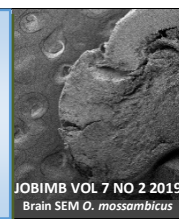


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Screening of Anti-Quorum Sensing Activity from Selected Chinese Herbs Against *Chromobacterium violaceum*

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ABSTRACT

Overuse of antibiotics was contributed to the increasing of bacterial infection resistance against antibiotics and caused a serious issue to the public health. Anti-quorum sensing is a new alternative ways or treatments to fight bacterial pathogenicity. Traditional Chinese herbs were screened of their anti-quorum sensing activities. Six selected traditional Chinese herbs were screened for a simple anti-quorum sensing activity by using *Chromobacterium violaceum* as the biomonitor. Two out of these herbs were found to be able to exhibit anti-quorum sensing properties; *Lycium barbarum* and *Zingiber officinale*. Extraction from *Lycium barbarum* has stronger anti-quorum sensing activity than *Zingiber officinale*. Colonies of biomonitor *C. violaceum* treated with *Lycium barbarum* almost fully loss its purple pigment. The loss and lack of purple colour from the colonies of *C. violaceum* indicated that quorum sensing activity was inhibited by the herb's extracted. It is believed that this herb contains rich source of compounds to fight or control pathogenic bacteria and potentially a new therapeutic way to reduce the development of antibiotic resistance.

INTRODUCTION

Many problems and diseases are caused by bacteria with the increased rate of resistance to antibiotic by formation of biofilm and decreased rate of discovery new antibiotics. These bacteria often cause morbidity, mortality and creates major public health problems around the world [1,2]. Many researches about pharmacological and pharmacognostical have been performed to discover new treatments to fight the infection caused by pathogenic bacteria which are resistant to antibiotic [3]. This study or discovery is about the interruption of the bacteria cell-to-cell communication system or anti-quorum sensing.

Quorum sensing is a cell-to-cell communication system used by bacteria to control their gene expression by signal molecules where the pathogenicity occurs after reaching certain level of cell-population density or quorum level [4,5]. In this system, bacteria require to accumulate certain level of signal molecule to form a signal-receptor complex binding to activate transcription for their pathogenicity [6,7]. Since most of the bacteria pathogenicity are controlled by quorum sensing system, an interruption of these communication system instead of killing them may possibly solve an antibiotic resistance problem and turn a pathogenic bacterium to become non harmful [8,9].

In recent years, there is an increasing interest in therapeutic and biological function of natural products towards microorganisms especially herbs in traditional medicine practise. The herbs in ancient times play a very important role for fighting and preventing an infectious disease. The anti-quorum sensing compounds that found in herbs were work differently in controlling diseases compare to other pharmaceutical drugs. Pharmaceutical drug is a chemical substance used to diagnose a disease (prevent, cure and treat). However, the uses of pharmaceutical drug may cause serious side effects. The most common side effects are diarrhoea, drowsiness, nausea, constipation, dizziness, pain and skin reactions while the serious side effects would cause allergies, physical debilitation, heart attack or heart failure, stroke, cancer or even death [10].

Anti-quorum sensing compounds from herbs extract do not directly target the host cell but they target the bacterial quorum sensing system by several different ways like degrading the quorum sensing signal molecules, interfere the signal molecules receptor and inhibit the production of signal molecules. That is why they are able to work differently compared to pharmaceutical drug and would not bring any side effects to the host or human body [11,12].

Traditional Chinese herbs have very important medicinal values and have been used for thousands of years in China and the popularity keeps increasing especially in the Western countries [13]. However, not many researches have been done about the effectiveness towards anti-quorum sensing system which is an alternative treatment to resistance of antibiotics in bacteria. The aim of this work is to screen traditional Chinese herbs that indicates the anti-quorum sensing activity against *C. violaceum*.

MATERIALS AND METHODS

Selected Chinese herbs

Six traditional Chinese herbs which are related to the improvement of immune system and blood were selected for anti-quorum sensing screening:

1. Lotus Seed (*Nelumbo nucifera*)
-Medicinal benefits: improve immune system, lowers blood sugar level, reduces hypertension, strengthen kidney and heart.
2. Ginkgo Nut (*Ginkgo biloba*)
-Medicinal benefits: strengthens lungs and kidneys, relieve cough and improve blood circulation.
3. Red Dates (*Ziziphus zizyphus*)
-Medicinal benefits: nourish the blood, increase metabolism, detoxify the body, improve immune system and rich in antioxidants.
4. Ginger Root (*Zingiber officinale*)
-Medicinal benefits: help digestion, detoxify the body, and lowers cholesterol.
5. Goji Berry (*Lycium barbarum*)
-Medicinal benefits: improve immune system, prevent iron deficiency, reduce cholesterol, lower blood pressure, strengthen liver and kidney and improve vision.
6. Female ginseng (*Angelica sinensis*)
-Medicinal benefits: balance hormone levels, detoxify the body, boost energy level and circulation, lower blood pressure, and protect the heart.

