Malaysian Journal of Microbiology, Vol 8(1) 2012, pp. 11-20
http://dx.doi.org/10.21161/mjm.34911

Anti-quorum sensing and antimicrobial activities of some traditional Chinese medicinal plants commonly used in South-East Asia

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Received 16 July 2011; received in revised form 25 August 2011; accepted 18 October 2011

ABSTRACT

Aims: Traditional Chinese medicine (TCM) has been used for relief and treatment of ailments dating back thousands of years and continues to the present day, with rapidly increasing interest in evidence-based evaluation of its efficacy. Studies of TCM plants have demonstrated that several have antimicrobial properties but few have explored their anti-quorum sensing potential. Quorum sensing (QS), also known as bacterial cell-to-cell communication, is used by a number of opportunistic pathogenic bacteria in the regulation of virulence expression. Compounds that interfere with QS signals and attenuate bacterial virulence without killing them may offer an alternative therapeutic solution with less pressure of antibiotic resistance developing. This study screened TCM plants for anti-quorum sensing properties and antimicrobial activities.

Methodology and Results: Twenty TCM plants commonly used in South-East Asia were screened for QS inhibitors using two biomonitor strains, *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* PAO1. Ten of these selected TCM plant (50%) were found to have QS inhibitory properties: *Angelica sinensis* (Umbelliferae), *Cnidium monnieri* (Umbelliferae), *Astragalus membranaceus* (Leguminosae), *Crataegus cuneata* (Rosaceae), *Dioscorea nipponica* (Dioscoreaceae), *Lilium brownii* (Liliaceae), *Aloe barbadensis* (Liliaceae), *Magnolia officinalis* (Magnoliaceae), *Ephedra sinica* (Ephedraceae) and *Panax pseudoginseng* (Araliaceae). Of these, six (30%) also showed varying antimicrobial activity against *C. violaceum* and *P. aeruginosa*.

Conclusion, significance and impact of study: The results suggest that traditional Chinese medicinal plants could be a prospective source to explore for useful compounds in the fight against bacterial infections.

Keywords: anti-quorum sensing, antimicrobial, traditional Chinese medicine, screening, plant extracts

INTRODUCTION

Interest and use of complementary and alternative medicine (CAM) is increasingly prevalent worldwide (Fisher and Ward, 1994; Ernst, 2000; Giovannini *et al.*, 2004). Among the therapies and interventions under CAM, traditional Chinese medicine (TCM) is among the better known (WHO, 2002). It has been used in the treatment of diseases for thousands of years and is a system of medicine that is popularly perceived to work (Wee and Keng, 1990). This however does not imply, as with other forms of medicines, every TCM therapy is effective and intensive research is ongoing to provide evidence of their efficacies. Studies on TCM for drug discovery have focused mainly on its antimicrobial potential (Chan *et al*., 2008; Seneviratne *et al*., 2008). Much less attention has been given to its other possible anti-pathogenic properties. The inhibition of quorum sensing (QS), or bacterial cell-to-cell communication, is one example of an anti-pathogenic effect of TCM that has been less investigated (Koh and Tham, 2011).

Quorum sensing is a mechanism used by many bacteria to detect their critical cell numbers (Greenberg, 2000; Parsek and Greenberg, 2005). Cell densities are indicated by concentration of signal molecules that regulate expression of specific genes for diverse cellular functions. The signal molecules released by bacteria on reaching a critical threshold concentration bind to receptors and trigger a concerted response from the bacteria as a whole population (Bassler, 2002). A major QS circuit primarily used by Gram negative bacteria is the LuxR/I system (Parsek and Greenberg, 2000). The LuxI family of proteins produces the signal molecule acyl-homoserine lactone (AHL), a low-molecular-mass molecule, that moves in and out of cell membranes through diffusion or active transportation (Fuqua and Greenberg, 2002). The LuxR family of receptor proteins induces the expression of QS-target genes when AHL binds to it. Different bacterial species produce AHLs that vary in length from 4-18 carbons and/ or have different substitutions of the fatty N-acyl chain but contain the same homoserine lactone moiety. AHL-mediated QS systems regulate common bacteria disease processes such as swarming, biofilm formation and secretion of virulence factors. QS systems have been shown to operate in important human and plant pathogens such as *Pseudomonas aeruginosa* and *Erwinia carotovora*, respectively (Cui *et al*., 1995; Parsek and Greenberg,

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

2000). The discovery that QS plays a critical role in bacteria virulence and survival makes this signalling pathway a novel and potential target for antimicrobial drugs that can act as QS inhibitors (QSI) to interfere with and attenuate QS-dependent bacterial pathogenicity (Hentzer and Givskov, 2003; Rasmussen and Givskov, 2006). Inhibitors disrupt QS in various ways; by acting as enzymes (e.g. AHL-lactonase or AHL-acylase) which destroy signal molecules, or as enzymes that degrade LuxR protein, or as AHL mimics that block signal molecules (Zhang and Dong, 2004).

