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Reduced expression of enterotoxin-encoding gene in four isolates of *Staphylococcus aureus* by crude alkaloids from young papaya leaves

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Abstract

Staphylococcal enterotoxin A (SEA) is one of enterotoxins produced by *Staphylococcus aureus* that widely involved in food intoxication. The *sea* gene is an enterotoxin-encoding gene that responsible for production of this toxin. The effect of crude alkaloids towards cells and expression of *sea* gene of four *S. aureus* isolates from food was studied. Crude alkaloid extracts were extracted from freeze-dried young papaya leaves. A short alkaloid exposure of 2 hours was conducted to generate an insignificant effect to the cell numbers. The relative quantification of the expressed gene was carried out using qRT-PCR technique. The results showed that exposure of crude alkaloid at concentration of 0.25 mg/mL and 0.5 mg/mL for 2 hours did not significantly reduce the cell numbers of *S. aureus*. The relative expression of *sea* gene, however, decreased varied among isolates, in a range of 1.8 to 27 times after exposure to 0.25 mg/mL and in a range of 13.6 to 33.2 after exposure to 0.5 mg/mL. This study showed that young papaya leaves contained crude alkaloids that affected the *sea* gene expression consistently in four *S. aureus* isolates.

Introduction

Food safety issue is interrelated with the presence of pathogenic bacteria in food, one of which is *Staphylococcus aureus*. Approximately 70 to 95% of *S. aureus* strains can cause food poisoning by producing staphylococcal enterotoxins (Veras *et al.*, 2007). Staphylococcal enterotoxin A (SEA) was in large quantities produced by *S. aureus* which often involved in food intoxication (Clarisse *et al*., 2013).

Canini *et al*. (2007) reported that papaya leaves contained alkaloids such as carpaine, pseudocarpaine, dehydrocarpaine I and dehydrocarpaine II. Some studies indicated differences in the yields of alkaloid extracts of papaya leaves. Alkaloids from papaya leaves showed antimicrobial activity as demonstrated by Anibijuwon and Udeze (2009) and Suresh *et al.* (2008). Alkaloids were also reported to have the ability to interact with the cell walls and DNA (Cowan, 1999). Moreover Cao *et al.* (2007) reported that alkaloids were effective as antimicrobials by intercalating into double-stranded DNA and affecting topoisomerase enzymes and DNA-repairing enzymes.

Handayani *et al.* (2014) reported that *S. aureus* showed less relative expression of *sea* gene after the cells were exposed to crude alkaloids extracted from mixtures of young and old papaya leaves. However, only one isolate was used on the study. More isolates should be included to show consistent effect of the crude alkaloids to the expression of *sea* genes. Furthermore, some studies reported that alkaloids were found more in young papaya leaves (Elgadir *et al*., 2014; Boshra and Tajul, 2013; and Anjum *et al*., 2013).

This research was subjected to study the effect of crude alkaloids, extracted from young papaya leaves, towards *S. aureus* cells and relative expression of *sea* gene in four *S. aureus* isolates from food, i.e. raw milk, egg dishes, sautéed chicken cuts and chicken satay. The *sea* gene expression was calculated by relative quantification using quantitative reverse transcription PCR (qRT-PCR) method. The use of real-time PCR (qPCR) is expected to provide results with a high level of sensitivity so that the determination of DNA or RNA content in a cell will be accurate.

Materials and Methods

Materials

Young papaya leaves of Calina papaya tree were

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obtained from the Center for Tropical Horticulture Studies, Bogor Agricultural University, Indonesia. Young leaves were the first upper three layers of the leaves. Bacterial isolates used were the previously-isolated *S. aureus* by Handayani *et al.* (2014) originated from raw cow's milk (S10), egg dishes (TBI), sautéed chicken cuts (UA13) and chicken satay (SJI). *S. aureus* subsp. *aureus* ATCC 25923 was used as a reference strain.

