Tropical Natural History 19(2): 103–112, October 2019

Antibacterial Activity of the Mucus Extract from the Giant African Snail (*Lissachatina fulica*) and Golden Apple Snail (*Pomacea canaliculata*) Against Pathogenic Bacteria Causing Skin Diseases

NATTAWADEE NANTARAT[1, 2, 3,4*], YINGMANEE TRAGOOLPUA[1, 2, 3] AND PATIPAN GUNAMA[1, 2]

[1]*Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, THAILAND*
[2]*Applied Parasitology Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, THAILAND*
[3]*Environmental Science Research Center (ESRC), Faculty of Science, Chiang Mai University, 50200, THAILAND*
[4]*Science and Technology Research Institute, Chiang Mai University, Chiang Mai 50200, THAILAND*
[*] Corresponding author. Nattawadee Nantarat (n_nantarat@yahoo.com)
Received: 16 October 2018; Accepted: 20 September 2019

ABSTRACT.– There have been many cases of snails reported to be agricultural pests in Thailand, including the important invasive pests, giant African snail, *Lissachatina fulica*, and the golden apple snail, *Pomacea canaliculata*. These snails have rich mucus that covers their surface, which may serve in preventing moisture evaporation, reducing friction and providing resistant to infection by microorganisms. In this study, the antibacterial activity of aqueous extracts of *L. fulica* and *P. canaliculata* mucus were tested against four strains of Gram-positive bacteria, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Staphylococcus epidermidis* and *Corynebacterium* sp. Thirty adult snail samples of both *L. fulica* and *P. canaliculata* were collected, snail mucus was harvested, and a crude aqueous extract of the mucus (CME) prepared. The *in vitro* antibacterial activity of each CME was evaluated by the agar well diffusion method, while the broth dilution method was used to determine its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). CME from both *L. fulica* and *P. canaliculata* displayed antibacterial activity against all four strains of Gram-positive bacteria in the agar well diffusion assay. In the broth dilution assay, CME from *L. fulica* showed weak activity against all four bacterial strains, being highest against *S. aureus* and MRSA (MIC 12.5 µg/ml; MBC >50 µg/ml**),** followed by *S. epidermidis* and *Corynebacterium* sp. **(**MIC 25 µg/ml; MBC >50 µg/ml**);** however, that from *P. canaliculata* showed no antibacterial activity against these bacteria. Therefore, CMEs from these two snail species were somewhat effective against these pathogens, and might be useful for human health-related applications in the future, following further fractionation to isolate the active components and determination of their optimal concentrations, and whether or not they act synergistically.

KEY WORDS: *Lissachatina*, *Pomacea*, antimicrobial, snail slime, pathogens

INTRODUCTION

Bacterial skin infections are a major health concern in Thailand, as in other countries of the world Jantakee and Tragoolpua (2015). Skin and wound infections are commonly caused by pathogenic bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and methicillin resistant *S. aureus* (MRSA), *Staphylococcus epidermidis*, *Streptococcus pyogenes* and vancomycin resistant *Enterococci* (Halcon and Milkus, 2004; Jantakee and Tragoolpua, 2015). The development of resistant bacterial strains has led to decreasing and limited efficacy of currently available antibiotics. Thus, it is imperative new pharmaceutical agents are developed for the effective treatment of these pathogen-induced diseases.

In recent years, there have been many developments in dermal substitution and bacterial skin infection research, including studies on snail secretions and their active components (Iguchi et al., 1982; Otsuka-Fuchino et al., 1992; Ito et al., 2011; Pitt et al., 2015; Etim et al., 2016; Cilia and Fratini, 2018). Snails are covered by a rich mucus that may help in preventing moisture loss, reducing friction and so allowing their smooth movement across dry surfaces, and also in protecting their body from physically injuries Cilia and Fratini (2018). Moreover, snails can produce 'mucin' in abundance in their mucus secretion, which includes antimicrobial proteins, providing a degree of resistance against infection by microorganisms Adikwu and Alozie (2007).

