Assessment of the anti-virulence potential of extracts from four plants used in traditional Chinese medicine against multidrug-resistant pathogens

Zhonghui Pu 1,2*, Huaqiao Tang 1,2*, Nana Long 1,2, Min Qiu 1, Mingxiang Gao 3, Fenghui Sun 1,2* and Min Dai 1,2*

Abstract

Background: Multidrug-resistant pathogens are resistant to many antibiotics and associated with serious infections. *Amomum tsao-ko* Crevost et Lemaire, *Sanguisorba officinalis*, *Terminalia chebula* Retz and *Salvia miltiorrhiza* Bge, are all used in Traditional Chinese Medicine (TCM) against multidrug-resistant pathogens, and the purpose of this study was to evaluate the antibacterial and anti-virulence activity of extracts derived from them.

Methods: The antibacterial activity of ethanol and aqueous extracts from these four plants was examined against several multi-drug resistant bacterial strains, and their anti-virulence potential (including quorum quenching activity, biofilm inhibition, and blocking production of virulence factors) was assessed against different *S. aureus* strains. The chemical composition of the most effective extract was determined by LC-FTMS.

Results: Only extracts from *S. officinalis* and *A. tsao-ko* were shown to exhibit limited growth inhibition activity at a dose of 256 μg·mL⁻¹. The *S. officinalis* ethanol extract, the ethanol and aqueous extract of *A. tsao-ko*, and the aqueous extract of *S. miltiorrhiza* all demonstrated quorum quenching activity, but didn’t significantly inhibit bacterial growth. The ethanol extract of *S. officinalis* inhibited bacterial toxin production and biofilm formation at low concentrations. Chemical composition analysis of the most effective extract of *S. officinalis* showed that it mainly contained saponins.

Conclusions: The most active extract tested in this study was the ethanol root extract of *S. officinalis*. It inhibited δ-toxin production and biofilm formation at low concentrations and saponins may be its key active components. While the four plants showed no direct antibacterial effects, their anti-virulence properties may be key to fighting bacterial infections.

Keywords: TCM plants, Quorum sensing inhibition, Virulence, Biofilm
Background
The advent of antibiotics in the early twentieth century greatly reduced the mortality associated with infection. However, overuse or abuse of antibiotics has led to bacterial resistance becoming a serious global public health problem [1]. Drug-resistant bacterial infections have a huge burden on healthcare systems, veterinary practices, and society in general, and have an impact on a wide range of sectors, from farms to public health [2]. The antibiotic resistance levels of a large number of drug-resistant pathogens, including Enterobacteriaceae, Campylobacter, and Candida, are increasing [3]. Some bacteria are, or are about to become, resistant to almost all antibiotics, such as the Carbapenem-resistant Enterobacteriaceae and the multi-drug resistant Acinetobacter [4]. The consequences of drug-resistant bacteria infections are serious and can include an extended duration of illness, longer hospital stays, higher mortality rates, and increased hospitalization costs [5]. Bacterial resistance to antibiotics not only threatens health, but also brings economic losses.

Antibiotic abuse, including incorrect usage, inaccurate dosages, frequency of use, and improper treatment, will lead to bacterial resistance and eventually promote the emergence of super bacteria [6]. Fluoroquinolones are used in large quantities in China, and resistance to these antibiotics has reached 60% [7]. In the past, skin and visceral infections caused by Staphylococcus aureus were effectively treated with penicillin, but methicillin-resistant Staphylococcus aureus has now become the dangerous pathogens in nosocomial infections [8]. After carbapenem was introduced to China it became one of the main antibacterial drugs used to treat severe bacterial infections, but recently, there is an increasing in the number of carbapenem-resistant bacteria, with the most common being Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Streptococcus pneumoniae, followed by Salmonella [9]. A combination of the decline in the antibacterial efficacy of antibiotics and a lack of research on new antibiotics, means that a new anti-infection strategy should be established, and the antivirulence method is thought to be a potentially effective way [10].

