDR) and therapeutic options are rapidly becoming more limited as new resistant phenotypes surface. One approach to drug discovery for the treatment of MRSA is through natural products research. Most research on natural botanic products activity for MRSA is focused on growth inhibition, while some have focused on inhibition of the MDR mechanisms, such as efflux pumps [2–5]. No studies on the *agr*-inhibiting or quorum sensing inhibiting (QSI) activity of natural botanic products on MRSA have been conducted thus far. Inhibition of staphylococcal QS pathways could potentially limit the degree of pathogenicity posed by some MRSA strains by blocking the production of certain virulence factors. Moreover, the inhibition of staphylococcal pathogenesis could be accomplished without growth inhibition, thus potentially avoiding selective pressures for drug-resistance.

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The staphylococcal QS system is a cell-density-dependent mechanism for controlling protein expression, including the production of staphylococcal virulence factors such as the α-, β, and δ-hemolysins. It is encoded by the *agr* locus, which is a quorum-sensing gene cluster of five genes (*hld, agrA, agrB, agrC* and *agrD*) [6].

Staphylococcal δ-hemolysin, or δ-toxin, is a translational protein product of RNAIII. It is a 26-aminoacid polypeptide with surfactant-like properties [7]. Translation of *hld*, the gene for δ-hemolysin, occurs about one hour after transcription of RNAIII. There are two forms of δ-toxin that can be found in the culture supernatant: formylated (with an N-terminal methionine) and deformylated. These forms are represented by two distinct peaks in the RP-HPLC chromatogram (Figure 1). δ-Toxin accumulates in the culture medium in both forms, and is approximately 90% formylated and 10% deformylated. This ratio is due to the arrest of deformylated δ-toxin production during the post-exponential growth phase, whereas formylated δ-toxin continues to accumulate. Sommerville *et al.* [8] suggest that this change may be linked to iron availability in the culture medium.

Quantification of δ-toxin produced by *S. aureus* and found in the culture supernatants allows for the analysis of *agr* activity at the translational, rather than transcriptional, level. The identification of *agr*-inhibiting drugs, or staphylococcal QS-inhibitors, has been proposed by several research groups as a potential anti-staphylococcal therapy [9–13]. In 2000, Otto and Götz [7] provided a fast method for δ-toxin quantification using RP-HPLC techniques for the analysis of staphylococcal culture filtrates. We apply this method for the first time as a screening tool for identifying plant extracts with QSI activity for a strain of HA-MRSA known as pulsed-field type (PFT) USA500.

USA500 isolates are SCCmecIV and MLST ST8. They are highly multidrug-resistant and tend to be associated with nosocomial transmission [14,15]. USA500 is associated with the production of many virulence factors, including enterotoxins A and B, as well as δ-hemolysin, among others. There is a critical need for novel therapeutic options in the treatment of highly virulent, MDR staphylococcal infection, such as those caused by USA500.

We quantify the amount of δ-hemolysin found in the supernatant of MRSA cultures treated with plant extracts as a means of measuring the impact of plant products on the staphylococcal quorum sensing (QS) system. We examine 168 crude extracts made from 104 Italian plants, representing 44 plant families.

Materials and Methods

Plant material and extraction

Ethnobotanical surveys of plants used in the traditional pharmacopoeia of the Vulture-Alto Bradano region of Basilicata, southern Italy were conducted and results are described in previous works [16–19]. Bulk and voucher specimens were collected and identified in 2006 by C. Quave. Voucher specimens of plants were deposited at the *Herbarium Lucanum* (HLUC) in Potenza, Italy and Fairchild Tropical Botanic Gardens (FTG) in Miami, FL, USA.

Dry plant materials were ground into a fine powder using a homogenizer. Ethanolic extracts of all plant samples were made by soaking in 95% denatured EtOH using a ratio of 1g (plant material):10 mL (EtOH) for 72 h. Flasks were agitated daily. Water extracts were made by boiling 1g (plant material): 50 mL (dH_2O) for 30 minutes. Extracts were vacuum filtered and rotary-evaporated, then frozen and lyophilized. Stock concentrations of 10 mg/mL of dry extract in the excipient (DMSO or dH_2O) were prepared, sterile filtered (0.2 μm) and

stored in the dark at 4°C. The excipient (DMSO or dH_2O) made up less than 5.1% of the final test solution for MIC assays and less than 2.5% for δ-toxin assays.

