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Phytochemical and Bioactivity Study of *Sargassum duplicatum* as a Candidate Natural Biolarvicide Compound

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Abstract
Dengue hemorrhagic fever (DHF) is an endemic disease found in nearly all provinces of Indonesia. The incidence of DHF is closely associated with climatic factors at spatial, temporal, and spatio-temporal scales. The search for new methods to eliminate sources of dengue virus transmission is both urgent and essential. *Sargassum duplicatum*, a species of brown seaweed native to Indonesia, has been shown to possess potential antioxidant properties. This alga contains various bioactive compounds, including steroids, alkaloids, phenols, flavonoids, saponins, and tannins, which are suspected to exhibit insecticidal activity. This study aimed to evaluate the phytochemical composition and bioactivity of the ethanol extract from *Sargassum duplicatum* as a biolarvacidal. A completely randomized design (CRD) was used, consisting of five treatment groups with three replications each. A total of 300 third-instar *Aedes aegypti* larvae were introduced into treatment containers containing predetermined concentrations of the *Sargassum duplicatum* ethanol extract, with each container holding 20 larvae. The observational data were analyzed using ANOVA and probit analysis. Phytochemical screening of the ethanol extract confirmed the presence of flavonoids, saponins, tannins, and alkaloids. The extract demonstrated a concentration-dependent increase in larval mortality. A concentration of 75 ppm showed larvicidal efficacy comparable to that of 1% Abate. The LC_{50} and LC_{90} values of the ethanol extract were calculated to be 9.098 ppm and 20.485 ppm.

INTRODUCTION

Mosquitoes are flying insects commonly found worldwide, with over 3,700 known species distributed globally (CDC, 2024). Several mosquito species act as disease vectors, collectively causing infections in approximately 700 million people worldwide each year (Dhiman *et al*., 2022). One of the most significant species is *Aedes aegypti*, which is recognized as the primary vector responsible for the transmission of several vital arboviruses, including dengue fever, Zika virus, and chikungunya (Gloria-Soria *et al*., 2017; Ross *et al*., 2017; Silvério *et al*., 2020; Aungtikun and Soonwera, 2021). *Aedes aegypti* is a widely distributed mosquito vector and the primary contributor to arbovirus transmission in humans (Louise *et al*., 2015; Estallo *et al*., 2020). It is also the principal vector of the dengue virus (Wiemer *et al*., 2017; Muhammad *et al*., 2025).

According to the World Health Organization (WHO), dengue hemorrhagic fever (DHF) is a vector-borne disease caused by the dengue virus and transmitted through the bites of *Aedes* mosquitoes, primarily *Aedes aegypti* and *Aedes albopictus* (Moniharapon, 2019; Moniharapon, 2020; Ayal *et al*., 2021; Kaihena and Ukratalo, 2021; Ukratalo, 2024). In Indonesia, dengue fever has become a significant public health concern. Based on Indonesia's Health Profile from

2020 to 2023, the incidence of dengue has fluctuated over the years. In 2020, there were 108,303 reported cases with 747 deaths (IR: 40 per 100,000 population); in 2021, 73,518 cases with 705 deaths (IR: 27/100,000); in 2022, 143,266 cases with 1,237 deaths (IR: 52.1/100,000); and in 2023, 114,720 cases with 894 deaths (IR: 41.4/100,000) (Homer *et al*., 2025).

In the Maluku Province, particularly in Ambon City, the number of dengue cases has increased significantly. By June 2021, a total of 81 dengue cases had been recorded, with two fatalities, compared to 41 cases reported in 2020. The highest number of cases was reported in Sirimau District (43 cases), followed by Nusaniwe (18 cases), Baguala (11 cases), South Leitimur (6 cases), and Teluk Ambon (3 cases). The fatalities occurred in the Baguala District in April and June (Sahureka, 2023).