Preparation of papaya leaves

The leaves were washed 2-3 times with clean water, then dried with two techniques, i.e. oven drying (VWR A143 A-143, Sheldon Manufacturing, Inc., Oregon, USA) at 55°C for 22 hours and freeze-drying (Martin Christ Gamma 2-16 LSC) for 24 hours (Atlabachew *et al*., 2013). Dried leaves were pulverized into powder using an electric blender, passed through 40-mesh sieve, and stored in a sealed container.

Crude alkaloid extraction of young papaya leaves

Crude alkaloid was extracted following the extraction procedure described by Djilani *et al.* (2006). Ten grams of ground-dried young papaya leaves were suspended in 400 ml of 0.2% (m/v) sodium dodecyl sulfate (Merck and Co., New Jersey) in an Erlenmeyer flask and sonicated for 2.5 hours at 25-35°C with a frequency of 40 kHz in an ultrasonic bath (Bransonic Ultrasonic Cleaner B8510 MTH model, Branson Ultrasonic Corporation, Connecticut, USA). The extract was separated using a filter cloth, and the yield was washed with 20 mL distilled water. Afterwards, the extract was filtered using Whatman filter paper No. 1 (W and R Balston, England), followed by added 2% sulfuric acid solution (Merck and Co.) to the filtrate to obtain a pH of 3 to 4. Subsequently, the filtrate was precipitated with 15 mL of Mayer's reagent. Precipitates were separated by centrifugation (HermLe Z383K; HermLe Labortechnik GmbH, Wehingen, Saint Nom, Germany) at 2400 × g for 10 minutes, diluted with 5% sodium carbonate solution (Merck and Co.), and then extracted with chloroform (Merck and Co.). The formed organic layer was washed with distilled water until the pH became neutral and then passed through disodium sulfate (Merck and Co.). The solution was evaporated with rotary evaporator (Buchi Rotavapor R-210, BÜCHI Labortechnik, Flawil, Switzerland)) and dried with N_2 gas to obtain crude alkaloid extract. Alkaloids were dissolved in dimethyl sulfoxide (Merck and Co.) and sterilized with membrane filter (0.45 µm) (Minisart, Sartorius).

Exposure of S. aureus *to crude alkaloid extract of young papaya leaves*

The method referred to the procedure described by Handayani *et al.* (2014). Five mL of Tryptic Soy Broth (TSB, Oxoid Ltd. Hampshire, UK) was added with crude alkaloid extract of papaya leaves to obtain 0.25 and 0.5 mg/mL crude alkaloid concentrations. TSB medium without crude alkaloid mixture was used as a control. One hundred µL of 10^8 CFU/mL *S. aureus* isolate suspension or *S. aureus* ATCC 25923 were then pipetted into TSB medium, incubated at room temperature for 2 hours on orbital shaker at 735 x g. Then, the numbers of bacterial cells before and after 2-hour incubation were enumerated using spread plate method on Tryptic Soy Agar (Oxoid Ltd., Hampshire, UK).

DNA extraction of S. aureus

DNA isolation of *S. aureus* was conducted following Mason *et al.*, (2001) with a slight modification, i.e. lysostaphin was replaced by 10 mg/mL of lysozyme solution (Bio Basic Canada Inc., Markham, Ontario, Canada), as reported by Handayani *et al.* (2014). *S. aureus* was grown on Tryptic Soy Broth media (Oxoid Ltd., Hampshire, UK) and incubated at 37°C for 18-24 hours. Two mL of *S. aureus* culture in TSB media were centrifuged at 21,000 × g for 1 minute. The supernatant was discarded and the pellet was resuspended in 560 µL of TE buffer (10 mM Tris pH 7.5 and 1 mM EDTA). Then, 100 µL of lysozyme (10 mg/mL) was added, mixed until homogeneous and incubated at 37°C for 1 hour (tube was inverted every 15 minutes). Thirty µL of 10% SDS and 10 µL of proteinase K (Thermo Fisher Scientific, Massachusetts) solutions (10 mg/mL) were added and incubated back at 37°C for 1 hour (tube was inverted every 15 minutes). Subsequently, 100 µL of 5 M NaCl and 80 µL of CTAB-NaCl (Merck and Co.) solution (10% CTAB in 0.7 M NaCl) that has been heated at 65°C were added. Incubation was conducted at 65°C for 10 min. Chloroform with the same volume as the suspension was added then vortexed and centrifuged at 21,000 × g for 5 minutes. The supernatant was transferred into a new tube and washed 2 times with solution of phenol (MP Biomedicals, LCC, Illkirch, France), chloroform, and isoamyl alcohol (Applychem, Darmstadt, Germany) in a concentration of 25:24:1; followed by 1 time with chloroform, by the same volume with the suspension. The upper part of the aqueous phase was transferred into a new tube and DNA precipitation was conducted using 0.7 supernatant volume of ice-cold absolute isopropanol (Merck and Co.). The mixture was incubated at -20°C