The first QSI was characterized in a seaweed (Rasmussen *et al*., 2000), and since then, QSI have also been found in six medicinal plant species from South Florida (Adonizio *et al*., 2006; 2008) and even in dietary plants such as vegetables, fruits and spices (Bauer and Teplitski, 2001; Vattem *et al*., 2007) and fungi (Zhu and Sun, 2008). Most recently, there has also been indication that herbs used in TCM have QSI properties and could be targeted for further screening (Koh and Tham, 2011). As QSI does not kill or inhibit bacteria growth, QSI drugs that interfere with signaling may have an advantage because they do not impose strong selective pressure for development of resistance, as would antibiotics (Rasmussen and Givskov, 2006). The objective of this study is to screen several TCM herbs commonly used in South-East Asia for QS-inhibitory properties in bioassays using two bioreporter species, CV026 and PAO1. CV026 is a derivative of the Gram-negative bacterium *Chromobacterium violaceum* (McClean *et al*., 1997). Wild type *C. violaceum* produces a purple pigment, violacein, when AHL reaches a threshold level. CV026 harbours a LuxR homologue and a corresponding QS-controlled promoter fused to a purple pigment (violacein)-producing gene cluster. However, its *luxI* homologue has been inactivated and it is unable to produce violacein like the wild type unless exogenous AHLs are supplied. In the presence of a mixture of exogenous AHLs and QSIs, transcription from the AHL-induced promoter is diminished and violacein production abolished, thus indicating the presence of a QSI. PAO1 is a strain of *Pseudomonas aeruginosa*, a Gram-negative swarming bacterial species. Swarming in this strain is QS dependent and inhibition of swarming would suggest possible presence of a QSI. TCM extracts revealed by the CV026 bioassay to have antibacterial properties were also further tested using Kirby-Bauer agar diffusion method for confirmation.

MATERIALS AND METHODS

Plant materials

Twenty different herbs used in TCM were selected for screening (Table 1). The criteria for selection were based on 1) ethnobotanical use in treatment against coughs, colds, diarrhoea, dysentery, inflammation and respiratory problems, and conditions with some indication of presence of microbial infection, and 2) general recognition and common usage among the Chinese population in South-East Asia. The information on the medicinal uses of the TCM herbs as indicated in Table 1 was obtained from published literature on Chinese Traditional Medicines (Wee and Keng, 1990; Zhao, 2004) and from various communications (both oral and printed) from dispensers of Chinese herbs and medicines in Singapore. TCM plants were purchased from Chinese medical halls in Singapore and voucher specimens stored in the Microbiology Lab, Natural Sciences and Science Education Department, Nanyang Technological University, Singapore. In general, for the TCM tested, 50 g of each dried sample purchased was used for extraction. Dried ground plant matter was soaked overnight in a 1:1 acetone:water mixture with shaking before filtration with Whatman No. 1 filter paper to remove particulate matter. The plant material weight to solvent volume ratio was 1/6. The filtrate was evaporated to dryness using a Heidolph rotary evaporator (Laborota 4000, Germany), freeze-dried and stored at 4 °C. For testing, 0.5 g of freeze-dried extract was reconstituted in 2 mL of sterile distilled water. A few extracts, however, remained in an oily, liquid form and could not be evaporated to dryness and were used directly in bioassays (Table 1). All extracts were filtered into autoclaved glass vials using a 0.22 µm (pore size) Iwaki filter disk to ensure sterility of the samples.

Bacterial strains

CV026, a mutant of the saprophytic bacterium species *C. violaceum* that is unable to produce the purple pigment violacein unless exogenous AHLs are supplied, was used as a reporter organism to test for QSI. The other bioreporter used was PAO1 (ATCC 27853), a wild type strain of *P. aeruginosa*. *P. aeruginosa* is an opportunistic nosocomial pathogen which uses QS signals to activate several genes involved in swarming, and colonization and persistence within immunocompromised individuals (Parsek and Greenberg, 2000). These bacterial species were also used for testing for antimicrobial activity of the herbal extracts. All strains were maintained on Luria-Bertani (LB) agar plates. Overnight cultures were grown in LB broth at 37 °C (or 30 °C for CV026) with shaking.

Bioassay for QSI using CV26

Five millilitres of molten Soft Top Agar (STA) (1.3 g agar, 2.0 g tryptone, 1.0 g sodium chloride, 200 mL deionised water) were seeded with 100 µL of an overnight LB culture of CV026, together with 20 µL of 100 µg/mL C6HSL as an exogenous AHL source. This was gently mixed and poured immediately over the surface of a solidified LBA plate as an overlay. Wells of 5 mm in diameter were made on the solidified agar of each plate. Each well was filled with 50 µL of filter-sterilised TCM extract. A positive control well contained 10 µL of 100 µg/mL C10HSL (*N*-decanoyl-L-homoserine lactone DHL, a reported antagonist of violacein synthesis (McLean *et al*., 2004) and 40 µL LB broth. Plates were incubated at room temperature (25 ± 2 °C) for 3 days. QS inhibition of violacein synthesis was detected by a turbid or creamy

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 1: Traditional Chinese medicines screened for anti-quorum sensing activity.