Current prevention efforts have primarily focused on controlling vector populations and preventing mosquito bites, typically through the use of aerosol sprays, mosquito coils, and repellents (Zen & Triana, 2017). However, these approaches often rely on synthetic insecticides, which, when used continuously, may have adverse effects on human health and the environment. Therefore, the use of botanical insecticides derived from plant-based compounds offers a promising alternative to reduce the negative impacts of synthetic chemicals.

The Maluku Islands are characterized by vast and deep marine areas, with a total administrative area of approximately 712,480 km², consisting of 658,295 km² (92%) of marine territory and 54,185 km² (8%) of land area. This geographic condition endows Maluku with rich and abundant marine biodiversity (Ukratalo *et al*., 2023). According to Ward *et al*. (2022), marine biological resources are valuable assets that support ecosystem sustainability and biodiversity conservation. Furthermore, the chemical diversity found in marine organisms constitutes an essential component of this biodiversity, offering opportunities for the discovery of bioactive compounds with pharmaceutical potential (Suleria *et al.,* 2016; Ahmed *et al*., 2022; Ukratalo *et al*., 2025). Marine organisms, recognized for their rich and unique chemical compositions, exhibit a wide range of biological activities (Macedo *et al*., 2021).

Sargassum duplicatum, a species of brown seaweed native to Indonesia, has been identified as a potential source of antioxidants due to its active compounds, including fucoidan and phenolic components (Ukratalo and Lateke, 2023; Ukratalo *et al*., 2024; Kaihena *et al*., 2024; Maatita *et al*., 2024). The primary phenolic compound found in brown seaweed is phlorotannin, which ranges from 0.74% to 5.06% (Zheng *et al.,* 2022). This seaweed also contains flavonoids, tannins, saponins, and terpenoids (Maatita *et al*., 2024). The flavonoids and saponins in *Sargassum duplicatum* are believed to damage larval membranes, inhibit endocrine functions, trigger chemical reactions that disrupt larval metabolism, and impair the respiratory system, ultimately reducing growth and causing mortality in mosquito larvae.

Several studies have explored the biolarvicidal potential of marine-derived natural compounds. For instance, Reegan *et al.,* (2015) reported that the marine sponge *Cliona celata* exhibited significant larvicidal activity. Espiegle *et al.,* (2019) also demonstrated larvicidal effects from sea cucumber (*Holothuroidea*) extracts. Additionally, biolarvicidal activity has been observed in extracts of *Curcuma xanthorrhiza* (temulawak) rhizomes. This study aimed to

evaluate the phytochemical profile and bioactivity of the ethanol extract from *Sargassum duplicatum* as biolarvacidal.

RESEARCH METHODS

Type of Research

This study employed an experimental design with five treatments and three replications, using a post-test-only control group design.

Time and Location of Research

The sampling of *Sargassum duplicatum* was conducted in the conservation area of Pombo Island, Central Maluku. The extraction process was carried out at the Basic Chemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pattimura University. The biolarvicidal activity tests were conducted at the Ecology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Pattimura University, over a period of three weeks.

Experimental Design

This study employed a Completely Randomized Design (CRD) with five treatment groups and three replications. The treatment groups were as follows:

- Group I: No extract treatment (negative control)
- Group II: Treated with 1% Abate (positive control)
- Group III: Treated with 25 ppm ethanol extract of *Sargassum duplicatum*
- Group IV: Treated with 50 ppm ethanol extract of *Sargassum duplicatum*
- Group V: Treated with 75 ppm ethanol extract of *Sargassum duplicatum*

Tools and Materials

The tools used in this study included pipettes, plastic trays, 18 plastic containers, beakers, an analytical balance, spatulas, measuring glasses, test tubes, protective cloth (to prevent adult mosquitoes from escaping), a blender, a loop, glass stirring rods, labeling paper, a rotary evaporator, and knives.

