# Antimicrobial Activity of Excretory and Secretory Products from *Chrysomya megacephala* (Diptera: Calliphoridae) Larvae

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***Background:*** *Excretory and secretary products (ES) from blow fly larvae are alternative sources for the discovery of new antimicrobial agents and may be a solution to an increasing global drug resistance problem. Chrysomya megacephala (Diptera: Calliphoridae) is recognized as a medically important blow fly species and it is the most prevalent blow fly species of Thailand. However, study of antimicrobial activity of this species is limited.*
***Objective:*** *To determine the antimicrobial activity of ES products from the third stage larvae of Chrysomya megacephala and morphological changes of pathogens after treatment with ES products.*
***Material and Method:*** *Five hundred 3-day-old larvae of Chrysomya megacephala were used to collect the ES products. The disc diffusion method and minimum inhibitory concentration (MIC) assay were used for the determination of the antimicrobial activity of the ES products against pathogenic bacteria (Staphylococcus aureus ATCC 25923, Bacillus cereus ATCC 1178, Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 25983), and fungi (Candida albicans ATCC 90028 and Malassezia furfur CBS 1878T). The morphological changes of the pathogens after treatment with the ES products were observed under scanning electron microscope (SEM).*
***Results:*** *The results of the disc diffusion method showed that the ES products can inhibit the growth of Pseudomonas aeruginosa. The MIC of the ES products against Pseudomonas aeruginosa was 150-200 µg/ml. Pseudomonas aeruginosa cells treated with the ES products displayed longer and larger sizes than non-treated cells and some of them had atrophy forms with irregular shapes. No fungicidal activity was found in this study.*
***Conclusion:*** *The ES products of this fly species are good sources for the discovery of anti-P. aeruginosa agents in the future.*

***Keywords:*** *Antimicrobial activity, Excretory and secretory products, Larvae, Chrysomya megacephala*

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Drug resistance of pathogens is a major healthcare problem worldwide and is increasing steadily[1] and new drugs or alternative treatments are required. Antimicrobial peptides (AMP) from insects are alternative sources for the solution of this problem. Anti-bacterial substances exist within many insects, due to their intimate contact with abundant microorganisms in their natural habitats and/or with injured or infected insects[2-4]. Currently, there are many reports concerning AMP from insect sources[5]. For example, Lucifensin I is a novel insect defensin extracted from the blow fly larvae of *Lucilia sericata* (Diptera: Calliphoridae)[6]. This peptide has the ability to inhibit growth of gram positive bacteria[7,8]. Lucifensin II is another insect defensin purified from hemolymph extract of *Lucilia cuprina* (Diptera: Calliphoridae) larvae[9]. This peptide demonstrated antibacterial properties against positive bacteria (*Micrococcus luteus* and *Staphylococcus epidermidis*) and gram negative bacteria (*Escherichia coli*)[10].

*Chrysomya megacephala* (Diptera: Calliphoridae), is a medically-important blow fly species

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distributed worldwide and in numerous near human habitations and it is the most prevalent blow fly species found in Thailand[11]. Larvae of this species are scavengers that live in microbial environments and possess efficient protection mechanisms which come from immune responses that allow survival in these kinds of environments[12]. Therefore, these larvae may be potential candidates in a search for AMP. However, studies of AMP related to this blow fly species are limited. Sahalan et al[12] reported that the hemolymph of *C. megacephala* larvae showed only bactericidal effects against gram positive bacteria (*Bacillus subtilis* and *Staphylococus aureus*) but there was no effects against the gram negatives. On the contrary, the hemolymph of pupae only lysed *Escherichia coli*. Antimicrobial activity of the ES products of this fly larvae has not been reported. To address this situation, the aims of this study were to determine the antimicrobial activity of the ES products isolated from larvae of *C. megacephala* against selected pathogens and to determine the morphological changes of the pathogen after treatment with the products under scanning electron microscope (SEM).