Bacteria and culture conditions

HA-MRSA PFT USA500 (NRS385) was obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) repository [14]. Bacteria were grown on Tryptic Soy agar plates for 18 h at 37°C. A 1:20 dilution of a standardized inoculum (0.5 McFarland Standard) was used to create final inoculum densities of 5–8 × 10^5 CFU/mL from overnight cultures using the direct suspension method [20] for MIC and δ-toxin assays. Inoculum densities were confirmed by taking colony counts using the spread plate method at the time of inoculation.

Determination of minimum inhibitory concentrations (MICs)

MICs were determined by the microtiter broth method [21] in sterile flat-bottom 96-well polystyrene plates. We used serial dilution techniques to determine the MIC_{50} and MIC_{90} of extracts at concentrations of 8–512 μg/mL after 18 h growth. We included negative controls (cells + TSB), positive controls (cells + TSB + antibiotics − vancomycin, ampicillin, and trimethroprim-sulfamethoxazole), vehicle controls (cells + TSB + DMSO), and media controls (TSB). All tests were performed in triplicate. Optical density readings were taken using a KC4 microplate reader at 600 nm at 0 and 18 hours post-inoculation. Results are reported as the MIC for growth at 18 hours post-inoculation. To account for the effect of extract color on the OD_{600nm} reading, a formula for calculating percent inhibition was used. The mean % inhibition of replicate tests was used to determine the final MIC values.

$$\%\ \text{inhibition} = \left(1 - \left(\frac{ODt18 - ODt0}{ODgc18 - ODgc0}\right)\right) \times 100$$

*ODt*18 = optical density (600 nm) of the test well at 18 hours post-inoculation

*ODt*0 = optical density (600 nm) of the test well at 0 hours post-inoculation

*ODgc*18 = optical density (600 nm) of the growth control well at 18 hours post-inoculation

*ODgc*0 = optical density (600 nm) of the growth control well at 0 hours post-inoculation

Quantification of δ-toxin production

Polystyrene 24-well culture plates were prepared with a total volume of 1 mL per well of TSB, an initial sub-MIC test concentration of 64 μg of extract suspended in DMSO (<1% DMSO in total well volume) and bacteria. Extracts demonstrating significant activity, as exhibited by lower δ-toxin levels, were also investigated at a range of test concentrations from 8–256 μg/mL. Controls for media, growth, and growth in the carrier solvent (DMSO) were also performed. Liquid test cultures were grown for 15 hours at 37°C and aerated by shaking at 150 rpm. All tests were performed in triplicate.

Aliquots of bacteria (2 mL) were centrifuged for 5 min at 14,000 × g with a microcentrifuge. Supernatants were removed and stored at −20 °C until HPLC analysis. The concentration of δ-toxin was measured by RP-HPLC with a 1-mL Resource PHE column (GE Healthcare, Uppsala, Sweden) as previously described [7], except that 200 μL of supernatant (as opposed to 500 μL) was injected onto the column using a Thermo Spectra-System HPLC

apparatus, equipped with a Diode Array Detector and autosampler (Thermo Electron Corporation, San Jose, CA) and ChromQuest 4.1 software.

δ-Hemolysin elutes at a retention time of about 6.4 minutes (deformylated) and 6.8 minutes (formylated) after sample injection as two distinct peaks. Integration of the δ-toxin peak area was performed at 280 nm. We confirmed the identity of δ-toxin peaks by peak fractionation and LC-mass spectrometry (Fig. 1S) using a Thermo Finnigan Deca XP max ion trap mass spectrometer and surveyor LC with autosampler and diode array detector (Thermo Electron Corporation, San Jose, CA) using conditions previously described [8]. The peak areas were calculated using ChromQuest software and the mean percent inhibition of δ-toxin production for the replicate tests was calculated in relation to the mean peak area of the excipient (DMSO) growth controls.