The materials consisted of *Sargassum duplicatum*, ethanol, clean water, 1% Abate, *Aedes aegypti* larvae, fish food, 2N H_2SO_4, 1% $FeCl_3$, 2N HCl, and Mg.

Research Procedures

Mosquito Larvae Preparation

Aedes aegypti eggs were hatched in plastic trays containing approximately 1000 cc of clean water. Before hatching, the water was conditioned by adding food for six hours. The eggs were then placed in the trays and allowed to hatch over a period of 2 - 3 days. The hatched larvae were fed fish food daily and reared until the second instar stage (approximately two days), at which point they were used for the experiment.

Test Material Preparation

1. Sampling of *Sargassum duplicatum*
   Samples of *Sargassum duplicatum*, approximately five months old, were collected from the waters of the Pombo Island Conservation Area, Central Maluku Regency, Maluku Province. The samples were then transported to the laboratory for further processing.
2. Preparation of Simplicia
   The collected samples were thoroughly washed to remove any adhering debris. The algae were dried in a shaded area, protected from direct sunlight. Once dried, the samples were ground using a blender to produce a fine powder, yielding 215 g of *Sargassum duplicatum* powder.
3. Extraction Method
   A total of 150 g of the dried powder was weighed and placed into a flask. Then, 1.5 liters of 95% ethanol were added to completely submerge the powder. The mixture was soaked for 24 hours with occasional stirring, followed by refluxing for 6 hours (Sadasivam, 1996). The refluxed solution was filtered and transferred to another flask, and the residue was re-refluxed using the same method. The combined filtrates were concentrated using a rotary evaporator to obtain a thick extract, yielding 9.26 g of *Sargassum duplicatum* extract.

Phytochemical Screening

The identification of chemical compounds in the *Sargassum duplicatum* extract was performed qualitatively using phytochemical screening methods (Sobuj *et al*., 2024). The compounds tested included flavonoids, steroids, triterpenoids, saponins, tannins, phenols, and alkaloids.

1. Flavonoid Test
   A concentrated extract of *Sargassum duplicatum* (1 mg) was placed into a test tube and dissolved in 1-2 mL of 50% hot methanol. Magnesium metal and 0.5 mL of concentrated HCl were then added. The appearance of a red, yellow, or orange coloration in the solution indicated the presence of flavonoids.
2. Steroid and Triterpenoid Test
   A concentrated extract of *Sargassum duplicatum* (1 mg) was placed into a test tube, dissolved in 0.5 mL chloroform, and mixed with 0.5 mL of anhydrous acetic acid. Subsequently, 1–2 mL of concentrated H_2SO_4 was carefully added along the inner wall of the test tube. The formation of a brown or violet ring at the interface between the two solvents indicated the presence of triterpenoids, whereas a bluish-green coloration in the solution indicated the presence of steroids.
3. Saponin Test
   A concentrated extract of *Sargassum duplicatum* (1 mg) was placed into a test tube, to which 10 mL of distilled water was added and shaken vigorously for 10 minutes. If foam formed, 2-3 drops of 1 N HCl were added. The presence of saponins was confirmed if the foam persisted for at least 10 minutes with a height of 1-3 cm.

4. Tannin Test
   A concentrated extract of *Sargassum duplicatum* (1 mg) was placed into a test tube, followed by the addition of 2–3 drops of 1% $FeCl_3$ solution. The appearance of a dark green or ink-blue coloration indicated the presence of tannins.
5. Phenol Test
   A concentrated extract of *Sargassum duplicatum* (1 mg) was placed into a test tube, and 10 drops of 1% $FeCl_3$ solution were added. The presence of phenols was indicated by the development of green, red, purple, blue, or dark black coloration.
6. Alkaloid Test
   A concentrated extract of *Sargassum duplicatum* (1 mg) was placed into a test tube, followed by the addition of 0.5 mL of 2% HCl. The solution was then divided into two test tubes. To test tube 1, 2-3 drops of Dragendorff's reagent were added, while to test tube 2, 2-3 drops of Mayer's reagent were added. The presence of alkaloids was confirmed by the formation of an orange precipitate in test tube 1 and a yellowish precipitate in test tube 2.

