Potency of *Andaliman* (*Zanthoxylum acanthopodium* DC.) Extracts as Quorum-sensing Inhibitor to *Serratia marcescens*

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Abstract: Quorum-sensing is a specific communication type among microbial species, exposing several virulence factors to host or internal environment. The phenomenon is currently being studied as potential target for drug discovery and development. Natural product derived from plant source may be evaluated as potential quorum-sensing inhibitor (QSI). The study aimed to determine the optimum concentration of *Andaliman* methanolic (MeOH) and ethyl acetate extract (EtOAc) against prodigiosin synthesis by *Serratia marcescens* as one of phenotypes controlled by quorum sensing. Based on optical density (OD_{600}), both extracts did not interfere with the growth of microorganism. The optimum concentration of MeOH extract in inhibiting the prodigiosin synthesis was at concentration of 0.05% (w/v) in the end of incubation period (30 h). Meanwhile, the EtOAc extract inhibit the prodigiosin synthesis at concentration of 0.4% in the end of incubation period. Our results showed that *Andaliman* possess natural products as QSI which needed further evaluation in the future.

1 INTRODUCTION

Qourum sensing (QS) is a bacteria communication type based on genetic expression in a population which impact on development of biofilm, pigment production, toxin synthesis and other virulence factors. The communication used a molecule signal called autoinducer. It produced when microbial population density reached an optimum state (Bassler, 1993; Hentzer & Givskov, 2003). Autoinducer commonly synthesized by Gram negative bacteria is N-acyl-homoserine lactone (Blaschek, 2007; Bai & Vittal, 2011).

Serratia marcescens is an opportunistic pathogen from gram negative group. The species is able to produce N-hexanol homoserine lactone (C6-HSL), form biofilm and synthesis prodigiosin, a red pigmented compound. Strains of *Serratia* are known to exhibit QS to control their genetic expression that code for extracellular virulence factor (Morohoshi *et al.*, 2007). Previous study had revealed a decrease in AHL level to less production of exoenzymes and prodigiosin leading to an immature biofilm formation (Kievit *et al.,* 2000). Several strains are known as nosocomial infection factor due to antibiotic resistance (Traub, 2000). Therefore, a strategy is needed to combat this infection without dependence on antibiotics.

The alternative way to manage virulence factors is by using Quorum-sensing inhibitor (QSI) from various plant phytochemicals (Bai & Rai, 2011; Packiavathy *et al.*, 2012; Bai & Vittal, 2014). *QSI* role to decrease the level of virulence factor without producing any bacteriostatic and bacteriocidal effect as usual factor to trigger antibiotic resistance (Yarmolinsky *et al.*, 2015; Chang *et al.*,2017).

One of commonly utilized plant commodity in North Sumatera is *Andaliman* (*Zanthoxylum acanthopodium DC.*). *Andaliman* is one of native plants in North Sumatera, commonly used as spices in Bataknese traditional dishes. Extract of *Andaliman* fruits have been reported to contain α-pinene, limonene, geraniol, citronella, gerany-aceate, and monoterpeneoids (Wijaya *et al.*, 2002).

Study of *Andaliman* has focused on antimicroial, antioxidant, anti-inflammation, inhibition of xanthine oxidase and cytotoxic properties (Kristanty & Suriawati, 2015). However, information of their ability as potential QSI is still limited. The ability of *Andaliman* as QSI will be evaluated in this study as inhibitor to prodigiosin synthesis by *S. marcescens.*

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2 RESEARCH METHODOLOGY

2.1 Inoculum preparation

Isolate of *S. marcescens* was firstly sub-cultured in Luria Bertani agar prior laboratory test. Isolate was obtained from collection of Department of Microbiology, Faculty of Mathematics and Natural Sciences, USU.

2.2 Phytochemical extraction

Fresh fruits and seeds of *Andaliman* (*Z. acanthopodium* DC.) were washed and dried under open air environment. Dried *Andaliman* fruits and seeds were crushed using blender to obtain simplisia powder. Simplisia powder was extracted using ethyl acetate solvent (w/v) (1:6) as semi-polar fraction and macerated for 3 d under agitation. Macerates were obtained by filtering and simplisia was further extracted using methanol solvent as polar fraction. Each macerates were concentrated using rortary-evaporator at 45ºC. Both concentrated extracts were diluted in various concentrations using Dimethyl Sulfoxide (DMSO) (Muzafri *et al.*,2018)

