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**Short Communication**

# The ethanol extract of *Sargassum duplicatum* as an ovicidal agent against *Aedes aegypti*

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## Abstract

Dengue hemorrhagic fever (DHF) is a major health concern in tropical and subtropical countries. Indonesia has DHF cases perennially every year. On the other hand, Indonesia is abundant with seaweed *(Sargassum duplicatum),* which can be found across its seashore. The macroalgae contains secondary metabolites with ovicidal activity; hence, it has the potential to be utilized in suppressing the *Aedes aegypti*. The aim of this study was to determine the ovicidal activity of *S. duplicatum* against the *Ae. aegypti* eggs. The algae were macerated with ethanol 70% before being subjected to qualitative phytochemical screenings. The ovicidal tests were conducted with an extract concentration of 50 ppm, 100 ppm, 500 ppm, 1000 ppm, and 1500 ppm, while distilled water was used as the control. The hatchability of *Ae. aegypti* was observed 24 hours a day for 4 days and the larval development was investigated under a microscope. Phytochemical screenings revealed that the extract was positive containing alkaloids, flavonoids, steroids, saponins, and phenols. The hatchability of *Ae. aegypti* eggs were significantly reduced following the *S. duplicatum* extract exposure for four days ($p$=0.000). The extract had $LC_{50}$ of 828.653 ppm and $LC_{50}$ of 1786.09 ppm for the ovicidal activity against *Ae. aegypti* eggs. The concentration of ethanol extract of *S. duplicatum* did not affect the mosquito development from larvae to adult stage ($p$=0.263). Further research is needed to explore the effect of specific compounds contained in the *S. duplicatum* and investigate their ovicidal potential.

**Keywords**: *Aedes aegypti,* phytochemical*, Sargassum duplicatum,* ovicidal, secondary metabolite

## Introduction

In Indonesia, more than 456 mosquito species belong to 18 genera and 48 subgenera. A total of 287 mosquitoes were belonged to the genus *Aedes, Anopheles*, or *Culex* [1]. These three genera are of concerns because they are zoophilic and anthrophilic, having significant potential as disease vectors [2]. Common diseases carried by these mosquitoes are dengue hemorrhagic fever (DHF) (*Aedes*), malaria (*Anopheles*), and filariasis (*Culex*) [3]. According to the World Health Organization, as of April 30, 2024, dengue cases have reached more than 7.6 million, indicating a global increase from the last five years [4]. The prevalence of DHF increases along with the mobility and population density [5,6]. DHF in Indonesia remains endemic, posing a significant public health challenge and necessitating comprehensive strategies from multiple sectors [7,8].





Received: June 13, 2024 - Accepted: August 27, 2024 - Published online: September 26, 2024

One of the strategies to control this disease is by preventing the growth of the mosquito population through environmental engineering, biological control, and chemical insecticides [9]. In common practice, temephos and fogging are frequently used to control mosquito population [10]. Despite being effective in its long-term use, it could induce resistance among *Aedes* spp. [11]. For example, a study from Brazil demonstrated that *Ae. aegypti* has been mostly resistant to organophosphate and pyrethroid insecticides [12]. Similarly, in Indonesia, the use of cypermethrin, malathion, and temephos is no longer effective as the mosquitos have developed resistance toward the chemicals [13]. Therefore, there is an urgency to explore new insecticides, particularly from natural products [14].

Despite being easily cultivated, highly abundant, and rich with bioactive secondary metabolites, macroalgae is still under-utilized [15-19]. For example, *Sargassum duplicatum*, the brown seaweed, has not been extensively used despite being rich with alkaloids, saponins, phenolics, flavonoids, and tannins [20-22]. These secondary metabolites, particularly flavonoids and alkaloids, have been reported to have ovicidal and larvicidal activities against *Ae. aegypti, Ae. albopictus, An. arabiensis, An. gambiae, An. stephensi,* and *Cx. pipiens* [14]. Previously, phytocompounds such as alkaloids, anthraquinones, flavonoids, cardiac glycosides, coumarins, phenols, saponins, tannins, and triterpenoids were reported to act as ovicidal and larvicidal against *Anopheles* mosquitoes. Hence, the aim of this study was to unveil the potential of *S. duplicatum* as a mosquito control agent, particularly in the egg stage.

# Methods

## Study design

The design of this research was a laboratory experiment using a completely randomized design (CRD) consisting of experimental groups exposed to *S. duplicatum* extract at different concentrations and a control group without concentration. This research was conducted from March to July 2020.

