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Original Research Article

Evaluation of antibacterial activity of hexanedioic acid isolated from *Hermetia illucens* larvae

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ABSTRACT

Hermetia illucens larvae have been used in Europe and America as a medical resource and medicinal insect for the treatment of skin damage such as burns and wound healing. This study was carried out to evaluate the effect of a new substance causing antibacterial activity against various pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). The antibacterial activity of hexanedioic acid was determined using different antimicrobial indicators such as turbidometric assay, resazurin assay, and agar disk diffusion. Hexanedioic acid showed the selective-growth inhibitory effects against the growth and proliferation of *Staphylococcus aureus*, MRSA, *Klebsiella pneumonia*, and *Shigella dysenteriae* in a concentration dependent manner. The growth inhibitory zones of bacteria treated with 80 μg/ml of hexanedioic acid for 24 h were measured as 18.27 ± 0.18, 23.35 ± 0.15, 16.62 ± 0.18, and 12.96 ± 0.24 mm, respectively. The minimum inhibitory concentration (MIC) values of hexanedioic acid against the viability of these bacteria for 24 h were measured as 140.377, 137.369, 139.117, and 139.704 μg/ml, respectively. These results demonstrate that hexanedioic acid has antibacterial properties that effectively inhibit the growth/proliferation of pathogenic bacteria. Furthermore, this study suggests novel aspects for the utilization of hexanedioic acid as a medicinal substance.

Introduction

Larvae of *Hermetia illucens* (Diptera: Stratiomyidae), a well-known species of fly belonging to the genus *Hermetia*, are found in carrion and food waste. Among diverse biological resources, arthropods/annelids such as the centipede and leech have been used extensively as biological resources in oriental medicine to treat human diseases in Asia (Feng et al., 2007; Calvino and Szczupak, 2008; Xu et al., 2010; Zhou et al., 2011). In addition, *H. illucens* and *Lucilia sericata* (Diptera: Calliphoridae) larvae have been utilized in medical fields for the treatment of skin damage as medical resources/medicinal insect in Europe and America (Mumcuoglu et al., 1999; Armstrong et al., 2002; Chambers et al., 2003; Wang et al., 2006). In accordance with the appearance of diverse pathogens, various antibiotics including rifampicin, cycloserine, cephalosporins, fluoroquinolones, tetracycline, vancomycin, gentamycin, erythromycin, and ciprofloxacin were

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developed to treat bacterial infections such as tuberculosis, pneumonia, diphtheria, typhoid fever, and sepsis for decades (Weinstein et al., 1963; Calvori et al., 1965; Drusano et al., 1986; Ednie et al., 1996). In addition, clinical guidelines for the treatment were developed by clinicians and researchers, and by various organizations including U.S. government agencies (Blumberg et al., 2003; American Thoracic Society et al., 2005; Mandell et al., 2007). However, despite these intensive drug developments, pathogenic bacteria such as *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Shigella dysenteriae* not only cause various/serious infectious diseases but also exhibit multi-drug resistance to antibiotics in humans (Boyanova and Mitov, 2013; Hamoud et al., 2014; Mathur and Singh, 2013). In particular, *S. aureus* and MRSA cause serious infectious symptoms such as meningitis, endocarditis, and sepsis in patients with burns and chronic wounds (Washida et al., 2011; Fernandez Guerrero et al., 2012; Glik et al., 2012). *K. pneumoniae* is a non-motile, encapsulated rod-shaped bacterium found on the skin and in the respiratory system, including the mouth, lower respiratory tract, and lungs (Goto et al., 2006). In addition, *S. dysenteriae* causes severe dysentery (shigellosis) through the release of the deadly shiga toxin as well as colitis, rectal prolapse, and central nervous system (CNS) damage (Harrison et al., 2005; Sipetic-Grujicic et al., 2010; Tesh, 2010; El-Gendy et al., 2012). According to previous studies, the excretion/secretion of *L. sericata* larvae effectively inhibited the growth of bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus carnosus*, *S. aureus*, and MRSA strains (Thomas et al., 1999; Kerridge et al., 2005; Daeschlein et al., 2007; Bexfield et al., 2008; Jaklic et al., 2008; Andersen et al., 2010). Furthermore, the extracts of *Musca domestica* (Diptera: Muscidae) larvae induced antibacterial activities against Gram-positive bacteria (*S. aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*E. coli*, *S. dysenteriae*, *Salmonella typhimurium*, and *Bacterium pyocyaneum*) in a dose-dependent manner (Hou et al., 2007). Until recently, maggot therapy has been known as an effective method for treating chronic, necrotic, and infected wounds, as well as for healing burns ''*in vivo*'' (Prete, 1997; Feng et al., 2010). Moreover, these studies demonstrate not only the effects of larvae therapy both ''*in vitro*'' and ''*in vivo*'' but also various biological functions and effects of the larvae. Nevertheless, the effects of the active substance/compounds isolated from *H. illucens* larvae and their potential pharmacological functions have not yet been reported in various bacteria, cells, and animals. In a previous study, we reported that the extract of *H. illucens* larvae induced antibacterial activity against Gram-negative bacteria (Choi et al., 2012). For this reason, the main objective of the present study was to evaluate the pharmacological effects and functions of hexanedioic acid isolated from *H. illucens* larvae, and also to demonstrate the antibacterial effects of a potential candidate substance against various pathogenic and drug-resistant bacteria, such as MRSA.