Bio-Larvicidal Activity Testing

A total of 360 third-instar *Aedes aegypti* larvae were distributed into containers, each containing a different concentration of the ethanol extract of *Sargassum duplicatum*. Each container received 20 larvae. Each treatment was performed in triplicate. Time measurement began immediately after the larvae were introduced into the containers. The observation was conducted for 24 hours

Data Collection

Data were collected by recording the number of dead larvae in each container. Mortality was observed and recorded in a tabular format. Dead larvae were identified as those that sank to the bottom of the container, showed no movement, were separated from the active larvae, and did not respond to stimuli.

The larval mortality rate of *A. aegypti* was calculated using the following formula (Rumasukun *et al.,* 2025):

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae tested}} \times 100\%$$

Data Analysis

The observational data were analyzed using Analysis of Variance (ANOVA) in the SPSS software. If significant differences were found, post hoc testing was conducted using the Least Significant Difference (LSD) test at a 0.05 significance level. Probit regression analysis was employed to determine LC_{50} and LC_{90} values Moniharapon & Ukratalo, 2021).

RESULTS AND DISCUSSION

Phytochemical Screening of *Sargassum duplicatum* Extract

The identification of secondary metabolite compounds was carried out qualitatively using phytochemical screening to determine the classes of bioactive compounds present in the *Sargassum duplicatum* extract (Kaihena *et al*., 2024). The phytochemical analysis was performed using the test tube method, where a small amount of the extract was treated with specific reagents targeting each type of compound. The qualitative results of the secondary metabolite analysis of *Sargassum duplicatum* extract are presented in Table 1.

Table 1. Phytochemical screening results of secondary metabolites in *Sargassum duplicatum* extract

No.	Compound Class	Color / Precipitate Formed	Result
1	Flavonoids	Bluish green	++
2	Steroids	Red	-
3	Triterpenoids	Green	-
4	Saponins	Green and foamy	+
5	Tannins	Green	+
6	Phenols	Dark green	-
	Alkaloid		
7	a. Mayer	Yellowish white precipitate	+
	b. Dragendroff	Orange precipitate	+

Notes:
++ : strongly positive (distinct color)
+ : positive (faint color)
- : not detected

The results shown in Table 1 indicate that *Sargassum duplicatum* extract tested positive for flavonoids, saponins, tannins, and alkaloids.

Flavonoids are phenolic compounds consisting of two aromatic rings with multiple hydroxyl groups. The greater the number of hydroxyl groups, the more easily the compound dissolves in polar solvents (Dias *et al*., 2021). According to Putra *et al*. (2016), flavonoids in plants are involved in photosynthesis and possess antimicrobial and antiviral properties. Certain flavonoid compounds also exhibit antioxidant activity and function as antihemorrhagic and antiscorbutic agents. In humans, flavonoids act as natural antibiotics, particularly in the treatment of cancer and kidney disorders. Some flavonoids, such as silymarin and silybin, have been shown to support liver function and inhibit prostaglandin synthesis, thereby serving as hepatoprotective agents. Flavonoids also help reduce blood clot formation. At low doses, flavones act as cardiac stimulants in humans, while hydroxylated flavones may function as diuretics and lipid antioxidants.

According to Wahid and Safwan (2020), saponins are surface-active compounds that can be easily identified by their ability to produce foam. The glycosidic bonds present in saponins make them relatively polar (Sangi *et al*., 2019). Saponins can promote tissue regeneration and re-epithelialization due to their immunostimulatory properties, which trigger the host immune response.