Chinese herbs extract preparation

The methods for herbs extraction followed the method of Adonizio *et al.* [14] with minor modification. Six of the selected Chinese herbs were cut into smaller pieces and then ground to suitable size manually by using ceramic mortar and pestle. In ethanol extract, approximately 20 g of the grounded herbs were added into 100 ml 95% ethanol and kept for 24 h. While in water extraction, approximately 20 g of the grounded herbs were boiled in 100 ml of distilled water for 30 min. Both aliquots were ready to use for anti-quorum sensing screening at the 0.2 g/ml concentration.

Biomonitor *Chromobacterium violaceum*

Chromobacterium violaceum is a wild type strain taken from Agrobiotechnology laboratory culture collection of the Department of Agriculture Technology, Universiti Putra Malaysia. It is able to produce violacein, a purple pigment by signal molecule N-hexanoyl-L-homoserinylactone (C6-HSL) under control of quorum sensing system. The production of purple pigment makes this bacterial strain excellent for anti-quorum sensing screening [15].

Bioassay

Each of the aliquot from ethanol and water extract (0.5 ml) were spread on a prepared LB agar plates by an alcohol-flamed glass rod and allowed it to evaporate slowly from the surface of LB agar. A single colony from each biomonitor *C. violaceum* strains were streak on the surface of LB agar plates. The plates were incubated in 29 °C overnight and the quorum sensing inhibition was detected by the colour of the colonies.

RESULTS AND DISCUSSION

The loss and lack of purple colour from the colonies of *Chromobacterium violaceum* indicated that quorum sensing was inhibited by the herb's extracts. Out of six selected chinese herbs for screening, two type of herbs Ginger Root (*Zingiber officinale*) and Goji Berry (*Lycium barbarum*) from ethanol and water extract were found showing an anti-quorum sensing activity from biomonitor *C. violaceum* (Table 1). However, traditional herbs extracted from ethanol were found decrease the growth rate of *C. violaceum* which may affect the result of screening. Water extract was still found best to use for screening in this study.

From the results, the extraction from *Lycium barbarum* has stronger anti-quorum sensing activity than *Zingiber officinale*. Colonies of *C. violaceum* treated with *Lycium barbarum* almost fully lost the purple colour (Fig. 1) while treatment by *Zingiber officinale* extract moderately reduce the purple colour concentration. *Zingiber officinale* was reported before that able to decrease violacein formation by 40% [16-18]. These indicated that *Lycium barbarum* inhibited more violacein production and exhibited more anti-quorum sensing activity than *Zingiber officinale*.

Table 1. Results of inhibition activity by selected chinese herbs extracts.

Herb	Inhibition
<i>Nelumbo nucifera</i>	nil
<i>Ginkgo biloba</i>	nil
<i>Ziziphus zizyphus</i>	nil
<i>Angelica sinensis</i>	nil
<i>Zingiber officinale</i>	moderate
<i>Lycium barbarum</i>	strong

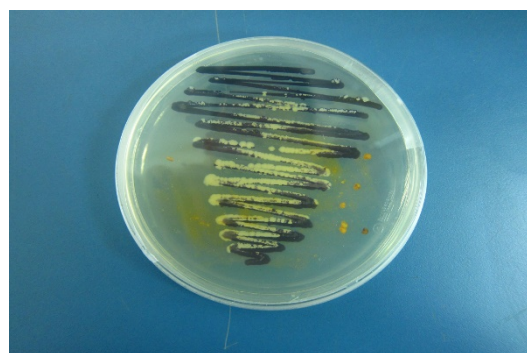


Fig. 1. Water extract of *Lycium barbarum* inhibited the production of violacein from *C. violaceum*.

The biological compounds extracted from traditional Chinese herbs were potentially target the bacterial AHL-Quorum Sensing system through 3 different methods. The first is to interrupt the signal molecules from being produced by the *luxI*, second is encoded the AHL synthase and last is to degrade the signal molecules or the receptor of LuxR [18,19]. In this study, the exact way or the mechanism of the inhibition violacein production from *C. violaceum* is still unknown. For a better use

of the current result, detailed studies of mechanism about these herbs and anti-quorum sensing need to be carry out.

CONCLUSION

Two out of the six traditional Chinese herbs screened exhibited an anti-quorum sensing activity by inhibition of violacein production indicating that anti-quorum sensing compounds are present in traditional Chinese herbs and these herbs may contain a rich source of compounds to fight or control pathogenic bacteria and are able to reduce the development of antibiotic resistance. However, further studies are needed to understand more about the mechanism so that the optimum levels of anti-quorum sensing from these herbs can be applied safely.

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