## Material and Method

### *Rearing of blow fly*

Adults of *C. megacephala* used in this study were maintained in a rearing cage (30x30x30 cm) and fed with 2 kinds of food, fresh pork liver (food source and oviposition site) and a mixture of 10% (w/v) sucrose solution with 5% (v/v) multivitamin syrup (SEVEN SEA, England) solution (food source). When oviposition occurred, fly eggs were gently transferred from the rearing cage to a new rearing box containing small pieces of fresh pork liver (40 g) as food for the larvae. Each rearing box consisted of 150-200 larvae. Fresh pork liver (40 g) was changed daily until they were 3 days old. They were then collected from the rearing boxes for use in the experiment. All flies were maintained under room temperature conditions (24-28°C) in the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand.

### *Preparation of the extract of the excretory and secretory products from larvae*

The method for the collection of the ES products used in this study was modified from Van der Plas et al[13]. Briefly, five hundred 3-day-old larvae were collected from the rearing box. They were then washed with 70% alcohol and then with sterile Milli-Q water three times. The larvae were divided into 10 groups and each group was kept in a sterile centrifuge tube (15 ml) with 200 microliter of sterile Milli-Q water and then incubated for 60 min at room temperature in the dark. Next, the ES products were collected and pooled in a new sterile micro-centrifuge tube. Protease inhibitor cocktail (1X) (AMRESCO, USA) was added to inhibit degradation of proteins/peptides. The ES products were then centrifuged at 1,300 xg for 5 min at 4°C to remove particulate material. After that, the supernatant was filtrated by the use of 0.45 µm syringe filter sterilization. The sterile supernatant was kept in a new sterile micro-centrifuge tube at -80°C. The ES products were checked for sterility before use in the experiments by spreading on a nutrient agar (NA) and then incubated at 37°C. If any bacterial colony was found on NA, the ES products were discharged from the experiment.

### *Determination of protein concentration*

The amount of protein in the ES products was determined using the BioRad Protein assay kit (BioRad, USA) following to the manufacturer's instructions. Protein concentration was calculated from standard albumin.

### *Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of protein*

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used for analysis of protein in this experiment. The samples with 4x loading dye were heated at 94°C for 5 minute by use of the Multi-Block Heater (LAL-LINE PLAZA, USA). Samples of the ES products (20 µg/sample) and protein markers were individually loaded into each lane. They were then run on 15% SDS-PAGE (Amresco, USA) in reducting of the condition at 180 V for 45 min. The gel was stained in 0.1% coomassie blue G-250 for 1 hour. Consequently, gel was de-stained with acid methanol for 5 minute and then left in distilled water overnight. The protein profile was observed and photographed by use of a digital camera.

### *Determination of the antimicrobial activity of the excretory and secretory products by using the disc diffusion method*

Microorganisms used in this study consisted of bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 1178, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 25983), and fungi (*Candida albicans* ATCC 90028 and *Malassezia furfur* CBS 1878T). Bacteria, *C. albicans*,

and *M. furfur* were grown on nutrient agar, Sabouraud dextrose agar, and Dixon agar respectively at 37°C for 18 hour before use. After that, each microorganism was prepared as concentration $1.5x10^8$ cells/ml and then swabbed on a testing medium. Muller Hinton agars (MHA), Sabouraud dextrose agar, and Dixon agar were used as testing media for testing antimicrobial activity against bacteria, *C. albicans*, and *M. furfur* respectively. After that, the ES products (20 µl) were dropped onto individually sterile paper discs (4 mm in diameter) placed on the testing media[15]. The protein concentration on each disc was approximately 60 µg. Reference drugs and sterile ultra-pure water were used as positive and negative controls respectively. The reference drugs were selected by following the Clinical Laboratory Standard Institute guidelines M100 document[16]. All plates were incubated at 37°C for 24 hou. The antimicrobial activities were measured from the zone of inhibition. This experiment was repeated 3 times.

***Minimum inhibitory concentration assay***

*P. aeruginosa* ATCC 25983 was selected to determine the MIC of the ES products according to the results from the disk diffusion method. This assay was determined in 96-well U bottom microtiter plate followed method of Teh et al[17].

***Determination of morphological changes of Pseudomonas aeruginosa after treated with excretory and secretory products by use of a scanning electron microscope***

The morphology of *P. aeruginosa* after treatment with the ES products was observed and compared with the normal cells. The samples from the growth control and the first well of MIC assay were used as normal and treated cells respectively. Each sample was dropped onto a sterile paper filter and individually processed by following the method of Kalab et al[18]. All samples were observed under SEM (JEOL: Japan) at the College of Medicine and Public Health, Ubon Ratchathani University, Ubon Ratchathani province in Thailand.