The phytochemical test for tannins in *Sargassum duplicatum* extract also yielded positive results. Ferric chloride ($FeCl_3$) was used to determine the presence of phenolic groups. A positive result is indicated by a greenish-black or dark blue color upon the addition of $FeCl_3$, suggesting the presence of phenolic compounds, likely tannins, which are polyphenolic. Nagori *et al.,* (2025) explained that the classic method for detecting simple phenols involves adding a 1% $FeCl_3$ aqueous solution, which induces intense green, red, purple, blue, or black coloration. These findings align with those of Putra *et al*. (2016), who reported the formation of a greenish or bluish-black color due to the complexation of tannins with Fe^{3+} ions.

Alkaloids tested with Mayer's reagent produced a yellowish-white precipitate. The addition of hydrochloric acid is intended to extract basic alkaloid compounds into the acidic aqueous phase (Wahid and Safwan, 2020). The presence of precipitate in the screening confirms the presence of alkaloids. Alkaloids are among the most abundant organic compounds found in nature, with most being derived from plants. Alkaloids typically contain at least one basic nitrogen atom as part of a heterocyclic ring structure. Based on the heterocyclic nitrogen configuration, alkaloids can be classified into several types, including pyrrolidine, piperidine, isoquinoline, quinoline, and indole alkaloids (Mahmiah *et al*., 2023).

Average Mortality of *Aedes aegypti* Larvae

The calculated average mortality rates of *Aedes aegypti* larvae in the control group, 1% abate treatment, and various concentrations (25 ppm, 50 ppm, and 75 ppm) of the brown algae (*Sargassum duplicatum*) extract are presented in Table 2.

Table 1**.** Average mortality of *Aedes aegypti* larvae for each treatment group

Treatment	Average Mortality (%) ± SD
Control	0.00 ± 0.00^{a}
Abate 1%	100.00 ± 4.52^{b}
25 ppm extract	68.33 ± 5.40^{c}
50 ppm extract	91.67 ± 6.71^{d}
75 ppm extract	100.00 ± 6.91^{e}

Note: Superscript letters denote significant differences (P > 0.05) among treatment groups.

As shown in Table 2, the control group (untreated with either Abate or extract) exhibited no larval mortality, indicating that external factors did not influence natural death. The 1% abate treatment resulted in complete mortality (100%), demonstrating its high efficacy as a chemical larvicide. Conversely, treatments using *Sargassum duplicatum* extract showed a concentration-dependent increase in larvicidal activity. Mortality rates were 68.33% at 25 ppm, increased to 91.67% at 50 ppm, and reached 100% at 75 ppm. These findings suggest that the algae extract has potential as a natural larvicide, particularly at higher concentrations, which may match the efficacy of 1% Abate.

One-way analysis of variance (ANOVA), performed using SPSS version 24.0, revealed that administration of the ethanolic extract of *Sargassum duplicatum* significantly affected the average larval mortality of *Aedes aegypti*. Post hoc analysis using the Least Significant Difference (LSD) test confirmed significant differences among all treatment groups. The ANOVA

results also indicated that neither the extract concentration nor the duration of exposure significantly influenced the average larval mortality (F_calculated > F_table).

According to Table 2, no mortality was observed in the control group (untreated). In the 1% abate group, larval mortality reached 18.46 ± 5.40. Observations showed that abate began to kill larvae within 5 minutes of exposure, with maximum effectiveness reached at 39 minutes post-treatment. Abate (temephos) is an organophosphate pesticide (Khaer *et al*., 2021) that works by inhibiting cholinesterase enzymes. This inhibition leads to the accumulation of acetylcholine at the synaptic junction, disrupting nerve activity and causing prolonged muscle contraction, ultimately resulting in convulsions (Marisa and Pratuna, 2018).

When the insect nervous system receives a stimulus, acetylcholine is released, acting as a neurotransmitter between the nerve and muscle tissue. After muscle contraction, acetylcholine is usually broken down by acetylcholinesterase into choline, lactate, and water. If acetylcholine is not broken down promptly, sustained muscle contraction occurs, leading to convulsions. Organophosphate pesticides, such as Abate, inhibit cholinesterase, causing continuous muscle spasms that ultimately result in insect death. Thus, like other organophosphates, abate exhibits anticholinesterase activity (Putri *et al*., 2023).