Traditional Chinese medicine (TCM) is a unique natural resource in China, with a long history in preventing and treating infectious diseases [11, 12]. The biggest feature and advantage of its anti-infection medicine is that it exerts anti-infective effects at an overall level. While TCMs have anti-infective properties, many also have antipyretic and anti-inflammatory effects [13]. TCMs anti-infective properties are strengthened by their functional enhancement of the body’s immune system [14]. Studies have shown that the bacteriostatic effect of TCM is related to the molecular structure of its active ingredients [15]. However, there have been minimal studies on the use of TCM to combat infection through anti-virulence mechanisms [16].

In this study, we selected four plants commonly used in TCM; Amomum tsako Crevost et Lemaire, Sanguisorba officinalis, Terminalia chebula Retz, and Salvia miltiorrhiza Bge. We examined the antibacterial activity of ethanol and aqueous extracts from these plants against the following multi-drug resistant bacterial strains, A. baumannii, E. aerogenes, E. cloacae, E. faecium, K. pneumoniae, P. aeruginosa, and S. aureus, and tested their anti-virulence potential against various S. aureus strains. We used S. aureus biosensor strains to assess the quorum sensing inhibition potential of the extracts, and to evaluate the δ-toxin synthesis as and biofilm formation inhibition effects of the most active extracts.

Methods
Preparation of the different extracts
Four plants commonly used in TCM (fruits of Amomum tsako Crevost et (#:20180705), root of Sanguisorba officinalis (#:1811096), fruits of Terminalia chebula (#:1811035) and root of Salvia miltiorrhiza (#:1811035) were purchased from Sichuan Neo-Green Pharmaceutical Technology Development Co., Ltd. (Pengzhou, China), in September 2018 and identified by Prof. Min Li.

Material from A.tsaoko, S. officinalis, T. chebula, and S. miltiorrhiza were air-dried and ground into a powder. To prepare the ethanol raw extracts, the powder was macerated at a ratio of 1:10 (w / v) in 1 L flasks for 72 h in 70% ethanol with regular agitation. The macerate was filtered and stored, and the residual plant material was reprocessed in 70% ethanol. The two macerates were combined and evaporated under reduced pressure using a rotary evaporator at ≤40°C to obtain a dark brown residue, which were dissolved in dH2O and shell frozen in a dry ice-acetone bath before being lyophilized. Dried extracts were stored in scintillation vials at –20°C. Each plant sample (30 g) was subjected to distilled water (1:10 ratio w/v) for 20 min on a hot plate, followed by centrifugation and vacuum filtration to obtain aqueous extracts. The all extracts were dissolved to 10 mg mL-1 in DMSO for biological assays.

Bacterial strains and culture conditions
The multi-drug resistant bacteria used in this study (Table 2 and Table S1): Acinetobacter baumannii (EU-24, CDC-33), Enterobacter aerogenes (CDC-7) and Enterobacter cloacae (CDC-32); Enterococcus faecium (EU-49, EU-44), Klebsiella pneumoniae (EU-32, CDC-76), Pseudomonas aeruginosa (PAO1, CDC-54), and Staphylococcus aureus (AH845, NRS249, NRS232,
NRS252), which were stored in Quave’s Lab (Emory University, Full details in Supplementary Table S1). All strains were grown on trypticase soy agar (TSA) plates and incubated at 37 °C for 12 h, then single colonies were picked and cultured in either Cation-adjusted Mueller Hinton II broth (CAMHB) or tryptic soy broth (TSB). All antibacterial tests were repeated three times and multiple independent experiments performed.

**Growth inhibition analyze**

Extracts were screened for growth inhibition activity as previously reported [17]. Overnight liquid cultures grown in CAMHB were diluted to a confluence of 5 × 10^5 CFU mL^{-1} as determined by on their optical density (OD_{600}). Assays were conducted in 96-well plates (CELL STAR 655–185). Plates were incubated for 24 h and the OD_{600} were read by a Cytation-3 multimode plate reader (Biotek), after which, the percentage of growth inhibition was calculated. The OD was also used to calculate the IC_{50}, and where the IC_{50} ≤ 256 μg·mL^{-1}, the dose-dependent anti-bacterial activity of the extract was tested.