## Biological specimen collection

*Sargassum duplicatum* J. Agardh was collected from Bandengan Beach, Jepara Regency, Central Java, Indonesia. The algae were cleaned, air dried for 2–3 days, and crushed into fine powder. *Aedes aegypti* eggs were obtained from the Salatiga Center for Disease Research and Development (B2P2VRP) and reared in the Animal Systems Laboratory, Parasitology Section, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

## Extraction of *Sargassum duplicatum*

The dried fine powder of *S. duplicatum* (100 g) was macerated with 1000 mL ethanol 70% (ratio 1:10, g/mL) and left still at room temperature. After 48 hours, the macerate was separated from the residue, and the process was repeated with the remaining residue. The macerate collected was combined and concentrated at 45°C. The viscous extract was evaporated in a water bath to remove the solvent completely.

## Phytochemical screening

Phytochemical screenings were carried out to determine qualitatively the presence of bioactive components of *S. duplicatum* ethanol extract based on the changes in shape or color. Phytochemical tests were carried out according to a previously described method, including the examinations of alkaloids, saponins, flavonoids, steroids, triterpenoids, phenols, and quinones [23]. Briefly, alkaloids were detected by a reaction with Dragendorf reagent, flavonoids – with Mg and HCl mixture, and steroids and triterpenoids – with sulfuric acid and anhydrous acetic acid. To identify saponins, the extract was dissolved, boiled, and shaken vigorously until the foam was formed. As for phenol and quinone, the reaction involved $FeCl_3$ and NaOH, respectively.

## Ovicidal test

The ovicidal test was performed in accordance with the published protocols [24]. Initially, the extract was prepared in different concentrations (50 ppm, 100 ppm, 500 ppm, 1000 ppm, and 1500 ppm). A 100 mL portion of each concentration was added to test tubes containing 25 *Ae.*

*aegypti* eggs. As a control, eggs were exposed to distilled water only. The eggs were observed every 24 hours for four days using a magnifying glass, noting their bell shape, lack of shrinkage, and intact structure. To promote larval development, the eggs were supplemented with chicken liver. Hatched eggs were transferred to separate containers for further observation of larval and adult development. Adult mosquitoes were immobilized by placing them on a glass slide, cooled in the freezer for five minutes, and then examined under a microscope connected to an Optilab. The hatching eggs were counted and observed to determine the development of larvae. The hatchability was calculated by the following equation: Hatchability = (Hatched larvae count / initial egg count) × 100%.

The effects of ovicidal activities on the development of larvae into adult mosquitoes were evaluated based on their flying behavior and morphology, specifically examining the thorax and ventral abdomen. Morphological assessments were conducted using a microscope and an Optilab tool to detect any defects or incomplete development in these areas. Flight behavior was evaluated by observing mosquitoes in a cage, focusing on their agility during flight, resting behavior, and ability to collect blood or nectar. The pH and temperature were monitored throughout the observation period, ranging from 7.25 to 6.69 and 27.5°C to 28.5°C, respectively (**Table 1**).

Table 1. PH and temperature conditions when testing the hatchability of mosquito eggs

| Environmental parameters of hatching eggs | | | | | |
|---|---|---|---|---|---|
| Concentration | pH | Temperature (°C) | | | |
| | | Day-1 | Day-2 | Day-3 | Day-4 |
| Control | 7.25 | 27.5 | 28 | 28.5 | 28 |
| Extract 50 ppm | 7.18 | 27.5 | 28 | 28.5 | 28 |
| Extract 100 ppm | 7.04 | 27.5 | 28 | 28.5 | 28 |
| Extract 500 ppm | 6.97 | 27.5 | 28 | 28.5 | 28 |
| Extract 1000 ppm | 6.77 | 27.5 | 28 | 28.5 | 28 |
| Extract 1500 ppm | 6.69 | 27.5 | 28 | 28.5 | 28 |

### Data analysis

The normality of the data distribution was analyzed based on the Shapiro-Wilk test and the data homogeneity was analyzed using the Levene test. Normally distributed data were then subjected to one-way ANOVA, followed by the Duncan Multiple Range Test (DMRT), performed on SPSS (IBM, New York, USA). The $p<0.05$ was set as the statistical significance. Probit analysis was performed to identify toxicity by calculating the lethal concentration (LC) in larvae at the 50% level ($LC_{50}$) and 90% level ($LC_{90}$) using non-linear regression.