Materials and Methods

Materials

Liquid nutrient broth was purchased from Duchefa Biochemie (Haarlem, Netherlands), and nutrient Mueller Hinton agar powder was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Petri-dishes (87 mm) were purchased from Green Cross Corp (Seoul, Republic of Korea), and paper discs (6 mm) were purchased from Roshi Kaisha., Ltd. (Tokyo, Japan). The *n*-hexane, chloroform, ethanol, methanol, and distilled water were purchased from J.T. Baker® chemicals (USA). All other chemicals and reagents were purchased from Merck Chemical Co., Ltd. (Darmstadt, Germany) and Sigma Chemical Co., Ltd. (St. Louis, MO, USA).

Microorganisms

Bacterial strains used in this study were *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 13883), and *Shigella dysenteriae* (ATCC 9750). The bacteria were purchased from American Type Culture Collection (USA). A clinical strain of MRSA isolated from a patient with infected chronic wound was provided by the Department of Microbiology, Kyung Hee University School of Medicine.

Preparation of extracts and fractions of H. illucens larvae

Fourth and sixth-instar larvae of *H. illucens* were used for this study. Colonies of *H. illucens* were maintained under a lighting regime of 16:8 (L:D) hours to mimic photoperiod at room temperature (25 ± 2 °C). Light was supplied by full-spectrum light bulbs controlled by plug-in timer. The larvae were maintained in electric propagators with plug-in thermostats at room temperature. The larvae were washed five times with distilled water at room temperature, and then dried with air on a clean bench at room temperature for 6 h. Next, *H. illucens* larvae (500 g) were independently extracted three times with 4 L of *n*-hexane, chloroform, ethanol, methanol, and distilled water at room temperature for 24 h, respectively (Fig. 1). The extracts were filtered using filter paper and a vacuum pump, then evaporated under reduced pressure using a rotary evaporator in a vacuum at 40 °C, and dried under a deep-freeze dryer after evaporation. Among the extracts, the methanol extract of *H. illucens* larvae strongly inhibited the growth and proliferation of bacteria compared with other extracts. The methanol extract was further diluted in methanol and mixed with silica gel. The mixed solution was evaporated under reduced pressure using a rotary evaporator in a vacuum at 40 °C, and then the silica gel was coated with methanol extract. The methanol extract-coated gel was loaded onto a cotton wool pad at the top of a silica gel column and divided into several fractions by silica gel column chromatography method with *n*-hexane–$CHCl_3$–MeOH eluent. All fractions were analyzed by thin layer chromatography, and finally divided into ten column-fractions, including F4/ME (fraction 4 obtained from the methanol extract). Among these fractions, F4/ME effectively inhibited the proliferation of bacteria compared with other fractions, and was used for high performance liquid chromatography (HPLC) analysis. All extracts and fractions were filtered using a 0.20 μm syringe filter (Roshi Kaisha, Ltd., Tokyo, Japan) and stored at −80 °C until use.

HPLC analysis

F4/ME was analyzed by a YL9100 HPLC system (Young Lin Instrument Co., Ltd., Korea) containing a C-18 reverse phase

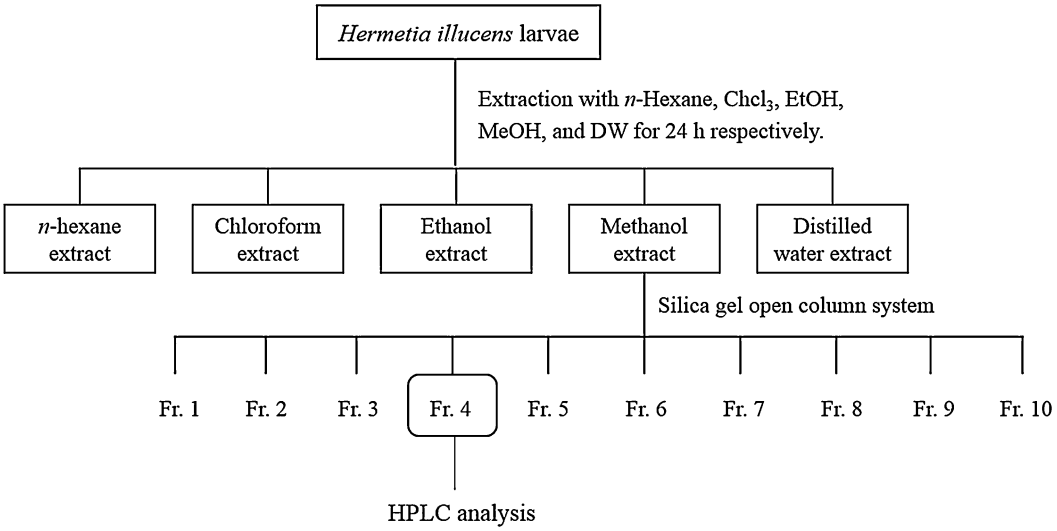

Fig. 1 – Extraction and fractionation process of *H. illucens* larvae. *H. illucens* larvae were extracted sequentially with ***n***-hexane, chloroform, ethanol, methanol and distilled water at room temperature three times for 24 h (total 72 h). Among these extracts, the methanol extract showed antibacterial activity against all bacteria tested. The methanol extract was divided into ten fractions by silica-gel column chromatographic method.

column (4.5 mm i.d. × 250 mm, 5.0 μm particle diameter) with an injection volume of 60 μl, a column flow rate of 1.0 ml/min, and a mobile phase of 100% methanol and 100% water (85:15 for 100 min). The analysis was performed at a temperature of 40 °C and a UV wavelength of 230 nm.