Plant species (Family)	Common name	Plant part	Voucher specimen number	Medicinal use[b]
Aloe barbadensis (Liliaceae)	Aloe	Leaf	TCM-SY17	As mild laxative and treatment for piles; abscesses, scabies; increases menstrual flow
Angelica sinensis (Umbelliferae)	Angelica	Root	TCM-SY1	As pain killer and treatment of cold, headache, fever; boils, abscesses, itching, diphtheria, blood in urine, vaginal discharge
Aster tataricus (Compositae)	Aster	Root	TCM-SY2	Treatment of cold, cough with sputum or blood, cough, painful menstruation
Astragalus membranaceus (Leguminosae)	Milk vetch	Root	TCM-SY3	Treatment of cold, arthritis, loss of appetite, weakness, sweating at night, numbness of muscles, boils, diarrhoea, asthma, nervousness
Atractylis ovata (Compositae)	Atractylis	Root	TCM-SY15	Treatment of indigestion, skin problems, diarrhoea, fever, stomach disorders, night blindness
Chrysanthemum indicum (Compositae)	Chrysanthemum	Flower	TCM-SY4	Treatment of skin infections, high blood pressure
Cnidium monnieri (Umbelliferae)	Cnidium	Seed	TCM-SY6	Treatment of wounds, yellow discharge from vagina, piles, scabies, kidney problem, rheumatism; increases menstrual flow
Crataegus cuneata (Rosaceae)	Hawthorn	Fruit	TCM-SY5	Treatment of diarrhoea, dysentery, stomachache after birth
Dioscorea nipponica (Dioscoreaceae)[a]	Yam	Tuber	TCM-SY7	As anti-inflammatory and treatment of rheumatoid arthritis
Dolichos lablab (Leguminosae)	Indian bean	Seed	TCM-SY14	As antidote against poisoning and treatment of colic, cholera, diarrhoea, rheumatism, sunstroke

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Ephedra sinica (Ephedraceae)	Joint fir	Branch	TCM-SY13	Teatment of asthma, influenza, coughs, fever, chronic bronchitis, rheumatism, whooping cough, sweating in the night
Gentiana scabra (Gentianaceae)	Japanese Gentian	Root	TCM-SY8	Treatment of eye inflammation, rheumatoid arthritis, jaundice, fevers, cold
Ginkgo biloba (Ginkgoaceae)	Ginkgo	Seed	TCM-SY11	Treatment of intestinal worms, coughs, asthma, bronchitis, kidney and bladder disorders, vaginal discharge, gonorrhoea
Hedyotis corymbosa (Rubiaceae)	Oldenlandia	Whole plant	TCM-SY9	Treatment of inflammation; improve circulation
Lilium brownii (Liliaceae)[a]	Lily bulb	Bulb	TCM-SY10	As sedative and tonic and for treatment of cough, lung disorders, urinary disorders, deafness, earache, nervousness, excessive gas in the system
Lycium chinense (Solanaceae)	Chinese wolfberry	Fruit	TCM-SY18	Treatment of impotence, backache, dizziness, weakness, fever, diabetes
Magnolia officinalis (Magnoliaceae)[a]	Magnolia	Bark	TCM-SY12	As antispasmodic and aphrodisiac and for treatment of phlegm, intestinal worms, spastic gastritis, peptic ulcer, diarrhoea, vomiting, typhoid fever, malaria, loss of appetite, shortness of breath, coughs
Panax pseudoginseng (Araliaceae)[a]	Ginseng	Root	TCM-SY16	As tonic, sedative, stimulant, aphrodisiac and treatment of anaemia, nervous disorders, shortness of breath, forgetfulness, excessive menstrual bleeding, impotence, fever, excessive sweating
Platycodon grandiflorus (Campanulaceae)	Chinese bell flower	Root	TCM-SY20	As tonic for indigestion and treatment of intestinal worms; stomach ulcers, dysentery, cholera, influenza, sore throat, asthma, colds, nausea, chest congestion, tonsillitis
Polygonum multiflorum (Polygonaceae)	Radix	Root	TCM-SY19	Treatment of dizziness, tuberculosis of the lymphatic glands, cancer, constipation, insomnia

[a] extracts remained in sticky or oily liquid form that could not be evaporated to dryness.
[b] Wee and Keng, 1990; Zhao, 2004; oral and printed communications from traditional Chinese medicine dispensers.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

ring of viable cells around the well against a purple background of activated CV026 bacteria (Fig. 1). A clear halo around the well would indicate antimicrobial (AM) activity. The limit of detection of activity was also determined by serial dilutions of extracts (1:1 to 1:64), using LB broth as diluent. Endpoints were estimated as the lowest dilution of the extract giving visible inhibition of violacein production. Each experiment was carried out in triplicate and all assays were repeated twice, except for dilution tests which were repeated once.