## Results

### Extract yield and phytochemical profile

Each dried fine of *S. duplicatum* was found to yield 2.98 grams of extract. The results from the phytochemical screening are presented in **Table 2**. The phytochemical test of alkaloids revealed a positive result by the formation of orange color. The saponin examination showed a positive result, indicated by the formation of stable foam after being shaken. The flavonoids test also showed orange color as the indication of positive result. The examination of steroid and triterpenoid showed positive and negative results, as indicated by the formation of turquoise and no color changes, respectively. The color of the extract turned red in the examination of phenol and no color changes in the quinones test, rendering the positive and negative results, respectively.

Table 2. The secondary metabolites contained in *Sargassum duplicatum*

| Secondary metabolites | Result | Observation | Criteria for positive result |
|---|---|---|---|
| Alkaloids | + | Orange deposits | The formation of orange, brown and white deposits |
| Saponins | + | Foam is formed | Foam is formed |
| Flavonoids | + | Formed orange | Red, yellow or orange colors are formed |
| Steroids | + | Bluish green color changes | Change color from purple to blue or green |

| Secondary metabolites | Result | Observation | Criteria for positive result |
|---|---|---|---|
| Triterpenoids | - | There is no red color | Change color from purple to red |
| Phenols | + | Formed red | It is formed in green, red, purple, blue or deep black |
| Quinones | - | No color changes | Formed red |

(+): present; (-): absent
The criteria for positive results are according to previous studies [25,26].

## The hatchability and the development of *Aedes aegypti*

Numbers of hatched and failed-to-hatch eggs from a single batch observation are presented in **Figure 1**. The hatching activities were mainly suppressed by the highest concentration of *S. duplicatum* extract (n=11). At 1000 ppm concentration, the number of hatched eggs was maintained three times more than 1500 ppm (n=30), while for 50 ppm, 100 ppm, and 500 ppm concentrations, the number of hatched eggs out of 100 eggs was 78, 72, and 62, respectively.

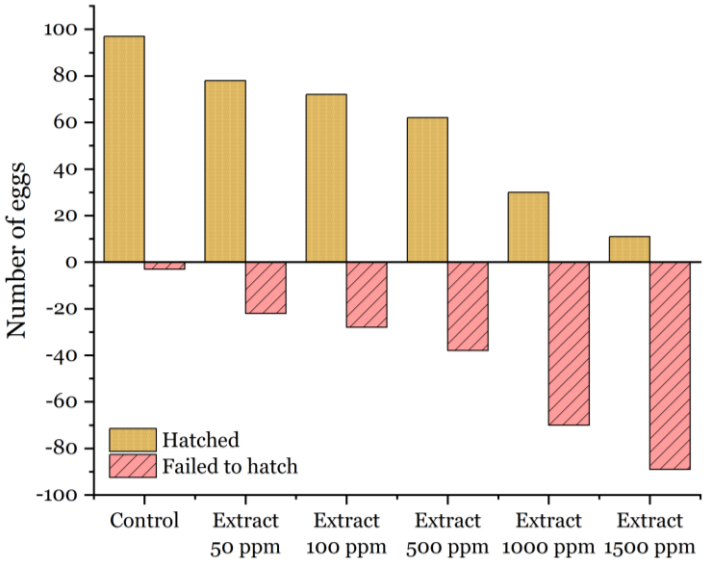


Figure 1. Number of hatched and failed to hatch eggs as exposed with *Sargassum duplicatum* extract in different concentrations for four days (n=25).

Duncan multiple range test demonstrated the significant difference among study groups, where the data are presented in **Table 3**. The values of Duncan analysis indicated that the higher concentration of the *S. duplicatum*, lowers the hatchability of *Ae. aegypti*. Exposure to the extract with 50 ppm concentration did not significantly affect the hatchability ($p$<0.215), yet increasing the concentration to 100 ppm gave a significant result ($p$=0.033). The probit analysis revealed the extract had $LC_{50}$ and $LC_{90}$ values of 828.65 ppm (95%CI: 685.55–950.75 ppm) and 1786.09 ppm (95%CI: 1,494.12–2,420.96 ppm), respectively.

Table 3. Duncan multiple range test analysis

| Treatment | Non-hatched eggs (%) | $p$-value |
|---|---|---|
| Control | 3±0.96 | Ref. |
| 50 ppm | 22±2.08 | 0.215 |
| 100 ppm | 28±1.63 | 0.033* |
| 500 ppm | 38±2.65 | 0.001 |
| 1000 ppm | 70±4.12 | <0.001 |
| 1500 ppm | 89±2.22 | <0.001 |

Data are presented as mean±standard deviation based on four repeated observations.
*Significant at $p$<0.05

The observation was continued until the mosquitos hatched and developed into adulthood, where the observation results are presented in **Table 4**. No apparent changes occurred on the hatched mosquitos following extract exposure. All mosquitoes exhibited normal flying behavior.