Gas chromatography/mass spectrometry (GC/MS) analysis

F8/HP (HPLC sub-fraction 8 isolated from F4/ME) isolated from F4/ME was analyzed by GC/MS using an Agilent 6890 Plus gas chromatograph equipped with a 5973N mass selective detector, quadrupole mass spectrometer system (Agilent Technologies, Inc., Palo Alto, CA, USA), and a DB-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific Inc., Folsom, CA, USA) after purification. The GC oven temperature was maintained at 60 °C for 2 min and then ramped to 320 °C at 20 °C/min. The sample was injected in the split mode at a splitting ratio of 1:10. Helium gas was used as a carrier, set at a constant flow rate of 1 ml/min.

Preparation and inoculation of the test bacteria

The bacteria were grown in 10 ml of LB broth at 37 °C for 24 h. Fresh LB broth solution (10 ml) was inoculated with 10 μl of this bacterial culture and incubated at 37 °C. Cell counts were determined by counting the colonies after petri dish plates (Ø 87 mm) were incubated at 37 °C for 24 h.

Turbidometric (TB) assay

The TB assay was used to determine the antibacterial effects of hexanedioic acid against the growth and proliferation of bacteria. As mentioned above, the bacteria were grown in 10 ml of sterile LB broth at 37 °C and adjusted to a density of 4×10^5 colony forming units (CFU)/ml in fresh LB broth. The phased active substances and hexanedioic acid were mixed with 200 μl of culture medium containing bacteria at 4×10^5 CFU/ml and placed in triplicate into wells of sterile flat-bottom, 96 well microtiter plates. The plates were incubated at 37 °C for 24 h, and the optical density (OD) was measured every 3 h using an ELISA reader at 590 nm. The percentage of cell viability (% of CV) was calculated as follows: $\%\ \text{of CV} = (OD_{\text{drug tested wells}} - OD_{\text{blank}})/(OD_{\text{control}} - OD_{\text{blank}}) \times 100$.

Resazurin assay

The *in vitro* antibacterial activities of hexanedioic acid were determined by resazurin assay using 96-well microtiter plates. Briefly, all strains were grown in 10 ml of fresh LB broth until the culture reached a turbidity equal to that of 0.5 McFarland standard (1.5×10^8 CFU/ml) at 37 °C. All bacteria were adjusted to a density of 4×10^5 CFU/ml in fresh LB broth. Finally, the bacterial suspensions were inoculated into all wells of a microtiter plate containing final concentrations (2.5–80 μg/ml) of hexanedioic acid and the plate was incubated at 37 °C for 24 h. Growth controls containing no antibiotic and blank controls without inoculation were also included. After 24 h of incubation, 30 μl of freshly prepared 0.01% resazurin solution was added to all wells of a microtiter plate. A change in color from blue to pink indicating cell viability was observed after 24 h of incubation. For quantitative analysis, the fluorescence intensity was measured by excitation at 560 nm and emission at 590 nm using a fluorescence mircotiter plate reader (Victor™ X5, Perkin Elmer, Waltham, MA, USA).

Agar disk diffusion assay

To evaluate the antibacterial activity of hexanedioic acid against various bacteria, we measured zones of growth inhibition of the test and control bacteria. The bacteria were incubated in 10 ml of

sterile LB broth at 37 °C and adjusted to a density of 4×10^5 CFU/ml in fresh sterile LB broth. Petri dishes with nutrient agar were inoculated with 50 μl of bacterial culture, and the culture was evenly mixed and immediately distributed on 87 mm petri dishes. Next, 60 μl of the test solution and antibiotics were added to each disk (Ø 6 mm) in a concentration-dependent manner, and the discs were dried on a clean bench at room temperature for 30 min. The discs were placed on the surface of the agar plates, and the plates were incubated at 37 °C. Penicillin–streptomycin (20 U/ml and 20 μg/ml/disk), vancomycin (20 μg/ml/disk), cephamycin (20 μg/ml/disk), and tetracycline (20 μg/ml/disk) were used as antibacterial positive controls. The diameters of bacterial inhibition zones surrounding the discs were measured visually after 24 h and 48 h.

Statistical analysis

All results were expressed as mean ± standard deviation of five independent experiments. Statistical analysis of the data was performed using the Student's *t*-test and one-way analysis of variance (ANOVA). $^*p < 0.05$ was considered to be statistically significant.

Results

Extraction and fractions of H. illucens larvae

The yields of *H. illucens* larvae extracts after solvent evaporation were 0.4% (*n*-hexane), 0.4% (chloroform), 2.5% (ethanol), 2.0% (methanol), and 0.5% (H_2O), respectively. In addition, we identified the most effective antibacterial substance which induces the antibacterial effects using different antimicrobial assays as follows: agar disk diffusion, turbidometric, and resazurin assays. Among the extracts, the methanol extract strongly inhibited the proliferation of bacteria in a dose-dependent manner, whereas *n*-hexane, chloroform, ethanol and H_2O extracts did not show any antibacterial activity against the tested bacteria. Ten fractions including F4/ME were obtained by column chromatography, and F4/ME showed the most effective antibacterial activity compared with other fractions. Furthermore, F4/ME strongly inhibited the growth and proliferation of bacteria in a concentration-dependent manner. The complete extraction and fractionation process is presented in Fig. 1.

HPLC and GC/MS analysis

F4/ME was analyzed by HPLC and showed various peaks in the HPLC chromatogram. The peaks were divided into fifteen sub-fractions based on HPLC analysis. To identify the active substance causing the most effective antibacterial activity, bacteria were treated with various concentrations of HPLC sub-fractions for 24 h. Among the HPLC sub-fractions, F8/HP strongly inhibited the proliferation of bacteria in a concentration-dependent manner compared with other HPLC sub-fractions. The HPLC chromatogram for F8/HP is shown in Fig. 2. The chemical components of the active substance causing the most effective antibacterial activity were identified using GC/MS analysis. F8/HP was identified as hexanedioic acid, and its profile is presented in Fig. 3.