Thailand has a diverse snail fauna; however, some of the snails are introduced (invasive) agricultural pests that cause damage to the economy and the natural ecosystem, especially the giant African snail, *Lissachatina fulica*, and the golden apple snail, *Pomacea canaliculata*. These two snail species are devastating agricultural pests, most notably of rice Yang et al. (2013). There have been attempts to control the snails and limit their spread, using physical, chemical, biological and agricultural methods (Salleh et al., 2012; Bhattacharyya et al., 2014; Chandaragi, 2014). However, these methods have not prevented major outbreaks of these snails in crops (Yang et al., 2013; Bhattacharyya et al., 2014;).

L. fulica (family Achatinidae) has been reported to produce a glycoprotein secretion that has biological effects, including antibacterial properties (Otsuka-Fuchino et al., 1992; Santana et al., 2012). A bactericidal glycoprotein of *L. fulica*, known as achacin, has been reported to kill both Gram-positive and Gram-negative bacteria by attacking their cell membrane (Iguchi et al., 1982; Otsuka-Fuchino et al., 1992; Santana et al., 2012; Etim et al., 2016). Furthermore, the mucus of this snail has been shown to improve dermal cicatricial repair of surgical wounds in experimental animals Santana et al. (2012). *P. canaliculata* (family Ampullariidae), is now widespread in many countries of eastern and southern Asia, including Japan, Korea, the Philippines, Thailand and Vietnam (Hayes et al., 2008; Yang et al., 2013). However, there are only a few reports on the chemical components and properties of its mucus (Takeichi et al., 2007; Wasiq-Hidayat and Parman, 2015).

While the price of synthetic antimicrobial drugs is relatively high and increasing, snails that can produce mucin-containing mucus secretions are widespread in Thailand. Hence, it is of medical and commercial interest to discover their potential use as an alternative source of antibacterial agents, as well as understanding how to control these agricultural pests. This study focused on the efficacy of crude aqueous mucus extracts (CMEs) from *L. fulica* and *P. canaliculata* in inhibiting the growth of four strains of pathogenic skin bacteria.

MATERIALS AND METHODS

Snail samples and obtaining the mucous secretion

Thirty adult *L. fulica* and 30 adult *P. canaliculata* were collected from Chiang Mai University, Mueang, Chiang Mai, Thailand (18°48'09.4" N, 98°57'05.5" E) and Sansai Noi Temple, San Sai, Chiang Mai, Thailand (18°49'03.1" N, 99°01'45.1" E), respectively. The snails were kept in two plastic boxes (45x37x30 cm, length/width/

height), with 30 snails in each box. The plastic boxes were sprinkled with water daily in order to maintain humidity. Subsequently, the snails were housed individually in plastic boxes and kept without food for 3 d to avoid contamination. The snails were then manually stimulated at their pedal glands, and approximately 2 ml of mucous secretion was collected per individual and pooled for each species.

Preparation of crude mucous extracts (CMEs)

Approximately 10 ml mucous extract of each snail species was kept at −20 °C for protein determination, while another 50 ml was filtered (Whatman No. 1), concentrated in a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) and then lyophilised (Snijders Scientific, Tilburg, The Netherlands), yielding 0.1 g CME. The dried CMEs were then resuspended in distilled water at an initial concentration of 100 µg total protein/ml, as calculated by the bicinchoninic acid (BCA) method, and kept at −20 °C until used.

Determination of protein concentration

A Novagen® BCA protein assay kit (Merck, Darmstadt, Germany) was used to determine protein concentrations, using bovine serum albumin (BSA) as the standard. All doses of snail mucus in the experiments were consequently stated as microgram of proteins per milliliter (µg/ml).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of mucus proteins

After the protein concentration of mucus from *L. fulica* and *P. canaliculata* was determined by the BCA method, undiluted mucus and a 1:2 dilution of both CMEs was electrophoresed on a 15% polyacrylamide gel. Separated proteins were stained with Coomassie Brilliant Blue R-250 (Bio-Rad, Hercules, CA, USA).