for 1 hour, and then centrifuged at 21,000 × g for 10 minutes. The supernatant was discarded and the pellet was washed with 1 mL of 70% ethanol (Merck and Co.) then centrifuged at 21,000 × g for 5 minutes. DNA pellets were dried and resuspended in 30 µL of double-distilled water (aqua-bidest).

Amplification of 16S rRNA- and sea-encoding genes

Amplifications of 16S rRNA and sea-encoding genes were conducted using Thermal Cycler 2720 (Applied Biosystems, Foster City, CA). Primers used for the detection of 16S rRNA-encoding gene were 16sF (5'-CCGCCTGGGGAGTACG-3') and 16sR3 (5'-AAGGGTTGCGCTCGTTGC-3'), while primers used for the detection of *sea* gene were SEA1 (5'-TTGGAAACGGTTAAAACGAA-3') and SEA2 (5'-GAACCTTCCCATCAAAAACA-3') (Lee et al., 2007). Temperature gradients were set during pre-denaturation (95°C, 5 min), 30 amplification cycles (denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min) and termination cycle (72°C, 5 min). PCR reaction mixture used was 25 mL, which consisted of 12.5 µL of DreamTaq Green master mix (Thermo Fisher Scientific, Waltham, MA), 1 µL of each primer (10 µM), 2 mL of DNA template, and 8.5 µl of nuclease-free water (Thermo Fisher Scientific). Amplification products were visualized on 2% agarose gel (Thermo Fisher Scientific, Massachusetts, USA) by electrophoresis (Bio-Rad, Bio-Rad Laboratories Pte. Ltd., Singapore) at 75 V for 40 minutes.

RNA extraction and complementary DNA (cDNA) synthesis

Two mL of bacterial culture suspension that have been exposed to crude alkaloid extract of young papaya leaves were centrifuged at 5000 × g for 5 minutes. Bacterial pellets were resuspended in TE buffer (PEQLAB Biotechnologie GmbH, Erlangen, Germany) and 30 µL of 10 mg/mL lysozyme (PEQLAB Biotechnologie GmbH). RNA extraction of S. aureus was conducted using peqGOLD Bacterial RNA Kit (PEQLAB Biotechnologie GmbH) referring to peqGOLD Bacterial RNA Kit protocol instruction. RNA obtained was treated with DNAse 1, RNase free (Thermo Fisher Scientific) to degrade DNA found in RNA extract. RNA concentration and purity (A_{260}/A_{280}) were measured by a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). RNA concentrations obtained were equalized to 100 ng/µL for cDNA formation. cDNA synthesis was conducted using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) referring to RevertAid First Strand cDNA Synthesis Kit protocol instruction.