Anti-swarming in PAO1

Fifty microlitres of sterile TCM extracts were mixed into 5ml of molten STA and poured immediately over the surface of a solidified LBA plate as an overlay. The plate was point inoculated with an overnight culture of PAO1 once the overlaid agar had solidified and incubated at 37°C for 3 days. The extent of swarming was determined by measuring the area of the colony using a leaf-surface area meter (Area Meter AM200, ADC Bioscientific Ltd).

Antimicrobic sensitivity testing

Standard disc-diffusion assays were used to test the anti-microbial activity of the TCM extracts. A modified Kirby-Bauer method was used in which bacterial cultures were seeded onto the plates using a sterile cotton swab (Benson, 1998). Twenty microlitres of each TCM extract was loaded onto sterile filter paper discs (6 mm diameter), air-dried in the laminar flow hood on sterile Petri plates and placed onto Mueller-Hinton (MH) agar plates seeded with overnight cultures of CV026 and PAO1, along with 8 standard antibiotic discs (diameter 6 mm) purchased from Oxoid (UK). They were: ampicillin (AMP10), chloramphenicol (C30), kanamycin (K30), gentamicine (CN10), penicillin (P10), neomycin (N30), streptomycin (S10) and tetracycline (T30). There were three replicates per test sample and the plates were incubated overnight at 37°C (or 30°C for CV026). Antimicrobial activity was determined by measuring the diameter of the zones of inhibition, a clear area devoid of bacterial growth.

RESULTS AND DISCUSSION

Using CV026 as a bioreporter, eight out of the twenty TCM herbs (i.e. 40%) screened demonstrated QS antagonistic activity (Table 2; Figs. 1 & 2). Inhibition of violacein or purple pigment production indicating QSI by the TCM extracts was clearly visible as a creamy-white halo in the CV026 bioassay (Figs. 1 & 2). With PAO1 as a reporter organism of QSI, four out of twenty (i.e. 20%) manifested a definite effect on swarming motility, a QS-related phenotype (Table 2; Figure 3). The remaining TCM plants screened did not show noticeable QSI activity with these two reporters. The percentage of herbs producing substances with QSI properties was much higher in the present study than reported from other ethnobotanical screenings whereby only six out of 50 plants (12%) screened showed QSI (Adonizio *et al.*, 2006). This contrast suggests that the anti-QS activity of TCM on bacteria may be more ubiquitous and diverse than previously thought and that there is distinct potential for further work on other TCM species in this area of research. Indeed, in a recent preliminary screen, seven out of 10 plants (70%) used commonly in TCM were shown to have QSI properties (Koh and Tham, 2011).

Extracts from *Astragalus membranaceus* (huang qi) had the strongest QSI properties, resulting in a cream-coloured zone, which was comparable to that observed in the control containing the known QS antagonist, C10HSL (Figure 2). *A. membranaceus*, also known as milk vetch, is one of fifty fundamental herbs used in traditional Chinese medicine (National Academy of Sciences, 1975; Wee and Keng, 1990). Recent research has shown that *Astragalus* could be a potential treatment for immune systems that are chemotherapy- or radiation-compromised and may help relief symptoms of heart diseases (Brush *et al*., 2006; Zhang *et al*., 2006). In the present investigation, extracts from *Astragalus membranaceus* continued to inhibit violacein production even at 1:16 dilution (Figure 2). For the eight TCM extracts that showed activity, the lowest dilution of extract that can give discernible inhibition of violacein synthesis ranged from 1:2 to 1:16.

In the PAO1 bioassay, two of the eight TCM herbs that showed QSI activity with CV026, i.e. *Lilium brownii* (bai he) and *Panax pseudoginseng* (ren shen), also contained substances that inhibited swarming (Table 2; Figure 3). In addition, two other TCM plants that did not demonstrate QSI in CV026 but revealed anti-swarming properties were *Cnidium monnieri* (she chuang) and *Aloe barbadensis* (lu hui) (Table 2; Figure 3). Compared to the control in which no herbal extract was present, extracts from *P. pseudoginseng* reduced swarming in PAO1 by 38.8%. The other TCM herbs *C. monnieri*, *L. brownii* and *A. barbadensis* inhibited swarming by 35.2%, 21.2% and 20.1%, respectively. Pyocyanin, a blue pigment that appears green on agar, was also noticeably produced on day 4 by the PAO1 colony growing on media supplemented with *P. pseudoginseng* (Figure 3). The roots of *P.pseudoginseng*, commonly known as ginseng, have long been regarded by the Chinese as a panacea and studies show it has effects on cardiovascular healing and cancer protection (Konoshima *et al*., 1999; Paul *et al*., 2002). Although *P. pseudoginseng* substantially inhibited swarming in PAO1, it was noticed that it also induced the production of pyocyanin in older cultures. This blue-green pigment is a quorum-controlled extracellular virulence factor. It appears there may be that more than one compound present in the ginseng extract, one of which is an agonist of swarming, with others that may affect a different signalling pathway in *Pseudomonas aeruginosa* PAO1, stimulating pyocyanin synthesis. Indeed, major pathogens such as *P. aeruginosa*, have evolved with numerous QS circuits, which modulate the production of various toxins and regulate parallel QS systems (Martin *et al*., 2008). Swarming motility in itself is a complex phenotype that involves several other traits (Overhage *et*

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 2: TCM plants showing quorum-sensing inhibition activity and antimicrobial activity as detected by CV026 and PAO1.

