Adulticide Efficacy of *Artemisia vulgaris* L. against *Aedes aegypti* L.

Potensi Adultisida dari Artemia vulgaris L. *terhadap* Aedesagypti L.

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ABSTRACT

Aedes aegypti is the vector of various arthropod-borne diseases such as dengue fever, chikungunya and currently, zika. This study aimed to evaluate *Artemisia vulgaris*as other adulticides for controlling *Ae. aegypti*. Indonesian ministry of health hasbeen reported that more than 70.000 dengue fever human cases have occurred in 2015 covering 34 provinces which mean that dengue fever has spread now into all national territories. Eradication of *Aedes* still largely depends on insecticides,which is the most cost-effective strategy, and often inefficient due to resistance development in exposed *Aedes* population This study was designed to use of Centers for Disease Control and Prevention (CDC) bioassay standard. CDC bottles were coated with the ethanolic solution of extract with a concentration of 10, 50, 100, 500, 1000, 5000, 10000, 50000, and 100000 µg per bottle. F0 mosquitoes were used for all experiments. Death and surviving mosquitoes were evaluated based on CDC standard assay. The test was performed with 10-25 adult mosquitoes every bottle and each concentration was repeated in triplicates. The results showed that LC_{50} and LC_{90} were 5790 µg and 52110 µg respectively after 120 minutes exposure to the extract. There was no mortality in ethanol control group. *A. vulgaris* significantly ($P < 0.05$) have adulticidal activity against *Ae. aegypti*. However, *A. vulgaris* have been reported to have larvacidal activity against *Ae. Aegypti.* These results indicated clearly that *A. Vulgaris* might act as the candidate of bioinsecticides for controlling *Ae. aegypti*.

Keywords: *Aedes aegypti*, *Artemisia vulgaris*, adulticide, bio insecticide.

INTRODUCTION

Mosquitoes are the vector of various disease such as malaria, chikungunya, dengue fever, yellow fever, Japanese encephalitis, filariasis, schistosomiasis and currently zika (Govindarajan *et al*., 2011). There are 111 genera and 137 subgenera containing 3,517 species of mosquitoes in worldwide (*Becker et al.,* 2010). *Aedes aegypti* is a major vector of arthropod-borne disease, primary vector responsible for dengue fever, yellow fever, chikungunya, and Zika fever (Ajaegbu *et al*., 2016). Dengue is major public health problem globally (Samuel & Tyagi, 2006) over 3.97 billion people in 128 countries at risk of disease. At 2010 approximately there were 390 million cases (Sayono *et al*., 2016). Indonesian ministry of health has been reported that more than 70.000 dengue fever human cases have occurred in 2015 covering 34 provinces which mean that dengue fever has spread now into all national territories(Hamid *et al.,* 2017). While vaccine of dengue fever is still developing with active research, the only way to prevent and control dengue virus transmission by controlling the vector (Wuliandari *et al*., 2015, Elumalai *et al*., 2016). The application of chemical and synthetic insecticide has been long utilized, there were several problems such as insecticidal resistance, environmental pollution, and harmful impact on human and other organisms (Cheah *et al*., 2013).

Mosquitoes have been resistance to synthetic and chemical insecticide in several places in Indonesia, as in Central Java province (Sayono *et al*., 2016), Yogyakarta province (Wuliandari *et al*., 2015), Bali (Hamid *et al*., 2017), Bandung, Surabaya, Palembang (Ahmad *et al*., 2007), and East Jakarta (Hardjanti *et al.,* 2015). Therefore, a new strategy of controlling mosquitoes need to develop. It has prompted the researcher to look for environment-friendly, safe for human and animal, target specific for mosquitoes, and cost effective. Another control method such as botanical and microbial insecticide has been applied for last few year (Elumalai *et al*., 2016). Due to limited use of it in vector control programs, no study reported about resistance.

Plant extract may be alternative source due to it has the abundant bioactive compound. *Artemisia vulgaris* is the member of the family of Asteraceae. *A. vulgaris* has height about 1-1.5 meter and release aromatic fragrant. Artemisia distributed throughout the northern temperate regions of Africa, Europe, Asia, and North America. The chemical studies on *A. vulgaris* indicate that thujone isomer and camphor were determined as the main components in *A. Vulgaris* from India (Judžentienė & Buzelyte 2006). Compounds like terpenoids and flavonoids are present in this plant. Artemisia extracts also contain artemisinin that has been reported to reduce *Plasmodium falciparum* gametocyte development, thus reducing transmission of malaria, this fact significant in preventing the spread of resistant strain (Masotti *et al.*, 2012). However, to our knowledge, the mosquito adulticidal activity of *A. vulgaris* extract has been little investigated.