Bacterial strains

The four strains of Gram-positive skin pathogenic bacteria, (*S. aureus*, MRSA, *S. epidermidis* and *Corynebacterium* sp., were obtained from the Microbiology Section, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. They were cultured in Mueller Hinton Broth (MHB) at 37 °C for 24 h before testing with the CMEs.

Agar well diffusion assay

The inoculum of each of the four strains of Gram-positive bacteria was adjusted to 1.5 x 10^8 colony forming units (CFU)/ml in 0.85% (w/v) NaCl solution, based on comparison with a McFarland scale tube No. 0.5. It was then swabbed onto Mueller Hinton agar (MHA), MHB with 2.1% (w/v) agar. After that, 100 µl CME from *L. fulica,* 100 µl CME from *P. canaliculata*, 100 µl distilled water alone (negative control) or 100 µl distilled water containing 10 mg/mL gentamicin or doxycycline (positive control) was added to each well. The plates were incubated at 37 °C for 24 h before inhibitory activity was determined by measuring the diameter of the inhibition zone (no bacterial colony growth) around each well.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each CME

The MIC was determined by the broth dilution method. Each sample of CME was diluted two-fold from 100 µg/ml in distilled water to 50, 25, 12.5, 6.25 and 3.125 µg/ml in sterile MHB. Each bacterial culture was adjusted to McFarland scale tube No. 0.5 (1×10^8 CFU/mL) and a dilution of CME was added to the culture and incubated at 37 °C for 24 h. Thereafter, the turbidity of the suspension was measure as optical density at a wavelength of 600 nm (OD_{600}), from

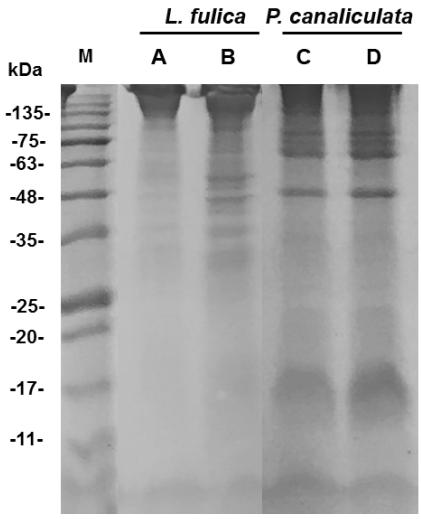

FIGURE 1. 15% SDS-PAGE of protein extracts. M) medium-range protein molecular weight markers (labelled in kDa); A-B) CME from *L. fulica*; A) 10 µg/lane CME; B) 20 µg/lane CME; C-D) CME from *P. canaliculata*; C) 10 µg/lane CME; D) 20 µg/lane CME.

which the MIC, the lowest concentration of CME that induced a detectable inhibition in bacterial growth, was determined. The cultures with no or very little bacterial growth from this assay were then streaked on MHA plates and incubated at 37 °C for 24 h to ascertain the colony count and to determine the MBC, the minimum concentration required to produce no viable bacteria.

Statistical analysis

Analysis of variance (ANOVA) was performed to evaluate the variance of data, while the significance of the differences between means was determined by multiple comparisons using Duncan's multiple range tests (DMRT), implemented with SPSS Statistics for Windows, version 22.0 software IBM Corp (2013). Statistical significance was accepted at the $p < 0.05$ level.

RESULTS

Physical properties of mucus secretions

The physical properties of the mucus secretions from both snail species were observed. Mucus and CME from *L. fulica* was a colourless, transparent and very viscous liquid, while those from *P. canaliculata* were slightly cloudy-white, with a low viscosity.