Relative quantification of sea *gene expression*

Relative quantification analysis was conducted using Swift Spectrum Thermal Cycler 48 (Esco Healthcare Pte. Ltd,. Singapore). qPCR reaction was conducted by making a 20 µL reaction mixture consisting of 1 µL of cDNA as a template, 0.4 µL of each primer (16S rRNA and SEA) (10 µM), 10 µL of KAPA SYBR® FAST qPCR Kit Master Mix (Kappa Biosystems, Woburn, MA), and nuclease-free water to a volume of 20 µL. PCR conditions used were pre-denaturation for 1 min at 95°C, 45 amplification cycles (denaturation for 1 min at 95°C, annealing for 1 min at 55°C, extension for 1 min at 72°C) and termination for 5 min at 72°C. Fluorescence readings were conducted after each extension step and followed by melting curve analysis at 70-90°C with an analysis every 0.5°C temperature increase and a constant time of 10 seconds (Lee *et al*., 2007). The *sea* gene expression was measured relatively towards the control by C_T ($2^{-\Delta\Delta CT}$) comparative method (Schmittgen and Livak, 2008), as also described by Handayani *et al.* (2014). The value of relative expression of *sea* genes was expressed as the $2^{-\Delta\Delta CT}$ value. The $\Delta\Delta C_T$ values were obtained from the subtraction of differences between ΔC_T *sea* genes with bacterial 16S rRNA gene of cells that were exposed to crude alkaloid extract to the differences between ΔC_T *sea* gene with bacterial 16S rRNA gene of cells without crude alkaloid exposure. The experiment was conducted three times.

Statistical analysis

Data were analyzed using One-Way ANOVA with IBM SPSS Statistics 22.0 software at 95% confidence level. Significantly different results, defined at $P < 0.05$, were further tested with Duncan's multiple range tests.

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Results and Discussion

Crude alkaloid extract of young papaya leaves

Extraction of freeze-dried papaya leaves resulted in a yield of crude alkaloid extract of 1.34±0.36%. This result was relatively higher than obtained by extraction of oven-dried leaves that yielded in 0.99±0.03% of crude alkaloid extract, although statistically they were not significantly different. Kaur *et al.* (2014) reported that the oven drying could reduce the level of alkaloid content. According to Schwarz *et al.* (2007), alkaloids are highly vulnerable and easily degraded by heat. Freeze-drying is an important process to maintain biological materials that are heat-sensitive and can increase the components activity (Ciurzynska and Lenart,

Table 1. Cell numbers of *S. aureus* before and after exposure to crude alkaloids of young papaya leaves for 2 hours at room temperature

Sample (alkaloid concentration)	Cell numbers* (Log CFU/mL)		Bacterial growth** (Log CFU/mL)	Δ Reduction of cell number (Log CFU/mL)
	0 hours	2 hours		
UA13 (0)	6.46 ± 0.34^{a}	7.08 ± 0.11^{b}	+0.61^{d}	-
UA13(0.25)	6.23 ± 0.19^{a}	6.29 ± 0.02^{a}	+0.05^{c}	0.55
UA13 (0.5)	6.26 ± 0.13^{a}	6.09 ± 0.18^{a}	-0.18^{c}	0.79
SJI (0)	6.45 ± 0.31^{a}	7.14 ± 0.14^{b}	+0.70^{d}	-
SJI (0.25)	6.19 ± 0.13^{a}	6.29 ± 0.09^{a}	+0.10^{c}	0.60
SJI (0.5)	6.40 ± 0.52^{a}	6.21 ± 0.63^{a}	-0.18^{c}	0.88
TBI (0)	6.69 ± 0.31^{a}	7.15 ± 0.04^{b}	+0.45^{d}	-
TBI (0.25)	6.30 ± 0.19^{a}	6.10 ± 0.13^{a}	-0.20^{c}	0.65
TBI (0.5)	6.63 ± 0.65^{a}	6.20 ± 0.45^{a}	-0.43^{c}	0.89
S10 (0)	6.62 ± 0.44^{a}	7.14 ± 0.05^{b}	+0.50^{d}	-
S10 (0.25)	6.39 ± 0.37^{a}	6.41 ± 0.43^{a}	+0.02^{cd}	0.48
S10 (0.5)	6.36 ± 0.22^{a}	6.15 ± 0.29^{a}	-0.22^{c}	0.72
ATCC (0)	6.47 ± 1.06^{a}	7.12 ± 0.71^{b}	+0.65^{d}	-
ATCC (0.25)	6.79 ± 0.60^{a}	6.59 ± 0.60^{a}	-0.20^{c}	0.85
ATCC (0.5)	6.92 ± 0.42^{a}	6.39 ± 0.50^{a}	-0.53^{c}	1.17

*Values on the same row followed by different small letters are significantly different.
** Values on the same column followed by different small letters are significantly different
Note: (-) decrease, (+) increase

2011). For further work, therefore, the crude alkaloid extracts from freeze-dried leaves were used.