**Agr reporters were used for quorum sensing assay**

The quorum sensing inhibition activity of the extracts was evaluated by using the accessory gene regulator (agr) reporters of *S. aureus*, including AH1672, AH430, AH1747, and AH1872, according to previously described [10]. Quorum sensing inhibition activity of the extracts was equal to the reporter strains fluorescent protein (YFP) signal. The agr reporter strains were grown in TSA and maintained TSB, supplemented with chloramphenicol (10 μg·mL^{-1}). All anti-quorum sensing assays were conducted in black 96-well microtiter plates (Costar 3603, final well volume: 200 μL). Plates were incubated in a humidified shaker at 37 °C at 1200 rpm (Stuart S1505). The OD_{600} and fluorescence (493 nm excitation, 535 nm emission) were measured by a plate reader at 0 h and 22 h incubation (BioTek Cytation3). The initial concentration of 256 μg·mL^{-1} were selected to against agr 1–4 reporter strains. Dose response curves were tested by 2-fold serial dilutions method for a final concentration range from 2 to 256 μg·mL^{-1}.

**Quantification of δ-toxin production**

The most active extracts determined by the agr reporter assay were selected to against the δ-toxin production of *S. aureus* strains (AH1262 and NRS249) as previously described [18]. The sub-inhibitory concentrations of the extracts with a final volume of 1.5 mL were used in the assay. The tubes were incubated at 37 °C/275 °C, 60 sheath gas, source voltage of 5.00 kV, source temperature of 275.0 °C, 60 sheath gas, source voltage of 5.00 kV, source current of 100.0 μA, and capillary voltage of −90.0 or +32.0 V. The presumed formula of extract components was determined using X-caliber software to perform isotope abundance analysis of the high-resolution mass spectrometry data and report the best matching empirical formula. Database searches were performed using Scifinder (American Chemical Society) and Natural Product Dictionary (Taylor & Francis Group) and compounds with molecular masses corresponding to the
above LC-FTMS data which had been previously identified from the same plant species were reviewed.

Statistical analysis
All of the data were analyzed with a two-tailed Student’s t-test by GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA). Cultures treated with DMSO or dH2O were used as vehicle controls, which were compared to extracts treated samples for all statistical analyses. *\( p < 0.05 \) or ** \( p < 0.01 \) was considered to be statistically significant.

Results
Limited growth inhibitory activity was confirmed in ESKAPE pathogens
To determine their growth inhibition activity, crude extracts (Table 1) were screened against each ESKAPE pathogen at 256 \( \mu \text{g} \cdot \text{mL}^{-1} \). Dose–response experiments were performed for extracts when the inhibition percentage above 40 for any individual strain. After the initial library screen, the EtOH and aqueous \( S. \text{officinalis} \) extracts and the EtOH \( A. \text{tsaoko} \) extracts showed significant activity and were therefore evaluated from further assay. The inhibitory activity of the EtOH and aqueous extracts is shown in Table 2.

Active extracts exhibited dose related antibacterial activity
As shown in Fig. 1, the \( S. \text{officinalis} \) ethanol extract inhibited \( A. \text{baumannii} \) strain (CDC-33) and \( S. \text{aureus} \) (AH845) growth with an IC50 of 256 \( \mu \text{g} \cdot \text{mL}^{-1} \). The \( S. \text{officinalis} \) aqueous extract inhibited \( A. \text{baumannii} \) strain (CDC-33) with an IC50 of 256 \( \mu \text{g} \cdot \text{mL}^{-1} \) and the ethanol extract inhibited \( A. \text{baumannii} \) strain (EU-24) and \( P. \text{aeruginosa} \) strain (CDC-54), with an IC50 of 128 and 256 \( \mu \text{g} \cdot \text{mL}^{-1} \) respectively.

