Journal of Applied Pharmaceutical Science Vol. 8(08), pp 021-025, August, 2018
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2018.8803
ISSN 2231-3354

Larvicidal and Adult Mosquito Attractant Activity of *Auricularia auricula-judae* Mushroom Extract on *Aedes aegypti* (L.) and *Culex sitiens* Wiedemann

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ARTICLE INFO

Article history:
Received on: 25/05/2018
Accepted on: 11/07/2018
Available online: 31/08/2018

Key words:
Auricularia auricula-judae, *Aedes aegypti*, *Culex sitiens*, larvicidal effect, mosquito attractant effect.

ABSTRACT

Mosquitoes are small insects that are major vectors for the transmission of many diseases to humans, including malaria, dengue fever, yellow fever, lymphatic filariasis, and Japanese encephalitis. These diseases are significant public health problems worldwide, especially in tropical and sub-tropical countries. In this study, we evaluated the effect of *Auricularia auricula-judae* mushroom extract on larvicidal and adult mosquito attractant activity of important vectors, including *Aedes aegypti* (L.) and *Culex sitiens* Wiedemann. Five concentrations of extract at 120, 12, 1.2, 0.12 and 0.012 mg/L for the larvicidal assay were used, while concentrations for the attraction of adult mosquitoes were 10^{-4} g/mL, 10^{-5} g/mL and 10^{-6} g/mL. The results of the larvicidal activity assay with the *A. auricula-judae* extract on both species investigated herein of mosquitoes did not affect *Ae. aegypti* larvae, though with *Cx. sitiens* mosquitoes, the mushroom extract slightly eliminated larvae at all concentrations. The results with the *A. auricula-judae* extract on adult mosquito attractant activity at three concentrations showed at 10^{-5} g/mL, the most attraction took place followed by 10^{-6} g/mL and 10^{-4} g/mL, respectively (11.66 ± 0.57 vs. 7.00 ± 1.00, 11.33 ± 0.57 vs. 6.66 ± 0.57, and 9.33 ± 0.57 vs. 6.00 ± 1.00 mosquitoes, respectively). However, statistical difference comparison of the number of mosquitoes attracted between A. *auricula-judae* extract and octenol were found to be different at all concentrations ($p > 0.05$). Although the performance of *A. auricula-judae* extract is not equal to that of octanol, it was effective in attracting more than half of *Ae. aegypti* mosquitoes as dengue vector (58.33%).

INTRODUCTION

Mosquitoes are small insects that are major vectors for the transmission of many diseases to humans, including malaria, dengue fever, yellow fever, lymphatic filariasis, and Japanese encephalitis (Service, 2008; Damapong *et al.*, 2016). These diseases are significant public health problems worldwide, especially in tropical and sub-tropical countries (Chaiphongpachara *et al.*, 2017). WHO estimates that every year, more than one million people die and nearly half of the world's population inhabit high-risk areas for mosquito-borne diseases (World Health Organization, 2014; World Health Organization, 2016). In 2014, dengue fever, which is mainly transmitted by *Aedes aegypti*, has increased 30-fold over the past 30 years, with more than 2.5 billion people in more than 100 countries now being at risk of infection and increasing numbers of countries reporting outbreaks of this disease (World Health Organization, 2014). These diseases are also a major problem in Thailand, with many people being infected each year, particularly with dengue fever. According to the Ministry of Public Health of Thailand, the number of patients with these diseases in 2017 was approximately 65,000 cases (Ministry of Public Health, 2014), indicating that these diseases are considerable health issues and should be resolved urgently.

The control of mosquito-borne diseases decreases the natural mosquito population (Roiz *et al.*, 2012). There are two ways

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to control vectors reducing the number of larvae and compromising the adult stage of mosquitoes. Temephos is an organophosphate larvicide that is a popular and widely used chemical product which effectively controls larval stage especially *Aedes aegypti*, and it is not toxic to humans or animals (Chaiphongpachara *et al.*, 2017). However, there are currently reports of resistance of mosquitoes to this chemical occurring in many areas around the globe, including Thailand, owing to the employment of this chemical for a long time (Jirakanjanakit *et al.*, 2007). This is a major obstacle that has created difficulties in controlling the mosquito population.