TCM plant species and plant part	Inhibition of violacein production[a]	Inhibition of swarming[b]	Antimicrobial activity[c]	Antimicrobial activity[c]
	CV026	PAO1	CV026	PAO1
Aloe barbadensis (leaf)	No	Yes (79.9)	No	No
Angelica sinensis (root)	Yes (13.5 ± 0.3)	No*	Yes (10.7 ± 0.7)	Yes (7.7 ± 0.7)
Astragalus membranaceus (root)	Yes (34.0 ± 0.0)	No*	No	No
Cnidium monnieri (seed)	No	Yes (78.8%)	No	No
Crataegus cuneata (fruit)	Yes (14.2 ± 0.4)	No	Yes (11.7 ± 0.9)	Yes (8.8 ± 0.9)
Dioscorea nipponica (tuber)	Yes (13.8 ± 0.2)	No*	Yes (11.8 ± 2.0)	Yes (7.8 ± 2.0)
Ephedra sinica (branch)	Yes (12.0 ± 0.0)	No*	Yes (8.3 ± 0.0)	No
Lilium brownii (bulb)	Yes (17.3 ± 0.3)	Yes (64.8)	Yes (15.0 ± 0.6)	Yes (9.5 ± 0.6)
Magnolia officinalis (bark)	Yes (23.7 ± 0.3)	No*	Yes (13.7 ± 0.9)	Yes (6.7 ± 0.9)
Panax pseudoginseng (root)	Yes (12.7 ± 0.3)	Yes (60.2)	No	No
Standard antibiotics tested:				
Ampicillin (AMP10)	-	-	No	No
Chloramphenicol (C30)	-	-	Yes (26.3 ±6.2)	Yes (13.3 ± 5.6)
Gentamicin (CN10)	-	-	Yes (18.5 ± 2.6)	Yes (20.7 ± 2.6)
Kanamycin (K30)	-	-	No	No
Neomycin (N30)	-	-	Yes (15.3 ± 0.7)	Yes (9.7 ± 1.2)
Penicillin (P10)	-	-	No	No
Streptomycin (S10)	-	-	No	Yes (9.7 ±0.7)
Tetracyclin (T30)	-	-	Yes (33.7 ± 0.7)	Yes (21.0 ± 4.5)

[a] Presence of quorum-sensing inhibition of violacein synthesis in *Chromobacterium violaceum* CV026 observed as a cream-white halo. Diameter of zone of quorum-sensing inhibition in millimeter ± SD in parentheses.
[b] Percentage reduction of area of colony (indicating inhibition of swarming) in *Pseudomonas aeruginosa* compared with control in parentheses. The percentage of reduction was calculated by comparing the area of the swarming colonies on medium supplemented with TCM with reference to that of the control on medium without extract. Extracts marked with an asterisk increased the area of the colony (promoted swarming) compared with the control.
[c] Presence of antimicrobial inhibition observed as a clear halo. Diameter of zone of antimicrobial inhibition in millimeter ± SD in parentheses.

al., 2007) and our preliminary results with ginseng points out that more specific traits need to be examined using additional indicators to further confirm the QSI effect of the TCM in *P. aeruginosa*.

Interestingly, of the eight TCM extracts that had marked QSI in the CV026 assay, six also showed antibacterial activity (Table 2 and Figure 4). These six TCM were further tested for anti-microbial properties against CV026 and PAO1 on Mueller-Hinton Agar (Figure 5) using standard disc-diffusion assay together with eight commercially available antibiotic discs included as a comparison. A clear zone of inhibition suggesting bactericidal activity was observed with antibiotics that were effective against the specific bacteria (Figure 5). In general, the TCM extracts produced smaller zones of inhibition compared to the standard commercial antibiotics. However, the extract from *L. brownii* showed a similar level of antimicrobial activity against CV026 and PAO1 to that of the antibiotic neomycin (Table 2). Various *Lilium* species used in herbal medicines have been reported to elicit antibacterial effects (National Academy of Sciences, 1975). Indeed, *L. brownii*, also commonly known as lily bulb, has been shown to contain compounds that significantly inhibit the growth of melanoma B16 cancer cell line and lung cancer in mice (Zhao *et al*., 2002). The other TCM herb that showed an inhibition level of CV026 near that of neomycin is *M. officinalis* which has also been reported to have antimicrobial effects on the bacterial species *Staphylococcus aureus, Bacillus subtilis* and *Mycobacterium smegmatis* but as in our present investigation, little or no antimicrobial effect on *P. aeruginosa* (Chan *et al*., 2008). There have been many ethnobotanically-directed searches for agents to treat infections. However, most studies focus on the bactericidal effects of the TCM used to treat infection (Janovska *et al*., 2003; Chan *et al*., 2008; Seneviratne *et al*., 2008). As seen from the present screening, the TCM herbs traditionally used in treatment of possible infections

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

did not show as substantial bactericidal activity compared to QS inhibition, and it may be that as a consequence, many potentially useful TCM in evidence-based evaluations may have gone unnoticed. From the current investigation, quorum inhibition, appears to be a potential mode of action of some of these herbs to control pathogenicity and may be as important as, and even more so, than bactericidal effect.