Determination of protein concentration and SDS-PAGE

CME from *L. fulica* and *P. canaliculata* contained 119.60 ± 4.06 µg/ml and 225.00 ± 3.11 µg/ml total protein, respectively. Electrophoresis resolved six major protein bands ranging from 17 to >105 kDa in both snail species (Fig. 1). The protein band sizes for *L. fulica* were 35, 48, 55, >70 and >100 kDa, while those of *P. canaliculata* were 17, 48, >70 and >100 kDa.

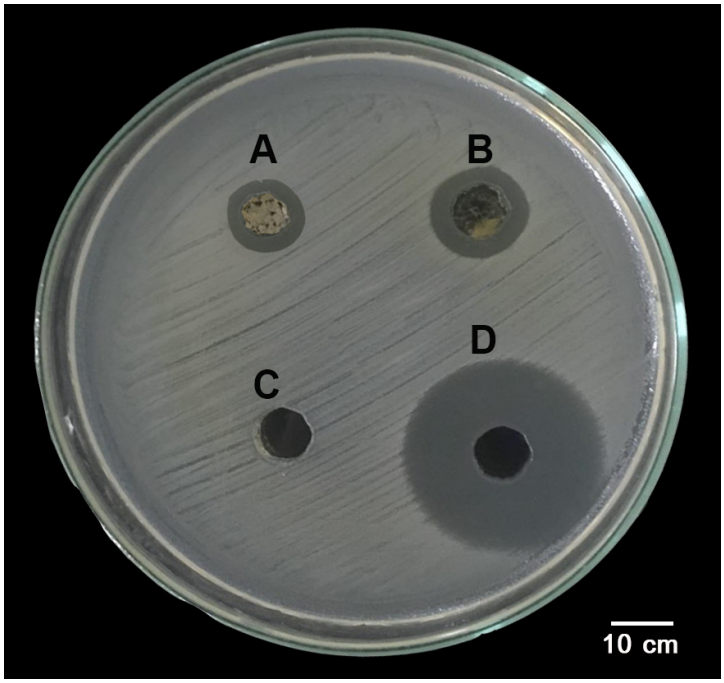

FIGURE 2. Antibacterial activities of mucus extract against *S. epidermidis,* as determined by agar well diffusion method. A) *P. canaliculata*; B) *L. fulica*; C) negative control, distilled water; D) positive control, gentamicin (10 µg/mL).

Anti-bacterial activity of CMEs (agar well diffusion assay)

The antibacterial activities of CMEs, as determined by agar well diffusion assay (Fig. 2), are summarised in Table 1. CME of both species inhibited all four tested strains of Gram-positive bacteria, where that from *L. fulica* was numerically and significantly higher than that from *P. canaliculata* at $P<0.05$. The CMEs were most active against MRSA followed by *S. epidermidis*, *Corynebacterium* sp. and *S. aureus*. However, antibiotics gentamicin (not MRSA) and doxycycline (MRSA) at 10 µg/ml had a significantly higher inhibitory activity, as determined by the diameter of zones of inhibition.

MIC and MBC values of CME from L. fulica and P. canaliculata against four strains of Gram-positive bacteria, as determined by broth dilution assay

The MIC and MBC values of CMEs from *L. fulica* and *P. canaliculata* were evaluated against the four bacterial strains using the broth dilution method. CME from *L. fulica* showed a weak antibacterial activity (12.5–25 µg/ml) against all the pathogenic bacteria, compared to that of the positive controls (doxycycline and gentamicin at 10 µg/ml), while CME from *P. canaliculata* had too low an antibacterial activity to determine MIC and MBC values (Table 2).

TABLE 1. Antibacterial properties of the CMEs from *L. fulica* and *P. canaliculata* against four strains of skin pathogenic bacteria, as determined by agar well diffusion assay.