Table 1 The parts used, extract ID, extraction solvent and yields of the plant species

| Plant species | Part used | Extract ID | Extract solvent | Yield (%) |
|---------------|-----------|------------|-----------------|-----------|
| \( T. \text{chebula} \) | Fruits | CDY 1 | EtOH | 7.64 |
| | | CDY 2 | dH2O | 9.35 |
| \( S. \text{officinalis} \) | Root | CDY 3 | EtOH | 10.46 |
| | | CDY 4 | dH2O | 7.82 |
| \( A. \text{tsaoko} \) | Fruits | CDY 5 | dH2O | 7.56 |
| | | CDY 6 | EtOH | 7.38 |
| | Fruits peel | CDY 7 | dH2O | 8.93 |
| \( S. \text{miltiorrhiza} \) | Root | CDY 8 | dH2O | 8.25 |
| | | CDY 9 | EtOH | 7.84 |

Extracts exhibited quorum quenching activity in \( S. \text{aureus} \)
In \( S. \text{aureus} \), the accessory gene regulator (agr) system plays an important role in the production of virulence factors by quorum-sensing component pathways [22]. The four allelic groups on the agr gene locus, agr I–IV [23], have been confirmed by genetic and agr-inhibiting methods [24]. We screened the quorum quenching activity of these extracts against \( S. \text{aureus} \) and the results are shown in Fig. 2. The \( S. \text{officinalis} \) EtOH extract exhibited dose dependent quorum quenching activity against Agr 1–4, but showed no anti-bacterial activity against Agr 2–4. The organic and aqueous extracts of \( A. \text{tsaoko} \) showed no anti-bacterial activity against the Agr strains, but significantly inhibited the quorum sensing of Agr 1–3. All other extracts, except for the \( S. \text{miltiorrhiza} \) aqueous extract, showed quorum quenching activity against Agr 1.

Table 2 The growth inhibition results of selected ESKAPE pathogens by four medicinal plant samples

| Plant Species | Extract ID | \( A. \text{baumannii} \) | \( E. \text{aerogenes} \) | \( E. \text{cloacae} \) | \( E. \text{faecium} \) | \( K. \text{pneumoniae} \) | \( P. \text{aeruginosa} \) | \( S. \text{aureus} \) |
|---------------|------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| \( T. \text{chebula} \) | CDY 1 | – | – | – | – | – | – | – |
| | | – | – | – | – | – | – | – |
| \( S. \text{officinalis} \) | CDY 3 | + | – | – | – | – | – | + |
| | | – | – | – | – | – | – | – |
| \( A. \text{tsaoko} \) | CDY 5 | – | – | – | – | – | – | – |
| | | – | + | – | – | – | + | – |
| \( S. \text{miltiorrhiza} \) | CDY 8 | – | – | – | – | – | – | – |

Note: ‘+’ = growth inhibition ≥40% vs. vehicle control; ‘−' = <40% growth inhibition vs.vehicle control
Extracts block production of virulence factor δ-toxin in *S. aureus*

The strains AH 1263 and NRS 249 produce high levels of δ-toxin. Toxin levels were measured after strains were treated with CDY 3, 5, 6, and 9 (extracts which exhibited quorum quenching activity). CDY 3, 6 and 9 significantly inhibited δ-toxin production in *S. aureus* (NRS 249) and CDY 3, 5, 6, and 9 showed dose dependent inhibition of δ-toxin produced by *S. aureus*. All inhibitory effects were produced without inhibiting bacterial growth (Fig. 3). CDY 3 exhibited the most effective anti-δ-toxin activity at a concentration of 128 μg·mL⁻¹.

Extracts exhibit inhibition of *S. aureus* biofilm

The inhibition activity of the extracts against biofilm formation was tested with *S. aureus* strains UAMS-929 and UAMS-1. Sar is a winged helix transcription factor that can regulate biofilm formation. UAMS-929 has the *sar*
gene knocked out and cannot produce biofilms and thus is used as a control. CDY3 showed the best biofilm inhibition activity in a dose related manner, CDY6 and CDY9 also inhibited biofilm formation, but only at higher doses, and CDY5 exhibited limited biofilm inhibition. The results are shown in Fig. 4.