Mosquito traps are another tool with which to reduce adult mosquito populations, especially *Culex* and *Anopheles* mosquitoes (Hock, 2004). Nowadays, there is the use of smell for optimization based on female mosquitoes requiring a blood meal to provide protein for egg production. Octenol (1-octen-3-ol) is a volatile organic compound found in the sweat and breath of humans that attracts mosquitoes (Takken and Kline, 1989). Although octenol attracts mosquitoes, it is quite expensive; therefore it is not popular in Thailand despite its high performance. Octenol is found in mushrooms and it is reportedly toxic to insects (Thongwat *et al.*, 2015; Inamdar and Bennett, 2014).

From all mentioned to this point, we selected *Auricularia auricula-judae* mushrooms as an edible and commercially available mushroom species grown in Thailand. The aim of this study was to assess larvicidal and adult mosquito attractant activity of this mushroom extract with respect to *Aedes aegypti* (L.) as a dengue vector and *Culex sitiens* Wiedemann as a filariasis and Japanese encephalitis vector (predominantly in costal habitats) (Chaiphongpachara and Sumruayphol, 2017). The results from this research revealed the ability of *A. auricula-judae* mushrooms to be utilized in controlling mosquitoes, leading to the creation of new additional new products.

METHODS

Collection of *A. auricula-judae* mushroom

A. auricula-judae mushrooms were collected from the Talat Thai market, Klong Luang Distinct, Pathum Thani province in Thailand (14°4'54.51"N 100°37'53.06"E) (Figure 1) during September 2016. Afterwards, mushroom samples were brought to the College of Allied Health Sciences, Suan Sunandha University, Samut Songkhram province and identified morphologically by characteristics through making use of the mushroom taxonomic keys (Largent and Thiers, 1977; Largent *et al.*, 1977; Stuntz, 1977; Largent, 1986; Largent and Baroni, 1988).