METHODS

Plant collection

The plant *A. vulgaris* were collected from Tlahap, Kledung, Temanggung, Central Java (7°19'30' S and 110°14' 88' E) in March 2017. Taxonomic identification was issued by Department of Plant Systematic, Biology Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Figure 1. *Artemisia vulgaris* from Tlahap, Kledung, Temanggung, Central Java

Mosquitoes rearing

The eggs of *Ae. Aegypti* was collected using about 200 ovitraps in several places in Sleman, Yogyakarta. Eggs were transferred to laboratory Parasitology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Eggs were hatched using plastic container 20 x 15 x 5 cm3 filled with 800 ml tap water within ± 24 hours. Larvae were fed with chicken liver and maintained at 28ºC, 70-85% relative humidity, with a photoperiod of 14 h light and ten h dark. Pupae were transferred to a cup (12 cm x diameter 9.8 cm) containing 200 ml clean water covered with a net for adult emergence. The adult was moved into the cage (20 x20x20 cm) and fed using 10% sucrose solution soaked in cotton.

Extraction

A total of 5 kilograms *A. vulgaris* were cleaned and dried using the oven for seven days with temperature 55ºC.Total 300 grams dried leaves were grounded into powder by grinding machine. Ethanol 95% (Emsure, Germany) was added to the powder for *A. Vulgaris*maceration. The mixture of *A vulgaris*and ethanol is homogenized for 30 minutes and kept for one day at room temperature. Complete removal of the filtrate was accomplished in vacuum rotary evaporator. The extract then is heated in the water bath at a temperature of 70ºC. The final extract was in the pasta form and kept in 4ºC until further use.

Adulticidal test using Center for Diseases Control Prevention (CDC) Standard

Weighing one gram of extract dissolved in 100 ml of ethanol for making 1% of the stock solution. To make 10, 50, 100, 500, 1000, 5000, 10000, 50000, and 100000 µg per bottle. Stock solution diluted using ethanol and transferred to a bottle according to the concentration required. The bottle used has a volume of 250 ml, the bottle was washed using soapy water then rinsed three times and placed in an oven for 20 minutes until dry. One ml of each concentration solution added to the bottle including for control using only ethanol; the bottle rotated gently to make all side coated with the solution, the caps were removed and continuously rolled until the solution completely dry. The bottle was kept from direct sunlight.

Bioassay procedure

The assay was performed using 10-25 F0 mosquitoes (2-5 days old sucrose fed) put into the previous bottle using the aspirator. Every 15 minutes the mosquitoes were observed until two hours long. Mosquitoes were considered died if they could no longer stand, the dead and alive mosquitoes were recorded. This procedure was performed triplicates include the control.

Statistical analysis

The average mortality was subjected to ANOVA using GraphPad Prism 7.00 and probit analysis for calculating lethal dosages causing 50% and 90% mortality (LC50 and LC90) also 95% upper and lower confidence limit.

RESULTS AND DISCUSSION

The adulticidal activity of *A. vulgaris* in the ethanolic extract at various concentration against *Ae. aegypti*was illustrated in figure 2. Observation of present study showed that mortality was increased as the concentration increased in 90, 105 and 120 minutes. For example in 100 mg concentration, mortality

was measured 6,7% and increased to 15% at 500 µg in 120 minutes. 100% mortality was reached at 10000µg. The LC50 and LC90 were determined by using probit analysis (GraphPad Prism 7.0.). The results given in figure 3 showed that LC50 and LC90 were 5790 µg and 52110 µg respectively after 120 minutes exposure to the extract. The 95 % lower confidence limit is 3331 µg and upper confidence limit is 9961 µg. There was no mortality in ethanol control group. *A. vulgaris* significantly ($P < 0.05$) have adulticidal activity against *Ae. aegypti.*