Chemical characterization of active extracts
Characterization of the major constituents of the ethanol extract of *S. officinalis*. All peaks correspond to the data presented in Supplementary materials. Table S2 and S3, and putative structural matches are listed by peak number (Fig. 5). Peak 1 was determined to be C_{36}H_{58}O_{9} and putative structural matches include; Olean-12-en-28-oic acid, 3,19-dihydroxy-, β-D-glucopyranosyl ester, (3β, 19α). Peak 2 was determined to be C_{41}H_{64}O_{13} and putative structural matches include; β-D-Glucopyranose, 1-[(2S,4aS,4bR,6aR,8S,10aS,10bR)-8-(α-L-arabinopyranosyloxy)-2,3,4,4a,4b,5,6,6a,7,8,9,10,10a,10b-tetrahydro-4a,4b,7,7,10a-pentamethyl-2-[(3R)-3-methyl-4-oxopentyl]-2-chrysenecarboxylate]. Peak 5 was assayed to be C_{36}H_{56}O_{12} and putative structural matches include; Urs-12-ene-23,28-dioic acid, 2,3,19-trihydroxy-, β-D-glucopyranosyl ester, (2α, 3β, 4α). Peak 6 was determined to be C_{30}H_{62}O and putative structural matches include; D-Ribofuranoside, methyl 3-O-methyl-2-C-[(3,4,5-trimethoxybenzoyl) oxy] methyl]-, 5-(3,4,5-trimethoxybenzoate). Peak 9 was determined to be C_{35}H_{57}O_{8} and putative structural matches include; Olean-12-en-28-oic acid, 3-(α-L-arabinopyranosyloxy)-19-hydroxy-, (3β,19α)-. Peak 10 was determined to be C_{30}H_{60}O and putative structural matches include; 1-Triacontanol. Peak 11 was determined to be C_{36}H_{56}O_{10} and putative structural matches include; Urs-12-en-28-oic acid, 2,3,19-trihydroxy-, β-D-glucopyranosyl ester, (2α,3α). Peak 12 was determined to be C_{30}H_{45}O_{5} and putative structural matches include; Urs-12-en-28-oic acid, 19-hydroxy-3,11-dioxo-. This shows that the active ingredients of the EtOH extract mainly include saponins, flavonoids, phenolic glycosides, and lignins.

Discussion
Traditional Chinese Medicine and its compounds have comprehensive therapeutic effects including inhibiting virus replication, preventing virus-induced cytopathy, and regulating immune function, as well as analgesic and anti-inflammatory properties. It therefore has unique advantages and broad development prospects for the prevention and treatment of infectious diseases [25]. However, many studies have shown that the anti-infective effect of Chinese medicine may not depend on its anti-bacterial properties, and some traditional medicines used to treat infections do not exhibit any antibacterial effects. In this study, only the organic and aqueous extracts of *S. officinalis* and the ethanol extract of *A. tsaoiko* exhibited moderate anti-bacterial activity. The *S.
officinalis ethanol extract had better anti-bacterial activity than the aqueous extract [26] and was effective against both A. baumannii and S. aureus, whereas the aqueous extract was only active against A. baumannii. In previous studies, the S. officinalis extract exhibited weak antibacterial activity, and the polyphenolic ingredients possessed antibacterial activity with an MIC were from 0.78 to 25 mg/mL when tested against different pathogens, including Gram-negative bacteria (Escherichia coli and Salmonella typhimurium) and Gram-positive bacteria (Staphylococcus aureus, Listeria monocytogenes and Bacillus subtilis) [27]. The A. tsaoko ethanol extract exhibited anti-bacterial activity against A. baumannii and P. aeruginosa, whereas the aqueous extract did not. Most previous studies have examined the antibacterial activity of the extract or volatile oil of A. tsaoko, and although some results implied that they had antibacterial effects, this may be due to the larger concentrations used in these studies [28, 29]. S. officinalis is a TCM, which is used to treat hemorrhoids, wounds, and ulcers in Eastern Asian countries [30]. Previous research involving extraction and pharmacological studies of this herb have been carried out to assess its anti-viral, anti-inflammation, anti-bacterial, anti-tumor, and immunomodulation properties [31–33]. A. tsaoko is widely used in TCM to treat stomach disorders, malaria, throat infections, diarrhea, dyspepsia, nausea, and abdominal pain, and some researchers have reported it to have broad anti-bacterial, anti-tumor, and anti-inflammatory activity [34–36]. In this study, we have made the first evaluation the activity of these extracts on multidrug-resistant bacteria, and shown that they had limited growth inhibition activity. Some previous studies support our conclusion by showing that most of these extracts only have antibacterial activity when used at mg level concentrations.