## Results

***Protein profile analysis from excretory and secretory products***

The ES products gave average protein quantification of 3,007.85±21.58 µg/µl. Protein profile by SDS-PAGE of *C. megacephala* ES products consisted of several peptides with different molecular weights including a single band at 8-12, 16, 26, between 16-26, 26-34, 34-45, 45-55, 62-82, 82, and 120 kDa as shown in Fig. 1.

***Antimicrobial activity***

The antimicrobial activity of the ES products determined by the disc diffusion method is shown in Table 1. The ES products showed only an inhibition area of *P. aeruginosa* and its MIC against *P. aeruginosa* was 150-200 µg/ml. No fungicidal activity was found in this study.

***Determination of morphological change of treated bacteria by use of a scanning electron microscope***

The ultrastructure of treated and non-treated *P. aeruginosa* with the ES products is shown in Fig. 2. The sizes and shapes of non-treated cells were normal (Fig. 2A) and their cell surfaces were smooth. In contrast, the treated cells displayed longer and larger sizes than non-treated cells (Fig. 2B). Moreover, the atrophy form with irregular shape was observed in *P. aeruginosa* treated with the ES products (Fig. 2C).

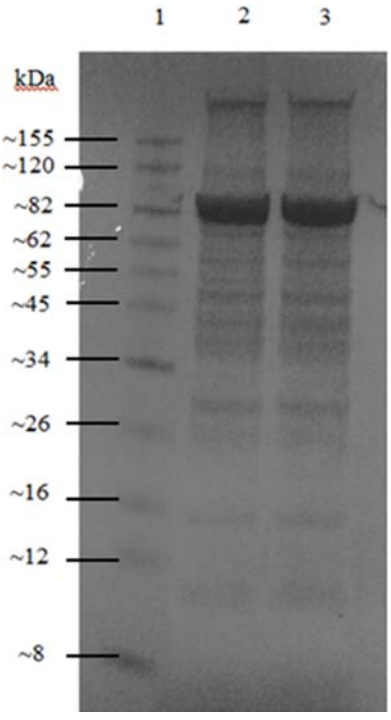


**Fig. 1** SDS-PAGE profile of *Chrysomya megacephala* ES products under reducing conditions; Lane 1: protein marker; Lane 2-3: profile of *C. megacephala* ES products; the numbers on the left indicate the masses of the molecular weight marker (kDa).

**Table 1.** Inhibition zone of the excretory and secretory products evaluated by the disc diffusion method

| Microorganisms | Inhibition zone (min ± SD; mm) | | | | | |
|---|---|---|---|---|---|---|
| | ES | G | P | S | N | Neg |
| *Pseudomonas aeruginosa* ATCC 25983 | 8.90±0.70 | ND | 15.15±2.29 | ND | ND | 0.00±0.00 |
| *Staphylococcus aureus* ATCC 25923 | 0.00±0.00 | 19.09±0.41 | ND | ND | ND | 0.00±0.00 |
| *Bacillus cereus* ATCC 1178 | 0.00±0.00 | 21.73±0.25 | ND | ND | ND | 0.00±0.00 |
| *Escherichia coli* ATCC 25922 | 0.00±0.00 | 17.86±1.59 | ND | ND | ND | 0.00±0.00 |
| *Candida albicans* ATCC 90028 | 0.00±0.00 | ND | ND | ND | 13.91±0.77 | 0.00±0.00 |
| *Malassezia furfur* CBS 1878T | 0.00±0.00 | ND | ND | 1.73±0.05 | ND | 0.00±0.00 |

ES = Excretory and secretory products; G = Gentamycin (10 µg); P = Pipericilin (100 µg); S = 2% Selenite; N = Nystatin (50 µg); Neg = Negative control; ND = not determined