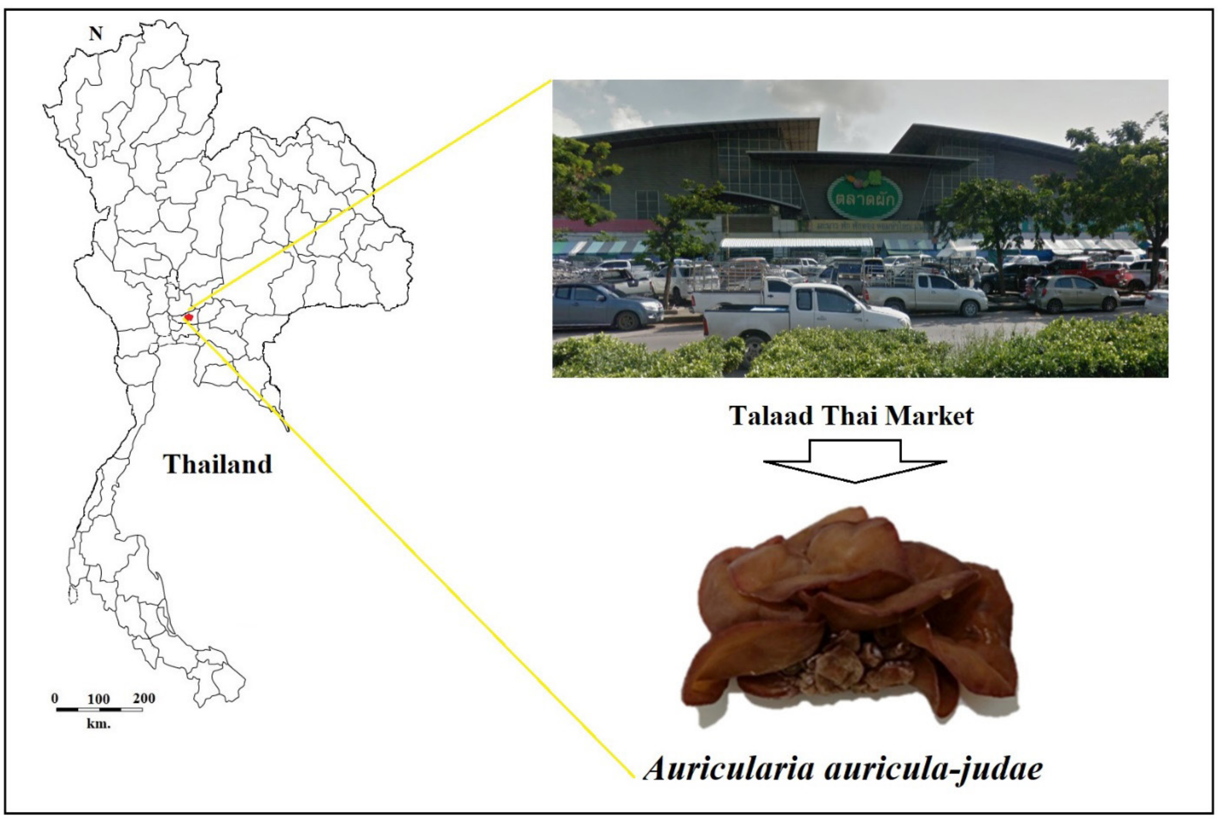

Fig. 1: Location of the collection site in Thailand.

Preparation of mushroom extract

A. auricula-judae samples were dried in the shade at environmental temperatures of approximately 37°C, ground to coarse powder and fermented with ethanol 95% at room temperature for 48 hours. The mushroom extract was filtrated by a Buchner funnel with Whatman number 1 filter paper and evaporated, whereas the water extract was lyophilized to dryness by a rotary evaporator. The yields of crude extract were weighed, recorded, dissolved in methanol for adult mosquito bioassay and distilled water for larvicidal bioassay and stored at -20°C before mosquito testing in the laboratory.

Mosquito rearing

In this work, we assessed the effect of *A. auricula-judae* extract against *Ae. aegypti* and *Cx. sitiens*. *Ae. aegypti* Bora Bora strain (WHO-susceptible strain) supported by the Faculty of Tropical Medicine, Mahidol University. *Cx. sitiens* were collected from the coastal field of Samut Songkhram province, which is 200 meters away from the sea (13°23'31.57"N 100°1'59.36"E) by a standard mosquito dipper in a water source with a salinity level of more than 0.05 ppt. We then added *Ae. aegypti* eggs and *Cx. sitiens* larvae to a tray containing filtered water (25 × 30 × 5 cm) by separating each species per tray at 25 ± 2°C with a 10:14 light:dark photoperiod and 0.1 g/mL dag food daily. During the pupae stage, they were transferred to a cage (30 × 30 × 30 cm) to facilitate adult mosquito emergence.

Bioassay for the larvicidal activity of *A. auricula-judae* extract

A larvicidal bioassay of this mushroom extract is recommended according to WHO (WHO, 2016). Five concentrations of extract at 120, 12, 1.2, 0.12 and 0.012 mg/L were used. Filtered water containing each substance concentration was poured into a six-ounce glass and 20 late third-instar larvae or early forth-instar larvae were added. After 24 hours, exposed dead larvae were counted and recorded for three replications. For the control group, we added extraction solvent into the testing glass. Octenol (1-Octanol EMPLURA® from Merck KGaA Company, Darmstadt, Germany) was used as a comparison group in all concentrations for testing against larvae and adult mosquitoes.

Bioassay for adult mosquito attractant activity of *A. auricula-judae* extract

Three concentrations of mushroom extract at 10^{-4} g/mL, 10^{-5} g/mL and 10^{-6} g/mL were employed for determining the attraction of adult mosquitoes. We conducted this bioassay with a modified Y-tube, according to Geier (Geier and Boeckh, 1999), using 20 mosquitoes per concentration performed in four replicates. This bioassay began with the release of female mosquitoes into the Y-tube, which has two intersections within the tube; the left side is where the mushroom extract was added, while the right side of the tube was where the extraction solvent was added. When mosquitoes flew to the end of the tube, we counted and recorded the results.

Statistical analyses

The numbers of dead larvae and numbers of mosquito attracted were presented using the mean (± standard deviation [S.D.]). Comparative analysis of the number of mosquitoes attracted between *A. auricula-judae* extract and oetenol was performed at all concentrations with a t-test with 95% confidence intervals ($p < 0.05$).

RESULTS AND DISCUSSION

In the work presented here, we assessed the effect larvicidal and adult mosquito attractant activity of *A. auricula-judae* mushroom extracts on *Ae. aegypti* and *Cx. sitiens*.