In this study, purple pigmentation and its inhibition in *C. violaceum* (McClean *et al*., 1997) provided a readily and easily observable phenotype that simplified and facilitated screening for QSI. However, it admittedly only provides qualitative results and does not give much insight as to the active chemical compounds. For future studies, fractionation of the crude extracts, and thin layer chromatography (TLC) or liquid chromatography-mass spectrometry (LC-MS) need to be carried out to isolate and characterise the anti-QS compounds in the screened efficacious extracts so as to determine the exact nature of the compounds that contribute to the anti-QS effect (Cseke *et al*., 2006). CV026 reporter strain was chosen here for ease of screening but it does have limitations as a model organism and further work will need specific study of activity against concrete pathological microorganisms that report on clearly defined QS-dependent virulence traits.

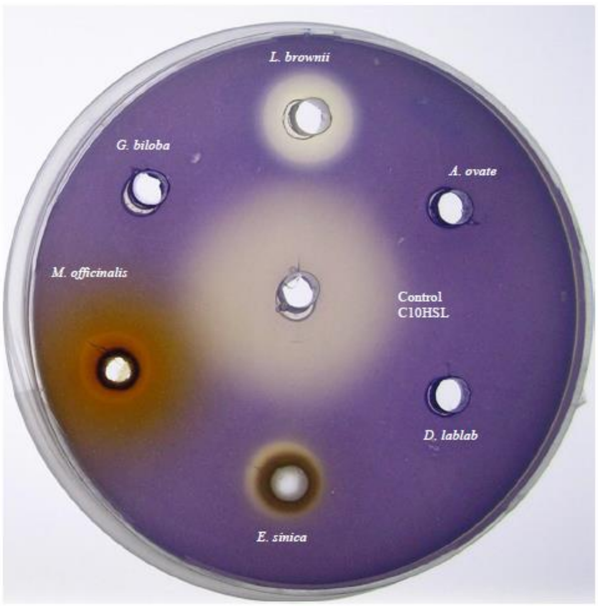

Figure 1: Bioassay using *Chromobacterium violaceum* CV026 bioreporter strain for testing extracts from traditional Chinese medicinal herbs. The extracts were from *Lilium brownii* (bai he), *Gingko biloba* (bai guo), *Magnolia officinalis* (hou pu), *Ephedra sinica* (cao ma huang), *Dolichos labab* (bian dou) and *Atractylis ovate* (bai shu). A turbid halo indicated an anti-QS effect and a clear halo indicated an antibacterial effect.

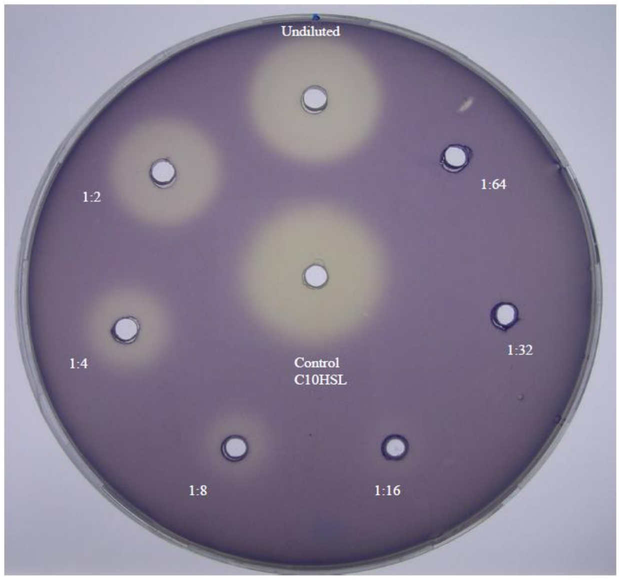

Figure 2: The TCM herb, *Astragalus membranaceus* (huang qi) showing dilution-dependent quorum-sensing inhibition on *Chromobacterium violaceum* CV026. Dilutions of 1:64 were carried out and the lowest dilution of *A. membranaceus* showing visibly detectable inhibition of violacein production was 1:16.