Statistical analysis

All experiments were carried out in triplicate. Data were analyzed using Microsoft Excel and SPLUS software. Differences between the means of the experimental and control groups were evaluated with two-sample t-tests using SPLUS software.

Results

There was a broad low level response of δ-toxin inhibition to the screening test concentration of 64 μg/mL. QSI activity was apparent in 90% of the extracts tested (Table 1). No QSI activity was apparent in aqueous extracts. This suggests that the active QSI components are predominantly nonpolar in nature. Extracts were not effective at inhibiting growth of this multidrug-resistant strain of HA-MRSA (USA500/NRS385). Only 6% of extracts demonstrated a MIC_{50} at concentrations of 256–512 μg/mL. None demonstrated a MIC_{90} at concentrations ≤ 512 μg/mL.

Three ethanolic extracts demonstrated significant δ-toxin inhibition, and come from the following plant species: *Ballota nigra*, *Castanea sativa*, and *Sambucus ebulus*. Interestingly, each of these species is applied in south Italian folk remedies for skin and soft tissue infection [19]. The HPLC chromatograms and graphs of the percent inhibition of δ-toxin (by measure of peak area) for these species demonstrate a strong dose-dependent response (Fig. 2S and 3).

Discussion

Quantification of δ-hemolysin in the supernatant of staphylococcal cultures can be used as a measure of *agr* system, or QS, activity [7–9]. The *agr* system controls approximately 150 genes and is critical to *S. aureus* virulence [22]. While the staphylococcal QS system is a useful target for the discovery and development of new anti-pathogenic drugs, the dynamic nature of the *agr* system must not be overlooked. A better understanding of the effect that *agr* manipulation can have on the development of infection *in vivo* is necessary. For example, inhibiting *agr* activity during certain times in the infection process can lead to deleterious effects, such as increased biofilm formation [23].

Based on analyses of δ-hemolysin production, we have offered the first reports of plant extracts interfering with QS pathways in MRSA. These results indicate that some degree of QSI activity is evident in 90% of the 168 Italian plant extracts screened, including those extracts with no growth inhibitory activity.

The validity of plant-based therapies for infection that do not exhibit activity in the standard *in vitro* bacteriostatic or bactericidal assays is oftentimes questioned. These data, however,

support the idea that other mechanisms of action may be in play, which do not necessarily impact bacterial growth, but virulence mechanisms, instead. These data give validity to the use of south Italian folk remedies incorporating *Ballota nigra, Castanea sativa,* and *Sambucus ebulus* for the treatment of skin and soft tissue infection. Further investigation, including the fractionation and isolation of active components from these three species is recommended.

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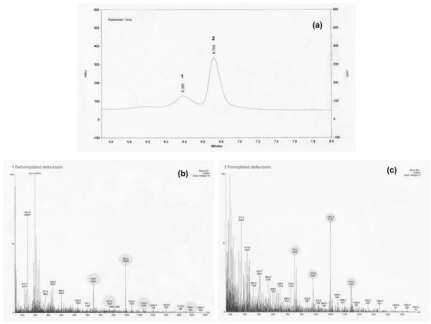

Fig. 1S.

Mass spectroscopic analysis of HPLC fractions containing derformylated and formylated δ-toxin. Peaks matching the spectrogram presented in the study by Somerville *et al.* [8] are highlighted. (**a**) Absorbance at 280nm of NRS385 (PFT USA500) supernatant fractionated by HPLC. (**b**) Mass spectrogram of peak 1, deformylated δ-toxin (molecular mass 2979.2 Da). (**c**) Mass spectrogram of peak 2, formylated δ-toxin (molecular mass of 3007.4 Da).