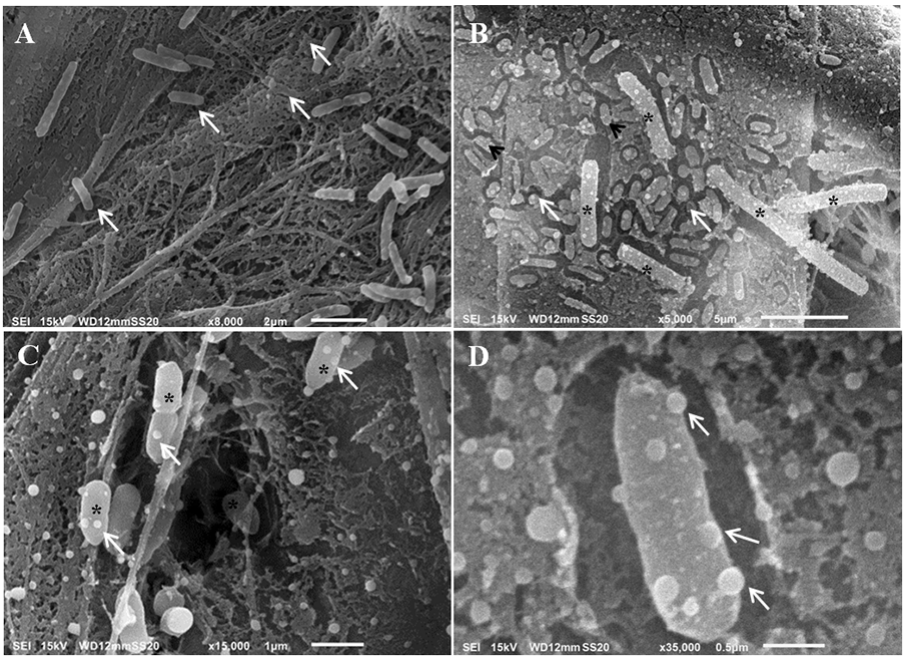


**Fig. 2** Scanning electron micrograph of *Pseudomonas aeruginosa* ATCC 25983. A) Non-treated cells showed the typical form (arrow). B) Treated cells by the ES products from *Chrysomya megacephala* larvae displayed atypical forms, including elongation (asterisks), oval shape (arrowheads), and atrophy form (white arrow). C) Higher magnification of atrophy cells indicating irregular shape (asterisks) covered with the ES products (white arrow). D) Higher magnification of treated *P. aeruginosa* cells showing ES (white arrow) attached to the cell surfaces.

Their surfaces were also covered with the ES products (Fig. 2D).

## Discussion

The study highlighted the potent antimicrobial property of the ES products from the third stage larvae of *C. megacephala* against *P. aeruginosa* by use of the disk diffusion method and revealed morphological changes of the treated cells by SEM. In a previous study by Taha et al[19], the healing property of the ES products of *C. megacephala* was observed on an artificial wound in a rabbit. Moreover, Ratcliffe et al[20] reported that the ES products of *C. megacephala* significantly inhibited the growth of *E. coli* and *S. aureus* by turbidometric assay without examining *P. aeruginosa*. However, differences of the procedures used for the extraction of the larval ES products and the techniques used for the determination of the

antibacterial activity may be involved in different antimicrobial activities[21,22].

Although the efficiency inhibition of the ES products against *P. aeruginosa* was less effective than pipericilin, the protein concentration of the ES products in each disk (~60 µg/disc) was also less than pipericilin (100 µg/disc). Therefore, their antimicrobial activity may increase when the active compounds in the ES products were isolated and purified. Moreover, the antimicrobial activity of these ES products against other strains of *P. aeruginosa* should be further investigated to prove the exact potential of these products.

Unfortunately, complete analysis of the composition of these ES products has not been done. Generally, the ES products of blow fly consisted of AMP, proteinase enzymes, and ammonia[23]. AMP are the main agents that fight with the microbes in the environment. Most of AMP have molecular weight mostly less than 10 kDa. For example, molecular weight of Lucifensin I, AMP from *L. sericata* is ~5 kDa[6,24]. The results from SDS-PAGE of this study were similar to that of Taha et al[19] and peptides having molecular weights less than 10 kDa were also found in this study. Therefore, these peptides should be purified and then their exact molecular weight and amino acid sequence should also be analyzed in the future. Recently, Lu et al[25] reported an ATPase inhibitory peptide which was purified from a whole body extract of *C. megacephala* larvae. This peptide can inhibit *P. aeruginosa* by the use of an agar well diffusion assay[25]. However, no evidence demonstrating that this peptide is available in the ES products has been reported.