2.3 Determination of QSI activity

Measurement of inhibitory activity of extract to prodigiosin synthesis is based on protocol by Morohoshi *et al.* (2007). Overnight culture of *S. marcescens* was inoculated into Luria Bertani broth supplemented with various concentration of *Andaliman* extracts 0.4, 0.2, 0.1, 0.05, 0% (control), and incubated for 30 hr. Indirect measurement of bacterial growth was obtained through OD_{600} of 5-hr interval sampling of aliquots. Prodigiosin was extracted from bacterial cells using acidified methanol solution (4% 1 M HCl in EtOH). Inhibition of prodigiosin was measured by quantifying absorbance value as A_{534} using following formula:

$$\%\text{inhibition} = \frac{\text{Absorbance of control-absorbance of treatment}}{\text{Absorbance of control}} \times 100\%:$$

3 RESULTS AND DISCUSSIONS

Potency of *Andaliman* extracts as QSI was evaluated from decrease of pigment quantity produced by reference strain. The first thing to consider in QSI testing was that extract did not inhibit bacterial growth.

Samples were taken at various times (up to 30 h) and analyzed to determine bacterial growth. The tested concentrations of both MeOH and EtOAc extracts did not show growth inhibitory activities as shown in Figure 1 and 2, as bacterial density proceed to increase following incubation time. This shows that the extract had condition as QSI.

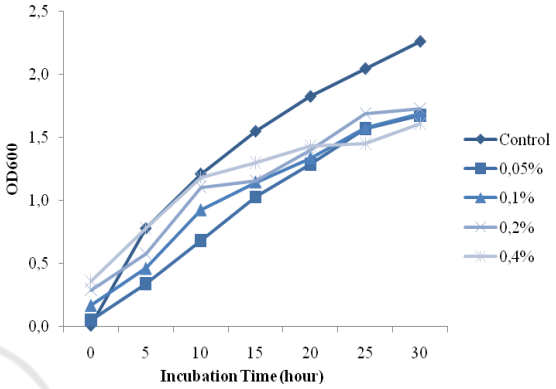

Figure 1: Effect of *Andaliman* MeOH extract on the growth of *S.marcescens* on 0 h to 30 h incubation.

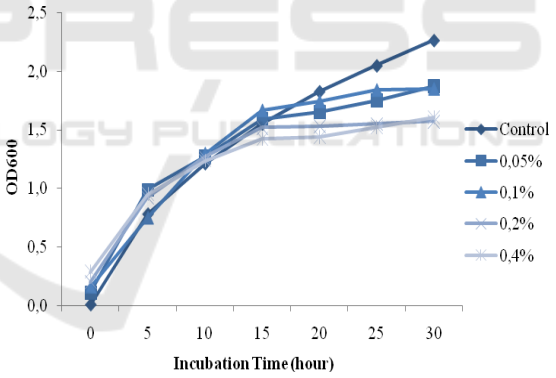

Figure 2: Effect of *Andaliman* EtOAc extract on the growth of *S.marcescens* on 0 h to 30 h incubation.

Prodigiosin production by *Serratia marcescens* was also analyzed at various times (up to 30 h) together with its growth. Intracellular production was extracted every 5 h. In the MeOH extract, the prodigiosin production had increased in the end incubation, and showed control was superior to the treatment shown in Figure 3.

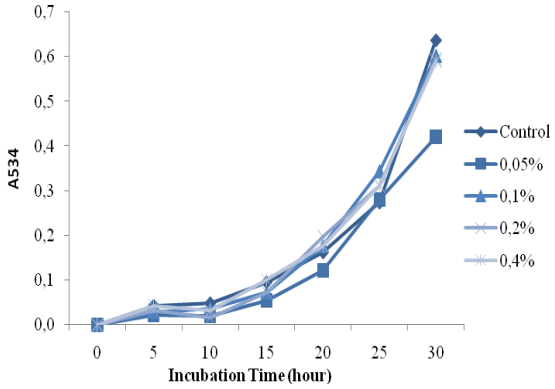

Figure 3: Effect of *Andaliman* MeOH extract on prodigios production by *S.marcescens* extracted every 5 hours (up 30 hours)

The same event is also shown in EtOAc extract, where prodigiosin production increases over time. In this analysis, prodigiosin production at the end of incubation showed that control was also superior to treatment shown in Figure 4.

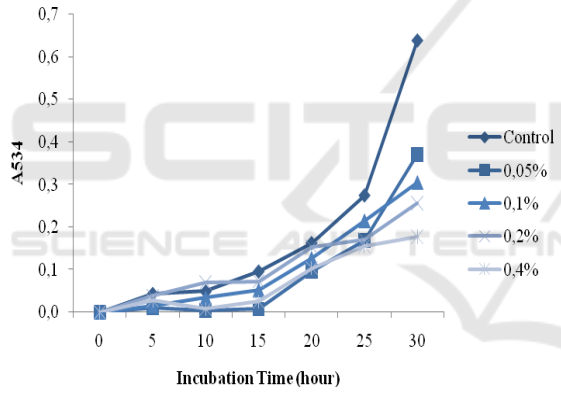

Figure 4: Effect of *Andaliman* EtOAc extract on prodigiosin production by *S.marcescens* extracted every 5 hours (up to 30 hours)

The highest inhibition was obtained in concentraion of 0.05% for MeOH and 0.4% for EtOAc with percentage of inhibition 43.4% and 72.0% respectively in the end of incubation period (30h). Packiavathy *et al* (2014) reported that *Curcuma longa* extract also exhibit potency in inhibiting prodigiosin production.