Activity of crude alkaloid extract of young papaya leaves towards S. aureus

S. aureus that was not exposed to crude alkaloid showed a slight increase in cell numbers after 2 hour incubation at room temperature, i.e. in a range of 0.45 Log CFU/mL to 0.70 Log CFU/mL (Table 1). This slight growth was possible, since the generation time of *S. aureus* was 37 minutes as reported by Novic and Katic (2009). After exposure to crude alkaloid at concentration of 0.25 mg/mL, the number of *S. aureus* was relatively constant. Furthermore, although the exposure to 0.5 mg/mL crude alkaloid for 2 hours led to slightly decrease of the cell numbers, i.e. in a range of 0.18 Log CFU/mL to 0.53 Log CFU/mL, the decreases were statistically not significant.

Some other studies showed that alkaloids could inhibit the growth of *S. aureus*. Handayani *et al.* (2014) reported that the crude alkaloids from mixtures of young and old papaya leaves at a concentration of 0.25, 0.5 and 1 mg/mL were able to inhibit the growth and to reduce significantly the cell number of *S. aureus* SJI. Anibijuwon and Udeze, (2009) found that alkaloids from papaya leaves at concentration of 1.2 mg/mL extracted by hot water and alkaloids from papaya leaves at concentration of 0.8 mg/mL extracted by ethanol could inhibit the growth of *S. aureus*. Alkaloid extract of papaya leaves at 25 µL concentration extracted by water showed a 3.6 mm zone of inhibition against *S. aureus* (Suresh *et al*., 2008). Generally, the growth inhibition test is conducted for 24 hours exposure. In this study, however, a short alkaloid exposure of 2 hours was performed to generate an insignificant effect to the cells numbers.

Crude alkaloid activity towards relative expression of sea *gene*

All isolates showed PCR products of 240 bp after amplification using primers 16sF and 16sR3 targeting 16S rRNA, as also showed by *S. aureus* ATCC 25923 (Figure 1). The presence of sea-encoding gene, however, only found in four isolates after amplification using SEA1 and SEA2 primers, revealed by the formation of 120 bp amplicon. This PCR product was not found in *S. aureus* ATCC 25923 (Figure 1), indicating that this reference strain did not produce enterotoxin A. Moreover, the obtained *sea* gene bands were relatively thinner than the 16S rRNA gene bands, suggesting that the concentration of *sea* gene in S10, UA13, SJI and TBI were likely less than 16S rRNA. The 16S rRNA

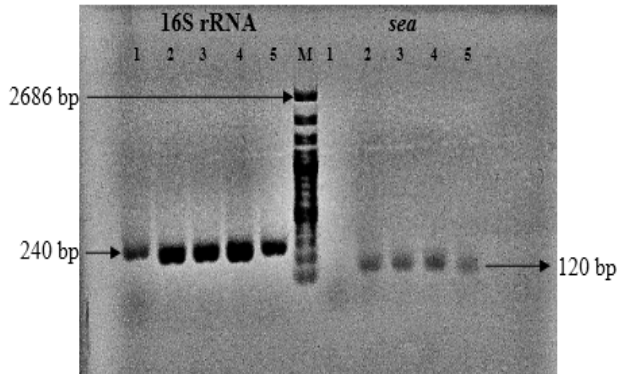

Figure 1. Amplifications of 16S rRNA- and *sea*-encoding genes.
Note: well M=100 bp DNA ladder, 1=ATCC 25923, 2=S10, 3=UA13, 4=SJI, 5=TBI

gene is known as a housekeeping gene that always expressed. According to Schelin *et al.* (2011), *sea* gene transcription is associated with the life cycle of sea-encoding prophage. Natural polymorphism in prophage is found to be affecting the amounts of *sea* gene produced by the prophage-carrier bacteria.