According to Reyes-Chaparro *et al.,* (2020), temephos undergoes metabolic conversion in the animal body, where the phosphorothioate group (P=S) is transformed into phosphate (P=O), which has a more substantial anticholinesterase effect. *Aedes aegypti* larvae can rapidly convert P=S to P=O esters. Over 99% of temephos present in the medium is absorbed within one hour after exposure. Once absorbed, abate is metabolized, and part of the resulting metabolites is excreted into the surrounding water (Dumeva *et al*., 2016). The rapid penetration of Abate causes acute organophosphate poisoning symptoms in insects, such as restlessness, hyperexcitation, tremors, convulsions, and ultimately, muscle paralysis. In mosquito larvae, death occurs due to an inability to breathe.

Observation of the group treated with 25 ppm ethanolic extract of *Sargassum duplicatum* revealed no mortality during the initial 0-8 hours. Mortality began at hour 10, with 0.33 individuals affected, and by 24 hours, the average mortality reached 13.67 individuals (68.33%). In the 50 ppm group, no mortality was observed during the first 2 hours. Mortality commenced at hour 4 (0.67 individuals on average), increasing to 18.33 individuals (91.67%) by hour 24. For the 75 ppm group, mortality was recorded as early as hour 2 (2.67 individuals), reaching complete mortality (100%) at hour 24.

Larval mortality in each treatment group increased with the extract concentration. These results are consistent with Jodynis-Liebert and Kujawska (2020), who stated that lower extract concentrations exert weaker effects, while higher concentrations lead to more substantial biological impacts due to the dose-dependent nature of chemical action. Aseptianova (2019) similarly noted that the potential toxicity or safety of a compound is determined by the relationship between chemical dose and its biological effect. Additionally, the interaction between toxic substances and biological systems is directly proportional to the concentration of the poisonous compound. Further emphasized that increased extract concentrations lead to a higher content of active ingredients, enhancing pesticidal efficacy and resulting in greater larval mortality.

Larval mortality caused by the ethanolic extract of *Sargassum duplicatum* in this study is attributed to its bioactive compounds, including flavonoids, saponins, tannins, and alkaloids, which act as larvicides. These compounds function as stomach poisons, entering the larvae's body through ingestion. Flavonoids, known for their insecticidal properties, target the nervous system and impair vital functions such as respiration, ultimately leading to death (Pereira *et al,* 2024). They are thought to inhibit mitochondrial respiration by disrupting electron transport, thereby affecting energy metabolism

Saponins act both as contact and stomach poisons, penetrating the larval cuticle or entering through the digestive tract (Rahmaddiansyah *et al.,* 2024). Their detergent-like nature enhances toxin absorption by emulsifying lipophilic substances, allowing for more effective removal. Saponins also irritate the digestive system, particularly the midgut, which is responsible for nutrient absorption and the secretion of enzymes. They disrupt the midgut's peritrophic membrane, cause swelling, and separate epithelial cells, leading to digestive tract damage and eventual larval deathc (Zhang *et al.,* 2024). Saponins are also suspected to act as respiratory poisons. When the larvae become weakened due to poisoning, either through direct contact or ingestion their ability to close the spiracles while diving is reduced. This decreased ability allows water to enter the spiracles. The larvae's uncontrolled movement further accelerates water entry. The presence of water in the respiratory tract obstructs respiration, ultimately leading to larval death due to oxygen deficiency. Under normal conditions, mosquito larvae keep their spiracles closed and only open them when exchanging gases with their environment.