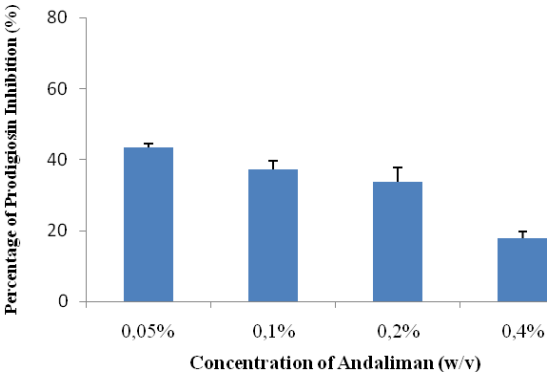

Figure 5. Percentage of prodigiosin inhibition on MeOH *Andaliman* extract in the end of incubation period.

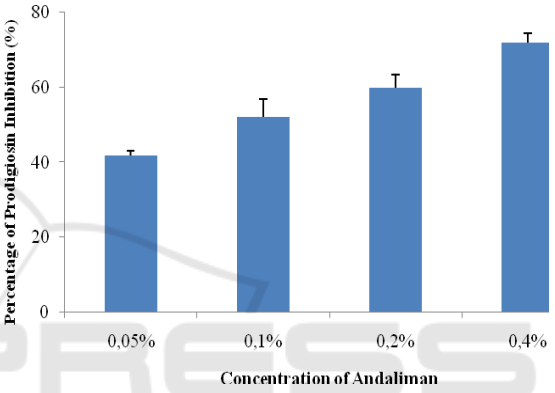

Figure 6. Percentage of prodigiosin inhibition on EtOAc *Andaliman* extract in the end of incubation period.

A decrease in red-color intensities were observed from concentration treatments. In the figure below shown that the intensity of red is more likely to be seen in cultures without the addition of *Andaliman* extract.

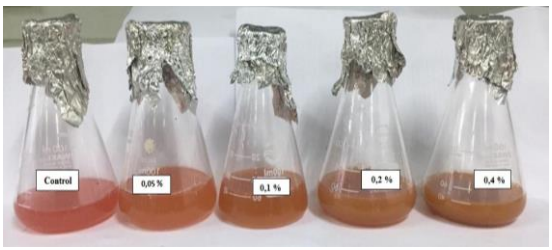

Figure 7. Red-pigmented colour intensity in LB medium with the addition of concentration variants extract andaliman.

In this study, the QSI potential of *Andaliman* fruit extract (*Zanthoxylum acanthopodium* DC.) is evaluated through synthesis of red pigments by *S. marcescens.* Quantitative analysis showed that

extracts reduced the prodigiosin production in percentage of 43.4 and 72.0% for methanol and ethyl acetate fraction, respectively. Prodigiosin synthesized by *S.marcescens* was known as one of essential virulence factors (Liu & Nizet, 2009). Two signals of the N-butanoyl homoserine lactone and HHL molecules are shownto regulate prodigiosin production (Morohoshi *et al*., 2007). Therefore, any interference with this QS system will leads to a reduction in prodigiosin production. A study by Packiavathy *et al.* (2014) reported that inhibition of prodigiosin using *Curcuma longa* was 58%. Bai & Vittal (2014) reported that inhibition of violacein production of *Chromobacterium violaceum* by *M. koenigii* essential oil is due to inhibition of CIRIR-dependent QS signaling either by preventing the reception of AHL signals or decreasing AHL gene expression.

A QS can be inhibited by certain compounds, with mechanical inhibition in the form of blocking the function of regulating transcription signals or receptors. Persson *et al.* (2005) dividing inhibitory compounds into two main groups, namely (a) molecules which are analogous structures of the original signal molecules; (b) other molecules with structures that are not similar to the original signal molecules. In this study the mechanism of inhibition of QS by Andaliman is still not known specifically.

4 CONCLUSIONS

Fruit extract of *Andaliman* from methanolic and ethyl acetate fractions showed inhibitory activities to prodigiosin production by *S.marcescens*. In this study, we evaluated that both extracts did not inhibit bacterial growth as concluded to become unique feature of QSI in combating and preventing microbial resistances.

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