Because these extracts exhibited limited anti-bacterial activity, this raises the question of why A. tsaoko, S. officinalis, T. chebula, and S. miltiorrhiza are still used in TCM to treat infectious diseases [37–39]. Recent studies have shown that information exchange occurs between bacteria. Many bacteria can synthesize and release a signaling molecule called autoinducer (AI), the extracellular concentration of which increases as bacterial density reaches a critical concentration. AI can activate the expression of related genes and regulate the biological behavior of bacteria, including the production of toxins, biofilms, antibiotics, spores, and fluorescence to adapt to...
changes in the environment. This phenomenon is called quorum sensing [40]. Since this induction phenomenon occurs only after the bacterial density reaches a certain threshold, some people also call this phenomenon cell density dependent control of gene expression [41]. Quorum sensing is closely related to the infectious capacity and pathogenicity of bacteria, and so quorum sensing inhibitors have attracted increasing attention from drug researchers [41]. Research on quorum-sensing inhibitors is considered to be a powerful direction for the study of new antibacterial drugs [42]. We therefore evaluated the quorum quenching activity of the nine extracts on Agr1–4 reporter strains. Most extracts exhibited dose related quorum quenching activity on at least one Agr strain. The S. officinalis ethanol extract, the A. tsaoko organic and aqueous extracts, and the S. miltiorrhiza ethanol extract all exhibited quorum quenching activity without significantly inhibiting bacterial growth. A recent study evaluated the anti-QS activity of the A. tsaoko extract using Chromobacterium violaceum a biosensor.
strain. They showed that it exhibited anti-QS activity in a dose-dependent manner at a concentration range of 0.5–4 mg/mL [28]. Our study is the first one to use agr reporter strains to evaluate the quorum quenching activity of these extracts.

Quorum sensing were used by bacteria to coordinate certain behaviors, including biofilm formation, virulence, and resistance to antibiotics, depending on their local population density [43, 44]. The extracts with quorum quenching activity (CDY3, 5, 6 and 8) were further evaluated for their ability to inhibit toxin production and biofilm formation. The ethanol extract of S. officinalis, the aqueous extract of A. tsako, and the ethanol extract of S. miltiorrhiza all inhibited δ-toxin production in both S. aureus strains NRS 249 and AH1263 without affecting bacterial growth. All four extracts with quorum quenching activity also inhibited the biofilm formation in UAMS-1, with the ethanol extract of S. officinalis and the aqueous extract of A. tsako exhibiting better inhibition activity. The anti-quorum sensing and anti-biofilm activities of A. tsako on food borne pathogens have been reported [28]. The ethanol extract of S. officinalis has been shown to inhibit the biofilm formation of MRSA [45]. Some of the polyphenolic compounds in S. officinalis have strong anti-bacterial activity [46]. Some polyphenolic compounds were found in our results and play an important role in inhibiting quorum sensing.

**Conclusion**

In conclusion, our study has shown that the ethanol extract of S. officinalis has considerable anti-infection capacity against five bacterial strains, and S. aureus in particular. It can inhibit bacterial toxin production and biofilm formation at low concentrations and does not promote bacterial proliferation, but can kill bacteria when used at high concentrations. The polyphenolic compounds were highly associated with its antibacterial and quorum quenching activities. However, the underlying mechanisms still need to be better elucidated in further studies. This is the first comprehensive study examining the anti-virulence activities of the S. officinalis extract via its quorum quenching mechanism, and our results show that it has potential as a novel, natural, anti-infection medicine.

**Supplementary information**

The online version contains supplementary material available at https://doi.org/10.1186/s12906-020-03114-2.

**Additional file 1**: Table S1. ESKAPE pathogens tested and their corresponding antibiotic resistance profiles as reported by the source provider. Table S2. Negative ESI Mass spectrometry (m/z) analysis of extract CDY3; peaks with > 0.5% relative abundance is listed. Table S3. Positive ESI Mass spectrometry (m/z) analysis of extract CDY3; peaks with > 0.5% relative abundance is listed.