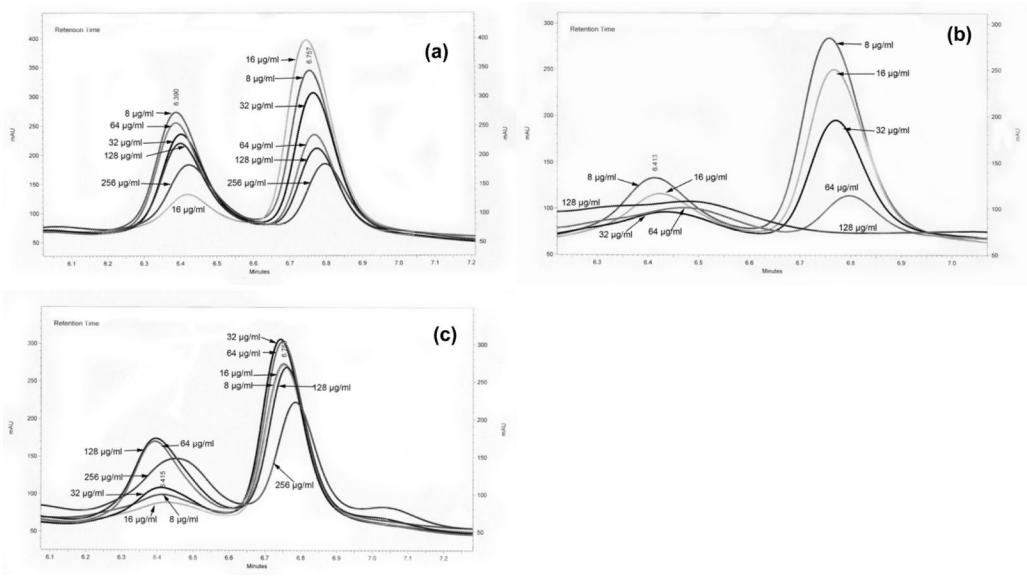

Fig. 2S.

HPLC chromatogram of δ-toxin after treatment with different concentrations of plant extract. (**a**) EtOH extract of *Ballota nigra* stems. (**b**) EtOH extract of *Castanea sativa* leaves. (**c**) EtOH extract of *Sambucus ebulus* leaves.

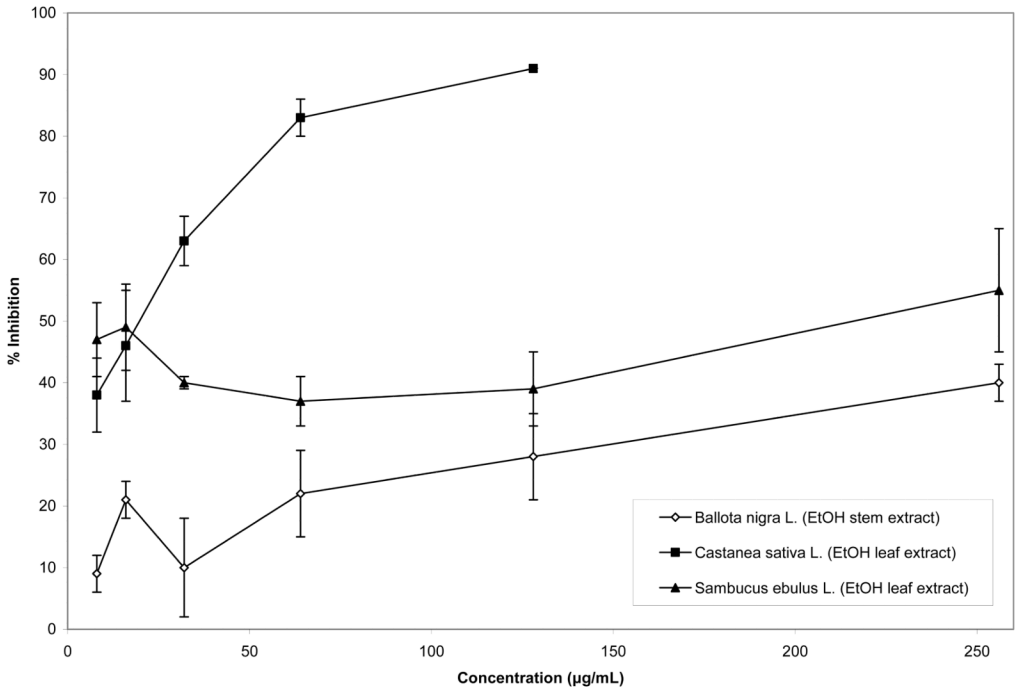

Fig. 3.