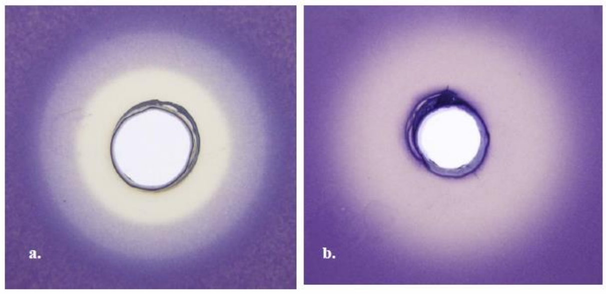

Figure 4: Bioassay of TCM extracts using *Chromobacterium violaceum* CV026 showed both anti-quorum sensing and antimicrobial activities. **a.** *Lilium brownii* (bai he) extract showing different active principles, i.e. antibacterial activity (inner clear ring) and QSI or quorum-sensing inhibition (outer creamy ring). **b.** *Panax pseudoginseng* (ren shen) extract exhibited only QSI (creamy ring) and no antibacterial activity.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

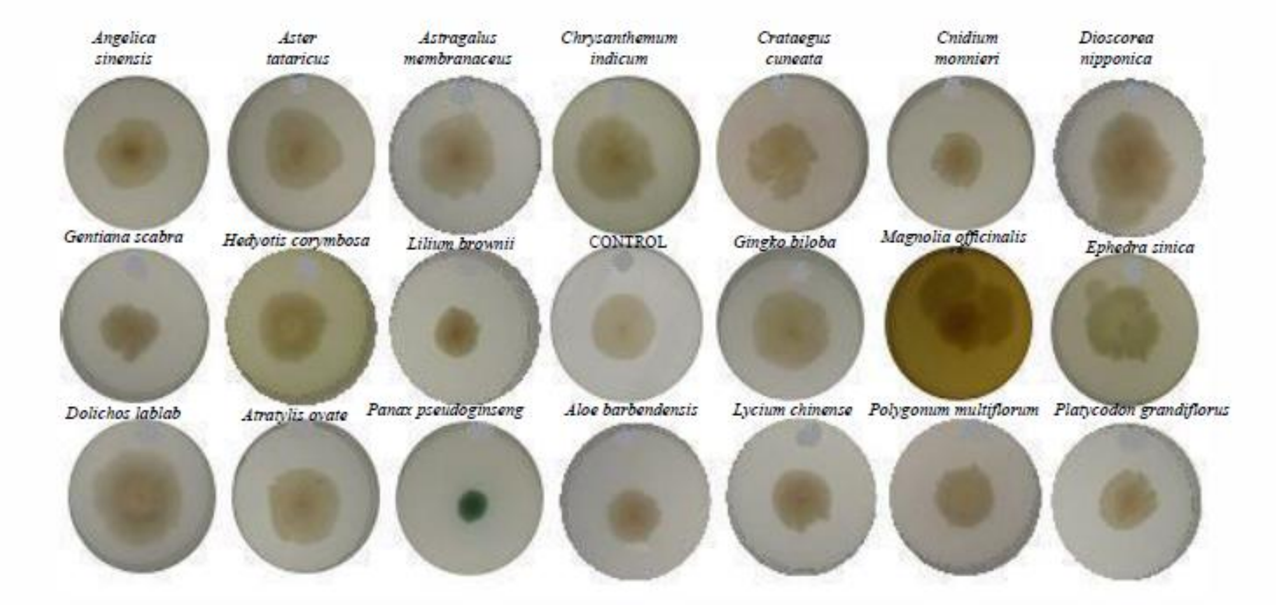

Figure 3: Effect of extracts of traditional Chinese medicinal herbs on swarming in PAO1. PAO1 colonies on LB media with extracts of *Panax pseudoginseng* (ren shen), *Lilium brownii* (bai he), *Cnidium monnieri* (she chuang) and *Aloe barbendensis* (lu hui) showed swarming inhibition compared to the control without traditional Chinese medicinal herb extract. *P. pseudoginseng* extract also induced pyocyanin synthesis in PAO1 observed as a green pigment.