**Abbreviations**

WHO: World Health Organization; MRSA: Methicillin-resistant *Staphylococcus aureus*; agr: Accessory gene regulator; HPLC: High Performance Liquid Chromatography

**Acknowledgements**

All the tests were conducted in Quave’s Lab (Emory University, USA). We really thank Dr. Quave’s kindly help in the methods and supply for necessary experiment materials.

**Authors’ contributions**

1. ZP, NL and HT; Methodology, FS and MT; Project administration, MQ and MG; Resources, Writing – original draft, HT; Writing – review & editing, ZP and HT; All authors have read and agreed to the published version of the manuscript.

**Funding**

This work was supported by the National Natural Science Foundation of China (No. 31970137), the Benefit People Project of Science and Technology of Chengdu Science and Technology Bureau (No.2016-HM01-00362-SF), the Scientific Research Fund of Chengdu Medical College (No. CYZ18-18) and the Development and Regeneration Key Laboratory of Sichuan Province, Chengdu Medical College (No.SYS19-08).

**Availability of data and materials**

The data used to support the findings of this study are available from the corresponding author upon request.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

None of the authors has any conflict of interests regarding this work.

**Author details**

1School of Laboratory Medicine, Chengdu Medical College, Chengdu, Sichuan, People’s Republic of China. 2Laboratory of Veterinary Drug Residue Prevention and Control Technology of Animal-derived Food, Chengdu Medical College, Chengdu, Sichuan, People’s Republic of China. 3School of Clinical Medical Sciences, Chengdu Medical College, Chengdu, Sichuan, People’s Republic of China.

**Received**: 9 May 2020 **Accepted**: 9 October 2020

**Published online**: 19 October 2020

**References**

1. Spellberg B, Bartlett JS, Gilbert DN. The future of antibiotics and resistance. N Engl J Med. 2013;368(4):299–302.
2. Frieti M, Kumar K, Boutin A. Antibiotic resistance. J Infect Public Health. 2017;10(4):369–78.
3. Banin E, Hughes D, Kuipers OP. Bacterial pathogens, antibiotics and antibiotic resistance. FEMS Microbiol Rev. 2017;41(3):450–2.
4. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelsson M, Monnet DL, Pulcini C, Kahlmeter G, Klymowska J, Carmeli Y. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):218–27.
5. Fernandes P, Martens E. Antibiotics in late clinical development. Biochem Pharmacol. 2017;133:152–63.
6. Z-F L, Zong Q. Super bacteria and their treatment. World Clin Drugs. 2010;11.
7. Liu X, Steele JC, Meng X-Z. Usage, residue, and human health risk of antibiotics in Chinese aquaculture: a review. Environ Pollut. 2017;223:161–9.
8. Kallen AJ, Mu Y, Bulens S, Reingold A, Petit S, Gerschman K, Ray SM, Harrison LH, Lynfield R, Dumyati G. Health care-associated invasive MRSA infections, 2005–2008. JAMA. 2010;304(6):641–7.
9. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med. 2012;18(5):263–72.
10. Muhs A, Lyles JT, Parlet CP, Nelson K, Kavanagh JS, Horwills AR, Quave CL. Virulence inhibitors from Brazilian peppertree block quorum sensing and abate dmemomcris in skin infection models. Sci Rep. 2017;7:42275.

11. Wong R, Hägg U, Samaranayake L, Yuen M, Seneviratne C, Kao R. Antimicrobial activity of Chinese medicine herbs against common bacteria in oral biofilm. A pilot study. Int J Oral Maxillofac Surg. 2010;39(6):599–605.

12. Wang, J, Cui M, Jiao H, Tong Y, Xu J, Zhao Y, Han M, Liu J. Content analysis of systematic reviews on the effectiveness of traditional Chinese medicine. J Tradit Chin Med. 2013;33(2):156–63.

13. Chen KC, Sun MF, Yang SC, Chang SS, Chen HY, Tsai FJ, Chen YC. Investigation into potent inflammation inhibitors from traditional Chinese medicine. Chem Biol Drug Des. 2011;78(4):679–88.