Percent inhibition of δ-toxin peak area after treatment with extracts of *Ballota nigra*, *Castanea sativa* and *Sambucus ebulus*.

Table 1

Inhibition of δ-toxin and minimal inhibitory concentrations of plant extracts against MRSA (strain I.D. NRS385/PFT USA500).

Family	Botanic Name	Voucher ID	Plant Part	Ethno-botanical Use*	Extract Solvent	Percent Inhibition of δ-toxin Production**	MIC_{50}***
Adoxaceae	*Sambucus ebulus* L.	CQ-168	inflorescence	N	EtOH	45	-
			leaves	S	EtOH	48	-
			stems	N	EtOH	28	-
	Sambucus nigra L.	CQ-151	woody parts	R	EtOH	29	-
			leaves	S	EtOH	36	-
					dH_2O	-	-
			inflorescence	S; R	EtOH	38	-
					dH_2O	-	-
			infructescence	F	EtOH	34	-
Alliaceae	*Allium cepa* L.	CQ-206	leaves; bulbs; roots	S; M; F	EtOH	22	-
Apiaceae	*Daucus carota* L.	CQ-215	leaves; stems	N	EtOH	2	-
			inflorescence; infructescence	N	EtOH	39	-
	Foeniculum vulgare ssp. *piperitum* (Ucria) Coutinho	CQ-192	leaves; stems	M; F	EtOH	-	-
	Foeniculum vulgare ssp. *vulgare* Mill.	CQ-196	leaves; stems	M	EtOH	8	-
	Tordylium apulum L.	CQ-101	flowers; leaves; roots; stems	N	EtOH	25	-
Apocynaceae	*Vinca major* L.	CQ-117	flowers; leaves; roots; stems	M	EtOH	26	-
Aracaeae	*Arum italicum* Mill.	CQ-175	stems	N	EtOH	28	-
			fruits	N	EtOH	15	-
			stalks	N	EtOH	2	-
			leaves	S	EtOH	22	-
Asphodelaceae	*Asphodelus microcarpus*_Salzm. & Viv.	CQ-109	inflorescence	N	EtOH	19	-
			leaves	N	EtOH	17	-
Asteraceae	*Achillea ageratum* L.	CQ-219	leaves; stems; flowers	M	EtOH	66	512
	Achillea millefolium L.	CQ-176	inflorescence	M	EtOH	41	-
			leaves; stems	M	EtOH	23	-
			leaves; stems; flowers	M	EtOH	38	-
	Anacyclus tomentosus DC.	CQ-167	leaves; stems; flowers	N	EtOH	36	-