Efficacy of *A. auricula-judae* extract for larvicidal activity

The results surrounding the larvicidal effect of the mushroom against both mosquito species showed that *A. auricula-judae* extract did not affect larvae of *Ae. aegypti* and only very few of the *Cx. sitiens* larvae died at all concentrations (Table 1). While octenol, the highest concentration at 120 mg/L showed a profound ability to affect *Cx sitiens* (19.67 ± 0.58) but less with *Ae. aegypti* (9.33 ± 4.93). In the control group, with no *A. auricula-judae* extract, *Ae. aegypti* did not die but we did observe a number of deaths for *Cx. sitiens* (0.33 ± 0.58). These results are consistent with Thongwat *et al.* (Thongwat *et al.*, 2015), who screened 143 species of mushroom against *Ae. aegypti* larvae and found larvicidal activity in only *Thaeogyroporus porentosus*, *Xylaria nigripes, Chlorophyllum* spp. and *Steccherinum* spp., along with two unidentified species. In spite of this, it is reported that octenol can be used to eliminate small insects (Inamdar and Bennett, 2014). This is consistent with the results of octenol in this experiment that the maximum concentration (120 mg/L) can kill almost all larvae of *Cx. sitiens* (98.35%) and about half of all *Ae. aegypti* larvae (46.65%). Temephos has been used to control and is the most popular, highest performance and cheapest. Criteria for susceptibility to larvae are set at 0.012 mg/L temephos as recommended by the World Health Organization standard. The results of octenol substance compared to the criteria of temephos were that it had the ability to eliminate mosquitoes slightly (1.65% of *Ae. aegypti* and 25% of *Cx. sitiens*). Therefore, both *A. auricula-judae* extract and octenol are not suitable for use in the field because they are less powerful.

Table 1: Mean dead numbers of the larvae of *Ae. aegypti* and *Cx. sitiens.*

Concentrations (mg/L)	n	*A. auricula-judae*		Octenol	
		Ae. aegypti	*Cx. sitiens*	*Ae. aegypti*	*Cx. sitiens*
120	20	ND	2.00 ± 1.00	9.33 ± 4.93	19.67 ± 0.58
12	20	ND	3.67 ± 2.52	0.33 ± 0.58	9.67 ± 3.06
1.2	20	ND	2.67 ± 1.53	ND	6.00 ± 2.00
0.12	20	ND	2.67 ± 3.21	1.00 ± 1.00	5.00 ± 2.65
0.012	20	ND	2.00 ± 1.73	0.33 ± 0.58	5.00 ± 2.65
Control group	20	ND	0.33 ± 0.58	ND	ND

ND = No deaths of mosquito larvae.

Efficacy of *A. auricula-judae* extract for mosquito attraction

The effects of *A. auricula-judae* crude extract attracting adult *Ae. aegypti* and *Cx sitiens* at three levels of concentration showed that 10^{-5}g/mL attracted the most of both species of mosquitoes followed by 10^{-6} g/mL and 10^{-4} g/mL, respectively (11.66 ± 0.57 vs. 7.00 ± 1.00, 11.33 ± 0.57 vs. 6.66 ± 0.57 and 9.33 ± 0.57 vs. 6.00 ± 1.00 mosquitoes, respectively) (Table 2). While the effects of this extract attracted female *Ae. aegypti* more so than *Cx. sitiens* at all concentrations.

Octenol's scent powerfully attracts female mosquitoes, and several studies have reported octenol being contained in mushrooms (Dijkstra, 1976). The results of the assay with *A. auricula-judae* extract for adult mosquito attractant activity demonstrated the ability to attract adult mosquitoes by more than 50% (58.33%) of *Ae. aegypti* at 10^{-5}g /mL concentration, while *Cx. sitiens* was less attracted less *Ae. aegypti* at all concentrations. These findings are in line with previous research that found this concentration could best attract mosquitoes, especially *Aedes* mosquitoes (Cilek *et al.*, 2011). However, a comparison of the number of mosquitoes attracted to *A. auricula-judae* extract and oetenol were different at all concentrations ($p > 0.05$) (Figure 2).

Table 2: Mean number of adult *Ae. aegypti* and *Cx. sitiens* responses.