14. Ma H-D, Deng Y-R, Tian Z, Lian Z-X. Traditional Chinese medicine and immune regulation. Clin Rev Allergy Immunol. 2013;44(3):229–41.

15. Lau D, Plotkin BJ. Antimicrobial and biofilm effects of herbs used in traditional Chinese medicine. Nat Prod Commun. 2013;8(1):1617–20.

16. Khan MF, Tang H, Lyles JT, Pineau R, Quave CL. Antibacterial properties of medicinal plants from Pakistan against multidrug-resistant ESRAE pathogens. Front Pharmacol. 2018;9(15).

17. Schultz F, Anway G, Tang H, Chassagne F, Lyles JT, Garbe LA, Quave CL. Targeting ESRAE pathogens with anti-infective medicinal plants from the greater Mpi region in Uganda. Sci Rep. 2020;10(1):11193.

18. Quave CL, Horwills AR. Identification of Staphylococcal Quorum Sensing Inhibitors by Quantification of ω-Hemolysin with High Performance Liquid Chromatography. Methods Mol Biol. 2018;1673:363–70.

19. Quave CL, Horwills AR. Identification of staphylococcal quorum sensing inhibitors by quantification of ω-hemolysin with high performance liquid chromatography. In: Quorum Sensing edn. Springer; 2018:363–70.

20. Quave CL, Estévez-Camorna M, Camparde CM, Hobby G, Hendrickson H, Beenken KE, Blevins JS, Smeltzer MS. Ellagic acid derivatives from Rubus ulmifolius inhibit Staphylococcus aureus biofilm formation and improve response to antibiotics. PLoS One. 2012;7(1). https://doi.org/10.1371/journal.pone.0028737.

21. Beenken KE, E levins JS, Smeltzer MS. Mutation of sarA in Staphylococcus aureus limits biofilm formation. Infec Immun. 2003;71(7):4206–11.

22. Quave CL, Lyles JT, Kavanagh JS, Nelson K, Parlet CP, Crosby HA, Helli mann KP, Horwills AR. Castanea sativa (European chestnut) leaf extracts rich in Ursene and Oleane n derivatives block Staphylococcus aureus virulence and pathogenesis without detectable resistance. PLoS One. 2015;10(8): e0136486.

23. Robinson DA, Monk AB, Cooper JE, Feli EI, Enright MC. Evolutionary genetics of the access gene regulator (agr) locus in Staphylococcus aureus. J Bacteriol. 2005;187(24):8312–21.

24. Park J, Jagasisa R, Kaufman GF, Mathison JC, Ruiz DI, Moss JA, Meijler MM, Beenken KE, Blevins JS, Smeltzer MS. Rubus ulmifolius ellagic acid derivatives inhibit Staphylococcus aureus biofilm formation. Infect Immun. 2003;71(7):4206–11.

25. Gavron-Gzella A, Witkowska-Banaszczak E, Bylka W, Dudek-Makuch M, Odwrot A, Skrodzka N. Chemical composition, antioxidant and antimicrobial activities of Sanguisorba officinalis L. Extracts. Pharm Chem J. 2016;50(4):244–9.

26. Basset SL, Ng WL, Perez LL, Cong J, Semmelhack MF. Broad spectrum pro-quorum-sensing molecules as inhibitors of virulence in vibrios. In Google Patents; 2017.

27. Kalia VC, Patel SK, Kang YC, Lee J-K. Quorum sensing inhibitors as antho physiotical applications. Biotechnol Adv. 2019;37(1):158–72.

28. Chen X, Shang F, Meng Y, Li C, Cui Y, Zhang M, Qi K, Xue T. Ethanol extract of Sanguisorba officinalis L. inhibits biofilm formation of methicillin-resistant Staphylococcus aureus in an In vitro resistant manner. J Dairy Sci. 2015;98(2):8469–9.

29. Pinho E, Ferrreira ICF, Barros L, Carvalho AM, Soares G, Henriques M. Antibacterial potential of northeastern Portugal wild plant extracts and respective phenolic compounds. Biomed Res Int. 2014;2014:814550.