Family	Botanic Name	Voucher ID	Plant Part	Ethno-botanical Use*	Extract Solvent	Percent Inhibition of δ-toxin Production**	MIC_{50}***
	Cichorium intybus L.	CQ-106	basal leaves; roots	F	EtOH	23	-
					dH_2O	-	-
			leaves; stems; flowers	F	EtOH	8	-
	Matricaria recutita L.	CQ-118	flowers; leaves; roots; stems	S; M	EtOH	29	512
					dH_2O	-	-
	Scolymus hispanicus L.	CQ-199	leaves; stems; flowers	N	EtOH	23	-
	Tussilago farfara L.	CQ-202	leaves; stems; roots	S	EtOH	16	-
	Urospermum dalechampii (L.) Scop.	CQ-134	flowers; leaves; roots; stems	N	EtOH	14	-
Boraginaceae	*Anchusa officinalis* L.	CQ-128	leaves; stems; flowers	N	EtOH	34	-
	Borago officinalis L.	CQ-100	flowers; leaves; roots; stems	M	EtOH	54	-
					dH_2O	-	-
	Cerinthe major L.	CQ-110	flowers; leaves; roots; stems	N	EtOH	48	-
	Echium italicum L.	CQ-162	leaves; stems; flowers	N	EtOH	32	-
Brassicaceae	*Brassica rapa* subsp. *rapa*	CQ-104	flowers; leaves; roots; stems	F	EtOH	27	-
	Cardaria draba (L.) Desv.	CQ-140	flowers; leaves; roots; stems	N	EtOH	12	-
	Eruca sativa Mill.	CQ-102	flowers; leaves; roots; stems	N	EtOH	13	-
	Sisymbrium officinale (L.) Scop.	CQ-131	flowers; leaves; roots; stems	N	EtOH	20	-
Caprifoliaceae	*Lonicera alpigena* L.	CQ-213	woody parts	N	EtOH	28	-
			leaves	N	EtOH	25	-
Caryophyllaceae	*Saponaria officinalis* L.	CQ-210	leaves; stems; flowers	N	EtOH	4	-
	Silene alba (Mill.) E.H.L. Krause	CQ-123	leaves; stems; flowers	N	EtOH	43	-
	Silene nutans L.	CQ-125	leaves; stems; flowers	N	EtOH	43	-
Cucurbitaceae	*Ecballium elaterium* (L.) A. Richard	CQ-169	leaves; stems; flowers	S	EtOH	21	-
Dennstaedtiaceae	*Pteridium aquilinium* (L.) Kuhn	CQ-211	leaves	N	EtOH	-	-
			stems	N	EtOH	24	-
Dipsacaceae	*Dipsacus fullonum* L.	CQ-201	leaves; stems	N	EtOH	28	-
			flowers	N	EtOH	28	-
	Knautia arvensis Coult.	CQ-190	leaves; stems; flowers	N	EtOH	48	-
	Knautia lucana Lacaita & Szabo	CQ-166	leaves; stems; flowers	N	EtOH	6	-
Equisetaceae	*Equisetum arvense* L.	CQ-226	stems; leaves	N	EtOH	22	-

Family	Botanic Name	Voucher ID	Plant Part	Ethno-botanical Use*	Extract Solvent	Percent Inhibition of δ-toxin Production**	MIC_{50}***
Fabaceae	*Acacia dealbata* Link	CQ-115	inflorescence	O	EtOH	56	-
			stems	O	EtOH	38	-
			leaves; stems	O	EtOH	21	-
	Anthyllis vulneraria L.	CQ-147	leaves; stems; flowers	N	EtOH	28	-
	Astragalus monspessulanus L.	CQ-112	leaves; stems; flowers; roots	N	EtOH	36	-
	Coronilla emerus L.	CQ-137	leaves; flowers	N	EtOH	33	-
			woody stems	N	EtOH	14	-
	*Melilotus alba*_Medik.	CQ-193	leaves; stems; flowers	N	EtOH	43	-
	Robinia pseudoacacia L.	CQ-155	woody parts	N	EtOH	32	-
			leaves	N	EtOH	-	-
			inflorescence	N	EtOH	21	-
	Spartium junceum L.	CQ-144	leaves; stems; flowers	A	EtOH	22	-
	Trifolium repens L.	CQ-138	leaves; stems; flowers; roots	N	EtOH	4	-
	Vicia craca L.	CQ-149	leaves; stems; flowers; roots	N	EtOH	19	-
	Vicia faba L.	CQ-103	leaves; stems; flowers; roots	F	EtOH	14	-
	Vicia sativa subsp. *angustifolio*	CQ-124	leaves; stems; flowers	N	EtOH	22	512
	Vicia sativa subsp. *sativa*	CQ-119	leaves; stems; flowers	N	EtOH	29	-
	Wisteria sinensis (Sims) Sweet	CQ-126	inflorescence	O	EtOH	36	-
			stems	O	EtOH	39	-
			leaves	O	EtOH	41	-
Fagaceae	*Castanea sativa* Mill.	CQ-191	inflorescence	N	EtOH	20	-
			leaves	N	EtOH	70	512
			woody parts	A	EtOH	32	512
	Quercus cerris L.	CQ-228	leaves	N	EtOH	27	-
			stems; fruits	N	EtOH	37	-
Gentianaceae	*Centaurium pulchellum* (Sw.) Druce	CQ-217	leaves; stems; flowers; roots	N	EtOH	21	-
Geraniaceae	*Erodium ciconium* (L.) L'Hér.	CQ-142	leaves; stems; flowers; roots	N	EtOH	34	-
	Erodium malacoides (L.) L'Hér. ex Aiton	CQ-121	leaves; stems; flowers	N	EtOH	7	512
	Geranium columbinum L.	CQ-129	leaves; stems; flowers	N	EtOH	-	-
Hyacinthaceae	*Leopoldia comosa* (L.) Parl.	CQ-105	bulbs	M; F	EtOH	21	-
					dH_2O	-	-