Purity and concentration of the total RNA will affect the measurement of gene expression levels in qRT-PCR reaction (Bustin and Nolan, 2004). According to Barbas *et al.*, (2007), a good purity of RNA at A_{260}/A_{280} wavelengths was 2.0. The extracted RNA was in a good purity with values that ranged between 2.04-2.70 (Table 2). Table 2 also shows that the concentrations of RNA were varying from 160 ng/µL to 682 ng/µL. This variability was likely affected by the concentration of bacterial cells involved. The higher number of *S. aureus* cells would be resulted in higher RNA concentration.

Differences between RNA concentrations in *S. aureus* without crude alkaloid exposure (control) with RNA concentrations in *S. aureus* exposed to crude alkaloid (test samples) would affect the results of quantification of *sea* gene expression in qPCR. In order to address this problem, a strategy to reduce RNA variation (normalization) is necessary to increase the accuracy of gene expression measurement. According to Huggett *et al*. (2005), there are three strategies in RNA normalization, i.e. equalize the cell numbers, equalize RNA concentration and normalization towards the reference gene. Vandesompele *et al.* (2002) reported that the equalization of cell numbers is an ideal normalization process, but the inequality of cell numbers is often occur during the process, particularly cells derived from solid tissues, therefore the normalizations of RNA concentration and reference gene are the most appropriate choices in reducing RNA variations. In this study, differences were found in cell numbers between *S. aureus*

Table 2. Concentration and purity of RNA of *S. aureus* S10, UA13, SJI and TBI after exposure to crude alkaloids of young papaya leaves for 2 hours at room temperature

Sample (alkaloid concentration)	RNA concentration (ng/µL)	RNA purity
S10 (0)	372	2.70±0.00
S10 (0.25)	224	2.21±0.25
S10 (0.5)	160	2.50±0.26
UA13 (0)	426	2.40±0.23
UA13 (0.25)	180	2.18±0.20
UA13 (0.5)	180	2.18±0.20
SJI (0)	234	2.20±0.15
SJI (0.25)	272	2.18±0.20
SJI (0.5)	272	2.04±0.10
TBI(0)	682	2.20±0.06
TBI(0.25)	256	2.27±0.29
TBI(0.5)	312	2.07±0.24

exposed to crude alkaloid and did not expose to crude alkaloid after 2 hours of incubation. Therefore, for quantification using qPCR, the RNA concentration was equalized to 100 ng/µL.

Decrease of relative expression of *sea* gene can be seen from the comparison of the cycle threshold (C_T) value. C_T value is set when the amplification curves show the exponential phase. The lower the initial amount of DNA/cDNA targets, the slower the fluorescence increase, and will result in higher C_T value, and vice versa (Rodriguez *et al*., 2011). The results indicated that exposure to crude alkaloid of young papaya leaves caused a relative increase in the C_T value of *sea* gene in all isolates. The C_T values of 16S rRNA gene, however, did not significantly increase (Table 3). The 16S rRNA gene is a housekeeping gene whose expression will not change, stable and universally distributed. Ribosomal RNA (rRNA) comprises 85–90% of total RNA (Bustin, 2000).

Relative expression of *sea* gene was represented by the value of $2^{-\Delta\Delta CT}$ as described by Schmittgen and Livak (2008). This method was successfully carried out to examine the expression of *sea* gene quantitatively in isolate SJ1 in previous study (Handayani *et al.*, 2014). As showed in Table 3, the highest reduction of relative expression of *sea* gene by 0.25 mg/mL crude alkaloid was demonstrated in isolate UA13, i.e. 27 times of reduction, whereas the lowest was found in isolate TBI, i.e. 1.75 times of reduction. Crude alkaloid at concentration of 0.5 mg/