Tannins are known to reduce the activity of protease enzymes involved in the conversion of amino acids. As a result, cellular metabolic processes in larvae are disrupted, leading to nutritional deficiencies. In addition, tannins bind to digestive proteins required for larval growth (Huang *et al.,* 2022).

Alkaloids exert their effects by suppressing larval feeding behavior and acting as stomach poisons (Ukoroije & Otayor, 2020). They are believed to inhibit acetylcholine activity, causing its accumulation, which subsequently disrupts nerve impulse transmission to muscle cells. This disturbance induces convulsions, followed by paralysis and ultimately death of *Aedes aegypti* larvae.

Determination of Lethal Concentration 50 (LC_{50}) and Lethal Concentration 90 (LC_{90})

The larvicidal activity of the ethanolic extract of *Sargassum duplicatum* against *Aedes aegypti* larvae was quantified using LC_{50} and LC_{90} values, which represent the concentrations required to kill 50% and 90% of the larval population, respectively. These values were determined through probit analysis, and the results are presented in Table 3.

Table 3. Probit Analysis Results

Probability	95% Confidence Limits for Treatment		
	Estimate	Lower Bound	Upper Bound
50	9,098	7,162	12,444
90	20.485	13,935	30,874

According to the probit analysis presented in Table 3, the LC_{50} value of the ethanolic extract of *Sargassum duplicatum* was 9.098 ppm, with a 95% confidence interval ranging from 7.162 to 12.444 ppm. The LC_{90} value was determined to be 20.485 ppm, with a confidence interval of 13.935 to 30.874 ppm. These results indicate that the extract is capable of causing 50% mortality in *Aedes aegypti* larvae at a concentration of 9.098 ppm and 90% mortality at 20.485 ppm.

LC_{50} and LC_{90} are widely used parameters in toxicity testing, representing the concentrations of an insecticide required to cause 50% and 90% mortality in the test insect population, respectively. Lower LC_{50} or LC_{90} values indicate higher toxicity, whereas higher values indicate lower toxicity. The plant-based insecticidal treatment in this study exhibited toxic effects, with increasing concentrations resulting in higher larval mortality rates.

Insecticides may enter the insect's body through direct contact, walking across treated surfaces, or ingestion of contaminated plant material. Contact insecticides primarily enter through the insect's cuticle, whereas ingested insecticides are absorbed via the digestive tract. While multiple routes of entry exist, contact insecticides rely predominantly on cuticular penetration.

Based on the findings in Table 3, the LC_{50} and LC_{90} values required to cause 50% and 90% mortality in *Aedes aegypti* larvae after 24 hours of exposure were 9.098 ppm and 20.485 ppm, respectively. The LC_{50} value was selected as a benchmark for assessing the larvicidal potency of the *Sargassum duplicatum* extract. Since LC_{50} and LC_{90} are standard parameters for evaluating insecticidal efficacy, the lower the values, the higher the toxicity of the extract.

Several factors can influence LC_{50} and LC_{90} values, including species, strain, sex, age, body weight, nutritional status, and gut content of the test organisms. Additionally, technical aspects of administration such as application time, ambient temperature, humidity, and air circulation, can also impact the outcome.

CONCLUSION

The ethanolic extract of the brown algae *Sargassum duplicatum* was confirmed to contain flavonoids, saponins, tannins, and alkaloids. The extract exhibited increasing larvicidal activity against *Aedes aegypti* larvae with higher concentrations, with the 75 ppm concentration showing comparable efficacy to 1% Abate. The LC_{50} and LC_{90} values, representing the concentrations required to cause 50% and 90% larval mortality after 24 hours of exposure, were determined to be 9.098 ppm and 20.485 ppm, respectively.

DECLARATIONS

N. P and N. A. P designed and conducted the study, analyzed the data, and wrote the manuscript.

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Declaration of Interest

The author declares no conflict of interest related to this work.

Data Sharing Statement

The data used in this research are available and can be accessed by interested parties upon reasonable request and in accordance with established procedures.

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