Family	Botanic Name	Voucher ID	Plant Part	Ethno-botanical Use*	Extract Solvent	Percent Inhibition of δ-toxin Production**	MIC_{50}***
			leaves; inflorescence	N	EtOH	31	-
Hypericaceae	*Hypericum perforatum* L.	CQ-183	leaves; stems; flowers	S	EtOH	36	-
Juglandaceae	*Juglans regia* L.	CQ-181	immature fruits	S; C	EtOH	-	-
			leaves	R	EtOH	39	-
			woody parts	N	EtOH	17	-
Juncaceae	*Juncus articulatus* L.	CQ-216	leaves; fruits	N	EtOH	32	-
Lamiaceae	*Ballota nigra* L.	CQ-160	stems	S; M	EtOH	76	-
			roots	N	EtOH	37	-
			leaves	S; M	EtOH	47	-
			leaves; stems; flowers	S; M	EtOH	47	-
					dH_2O	-	-
	Clinopodium vulgare L.	CQ-182	leaves; stems; flowers	N	EtOH	40	-
	Marrubium vulgare L.	CQ-170	leaves; stems; flowers	S; M	EtOH	40	-
					dH_2O	6	-
			roots	N	EtOH	40	-
	Mentha pulegium L.	CQ-200	leaves; stems; flowers; roots	F	EtOH	36	-
	Mentha spicata L.	CQ-224	leaves; stems; flowers	F	EtOH	28	-
	Origanum heracleoticum L.	CQ-207	leaves; stems; flowers	F	EtOH	30	-
	Phlomis herba-venti L.	CQ-168	leaves; stems; flowers	N	EtOH	16	-
	Rosmarinus officinalis L.	CQ-113	leaves; stems; flowers	F; S	EtOH	58	256
	Salvia pratensis L.	CQ-165	leaves; stems	N	EtOH	23	-
			inflorescence	N	EtOH	58	-
	Salvia virgata Jacq.	CQ-127	leaves; stems; flowers	N	EtOH	42	256
	Stachys tymphaea Hausskn.	CQ-189	leaves; stems; flowers	N	EtOH	41	-
Liliaceae	*Lilium candidum* L.	CQ-174	leaves; stems	N	EtOH	37	-
			inflorescence	N	EtOH	30	-
Malvaceae	*Alcea rosea* L.	CQ-205	leaves; stems; flowers; roots	O	EtOH	18	-
	Malva sylvestris L.	CQ-156	stems	S; M	EtOH	22	-
					dH_2O	-	-
			flowers	S; M	EtOH	53	-