Concentrations (g/mL)	n	*A. auricula-judae*		Octenol	
		Ae. aegypti	*Cx. sitiens*	*Ae. aegypti*	*Cx. sitiens*
10^{-4}	20	9.33 ± 0.57	6.00 ± 1.00	15.33 ± 0.57	11.00 ± 0.33
10^{-5}	20	11.66 ± 0.57	7.00 ± 1.00	16.33 ± 0.57	12.66 ± 1.15
10^{-6}	20	11.33 ± 0.57	6.66 ± 0.57	16.00 ± 0.00	11.66 ± 0.57

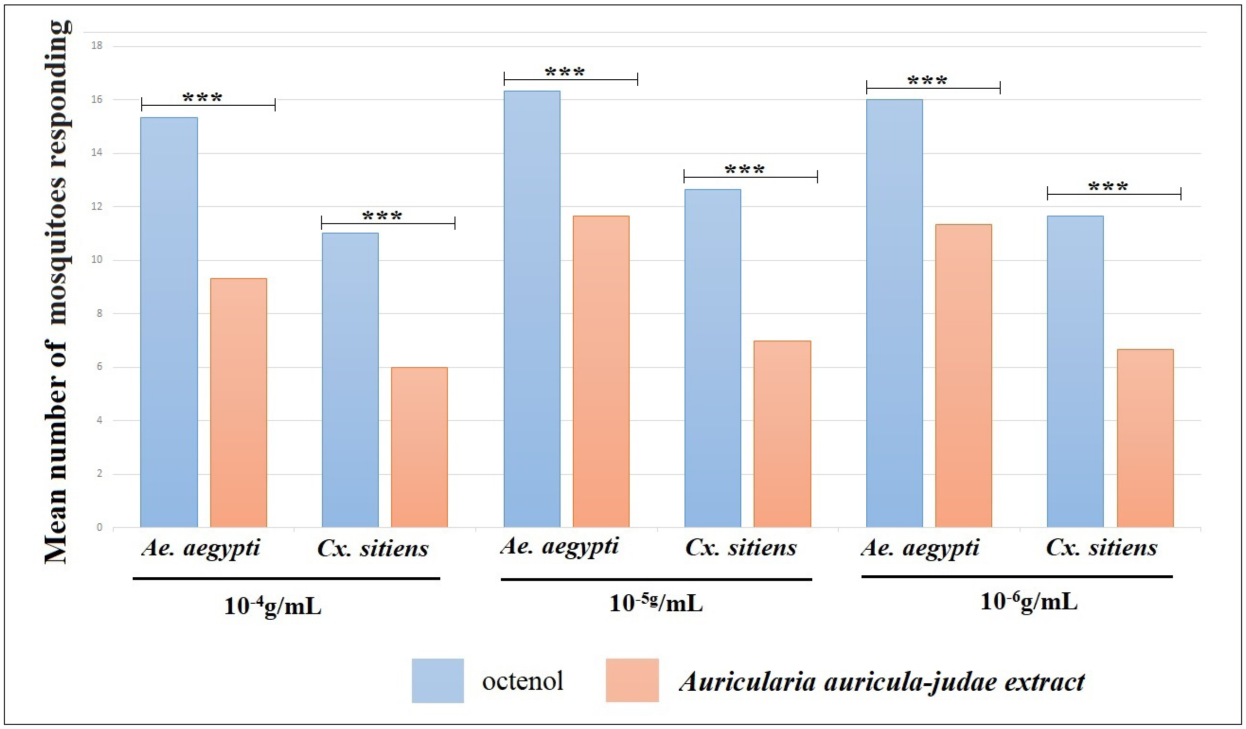

Fig. 2: Comparative analysis of the mean number of mosquitoes responding to *A. auricula-judae* extract and octenol.*** = Statistical difference is significant at the 0.05 level (two-tailed).

CONCLUSION

Although *A. auricula-judae* extract is not equal to octenol in terms of its ability to kill larvae or attract adult females, it is possible to develop a higher efficacy, such as incorporating other substances to attract mosquitoes, especially dengue vector according to our results, revealing a capacity to attract more than half of all *Ae. aegypti* mosquitoes. With this, *A. auricula-judae* extract has the compelling features of being inexpensive and eco-friendly and serves as a robust option for increasing the effectiveness of controlling mosquito-borne diseases.

ACKNOWLEDGMENTS

We would like to acknowledge and thank the College of Allied Health Science, Suan Sunandha Rajabhat University, Thailand, for their kind support of our research.

CONFLICT OF INTEREST

There are no conflicts of interest related to this research.

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How to cite this article:
Chaiphongpachara T, Sumchung K, Chansukh KK. Larvicidal and Adult Mosquito Attractant Activity of *Auricularia auricula-judae* Mushroom Extract on *Aedes aegypti* (L.) and *Culex sitiens* Wiedemann. J App Pharm Sci, 2018; 8(08): 021-025.