Treatment	Zone of inhibition[a] (mm)			
	S. aureus	**MRSA**	***S. epidermidis***	***Corynebacterium* spp.**
CME from *L. fulica*	$14.83 \pm 1.11^{b*}$	$17.51 \pm 0.30^{b*}$	$15.87 \pm 1.48^{b*}$	$15.73 \pm 0.53^{b*}$
CME from *P. canaliculata*	$12.03 \pm 0.17^{c*}$	$13.60 \pm 0.51^{c*}$	$11.07 \pm 1.11^{c*}$	$13.35 \pm 1.28^{c*}$
Distilled water	0	0	0	0
Gentamicin	$27.77 \pm 1.12^{a*}$	ND	$28.63 \pm 1.08^{a*}$	$36.76 \pm 0.65^{a*}$
Doxycycline	ND	$30.23 \pm 1.23^{a*}$	ND	ND

ND = Not determined [a]Data are shown as the mean ± 1 standard deviation (SD), derived from 3 independent trials. * Means followed by a different letter are significantly different ($p < 0.05$; DMRT).

DISCUSSION

Physical characteristics of the mucus secretions and CMEs

The physical characteristics of the two snail CMEs, in terms of colour and viscosity, were not the same, with that from *L. fulica* being very viscous and colourless, while that from *P. canaliculata* was slightly cloudy-white and of low viscosity. The high viscosity of the CME of *L. fulica* helped to provide a barrier, reducing moisture loss and protecting the snail from bacterial infection (Etim et al., 2016; Cilia and Fratini, 2018). The dissimilarities in these properties might reflect differences in the feeding behavior of the snails, which might affect their nutritional intake and the composition of their slime Fagbuaro et al. (2006). Moreover, their food intake will affect the volume of mucus they produce Ademolu et al. (2005).

Identification of proteins in CMEs from each snail species

The concentration of total protein in CME from *L. fulica* was 119.60 ± 4.06 µg/ml, and in CME from *P. canaliculata* was 225.00 ± 3.11 µg/ml. Electrophoresis revealed protein bands for *L. fulica* of 35, 48, 51, >70 and >100 kDa, while those of *P. canaliculata* were 17, 48, >70 and >100 kDa. In terms of molecular weight range, these resembled achasin protein, 59.086–150 kDa Dwi-Nugrahananto et al. (2014), and mytimacin-AF, 50.81, 15 and 11.45 kDa Berniyanti et al. (2015). The >70 kDa and ~51 kDa bands were presented in CME from *L. fulica* in this current study. This was also consistent with previous reports that suggested achasin (59.086–150 kDa) and mytimacin-AF (50.81, 15 and 11.45 kDa) were proteins of *L. fulica* which had antimicrobial activity (Otsuka-Fuchino et al., 1992; Dwi-Nugrahananto et al., 2014; Etim et al., 2016). This current study was the first identification of proteins in *P. canaliculata* mucus.

Antimicrobial activity

Based on the agar well diffusion assay, CME from both snail species had antibacterial activity against *S. aureus*, MRSA, *S. epidermidis* and *Corynebacterium* sp., with that from *L. fulica* being numerically greater in all cases. However, it was only significantly larger ($P < 0.05$) against MRSA, and the largest inhibition of growth zone by CME of both snail species was observed against MRSA. By comparison, mucous of *Helix aspersa* had a stronger antibacterial effect against several

TABLE 2. MIC and MBC values of CME from *L. fulica* and *P. canaliculata* against four strains of skin pathogenic bacteria, using the broth dilution assay.

Sample	Bacterial strain/ MIC and MBC (µg/mL)							
	S. aureus		MRSA		*S. epidermidis*		*Corynebacterium* spp.	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
CME of *L. fulica*	12.5	> 50	12.5	> 50	25	> 50	25	> 50
CME of *P. canaliculata*	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
Gentamicin	0.78	3.12	ND	ND	3.12	3.12	1.56	3.12
Doxycycline	ND	ND	1.56	6.25	ND	ND	ND	ND