Table 4. Observations on eggs that hatched into adult mosquitoes

| Observation | Control | | Extract 1500 ppm | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Flying behaviour | Good | Good | Good | Good |
| Ventral abdomen |  |  |  |  |
| Thorax |  |  |  |  |

## Discussion

The ethanol extract of *S. duplicatum* inhibited the *Ae. aegypti* eggs from hatching in a concentration-dependent manner. The DMRT test suggested a significant effect starting from a concentration of 100 ppm ($p$=0.033), but the probit analysis obtained results at $LC_{50}$ and $LC_{90}$ of 828.65 ppm and 1786.09 ppm, respectively. Similar to previous research, extracts from *Mangifera* spp. yield an effective inhibition after the concentration increased to 640 ppm [27]. Extracts of *Dodonaea viscosa, Lantana camara*, and *Ruta chalepensis*, at 500 ppm, reduced the hatchability up to 89.7%, 87.0%, and 69.8%, respectively [28]. In another study, *Delonix elata* extracted with hexane, benzene, chloroform, ethyl acetate, and methanol at a concentration of 450 ppm yielded 100% hatching inhibition [29]. Discrepancies between the findings in the present study and those previously reported could be affected by the variation of secondary metabolites biosynthesized across different plants [30]. Moreover, the types of solvent used for the extraction also determine the secondary metabolite contained in the extract [31-33].

In a previous study, hatching inhibition was associated with the presence of internal abnormalities in mosquito eggs [34]. The secondary metabolites contained in plants may also affect the bioactivity of *S. duplicatum* as an ovicidal agent. These secondary metabolite compounds may contribute together or by working independently as an ovicidal activity for the *Ae. aegypti* mosquito [35]. The embryo in the eggs of the *Ae. aegypti* is protected by a shell consisting of exocorion, endocorion, and serosal cuticle. The cuticle is responsible for preventing the desiccation of *Ae. aegypti* eggs and is also useful for the entry of substances, compounds, and gas exchange needed during the embryogenesis process [36]. As observed in the present study, *S. duplicatum* extract was found to contain alkaloids, saponins, flavonoids, steroids, and phenols. Flavonoid is a compound with a high level of polarity, which will easily pass through the cell membrane of the mosquito eggs, thereby causing hatching inhibition [37]. In a previous study, the secondary metabolites could induce endosmosis, leading to the shrinkage and death of the mosquito embryo [35].

The findings of the present study suggest that the ethanol extract of *S. duplicatum* is considered relatively low in its ability to inhibit the hatching of *Ae. aegypti* mosquito eggs, but its activity can still be improved with further research on certain compounds. Therefore, this study also requires further research on extracts using different solvents. In addition, a limitation of this study is the variability in *S. duplicatum* composition due to differences in collection time and location, which may affect the consistency of the results. In addition, this study was conducted in a controlled laboratory environment, which may not fully mimic natural conditions. Therefore, further studies are needed to validate these findings in various environmental settings and over a more extended period, which should also focus on ecological safety and the potential development of resistance in mosquito populations.

## Conclusion

The ethanolic extract of *S. duplicatum* contains alkaloids, flavonoids, saponins, steroids, and phenols. The extract has a low potential ovicidal agent against *Ae. aegypti* eggs ($LC_{50}$=828.65 ppm and $LC_{90}$=1786.09 ppm), yet the effect is found to be dose-dependent. Moreover, the extract does not affect the development of larvae into mosquitoes, as observed in the morphology and behavior of the hatched adult mosquitoes. Further research is recommended to use other solvents and elucidate the exact mechanisms by which the secondary metabolites exert their ovicidal effects. Future studies should validate these findings under natural environmental conditions and assess the long-term ecological impacts and potential for resistance development in mosquito populations.

### Ethics approval

Not required.

### Acknowledgments

We would like to thank the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, which has facilitated the laboratories related to research.

### Competing interests

The authors declare that they have no conflict of interest in the writing or publication of this article.

### Funding

This study received no external funding.

### Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

## How to cite

Giyantolin G, Subiakto Y, Poerwanto SH. The ethanol extract of *Sargassum duplicatum* as an ovicidal agent against *Aedes aegypti*. Narra J 2024; 4 (3): e990 - http://doi.org/10.52225/narra.v4i3.990.

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