Antibacterial activity of hexanedioic acid isolated from H. illucens larvae extracts

The antibacterial effects of hexanedioic acid isolated from *H. illucens* larvae were measured using the agar disk diffusion assay. In this assay, the antibiotics caused zones of growth inhibition against all tested bacteria, and hexanedioic acid

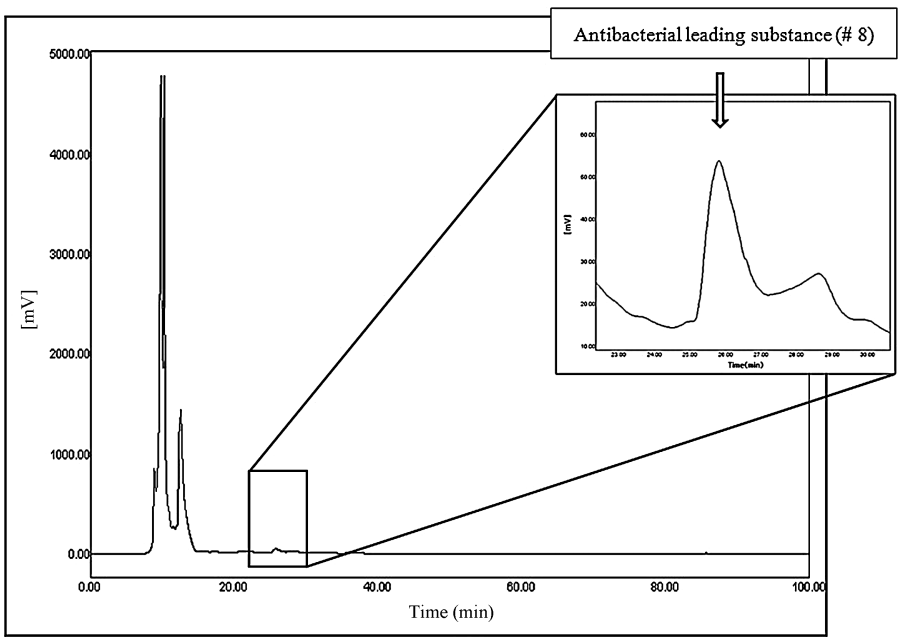

Fig. 2 – HPLC chromatogram of F8/HP isolated from F4/ME. F4/ME was analyzed by HPLC and was divided into fifteen sub-fractions including F8/HP.

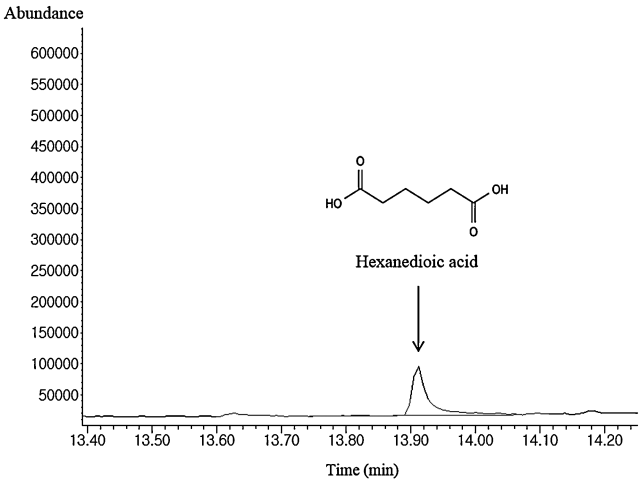

Fig. 3 – GC/MS profile of F8/HP isolated from *H. illucens* larvae. F8/HP was analyzed by GC/MS, and its composition was identified as hexanedioic acid.

effectively induced antibacterial effects against the Gram-positive bacteria (*S. aureus* and MRSA) as well as the Gram-negative bacteria (*K. pneumoniae* and *S. dysenteriae*) (Fig. 4). The differences in susceptibility among antibacterial activities were markedly observed when the plates were incubated for 24 h, and all bacteria showed greater zones of growth inhibition in 24 h than in 48 h (Table 1). However, the antibacterial activity of hexanedioic acid gradually decreased in a time-dependent manner. These results indicate that hexanedioic acid has antibacterial activity and properties that effectively inhibit the growth of bacteria in a concentration-dependent manner.

The effect of hexanedioic acid in TB and resazurin assays

The antibacterial activity of hexanedioic acid was further evaluated using the TB and resazurin assays (Table 2). After the bacteria were incubated with various concentrations (10–160 μg/ml) of hexanedioic acid for 24 h, their viabilities