ND = not determined. Data are from one trial representative of three independent repeats

strains of *P. aeruginosa* and a weaker effect against *S. aureus* than *L. fulica* mucus Pitt et al. (2015). The difference in the antimicrobial activity between snail species or extracts may be related to the amount of mucin contained in the mucus secretion Etim et al. (2016), or differences in its composition. Hence diffusion rates versus effective concentrations between different compounds may be critical factors. There are reports of antibacterial and antifungal activities of proteins extracted from seven different snails Ulagesan and Kim (2018), *Lisachatina fulica* (Bowdich, 1822), *Cryptozona bistrialis* (Beck, 1837), *Pila globosa* (Swainson, 1822), *Pila virens* (Lamarck, 1822), *Bellamya dissimilis* (Mueller, 1774), *Bithynia* (*Digoniostoma*) *pulchella* (Benson, 1836), and *Melanoides tuberculata* (Muller, 1774). They reported that proteins extracted from the terrestrial snail *C. bistrialis* showed the highest antimicrobial activity against pathogenic bacteria and fungi, compared to the other snail proteins. Mucous from *L. fulica* also inhibited the growth of *P. aeruginosa;* however, a broad spectrum of activity was not observed. Notably, with no reports about antimicrobial activities produced by *P. canaliculata*, this current study represented the first examination of this organism for such properties.

With some congruency, the broth dilution method, which was a more sensitive assay for quantifying antibacterial activity than the agar well diffusion assay Jantakee and Tragoolpua (2015), showed significant differences in MIC and MBC values for the CMEs from *L. fulica* and *P. canaliculata* against all four tested bacterial strains. CME from *L. fulica* was somewhat effective against all four strains of pathogenic bacteria, while that from *P. canaliculata* showed no antibacterial activity against these bacteria. The antibacterial activity of CME from *L. fulica*, in terms of MIC values, was *S. aureus* = MRSA > *S. epidermidis* = *Corynebacterium* sp. (Table 2). However, the MBC values were not determined (>50 mg/ml) for the CMEs of both snail species against the four bacterial species.

In the broth dilution assays the bacteria were in direct contact with the CME. By contrast, the interaction in agar is dictated by diffusion rates of compounds, favouring small hydrophilic molecules over large hydrophobic ones, but also potentially partitioning synergy between compounds. Notably, CME from *P. canaliculata* showed no antibacterial activity against the bacteria

in the current study in the broth dilution assay, while it showed activity against all four strains in the agar well diffusion assay. In addition, this differential response in the assays could reflect variation in various factors related to the CME, such as its pH, temperature and denaturation of protein. It is known that lyophilisation can induce aggregation of some proteins, and the relative importance of the protein structure, formulation and processing conditions are poorly understood Roughton et al. (2013).

Snails have specific proteins that help their survival in their environment, including preventing bacterial contamination. Their mucus consists of mucin, which includes antimicrobial proteins (Cilia and Fratini, 2018). The antibacterial activity of CME may well depend on various factors that function alone or synergistically (Iguchi et al., 1982; Ito et al., 2011; Pitt et al., 2015). The antibacterial activity of mucin found in the mucous secretions of *L. fulica* was found to be related to antibacterial factors in the protein component, instead of its activity on the cell surface of bacteria Etim et al. (2016).

The antibacterial protein achacin found in the mucus of *L. fulica* can bind to both Gram-positive and Gram-negative bacteria (Etim et al., 2016; Iguchi et al., 1982), but it gives a better inhibition of those that are Gram-positive Dwi-Nugrahananto et al. (2014). Therefore, it would be interesting to test both antimicrobial activity of *L. fulica* and *P. canaliculata* mucus on Gram-negative and fungi. Achacin is a member of the L-amino acid oxidase family, and is antibacterial through its production of hydrogen peroxide Ehara et al. (2002). *Achatina fulica* high molecular weight lectin (AfHML) was also secreted from the same collar tissue as achacin, and increased the local concentration of hydrogen oxides in the mucus and helped to accelerate the antibacterial activity of achacin Ito et al. (2011). In addition, an antibacterial glycoprotein in the mucus of snails has been reported. Therefore, antibacterial activity of snails may depend on the protein, higher-order structures of the protein and the protein moiety of the glycoprotein Etim et al. (2016). Further tests against Gram-negative bacterial and fungal pathogens should be done to demonstrate the presence of antimicrobial agents, and the role of snail mucus in antimicrobial protection.