Morphological changes of *P. aeruginosa* after treatment with the ES products of *C. megacephala* were found in this study by the use of SEM. These findings indicated that the ES products had a direct effect on cells of *P. aeruginosa*. Similarly, a previous study showed that the *E. coli* cells treated with antimicrobial lactoferricin B peptides were elongated and increased over fivefold in length[26]. Also, a swollen shape of *E. coli* after treatment with *Musca domestica* cercropin, was found[27]. As mentioned previously, an increased length of bacteria cells resulted from the inhibition of DNA synthesis by blocking or reducing DNA replication and/or transcription through DNA binding, which then led to the death of bacteria[27,28]. The ES products of this blow fly species may consist of active compounds affecting DNA synthesis. However, the mechanism of the ES products was not clarified in this study and further research is required.

**Conclusion**

The findings that the ES products derived from *C. megacephala* larvae inhibited the growth of only *P. aeruginosa* and had effects on cell morphology are of special interest and merit further investigation. Such investigation is urgently required in relation to the active compounds in the ES products and their mechanism. These ES products may be an alternative source for the development of antimicrobial agents or areas of research of a combination of treatments between the ES products and antibiotics for the management of *P. aeruginosa* infection in the future.

**What is already known on this topic?**

Some blow fly species have already been reported as a source of potential antimicrobial peptides, such as *Lucilia sericata* and *Lucilia cuprina*. Different insect species possess different antimicrobial activities. Excretory and secretory (ES) products are a good source of investigation of antimicrobial agents, especially antimicrobial peptides (AMP). Generally, the molecular weight of AMP is less than 10 kDa. *C. megacephala* is the most predominant blow fly species in Thailand and can be reared in a laboratory. The study on the ES products against bacteria is still limited and its fungicidal activity has not been documented.

**What this study adds?**

The researchers investigated the bactericidal and fungicidal activity of the ES products from *C. megacephala* larvae and determined the protein profile of the ES products by use of sodium dodecyl sulfate polyacrylamide gel electrophoresis. The results showed that these ES products inhibited only *P. aeruginosa* ATCC 25983 and contained peptides which had molecular weights ≤10 kDa. In addition, morphological changes of *P. aeruginosa* were found under observations using a scanning electron microscope. No fungicidal activity of the ES products against *C. albicans* ATCC 90028 and *M. furfur* CBS 1878T was observed in this study.

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**Potential conflicts of interest**

None.