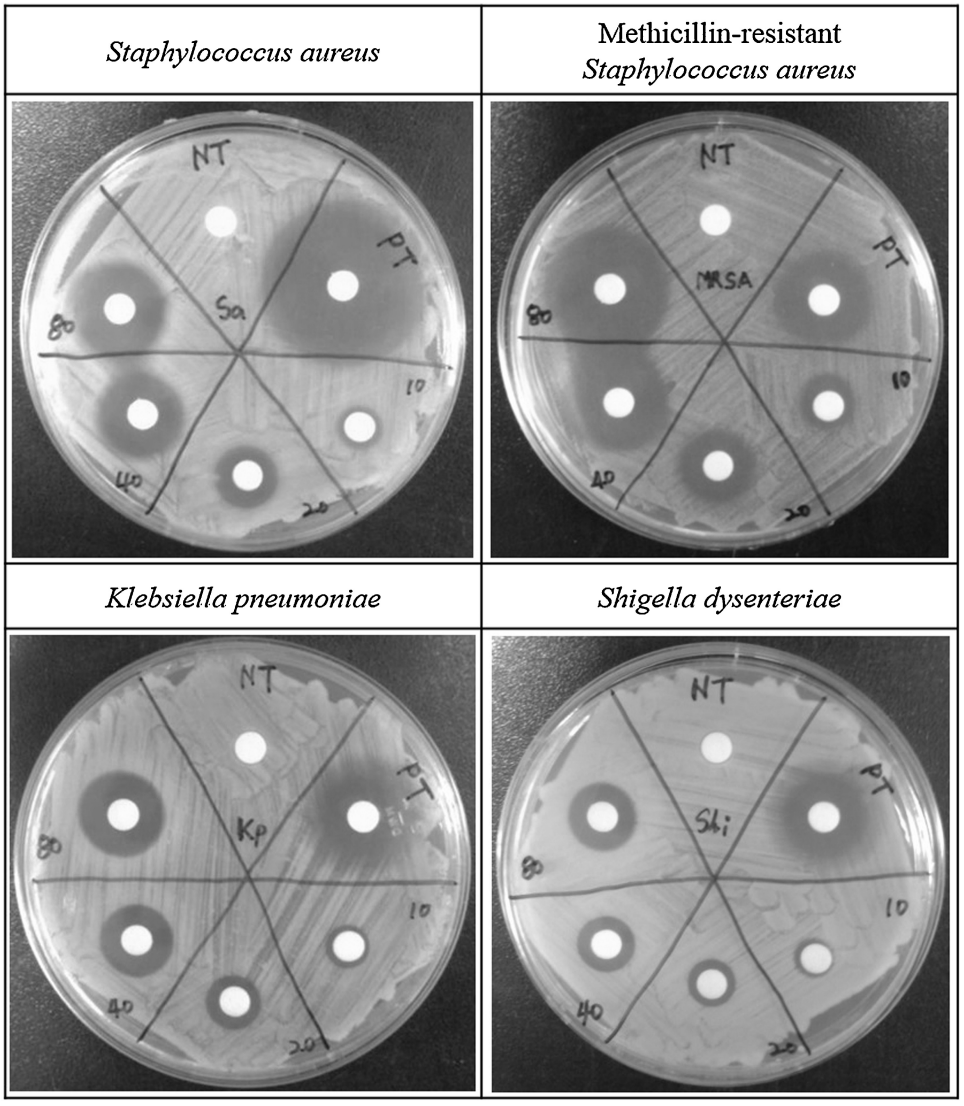

Fig. 4 – Zones of bacterial growth inhibition. The zones of bacterial growth inhibition show the antibacterial activity of hexanedioic acid on *S. aureus*, MRSA, *K. pneumoniae*, and *S. dysenteriae*. The diameters of bacterial inhibition zones surrounding the discs were measured after 24 h of incubation.

Table 1 – Zones of bacterial growth inhibition of hexanedioic acid isolated from *Hermetia illucens* larvae.

Concentration (μg/ml)	Test hours	Mean zone of inhibition of the tested bacteria (mm)[a]			
		Gram-positive bacteria		Gram-negative bacteria	
		Staphylococcus aureus	Methicillin-resistant *Staphylococcus aureus*	*Klebsiella pneumoniae*	*Shigella dysenteriae*
10	24 h	8.36 ± 0.23*	9.75 ± 0.27*	7.74 ± 0.21*	6.46 ± 0.17
20		12.41 ± 0.13*	14.35 ± 0.15*	10.34 ± 0.14*	9.46 ± 0.22
40		16.54 ± 0.20*	19.61 ± 0.24*	13.71 ± 0.26*	11.23 ± 0.19*
80		18.27 ± 0.18*	23.35 ± 0.15*	16.62 ± 0.18*	12.96 ± 0.24*

All results are expressed as means ± standard deviation of five independent experiments.
[a] Antibacterial activity against the bacterial growth was measured using the agar disk diffusion assay.
* $p < 0.05$ versus the control.

Table 2 – Antibacterial activity of hexanedioic acid isolated from *Hermetia illucens* larvae.

Test hour	Concentration (μg/ml)	The tested bacteria			
		Gram-positive bacteria		Gram-negative bacteria	
		Staphylococcus aureus	Methicillin-resistant *Staphylococcus aureus*	*Klebsiella pneumoniae*	*Shigella dysenteriae*
24 h	10	+	+*	+*	+
	20	+*	+*	+*	+*
	40	+	+*	+*	+*
	80	+*	+*	+*	+*

Antibacterial activity against the growth of bacteria was measured using the turbidometric (TB) assay. The bacteria were incubated with various concentrations (10–80 μg/ml) of hexanedioic acid at 37 °C for 24 h. The "+" sign indicates antibacterial activity. All results are expressed as mean ± standard deviation of five independent experiments.
* $p < 0.05$ versus the control.

Table 3 – Inhibitory concentration of hexanedioic acid isolated from *Hermetia illucens* larvae against bacterial growth.