CONCLUSION

In summary, this study supported the presence of antibacterial factors in mucous secretions of *L. fulica* and *P. canaliculata*, where the CMEs from these two snail species showed different inhibitory and bactericidal effectiveness against the pathogenic bacteria *S. aureus*, MRSA, *S. epidermidis* and *Corynebacterium* sp. This was also the first report on a CME from *P. canaliculata* with anti-microbial activities. The CME from *L. fulica* had a higher inhibitory activity against the bacteria in this current study, but it was still much weaker than that of gentamicin or doxycycline. However, the snail CMEs might still have potential as alternative therapeutic agents against pathogenic bacteria that cause skin disease, and may be useful in human health related applications in the future following fractionation to identify the active compounds and optimal doses, and whether or not they act synergistically.

ACKNOWLEDGEMENTS

We thank the applied parasitology and the microbial laboratory, Department of Biology, Faculty of Science, Chiang Mai

University for the provision of instruments, facilities and access to laboratories. This research work was partially supported by Chiang Mai University.

LITERATURE CITED

Ademolu, K., Idowu, A., Mafiana, C. and Osinowo, O., 2005. Performance, proximate and mineral analyses of African giant land snail (*Archachatina marginata*) fed different nitrogen sources, African Journal of Biotechnology, 3(8): 412–417 pp. https://doi.org/10.5897/AJB2004.000-2079.

Adikwu, M. and U. Alozie, B., 2007. Application of snail mucin dispersed in detarium gum gel in wound healing. Scientific Research and Essay, 2: 195–198.

Berniyanti, T., Waskito, E.B. and Suwarno, S., 2015. Biochemival Characterization of an Antibactrial Glycoprotein from *Achatina fulica* ferussac Snail Mucus Local Isolate and Their Implication on Bacterial Dental Infection, Indonesian Journal of Biotechnology, 12(1): 943–951 pp. https://doi.org/10.22146/ijbiotech.7765.

Bhattacharyya, B., Das, M., Mishra, H. and Nath, D.J., 2014. Bioecology and management of giant African snail , *Achatina fulica* (Bowdich), International Journal of Plant Protection, 7(2): 476–481.

Chandaragi, M., 2014. Integrated management of giant african snail, *Achatina fulica* (Ferussac) (Stylommataphora:Achatinidae) in agriculture and horticulture ecosystems 202 pp.

Cilia, G. and Fratini, F., 2018. Antimicrobial properties of terrestrial snail and slug mucus., Journal of complementary & integrative medicine, 15(3): 1–10. https://doi.org/10.1515/ jcim-2017-0168.

Dwi-Nugrahananto, H., Kriswandini, I. and R, E., 2014. Antimicrobial proteins of Snail mucus (*Achatina fulica*) against *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans*, Dental Journal, 47: 31–36. https://doi.org/10.20473/ j.djmkg.v47.i1.

Ehara, T., Kitajima, S., Kanzawa, N., Tamiya, T. and Tsuchiya, T., 2002. Antimicrobial action of achacin is mediated by L-amino acid oxidase activity., FEBS letters, 531(3): 509–512.

Etim, L., Aleruchi, C. and Obande, G., 2016. Antibacterial Properties of Snail Mucus on Bacteria Isolated from Patients with Wound Infection, British Microbiology Research Journal, 11(2): 1–9. https://doi.org/10.9734/BMRJ/2016/21731.

Fagbuaro, O., Oso, J.A., Edward, J.B. and Ogunleye, R.F., 2006. Nutritional status of four species of giant land snails in Nigeria, Journal of Zhejiang University. Science. B, 7(9): 686–689. https://doi.org/10.1631/jzus.2006.B0686.