**References**

1. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem 2014; 6: 25-64.
2. Ganz T. The role of antimicrobial peptides in innate immunity. Integr Comp Biol 2003; 43: 300-4.
3. Kavanagh K, Reeves EP. Exploiting the potential of insects for in vivo pathogenicity testing of microbial pathogens. FEMS Microbiol Rev 2004; 28: 101-12.
4. Hoffmann JA. Innate immunity of insects. Curr Opin Immunol 1995; 7: 4-10.
5. Ravi C, Jeyashree A, Renuka Davi K. Antimicrobial peptides from insects: an overview. RIB 2011; 2: 1-7.
6. Cerovsky V, Zdarek J, Fucik V, Monincova L, Voburka Z, Bem R. Lucifensin, the long-sought antimicrobial factor of medicinal maggots of the blowfly *Lucilia sericata*. Cell Mol Life Sci 2010; 67: 455-66.
7. Andersen AS, Sandvang D, Schnorr KM, Kruse T, Neve S, Joergensen B, et al. A novel approach to the antimicrobial activity of maggot debridement therapy. J Antimicrob Chemother 2010; 65: 1646-54.
8. Arora S, Baptista C, Lim CS. Maggot metabolites and their combinatory effects with antibiotic on *Staphylococcus aureus*. Ann Clin Microbiol Antimicrob 2011; 10: 6.
9. El Shazely B, Veverka V, Fucik V, Voburka Z, Zdarek J, Cerovsky V. Lucifensin II, a defensin of medicinal maggots of the blowfly *Lucilia cuprina* (Diptera: Calliphoridae). J Med Entomol 2013; 50: 571-8.
10. Troy TSE, Darren) CJK. Larval peptides a novel source of antibiotics from *Lucilia cuprina* maggots [Internet]. 2010 [cited 2015 Sep 15]: 1-11. Available from: http://ssrc.wiki.hci.edu.sg/file/view/SSEF+2010+MB16+Research+Paper.pdf
11. Bunchu N. Blow fly (Diptera: Calliphoridae) in Thailand: distribution, morphological identification and medical importance appraisals. Int J Parasitol Res 2012; 4: 57-64.
12. Sahalan AZ, Omar B, Mohamed AY, Jeffery J. Antibacterial activity of extracted hemolymph from larvae and pupae of local fly species, *Musca domestica* and *Chrysomya megacephala*. J Sains Kesihatan Malaysia 2007; 4: 1-11.
13. van der Plas MJ, van der Does AM, Baldry M, Dogterom-Ballering HC, van Gulpen C, van Dissel JT, et al. Maggot excretions/secretions inhibit multiple neutrophil pro-inflammatory responses. Microbes Infect 2007; 9: 507-14.
14. van der Plas MJ, Andersen AS, Nazir S, van Tilburg NH, Oestergaard PR, Krogfelt KA, et al. A novel serine protease secreted by medicinal maggots enhances plasminogen activator-induced fibrinolysis. PLoS One 2014; 9: e92096.
15. Ahamad MNU, Mojumder S, Asing, Sikdar D. Antimicrobial and cytotoxic activities of larval extract of *Musca domestica* (house fly). Int J Curr Res Rev 2012; 4: 63-9.
16. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: Approved standards-11thed. CLSI Document M02-A11. Wayne, PA; CLSI; 2012.
17. Teh CH, Nazni WA, Lee HL, Fairuz A, Tan SB, Sofian-Azirun M. In vitro antibacterial activity and physicochemical properties of a crude methanol extract of the larvae of the blow fly *Lucilia cuprina*. Med Vet Entomol 2013; 27: 414-20.
18. Kalab M, Yang AF, Chabot D. Conventional scanning electron microscopy of bacteria. Infocus 2008; 10: 44-61.
19. Taha N, Abdel-Meguid A, El-ebiarie A. Application of native excretory/secretory products from third larval instar of *Chrysomya megacephala* (Diptera:Calliphoridae) on an artificial wound. J Am Sci 2010; 6: 313-7.
20. Ratcliffe NA, Vieira CS, Mendonca PM, Caetano RL, Queiroz MM, Garcia ES, et al. Detection and preliminary physico-chemical properties of antimicrobial components in the native excretions/secretions of three species of *Chrysomya* (Diptera, Calliphoridae) in Brazil. Acta Trop 2015; 147: 6-11.
21. Barnes KM, Gennard DE, Dixon RA. An assessment of the antibacterial activity in larval excretion/secretion of four species of insects recorded in association with corpses, using

*Lucilia sericata* Meigen as the marker species. Bull Entomol Res 2010; 100: 635-40.
22. Diaz-Roa A, Gaona MA, Segura NA, Suarez D, Patarroyo MA, Bello FJ. *Sarconesiopsis magellanica* (Diptera: Calliphoridae) excretions and secretions have potent antibacterial activity. Acta Trop 2014; 136: 37-43.
23. Chambers L, Woodrow S, Brown AP, Harris PD, Phillips D, Hall M, et al. Degradation of extracellular matrix components by defined proteinases from the greenbottle larva *Lucilia sericata* used for the clinical debridement of non-healing wounds. Br J Dermatol 2003; 148: 14-23.
24. Valachova I, Prochazka E, Bohova J, Novak P, Takac P, Majtan J. Antibacterial properties of lucifensin in *Lucilia sericata* maggots after septic injury. Asian Pac J Trop Biomed 2014; 4: 358-61.
25. Lu J, Chen ZW, Wu Y, Zhang M, Ding JP, Cederlund E, et al. An ATPase inhibitory peptide with antibacterial and ion current effects. Biochem Biophys Res Commun 2014; 446: 519-22.
26. Ulvatne H, Samuelsen O, Haukland HH, Kramer M, Vorland LH. Lactoferricin B inhibits bacterial macromolecular synthesis in *Escherichia coli* and *Bacillus subtilis*. FEMS Microbiol Lett 2004; 237: 377-84.
27. Lu X, Shen J, Jin X, Ma Y, Huang Y, Mei H, et al. Bactericidal activity of *Musca domestica* cecropin (Mdc) on multidrug-resistant clinical isolate of Escherichia coli. Appl Microbiol Biotechnol 2012; 95: 939-45.
28. Hsu CH, Chen C, Jou ML, Lee AY, Lin YC, Yu YP, et al. Structural and DNA-binding studies on the bovine antimicrobial peptide, indolicidin: evidence for multiple conformations involved in binding to membranes and DNA. Nucleic Acids Res 2005; 33: 4053-64.