Inhibitory concentration	Test hour	Inhibitory concentration of the bacteria (μg/ml)			
		Gram-positive bacteria		Gram-negative bacteria	
		Staphylococcus aureus	Methicillin-resistant *Staphylococcus aureus*	*Klebsiella pneumoniae*	*Shigella dysenteriae*
IC_{90}	24 h	123.874	120.694	122.643	123.335
MIC		140.377	137.369	139.117	139.704

Antibacterial activity of hexanedioic acid was measured by the turbidometric (TB) assay. IC_{90}, the 90% inhibitory concentration against bacterial growth. MIC, minimum inhibitory concentration (MIC) against bacterial growth. All results are expressed as mean ± standard deviation of five independent experiments.

were markedly inhibited in a concentration-dependent manner and were 0% at a concentration of 160 μg/ml. Furthermore, hexanedioic acid not only showed higher antibacterial activity in 24 h than 48 h (Fig. 5) but also demonstrated its antibacterial effect through the bacterial color change observed in the resazurin assay (Fig. 6). These results showed similar tendencies to those of the agar disk diffusion assay. In addition, the 90% inhibitory concentration (IC_{90}) values of hexanedioic acid against the growth of *S. aureus*, MRSA, *K. pneumoniae*, and *S. dysenteriae* for 24 h were 123.874, 120.694, 122.643, and 123.335 μg/ml, respectively (Table 3). Notably, the antibacterial effects of natural hexanedioic acid isolated from *H. illucens* larvae were consistent with the results of commercial hexanedioic acid (Fig. 7). These results demonstrate that hexanedioic acid not only induces antibacterial activities which more strongly inhibit the growth of the tested bacteria in 24 h compared to 48 h, but also has the potential which could be used as an antibacterial agent.

Discussion

Until recently, medicinal plants (*Glycyrrhiza uralensis* Fischer, *Ginkgo biloba* L, *Corni fructus*, *Schizandra chinensis* Baillon, *Melia azedarach* L., ginger, and ginseng) have traditionally been used in oriental medicine for the treatment of various diseases and symptoms such as cough, gastroenteritis, headache, arteriosclerosis, and hypercholesterolemia. Traditional oriental medicine has used various/useful biological resources such as bee stings and centipedes in addition to medicinal plants to treat human diseases. Furthermore, various substances derived from medicinal plants and insects have shown

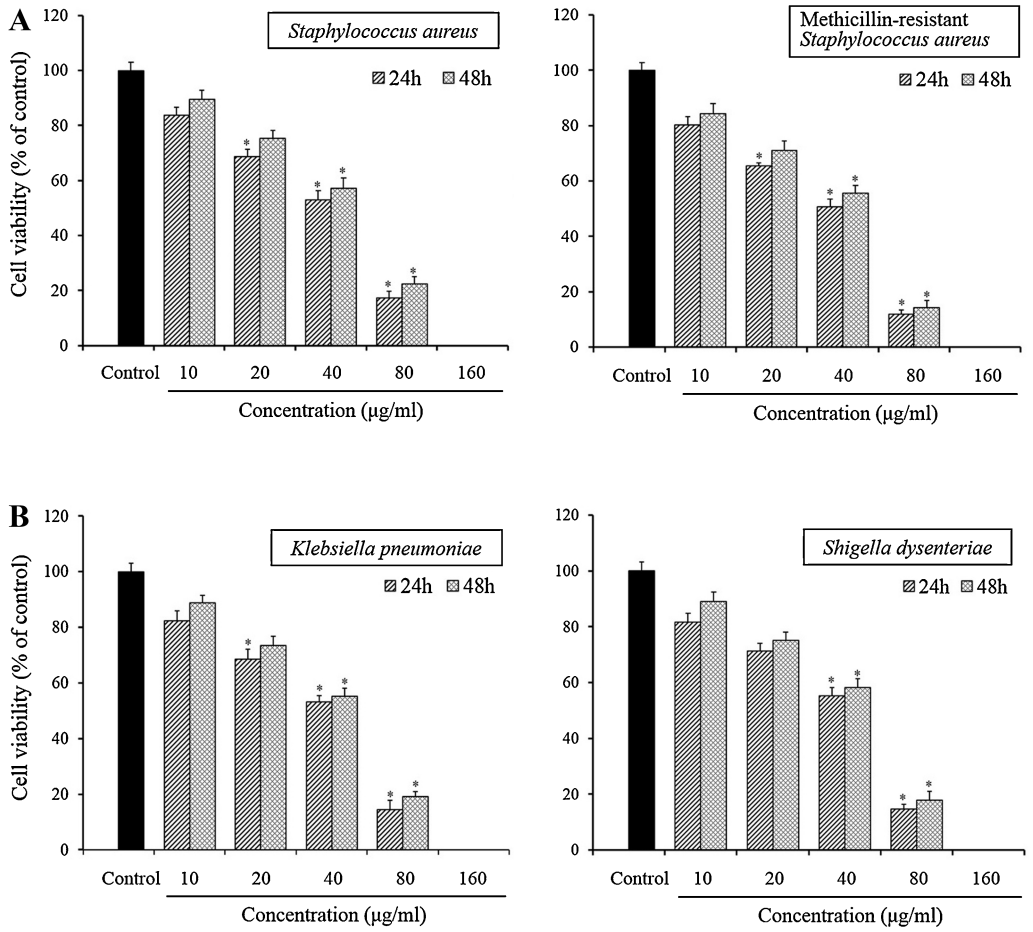

Fig. 5 – Antibacterial activity of hexanedioic acid against the viability of bacteria. The bacteria were incubated with various concentrations (10–160 μg/ml) of hexanedioic acid at 37 °C for 24 h and 48 h, and the bacterial survival rate was determined by TB assay. The bacterial viability was 0% at concentrations of 160 μg/ml. (A) *S. auerus* and MRSA. (B) *K. pneumoniae* and *S. dysenteriae*. All results are expressed as mean ± standard deviation of five independent experiments. $^{*}p < 0.05$ versus the control.