Halcon, L. and Milkus, K., 2004. *Staphylococcus aureus* and wounds: a review of tea tree oil as a promising antimicrobial., American journal of infection control, 32(7): 402–408. https://doi.org/10.1016/S0196655304003657.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. and Cowie, R.H., 2008. Out of South America: multiple origins of non-native apple snails in Asia, Diversity and Distributions, 14(4): 701–712. DOI: https://doi.org/10.1111/j.1472-4642.2008.00483.x.

IBM Corp, 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk (ed.). New York: IBM Corp.

Iguchi, S.M., Aikawa, T. and Matsumoto, J.J., 1982. Antibacterial activity of snail mucus mucin., Comparative biochemistry and physiology, 72(3): 571–574.

Ito, S., Shimizu, M., Nagatsuka, M., Kitajima, S., Honda, M., Tsuchiya, T. and Kanzawa, N., 2011. High molecular weight lectin isolated from the mucus of the giant African snail *Achatina fulica*., Bioscience, biotechnology, and biochemistry, 75(1): 20–25. https://doi.org/10.1271/bbb.100389.

Jantakee, K. and Tragoolpua, Y., 2015. Activities of different types of thai honey on pathogenic bacteria causing skin diseases, tyrosinase enzyme and generating free radicals, Biological Research, 48: 1–11. https://doi.org/10.1186/0717-6287-48-4.

Otsuka-Fuchino, H., Watanabe, Y., Hirakawa, C., Tamiya, T., Matsumoto, J.J. and Tsuchiya, T., 1992. Bactericidal action of a glycoprotein from the body surface mucus of giant African snail., Comparative biochemistry and physiology, 101(3): 607–613 pp.

Pitt, S.J., Graham, M.A., Dedi, C.G., Taylor-Harris, P.M. and Gunn, A., 2015. Antimicrobial properties of mucus from the brown garden snail *Helix aspersa*., British journal of biomedical science, 72(4): 174–81.

Roughton, B.C., Iyer, L.K., Bertelsen, E., Topp, E.M. and Camarda, K. V, 2013. Protein aggregation and lyophilization: Protein structural descriptors as predictors of aggregation propensity, Computers & chemical engineering, 58: 369–377.

Salleh, N.H.M., Arbain, D., Daud, M.Z.M., Pilus, N.

and Nawi, R., 2012. Distribution and Management of *Pomacea Canaliculata* in the Northern Region of Malaysia: Mini Review, APCBEE Procedia, 2: 129–134. https://doi.org/10.1016/j.apcbee. 2012. 06.024.

Santana, W.A., Melo, C.M. de, Cardoso, J.C., Pereira-Filho, R.N., Rabelo, A.S., Reis, F.P. and Albuquerque-Júnior, R.L.C. de, 2012. Assessment of Antimicrobial Activity and Healing Potential of Mucous Secretion of *Achatina fulica*, International Journal of Morphology, 30(2): 365–373. https://doi.org/10.4067/S0717-95022012000 200001.

Takeichi, M., Hirai, Y. and Yusa, Y., 2007. A water-borne sex pheromone and trail following in the apple snail, *Pomacea canaliculata*, Journal of Molluscan Studies, 73(3): 275–278. https://doi. org/ 10.1093/mollus/eym027.

Ulagesan, S. and Kim, H., 2018. Antibacterial and Antifungal Activities of Proteins Extracted from Seven Different Snails, Applied Sciences, 8(8): 1362. https://doi.org/10.3390/app8081362.

Wasiq Hidayat, J. and Parman, S., 2015. Utilization digestive tract of golden snail (*Pomacea canaliculata*) as lytic enzyme for protoplast isolation *Pichia manshurica* DUCC-Y15, European Journal of Experimental Biology, 5(10): 49–53.

Yang, T.B., Wu, Z.D. and Lun, Z.R., 2013. The apple snail *Pomacea canaliculata*, a novel vector of the rat lungworm, *Angiostrongylus cantonensis:* its introduction, spread, and control in China, Hawaii Journal of Medicine and Public Health, 72: 23–25.