## ฤทธิ์ต้านจุลินทรีย์ในสารสกัดหลั่งในตัวอ่อนแมลงวันหัวเขียว *Chrysomya megacephala (Diptera: Calliphoridae)*

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***ภูมิหลัง:*** *ปัจจุบันปัญหาการดื้อยาปฏิชีวนะพบเพิ่มมากขึ้นทั่วโลก สารสกัดหลั่งที่ได้จากแมลงวันหัวเขียวเป็นแหล่งหนึ่งสำหรับการค้นหาแหล่งต้านจุลชีพ ซึ่งแมลงวัน Chrysomya megacephala (Diptera: Calliphoridae) เป็นแมลงวันหัวเขียวที่มีความสำคัญทางการแพทย์และพบการกระจายตัวมากที่สุดในประเทศไทย แต่ยังมีการศึกษาฤทธิ์ต้านจุลชีพในแมลงวันชนิดนี้ค่อนข้างน้อย*

***วัตถุประสงค์:*** *เพื่อทดสอบฤทธิ์ต้านเชื้อจุลชีพจากสารสกัดหลั่งที่ได้จากตัวอ่อนระยะที่ 3 ของแมลงวันหัวเขียว Chrysomya megacephala และศึกษาการเปลี่ยนแปลงรูปร่างของเชื้อจุลชีพหลังการทดสอบด้วยสารสกัดหลั่งดังกล่าว*

***วัสดุและวิธีการ:*** *ทำการเก็บสารสกัดหลั่งจากตัวอ่อนระยะที่ 3 ของแมลงวันหัวเขียว Chrysomya megacephala จำนวน 500 ตัว นำสารสกัดหลั่งไปทดสอบฤทธิ์ในการต้านเชื้อแบคทีเรีย (Staphylococcus aureus ATCC 25923, Bacillus cereus ATCC 1178, Escherichia coli ATCC 25922, และ Pseudomonas aeruginosa ATCC 25983) และเชื้อรา (Candida albicans ATCC 90028 และ Malassezia furfur CBS 1878T) ด้วย disc diffusion method และ minimum inhibitory concentration (MIC) assay และทำการศึกษาสัณฐานวิทยาของเซลล์หลังจากที่ทดสอบด้วยสารสกัดหลั่ง*

***ผลการศึกษา:*** *สารสกัดหลั่งสามารถยับยั้งการเจริญของเชื้อ Pseudomonas aeruginosa ด้วยวิธี disc diffusion โดยมีค่าความเข้มข้นของสารสกัดหลั่งต่ำสุดที่สามารถยับยั้งเชื้อ Pseudomonas aeruginosa เท่ากับ 150-200 ไมโครกรัมต่อมิลลิลิตร โดยเซลล์ Pseudomonas aeruginosa ที่ทดสอบด้วยสารสกัดหลั่งมีความยาวมากกว่าและมีขนาดใหญ่กว่าเซลล์ที่ไม่ได้ทดสอบด้วยสารสกัดหลั่งและพบเซลล์เหี่ยวที่มีรูปร่างผิดปกติ แต่อย่างไรก็ตามไม่พบฤทธิ์ฆ่าเชื้อราในการศึกษานี้*

***สรุป:*** *สารสกัดหลั่งจากตัวอ่อนของแมลงวันหัวเขียวชนิดนี้เป็นแหล่งที่ดีสำหรับการค้นหาแหล่งต้านเชื้อ Pseudomonas aeruginosa ต่อไปในอนาคต*