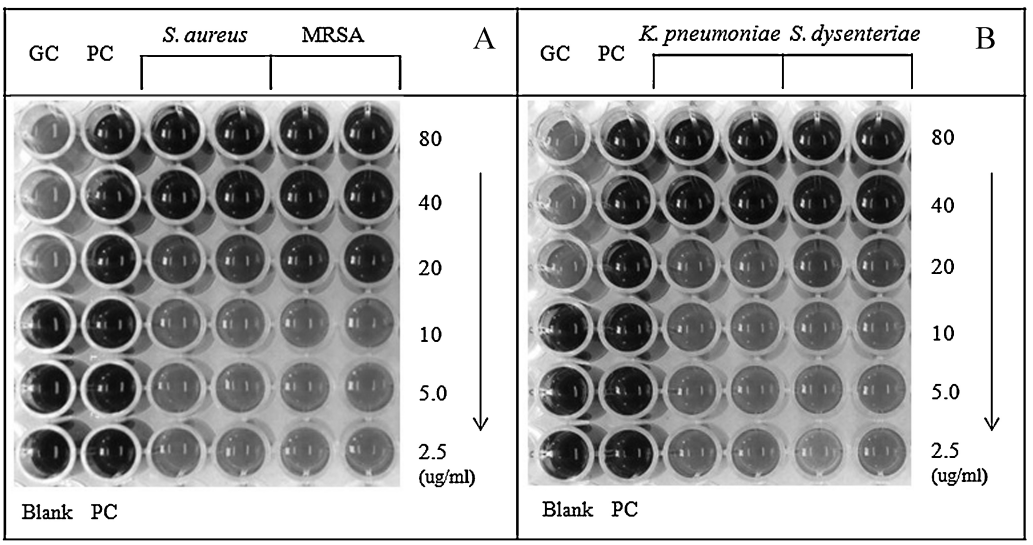

Fig. 6 – The *in vitro* antibacterial activity of hexanedioic acid determined by the resazurin assay. The bacteria were incubated with various concentrations (2.5–80 μg/ml) of hexanedioic acid at 37 °C for 24 h. A change in color of the bacteria from pink to dark blue indicates a reduction of bacterial growth. The color change shows the antibacterial effects of hexanedioic acid on (A) *S. aureus* and MRSA, and (B) *K. pneumoniae* and *S. dysenteriae* (GC, growth control containing no antibiotic; Blank, blank controls without inoculation; PC, positive control containing antibiotic). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

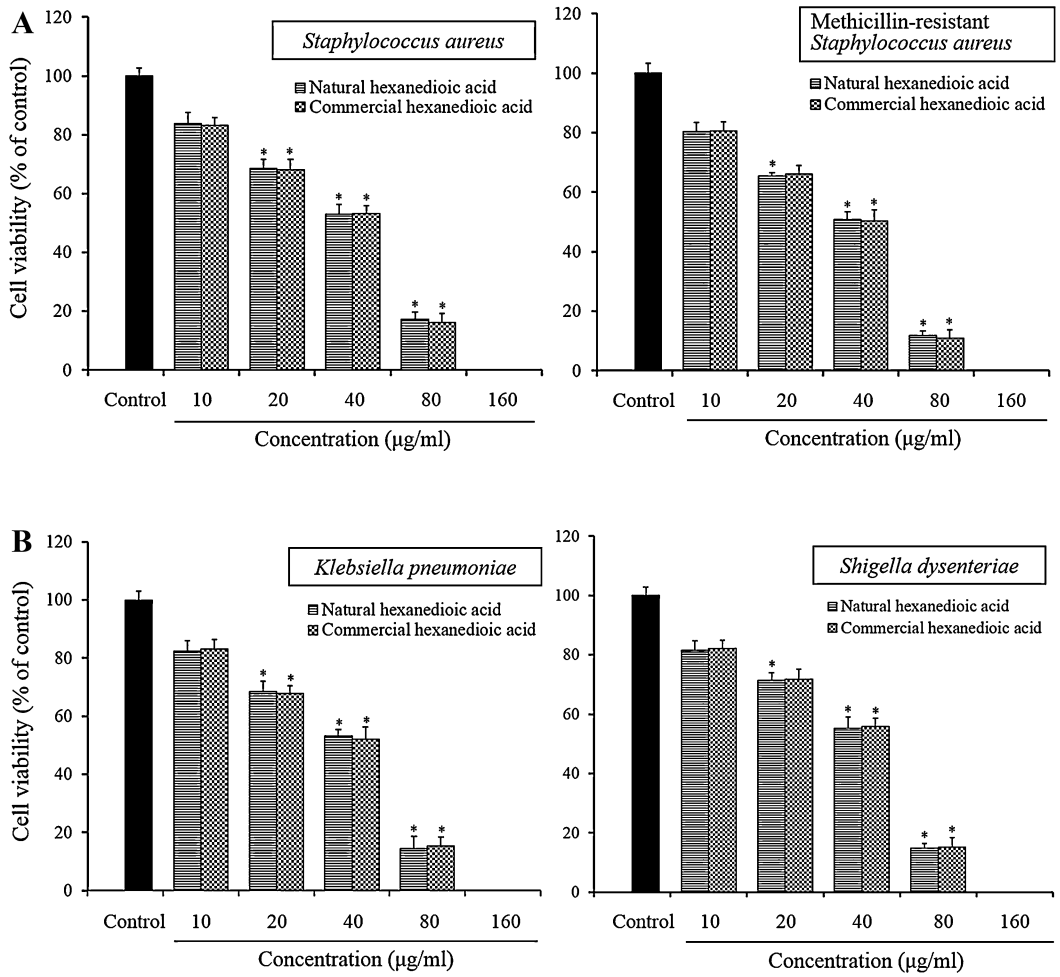

Fig. 7 – The antibacterial activity of hexanedioic acid isolated from *H. illucens* larvae and commercial hexanedioic acid. The bacteria were incubated with various concentrations (10–160 μg/ml) of both natural hexanedioic acid and commercial hexanedioic acid at 37 °C for 24 h. (A) *S. auerus* and MRSA. (B) *K. pneumonia* and *S. dysenteriae*.