Family	Botanic Name	Voucher ID	Plant Part	Ethno-botanical Use*	Extract Solvent	Percent Inhibition of δ-toxin Production**	MIC_{50}***
			leaves	S; M	EtOH	34	-
					dH_2O	-	-
Moraceae	*Ficus carica* L.	CQ-173	leaves	N	EtOH	24	-
			woody parts	N	EtOH	21	-
			immature fruits	S; F	EtOH	41	-
Myrsinaceae	*Cyclamen hederifolium* Aiton	CQ-186	tubers	M	EtOH	10	-
Nyctaginaceae	*Mirabilis jalapa* L.	CQ-222	leaves; flowers; fruits	N	EtOH	16	-
Oleaceae	*Olea europaea* L.	CQ-197	leaves	N	EtOH	40	-
			woody parts	A	EtOH	24	-
Orchidaceae	*Aceras anthropophora* R. Br.	CQ-153	leaves; stems; flowers; roots	N	EtOH	51	-
	Orchis italica Poir.	CQ-133	inflorescence; leaves; stems	N	EtOH	49	-
	Orchis purpurea Huds.	CQ-132	inflorescence; leaves; stems	N	EtOH	57	-
Papaveraceae	*Fumaria officinalis* L.	CQ-107	leaves; stems; flowers; roots	N	EtOH	44	-
	Papaver rhoeas subsp. *rhoeas*	CQ-145	leaves; stems; flowers; roots	F	EtOH	25	-
	Papaver somniferum L.	CQ-178	leaves; stems; flowers; roots	M; R	EtOH	36	-
Plantaginaceae	*Digitalis ferruginea* L.	CQ-227	leaves; stems; flowers	N	EtOH	32	-
	Linaria vulgaris Hill	CQ-223	leaves; stems; flowers; roots	N	EtOH	25	-
	Plantago major L.	CQ-225	leaves; stems; flowers; roots	S; M	EtOH	12	-
Poaceae	*Agropyron repens* (L.) P. Beauv.	CQ-208	leaves; stems; roots	M	EtOH	19	-
	Arundo donax L.	CQ-146	stem internodes	A; R	EtOH	22	-
			stem nodes	S	EtOH	35	-
					dH_2O	-	-
			leaves; stems	A; R	EtOH	10	-
Polygonaceae	*Rumex crispus* L.	CQ-171	leaves; stems; fruits	S	EtOH	25	-
Pottiaceae	*Syntrichia ruralis* (Hedw.) Web. & Mohr	CQ-229	whole plant	N	EtOH	45	-
Ranunculaceae	*Delphinium fissum* Waldst. & Kit.	CQ-187	leaves; stems; flowers; fruits	N	EtOH	29	-
	Ranunculus acris L.	CQ-135	leaves; stems; flowers	N	EtOH	34	-
Rosaceae	*Crataegus monogyna* Jacq.	CQ-116	leaves; stems; flowers	M	EtOH	57	-
	Prunus spinosa L.	CQ-163	woody parts; leaves	M	EtOH	33	-
			fruits	N	EtOH	29	512

Family	Botanic Name	Voucher ID	Plant Part	Ethno-botanical Use*	Extract Solvent	Percent Inhibition of δ-toxin Production**	MIC_{50}***
	Rosa canina var. *canina*	CQ-152	fruits	N	EtOH	16	-
			woody parts	N	EtOH	44	-
			leaves; stems	N	EtOH	14	512
	Rubus ulmifolius Schott	CQ-164	leaves; stems; flowers	S	EtOH	10	-
			leaves	S	EtOH	17	-
			roots	M	EtOH	21	-
			woody stems	N	EtOH	16	512
Rubiaceae	*Galium verum* L.	CQ-177	leaves; stems; flowers	N	EtOH	27	-
Scrophulariaceae	*Verbascum sinuatum* L.	CQ-218	leaves; stems; flowers	N	EtOH	34	-
	Verbascum thapsus L.	CQ-172	stems	M	EtOH	31	-
			leaves	M	EtOH	40	-
			inflorescence	M	EtOH	38	-
Ulmaceae	*Ulmus minor* L.	CQ-195	leaves	N	EtOH	23	-
			woody parts	M	EtOH	-	-
Urticaceae	*Parietaria diffusa* Mert. & Koch	CQ-212	leaves; stems; fruits; roots	M	EtOH	19	-
	Urtica dioica L.	CQ-179	leaves; stems; flowers	S; M; F	EtOH	36	-
Valerianaceae	*Centranthus ruber* (L.) DC.	CQ-143	leaves; stems; inflorescence	M	EtOH	31	-
Vitaceae	*Vitis vinifera* var. *aglianico*	CQ-209	wine	S; F		-	-
			stems	N	EtOH	-	-
			fruits	F	EtOH	21	-
			leaves	N	EtOH	22	-

*Ethnobotanical use of specific plant part(s) in the study region: S = medicinal application to skin; M = medicinal application not involving the skin; C = cosmetic applications; A = agricultural tool; O = ornamental; R = ritual or spiritual use; F = food; N = no reported use.

**percent inhibition for δ-toxin based on an initial screening concentration of 64 μg/mL for MRSA PFT USA500.

***MIC values are reported as μg/mL for MRSA PFT USA500.

"-"signifies no inhibitory activity