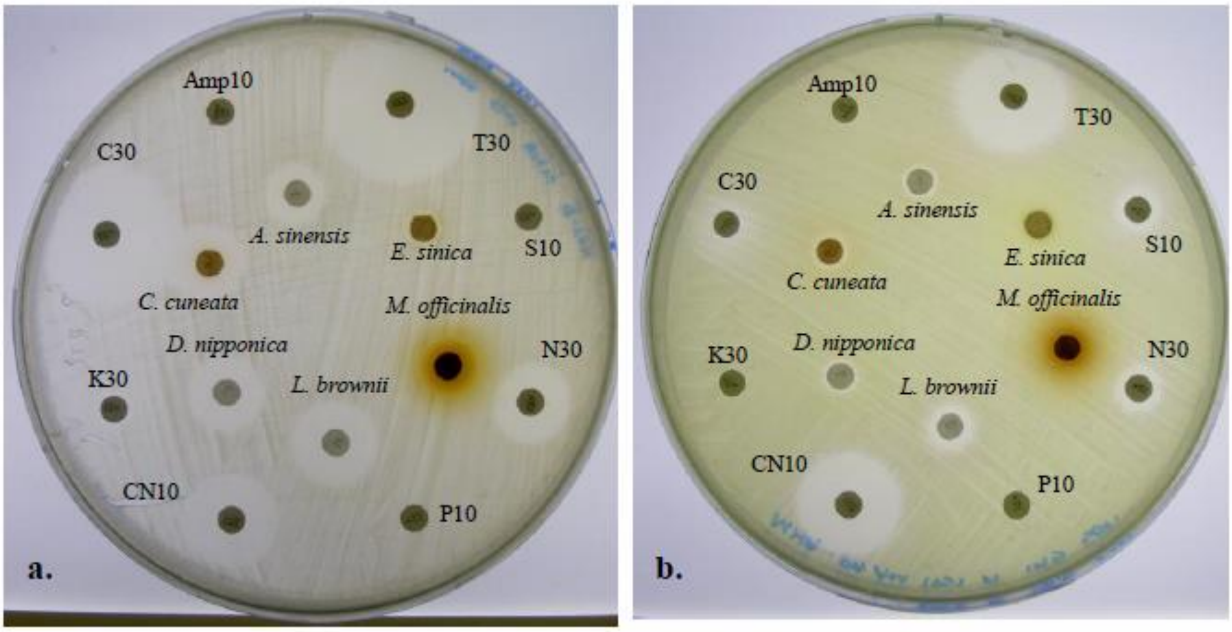

Figure 5: Antibiotic sensitivity testing of TCM extracts using disc diffusion assay on bacterial strains. a. *Chromobacterium violaceum* CV026 and b. *Pseudomonas aeruginosa* PAO1. The TCM extracts (inner circle of plate) were from *Angelica sinensis* (dang gui), *Crataegus cuneata* (ye shan zha), *Dioscorea nipponica* (chuan long shu yu), *Lilium brownii* (bai he), *Magnolia officinalis* (hou pu) and *Ephedra sinica* (cao ma huang). The 8 standard antibiotics (outer circle of plate) included were: Ampicillin (Amp10), Chloramphenicol (C30), Kanamycin (K30), Gentamicine (CN10), Penicillin (P10), Neomycin (N30), Streptomycin (S10) and Tetracycline (T30).

ISSN (print): 1823-8262, ISSN (online): 2231-7538

It is estimated that close to 80% of all the world's medicines are derived originally from plant sources (Cseke *et al*., 2006). Of the flowering plants or angiosperms, only 15% have been examined for medicinal value and there is definitely a large proportion that remains to be discovered and characterised. Many TCM products are herbal in nature (National Academy of Sciences, 1975). Research has shown anti-QS activity in the seaweed, *Delisea pulchra* (Rasmussen *et al*., 2000; Givskov *et al*., 1996), higher plants (Adonizio *et al*., 2006; 2008) and from the fungus, *Tremella fusiformis* (Zhu and Sun, 2008). Zheng *et al*. (2008) have also recently used virtual screening of TCM for QS inhibitors effective against *Pseudomonas aeruginosa*. This investigation is among the few studies we know of to report on the physical screening of TCM extracts for anti-QS activity.

CONCLUSION

Out of the directed sampling of twenty TCM herbs, the eight which exhibited anti-QS activity in CV026 bioassay were *Angelica anomala, Astragalus membranaceus, Crataegus cuneata, Dioscorea nipponica, Lilium brownii, Magnolia officinalis, Ephedra sinica* and *Panax pseudoginseng*. In addition, *Cnidium monnieri* and *Aloe barbadensis* also showed anti-swarming effect in PAO1 bioassay. Anti-QS potential of TCM could offer an alternative mode of action against opportunistic pathogenic bacteria that use QS to regulate virulence expression. It will be imperative and of interest to further investigate the nature of these QS inhibitor compounds and the mechanism by which they inhibit QS. Most of the TCM plants with activity belonged to different angiosperm plant families, and it appears that QS inhibitors are not unique to any group of plants. Although the data here which shows that several TCM herbs have QSI activity represents only preliminary data, it clearly identifies those potentially useful plants and their parts and forms the basis for more in-depth experiments to isolate and characterise the active compounds. It also provides possible insight into why some of these TCM plants might have been used successfully in the past for treatment of pathogenic infections and contributes towards our scientific understanding of their uses. The findings highlight that there lies a rich source of plants in traditional Chinese medicine that contain compounds able to inhibit QS and QS- related virulence processes. Continued systematic screening of more TCM with proper authentication and vouchering of plants as well as determination of the active principles in them and their interactions will yield additional means to combat pathogenic bacteria and help alleviate issues related to antibiotics resistance.

ACKNOWLEDGEMENTS

We thank Prof P. Williams, The University of Nottingham, UK, for supplying us with the homoserine lactones and the bacterial culture CV026 and Dr L.T. Tan, National Institute of Education, Singapore for the bacterial culture PAO1 and advice on extractions. Support of funds and facilities from the National Institute of Education, Nanyang Technological University, Singapore is also gratefully acknowledged.

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ISSN (print): 1823-8262, ISSN (online): 2231-7538