inhibitory effects against pathogens including bacteria, *Helicobacter pylori* (Campylobacterales: Helicobacteraceae), parasites, and viruses. Some of these natural products have also been used to treat other diseases such as cancers (Mesquita et al., 2005; O'Mahony et al., 2005; Ding et al., 2006; Choi et al., 2008; Ruddock et al., 2011; Qiu and Chen, 2013; Choi et al., 2013). Until recently, novel antibiotics derived from diverse biological resources and synthetic compounds have been developed to treat pathogenic microorganisms. However, the overuse of antibiotics has rapidly increased the incidence of multidrug-resistant bacteria, so that many patients were confronted with more difficult situation to treat serious bacterial infections such as MRSA and VRSA (vancomycin resistant *S. aureus*) in the present than the past. For these reasons, the development of new antimicrobial substances and drugs that are more effective than existing antibiotics is urgently needed. Nevertheless, studies to develop new drugs or compounds derived from insects/arthropods have been rarely reported. In this respect, the study of insect-derived compounds is an unexploited field for developing new substances, and its products involve the potential which can be used as a biological resource in a medical field. Recently, *H. illucens* larvae have been used to convert food wastes and to produce assorted feed in many countries (Myers et al., 2008; Diener et al., 2009; Li et al., 2011). In particular, the poultry industries in Europe and the USA have applied these larvae to grub containers that are used in poultry farms as a cost-cutting measure. The insects infected with bacteria, fungi, or viruses induce interconnected biological defense-signaling mechanisms such as proteolytic cascades, cellular defense-pathways, phagocytosis, synthesis of antibacterial peptides, and encapsulation (Hoffmann et al., 1996). In addition, various insects such as the house fly, centipede, honeybee, and ant have various compounds and peptides that might be developed as potent pharmaceuticals (Pemberton, 1999; Ang et al., 2009; Feng et al., 2010; Vercruysse et al., 2010; Zhou et al., 2011; Ratcliffe et al., 2011; Staljanssens et al., 2011; Rifflet et al., 2012).

S. aureus induces infectious syndromes such as bacteremia, which can be caused by postoperative wound infections in hospitals. *S. aureus* is also found on the skin, in respiratory diseases, and in endovascular infections. Furthermore, MRSA is a multidrug-resistant bacterium that does not respond to

typical antibiotics such as methicillin, dicloxacillin, and cephalosporin, and it can cause serious infectious diseases such as necrotizing pneumonia, fasciitis, and sepsis in patients with wounds and immunodeficiency. Additionally, *K. pneumoniae* causes serious symptoms such as hemorrhage and inflammation in the lung and lower respiratory tract in patients with weakened immune systems, which finally are proceed with pneumonia. The increased incidence of these infectious bacteria has stimulated the interest of researchers in various aspects for eradicating infectious diseases. For these reasons, biomedical researchers have focused on biodiversity and the effects of interactions between soil or marine microbes, viruses, bacteria, and insects to develop novel ways to inhibit or treat pathogens. Bacterial infections in the intestinal tract of maggots are eliminated by an antibacterial substance produced by the maggots (Mumcuoglu et al., 2001). In particular, maggot secretions show antibacterial activity against pathogenic bacteria such as *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Enterobacter cloacae*, *P. aeruginosa*, *K. pneumoniae*, MRSA, *S. aureus*, *E. coli*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Streptococcus agalactiae*. However, these secretions had no antibacterial effects against *Proteus mirabilis*, *Staphylococcus epidermidis*, *S. typhimurium*, and *Candida albicans* (Thomas et al., 1999; Bexfield et al., 2004, 2008; Kawabata et al., 2010). Nevertheless, maggots have been used in maggot debridement therapy (MDT) such as the debridement of necrotic tissue, the treatment in chronic wounds, and promotion of granulation tissue (Chan et al., 2007; Blueman and Bousfield, 2012; Marineau et al., 2011; Gilead et al., 2012). In general, the cell wall of Gram-positive bacteria consists of only a single-unit thick peptidoglycan layer, whereas Gram-negative bacteria include a thin peptidoglycan layer with an outer membrane containing components such as lipoprotein, lipopolysaccharide (LPS), and phospholipids. In this respect, differences in the susceptibility of Gram-positive and Gram-negative bacteria to hexanedioic acid may be associated with the inactivation of cell-signaling pathways, and degradation of an intracellular metabolic mechanism caused by the interaction of bacteria and hexanedioic acid.

In summary, hexanedioic acid isolated from *H. illucens* larvae effectively inhibited the growth and proliferation of both Gram-positive and Gram-negative bacteria in a concentration-dependent manner. In particular, hexanedioic acid showed antibacterial effects on *K. pneumoniae* that can causes severe pneumonia, especially in patients being treated with ampicillin or those with weakened immune systems as well as drug-resistant bacteria such as MRSA. Furthermore, the results of this study not only indicate the potential for utilization of hexanedioic acid in medical fields, but also demonstrate a new biological function and pharmacological effect regarding antibacterial activity of hexanedioic acid against pathogenic bacteria. Therefore, this study provides the potential that hexanedioic acid can be used as a novel antibacterial candidate substance for the treatment of bacterial infections.

Conflict of interest

The authors have no conflicts of interest.

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