Table 3. Relative expression of sea gene of *S. aureus* S10, UA13, SJI and TBI quantified by qRT-PCR and $2^{-\Delta\Delta CT}$ method

Sample (alkaloid extract concentration (mg/mL))	C_T value of *sea* gene	C_T value of 16S rRNA gene	ΔC_T	$\Delta\Delta C_T$	$2^{-\Delta\Delta CT}$	Reduction of *sea* gene relative expression (times)
S10 (0)	24.51±1.53[a]	19.52±0.31[c]	5.00	0.00	1.00	-
S10 (0.25)	26.03±0.10[a]	19.40±0.28[c]	6.63	1.63	0.32	3.1
S10 (0.5)	29.45±0.07[b]	19.55±0.07[c]	9.90	4.90	0.03	30.0
UA13 (0)	26.26±1.90[a]	19.62±0.30[c]	6.65	0.00	1.00	-
UA13 (0.25)	30.44±0.62[b]	19.04±0.06[c]	11.40	4.76	0.04	27.0
UA13 (0.5)	31.10±0.13[b]	19.40±0.55[c]	11.70	5.06	0.03	33.2
SJI (0)	27.07±1.04[a]	19.84±0.21[c]	7.23	0.00	1.00	-
SJI (0.25)	29.27±0.67[a]	19.75±0.21[c]	9.52	2.30	0.20	4.9
SJI (0.5)	30.75±2.37[a]	19.51±0.69[c]	11.24	4.02	0.06	16.2
TBI (0)	26.33±1.94[a]	19.55±0.49[c]	6.78	0.00	1.00	-
TBI (0.25)	27.08±2.30[a]	19.50±0.70[c]	7.58	0.81	0.57	1.8
TBI (0.5)	30.80±0.57[a]	19.46±0.22[c]	11.35	3.77	0.07	13.6

Values on the same column followed by different small letters are significantly different.

mL showed a higher activity in reducing the relative expression of *sea* gene, with the highest reduction of 33.2 times found in UA13 isolate, and the lowest was found in TBI by 13.6 times reduction.

Reduction of relative expression of *sea* gene in this study, however, was lower in comparison to the previous study conducted by Handayani *et al.* (2014). Crude alkaloid exposure at 0.25 mg/mL and 0.5 mg/mL extracted from the mixture of old and young papaya leaves was able to lower the relative expression of *sea* gene in isolate SJI by 28.5 times and by 40.7 times, respectively (Handayani *et al*., 2014). In recent study, 0.25 mg/mL and 0.5 mg/mL crude alkaloids of young papaya leaves were only able to reduce the relative expression of *sea* gene of isolate SJI by 4.91 times and by 16.17 times, respectively. Differences in capacity of reducing the relative expression of *sea* gene between both studies were probably due to different composition of alkaloids in young papaya leaves and in old papaya leaves. Papaya leaves contained four types of alkaloid such as carpaine, pseudocarpaine, dehydrocarpaine I and dehydrocarpaine II (Canini *et al.*, 2007). Saran and Choudhary (2013) reported that in old papaya leaves more carpaine and pseudocarpaine were found. The recent study found that although young papaya leaves yielded in higher amounts of crude alkaloids, but their activities as antimicrobials were lower than the activity of crude alkaloids from the mixture of old and young papaya leaves as reported by Handayani *et al.* (2014). However, in recent study, it has been proved that the crude alkaloids demonstrated consistent reduction of relative expression of *sea* gene in three isolates, i.e. isolate TBI, UA13 and S10, next to the isolate SJ1.

Conclusions

This study reconfirmed that all isolates were *S. aureus* carrying *sea* gene. The exposure of crude alkaloid from young papaya leaves for 2 hours slightly reduce the cell numbers of *S. aureus* but did not statistically significant. The crude alkaloid of young papaya leaves demonstrated a consistent reduction on the relative expression of encoding gene for staphylococcal enterotoxin A in four *S. aureus* isolates from food. This finding highlighted that the papaya leaves can be used as important natural resources to control the growth of *S. aureus* and formation of enterotoxin A.

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