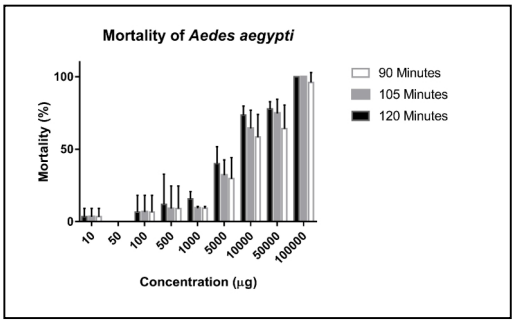

Figure 2. Adulticidal activity of *A. vulgaris* extract against *Ae. aegypti.*

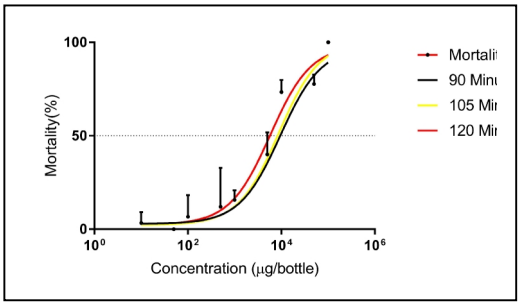

Figure 3. Graph showed result of probit analysis

Insecticides resistance tends to increase worldwide (Hamid *et al,.* 2017, Ishak *et al.* ,2015, Li *et al*., 2015, Lima *et al.,* 2011, Saavedra *et al,.* 2007). This problem becomes a dangerous nowadays since arthropod-borne diseases prevention strategy depends on the vector controls. Compounds from natural herb can be used as an alternative insecticide to control the vectors (Govindarajan *et al.,* 2011). Bioinsecticide of plant origin was used against several insect species due to safer to human, without toxic and no residues left in the environment.

The adulticide assay of this study showed activity against adult *Ae. aegypti*. We adapted CDC bottle assay in this experiment to demonstrate the ability of *A. vulgaris* in penetrating adult *Ae. aegypti* and killing the mosquitoes. According to literature survey, lack of published paper about the adulticidal activity of *A. vulgaris* on *Ae. aegypti*. However, *A. vulgaris* have been reported to have larvacidal activity against *Ae. Aegypti* (Mya *et al*., 2016, Sundararajan & Kumari, 2017). Another species of Artemisia, *A. nilagirica* have been reported to have adulticidal activity against *Ae. aegypti* (Panneerselvam *et al.,* 2012) indicated that *A. nilagirica* has adulticide activity the LC50 and LC90 values 242.52 and 523.73 ppm. *A. annua* in the previous report showed that have larvacidal activity, ovicidal and oviposition deterrent (Cheah *et al*., 2013), *A. abrotanum* and *A. pontica* also showed have larvacidal activity (Tabanca *et al*., 2011). Using *Spondia mombin* leaves in methanol extract (Ajaegbu *et al*., 2016) with CDC bioassay procedure showed the LC50 values of 4061.946 µg/bottle in 24 hours post treatment. *Ae. aegypti* showed restless movement, abnormal wagging, and died inside the bottle contain *A. vulgaris* extract only within 120 minutes.

Another plant has adulticidal activity using WHO protocol, essential oil *Piper retrofractum Vahl* in acetone showed that LD50 and LD99 of against *Ae. aegypti* (8.86%, 23.21%) and *Culex quinquefasciatus* (6.95% and 17.35%) (Subsuebwong*et al*., 2016).

Cassiatora leaf extracts against the adulticidal activity of (hexane, chloroform benzene, acetone, and methanol) *Ae. aegypti*LC(50) values were 329.82, 307.31, 287.15, 269.57, and 252.03 ppm and LC(90) values were 563.24, 528.33, 496.92, 477.61, and 448.05 ppm (Amerasan*et al*. 2012). *Citrus sinensis* orange peel extract against *Ae. aegypti* showed LC50 value was 320.38ppm (Murugan*et al*., 2012). C*lausena dentata* plant extract against *Ae. aegypti* showed the LC50 and LC90 4.1783 mg/ml and 9.3884 mg/ml (Ramkumar *et al*., 2015). The obtained results indicate that the extract of *A. vulgaris* has potential to be developed as an adulticide against *Ae. aegypti* mosquitoes. However, further studies to evaluate its toxicity need to be conducted. Studies aimed at isolation andidentification of active compounds must be performed. The results of the present study could be used in promoting research aimed at the development of new agents for mosquito

control based on bioactive chemical compounds from indigenous plant sources.

CONCLUSION

The results indicate that the extract of *A. vulgaris* has potential to be developed as an adulticide against *Ae. aegypti* mosquitoes with LC50 and LC90 were 5790 mg and 52110 mg. However, further studies to evaluate its toxicity need to be conducted.

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